Efficacy, safety and pharmacokinetics of dihydroartemisinin-piperaquine for perennial malaria chemoprevention in infants during routine health facility visits

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DECLARATION

I, Clifford George Banda, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work, nor any part of it, has been, is being, or is to be submitted for another degree in this or any other university. I authorise the University to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

I hereby declare that this thesis includes two journal manuscripts. One of the two manuscripts (Chapter 2) included in this thesis has been published in the British Journal of Clinical Pharmacology. The second manuscript (Chapter 3) has been submitted to Wellcome Open Research and is undergoing open peer-review. As the leading author, I conceptualised the ideas in these manuscripts, wrote the first drafts, and revised the manuscripts with inputs from all co-authors for all included publications. I provided responses to journal reviewers and revised the manuscripts accordingly.

I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publications in my PhD thesis, and where coauthorships are involved, my co-authors have agreed that I may include the publications:

1. **Banda CG**, Tarning J, Barnes KI. Use of population pharmacokinetic-pharmacodynamic modelling to inform antimalarial dose optimization in infants. Br J Clin Pharmacol. 2025 Apr;91(4):968-980. doi: 10.1111/bcp.16132. Epub 2024 Jun 10. PMID: 38858224; PMCID: PMC11992656 (**Chapter 2 of thesis**)
2. **Banda CG**, Kantonya MS, Munharo S *et al.* 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: 1

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2024, **9**:291 (https://doi.org/10.12688/wellcomeopenres.20355.1) (**Chapter 3 of thesis**)

ABBREVIATIONS

ACT	Artemisinin-based combination therapy
AE	Adverse event
AS	Artesunate
AQ	Amodiaquine
AUC	Area under the concentration-time curve
aIRR	Adjusted incidence rate ratio
$C_{day\ 7}$	Day 7 drug concentrations
$C_{day\ 28}$	Day 28 drug concentrations
CD	Chlorproguanil-dapsone
CL/F	Apparent clearance
C_{max}	Maximum drug concentration
DP	Dihydroartemisinin-piperaquine
DSMB	Data and safety monitoring board
IPTi	Intermittent preventive treatment of malaria in infants
IRS	Indoor residual spraying
ITN	Insecticide treated bednets
PBPK	Physiologically-based pharmacokinetic modelling
PMC	Perennial malaria chemoprevention
PD	Pharmacodynamics
PK	Pharmacokinetics
qPCR	Quantitative polymerase chain reaction
RR	Relative risk
SMC	Seasonal malaria chemoprevention
SP	Sulfadoxine-pyrimethamine
TS	Trimethoprim-sulfamethoxazole
WHO	World Health Organisation

Vd	Volume of distribution

ABSTRACT

Introduction

Monthly dihydroartemisinin-piperaquine for perennial malaria chemoprevention (PMC) has been shown to offer superior malaria prevention compared to three-monthly dosing. However, aligning its administration with routine health facility visits has the potential to enhance adherence. Additionally, rapid physiological changes in infancy may influence drug exposure. We hypothesised that increasing age in infancy would result in a reduction in piperaquine's exposure due to age-related increases in apparent clearance (CL/F) and volume of distribution (Vd). Consequently, this could impact the protective efficacy of dihydroartemisinin-piperaquine for malaria prevention in infants.

Methods

We assessed the efficacy and safety of dihydroartemisinin-piperaquine and piperaquine's exposure profile in Malawian infants receiving PMC aligned with routine health facility visits at 10 weeks, 14 weeks, 6 months, and 9 months of age, within a setting where the RTS,S/AS01 malaria vaccine became part of the standard of care. In a randomised, single-blind, placebo-controlled trial, infants were allocated to receive a three-day course of dihydroartemisinin-piperaquine (n = 110) or placebo (n = 110) starting at 10 weeks of age. Malaria incidence and adverse events were tracked until 12 months of age. In the dihydroartemisinin-piperaquine arm, piperaquine capillary blood samples were collected pre-dose and on days 3, 7, 14, and 28 following the 10-week and 6-month or 14-week and 9-month treatment courses. Nonlinear mixed-effects modelling was used to derive piperaquine's pharmacokinetic parameters. Geometric means (GM) and 90% confidence intervals (CI) were compared between 10 weeks and 14 weeks, 6 months or 9 months.

Results

Malaria incidence was 1 versus 4 cases per 1,000 person-years (0.001 and 0.004 episodes

per person-year) in the DP and placebo arms, respectively, corresponding to a non-significant 77% reduction with dihydroartemisinin-piperaquine (adjusted incidence rate ratio 0.23; 95% CI: 0.03 - 2.11; p = 0.196). All cases occurred within the first seven months, when nearly 50% of infants had received at least one malaria vaccine dose. Additionally, DP was associated with a 31% reduction in the prevalence of moderate–to–severe anaemia (18.3% and 12.5% in the DP and placebo arms, respectively; adjusted risk ratio 0.69, 95% CI 0.52– 0.93, p = 0.013). The frequency of adverse events was similar between groups (149 versus 134). At 9 months versus 10 weeks of age, piperaquine exposure was 58% lower (GM ratio 0.42; 90% CI 0.36-0.49) with corresponding CL/F and Vd that were 2.6 (GM ratio 2.61; 90% CI 2.52 – 2.70) and 3.2-fold (GM ratio 3.24; 90% CI 3.01–3.49) higher, respectively.

Conclusion

Dihydroartemisinin-piperaquine administration during routine visits, in the context of ongoing malaria vaccination, was safe and showed a non-significant trend towards reduced malaria incidence in early infancy. However, age-related declines in piperaquine exposure indicate the need for dosage regimen optimisation of this promising antimalarial to ensure effective malaria chemoprevention in older infants.

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I extend my sincere gratitude to my family and friends for their moral support.

This work would not have been possible without the participation of the women and infants who were keen to spend several hours at the study clinic.

Several people, detailed below, contributed to the clinical trial, which is the basis of this PhD thesis (as reported in Chapters 3 to 5):

- Dr Clifford George Banda, Prof. Joel Tarning, and Prof. Karen Barnes designed the study, developed the study protocol, and led the data analysis and interpretation of the findings.
- Dr Mphatso S Kantonya, Ms Marumbo E Chirwa, Ms Mayamiko D Kapulula, Mr Steven Munharo, Ms Hellen D. Chavula, and Mr Aubrey Chiyana supported sample and data collection, as well as the daily implementation of the study, led by Dr Clifford G. Banda.
- Mr Vincent Katunga-Phiri and Ms Diksha Patel coordinated data management for the study.
- Mr Vusumuzi Katangwe was responsible for the storage and quality control of the investigational products used in the clinical trial.
- Dr Elizabeth Allen supported the training of the study team and internal quality assurance of the study.

- Prof. Feiko ter Kuile, Prof. Victor Mwapasa, and Dr Dianne J. Terlouw supported the study's administrative activities and the infrastructure under which the clinical trial was conducted.
- Prof. Kamija S. Phiri provided coordination to obtain some of the investigational product (placebo) for the clinical trial.
- Dr Karl Seydel assisted with quantitative PCR of malaria dry blood spot samples in Malawi.
- The Pharmacology Laboratory at the Mahidol-Oxford Tropical Medicine Research Unit (MORU) in Thailand assisted with the assay of blood samples for piperaquine concentrations, under the leadership of Prof. Joel Tarning.

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CHAPTER 1

Introduction

Context

Malaria in infants and strategies to mitigate the burden

Sub-Saharan Africa is home to 94% of all malaria cases and 95% of all malaria-related deaths.[1] In this mostly moderate-high malaria transmission setting, 76% of the burden is seen in under-five children,[1] 20-36% of it falls on infants,[2] and *Plasmodium falciparum* malaria predominates. In infancy, the burden of malaria is highly dynamic, with the risk varying substantially across these first 12 months of life. Evidence consistently shows that the incidence of clinical malaria is relatively low in the first 3 months of life and increases progressively thereafter.[3,4] Several complementary mechanisms and behaviours contribute to this early period of reduced susceptibility. First, there are transplacentally acquired maternal antibodies, especially IgG targeting *Plasmodium falciparum* antigens, which confer partial protection that wanes rapidly over the first few months of life (Figure 1). [5,6] Second, there is reduced exposure to infectious mosquito bites as infants are less mobile in the first months of life and spend less amount of time outdoors. Third, there is seasonal, age, time-dependent and transmission intensity variation in malaria incidence. Infants born in different seasons may have varied exposure risk.[7] As a result, malaria incidence typically remains lowest in the first 2 - 3 months and increases sharply from approximately 4 - 6 months onward, coinciding with waning maternal immunity and behavioural changes (e.g., crawling, spending more time outdoors). Peak vulnerability often occurs between 6 - 12 months of age (Figure 1), when infants have high exposure but still immature immunity, making this a critical window to target preventive interventions.

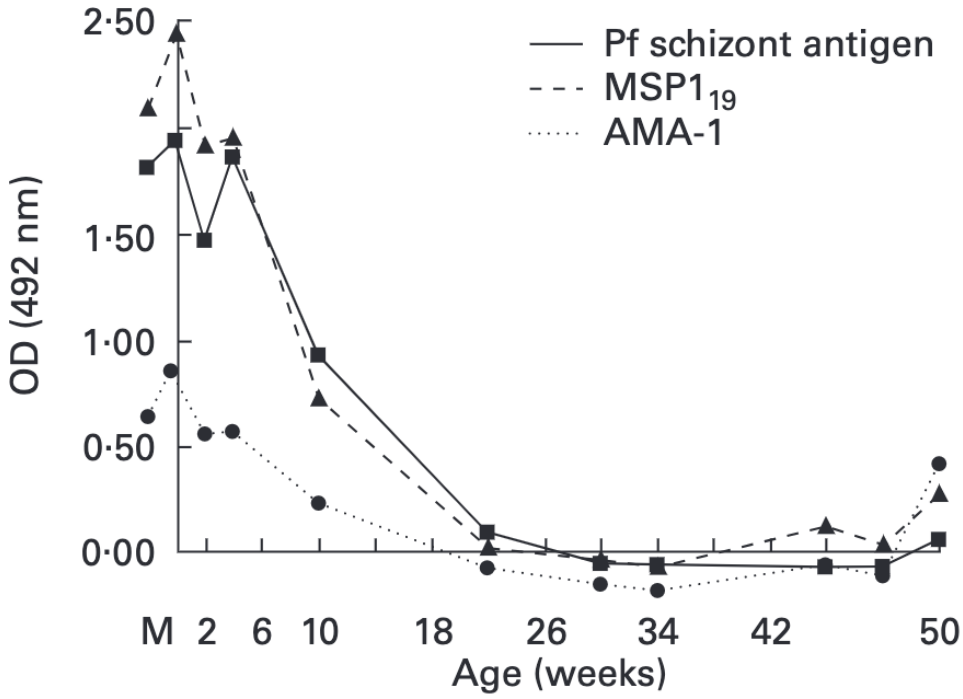

Figure 1: Decay kinetics of maternally derived IgG antibodies against three *Plasmodium falciparum* blood-stage antigens: AMA-1 and MSP1 (merozoite surface antigens), and a *Plasmodium falciparum* schizont extract antigen. Adapted from: Riley EM et al. *Parasite Immunol.*2001; 23(1):51-59

To mitigate the impact of malaria in the subpopulation of infants, the World Health Organisation (WHO) has, since 2010, highlighted the need to protect infants from malaria in areas of moderate to high malaria transmission in sub-Saharan Africa.[8] Initially, the WHO recommended the use of sulfadoxine-pyrimethamine (SP), administered at the time of routine immunisation visits, for intermittent preventive treatment of malaria in infancy (IPTi) [8]. However, although recommended by the WHO for over a decade, IPTi had limited uptake by most malaria-endemic countries,[9] in part due to concerns about the increasing resistance of malaria parasites to SP.[10,11]

Fortunately, monthly administration of a newer antimalarial, dihydroartemisinin-piperaquine (DP), an artemisinin-based combination therapy (ACT), has shown higher protective efficacy

than monthly SP. [12,13] However, implementing a monthly regimen for malaria chemoprevention is logistically challenging in programmatic settings.[14,15]

Recently, the WHO's recommendation has been revised to extend malaria protective treatment to the second year of life.[16] The intervention is now called perennial malaria chemoprevention (PMC). While acknowledging the potential for ACTs, such as DP, for malaria chemoprevention in young children, the WHO has called for more evidence of their safety, efficacy, and adherence to multi-day PMC regimens, and when administered during routine health facility visits.[17] Unfortunately, there is limited evidence to guide its optimal dosing in infants and inform PMC policy changes. Therefore, there is an urgent need to understand how best to use this promising antimalarial medication, DP, for this purpose in infants.

Malaria chemopreventive treatment in the context of other preventive strategies in moderate-to-high transmission settings

A multipronged approach to malaria control is crucial for reducing both morbidity and mortality. Preventive malaria treatment plays a critical role, particularly among vulnerable populations such as pregnant women, infants and young children. However, there are other preventive strategies that countries in sub-Saharan Africa have employed, including:

- Vector control strategies such as insecticide-treated nets (ITNs) and indoor residual spraying (IRS). Long-lasting insecticidal nets remain one of the most cost-effective interventions. In high-transmission settings, universal coverage campaigns and continuous distribution through antenatal and child health platforms are critical and have led to significant reductions in malaria incidence and mortality (68% of cases averted between 2000 and 2015).[18] Similarly, IRS has been effectively used in targeted regions to reduce indoor-resting mosquito populations and interrupt transmission in some malaria-endemic countries.[19] However, high operational costs

(particularly for IRS) and insecticide resistance limit its effectiveness. When used in combination with ITNs, IRS may have a synergistic effect.[20]

- Malaria vaccination. The RTS, S/AS01 and R21/Matrix-M malaria vaccines, which the WHO now recommends, have demonstrated efficacy against malaria (approximately 30% reduction in severe malaria for RTS, S/AS01[21] and 75% reduction in clinical malaria in the case of R21/Matrix-M[22]). Their use with other malaria preventive strategies, such as ITNs and chemoprevention, has the potential to provide additional protection.[21] Pilot malaria vaccine implementation in Ghana, Malawi, and Kenya has demonstrated its safety and operational feasibility.[23] In Malawi, the RTS, S/AS01 vaccine has recently been rolled out for use in areas with moderate to high malaria transmission.

Considering the various existing malaria preventive strategies and the promise of ACTs for chemoprevention, layering interventions, such as combining PMC with ITNs, vaccination, and case management, could potentially yield greater impact than any single strategy alone in moderate-to-high transmission settings, as suggested in a modelling study.[24] This has proven true for seasonal malaria chemoprophylaxis and the RTS, S/AS01 malaria vaccine, where concurrent use was 57% and 59% more protective against malaria than SMC or the malaria vaccine alone, respectively.[25,26] However, there is a paucity of evidence on the combined effect of malaria vaccines and PMC in programmatic settings. Additionally, there would be a need for service integration to align PMC with routine health facility visits.

This thesis will, in part, explore some of these questions in Malawi, where the RTS, S/AS01 malaria vaccine has recently been rolled out, and PMC with ACTs is being considered in infants.

The intersection between malaria prevention in infancy and immunological development

Naturally acquired immunity (NAI) to *Plasmodium falciparum* is acquired through repeated exposure to the blood-stage parasite; however, in early infancy, it is minimal. The timing of first parasite encounters and subsequent infections can influence both the magnitude and character of the immune response. A central concern when considering interventions such as chemoprevention in infancy is that suppressing early parasite exposure may impair immunological priming, potentially increasing susceptibility later. However, evidence suggests that chemoprevention does not necessarily blunt long-term immunity; antibody responses still develop despite chemoprevention,[27] and age at first malaria exposure may not critically determine immune acquisition rate.[28] This implies that preventing malaria in early life in infancy does not diminish the ability to acquire immunity against malaria in older children.

Concerns about antimalarial resistance when using ACTs for chemopreventive treatment in children

Despite the promise that ACTs hold for PMC, concerns exist that their use for chemoprevention may fuel the development of antimalarial resistance. Some argue that repeated use of ACTs in asymptomatic individuals may expose a larger parasite biomass to sub-therapeutic drug concentrations, thereby favouring the selection of resistant strains, a process partly driven by tolerance.[29] Additionally, if resistance emerges due to the use of preventive ACT, first-line treatment options may be compromised, especially in regions with limited alternatives.[30] However, symptomatic infections with high parasite numbers are the most likely source of resistance emergence, rather than asymptomatic infections.[31] When used for preventive treatment, ACTs are administered in treatment doses, and the artemisinin component eliminates initial, low-density, asymptomatic parasitaemia while providing long-term antimalarial suppression for sustained protection (post-treatment prophylaxis) through the longer-acting component.[32] This reduces the number of symptomatic malaria cases and

transmission in a population.[31] Thus, the use of ACTs may actually reduce the threat of antimalarial resistance emerging and spreading in treated populations.

Efficacy, safety and pharmacokinetics of ACTs for PMC in infants.

Despite growing evidence supporting the use of ACTs, such as DP, for malaria chemoprevention in children (for SMC or PMC), data remain limited regarding their efficacy, pharmacokinetics, safety, and optimal dosing schedules in infants.[11,17] Table 1 highlights selected studies on ACTs for PMC in children under five years of age.[12,13,33–35] Notably, only two studies have utilised ACTs for PMC in infants under six months of age,[34,35] and only one study has investigated the alignment of PMC with routine immunisation visits[34] (Table 1). Additionally, although monthly administration has demonstrated high efficacy,[12,13,33,35] concerns exist about feasibility, coverage and adherence, particularly when antimalarial dosing is not aligned with routine infant healthcare visits, such as those scheduled for immunisations [14,15] This highlights the need for integrated and practical dosing strategies of ACTs for PMC aligned with routine health facility visits to improve uptake and effectiveness.

Rationale and key research questions

Given the promising impact of malaria chemopreventive treatment, especially DP, there is a need to understand how the dosing of such interventions, when administration is aligned with routine health facility visits, can be optimised in a complex subpopulation that bears a significant disease burden. This thesis presents evidence that will help inform the treatment guidelines applicable to areas of moderate to high malaria transmission.

Table 1: Overview of clinical trials investigating use of artemisinin-based combination therapies (ACTs) for perennial malaria chemoprevention (PMC) in under-five children

Study	Country	Age	Indication	ACT used	Dosing schedule	Notes
Bigira V, 2014	Uganda	6 - 24 months	PMC	C: No chemoprevention	Not applicable	Monthly DP was efficacious and safe
				T: TS	Daily TS	
				T: SP	Monthly SP	
				T: DP	Monthly DP	
Kamya MR, 2014	Uganda	6 - 24 months	PMC (in HIV exposed children)	C: No chemoprevention	Not applicable	Monthly DP was efficacious and safe
				T: TS	Daily TS	
				T: SP	Monthly SP	
				T: DP	Monthly DP	
Sundell K, 2015	Uganda	6 - 24 months	PMC	C: No chemoprevention	Not applicable	Monthly DP was efficacious and safe
				T: DP	Monthly DP	
Odhiambo F, 2010	Kenya	2.5 - 12 months	PMC (at routine immunisation visits)	C: Placebo	10 weeks, 14 weeks and 9 months	Long-acting regimens (SP + AS and AS + AQ) provide protection against clinical malaria for up to 8 weeks
				T: SP + AS	10 weeks, 14 weeks and 9 months	
				T: AS + AQ	10 weeks, 14 weeks and 9 months	
				T: CD	10 weeks, 14 weeks and 9 months	
Muhindo MK, 2019	Uganda	2 - 24 months	PMC	C: DP	12 weekly	4 weekly DP was superior to 12 weekly DP
				T: DP	4 weekly	

Abbreviations: SMC, seasonal malaria chemoprevention; C, control; T, treatment group; SP, sulfadoxine-pyrimethamine; AS, artesunate; AQ, amodiaquine; TS, trimethoprim-sulfamethoxazole; DP, dihydroartemisinin-piperaquine; CD, chlorproguanil-dapsone

The thesis aims to address the following questions:

1. How can we best optimise the design of studies to understand antimalarial exposure in infants and generate data that informs the development of dosing regimens of ACTs for malaria preventive treatment?
2. What is the efficacy, safety and tolerability of DP given for perennial malaria chemoprevention when administration is aligned with routine health facility visits
3. What is the exposure profile of piperaquine in infants following administration of DP for perennial malaria chemoprevention during routine health facility visits, and does this change with increasing age from 10 weeks to 9 months?

Hypothesis and objectives of the PhD work

We hypothesised that physiological changes associated with increasing age in infancy (such as the maturation of CYP3A4 enzymes involved in piperaquine metabolism and an increase in fat-to-water body ratio that expands the volume of distribution for lipophilic drugs such as piperaquine in early infants) would result in increased piperaquine clearance and volume of distribution, subsequently resulting in a reduction in overall piperaquine exposure, despite weight-based dosing. This would inform whether optimisation of the DP dosing regimen for perennial malaria chemoprevention in infants is needed when dosing is aligned with routine health facility visits.

The above research questions and hypothesis will be addressed in this thesis through the following objectives:

1. To review the literature on the use of pharmacokinetic-pharmacodynamic modelling as a tool to optimise the design of studies and analysis of data to inform antimalarial dose optimisation in infants, in whom intensive blood sampling is generally not considered acceptable (**Chapter 2**).
2. To design a clinical trial aimed at collecting the efficacy, safety and sparse (as opposed to intense) pharmacokinetic data on the promising antimalarial DP, to help

inform its dosing in infants when administration for perennial malaria chemoprevention aligned with routine health facility visits (**Chapter 3**)

3. To describe the efficacy, safety and tolerability of DP for perennial malaria chemoprevention administered in infants during routine health facility visits (**Chapter 4**)
4. To evaluate the pharmacokinetic exposure profile of the long-acting partner drug, piperaquine, in infants following DP for perennial malaria chemoprevention during routine health facility visits between 10 weeks and 9 months of age (**Chapter 5**)

Brief description of the PhD clinical research project and setting

A pharmacokinetic, randomised, placebo-controlled, single-blinded study site was set up at a district hospital in Southern Malawi to investigate the efficacy, safety and pharmacokinetics of DP for perennial malaria chemoprevention in infants when dosing is aligned with routine health facility visits. In both the intervention and control arms, infants received DP or placebo, respectively, at routine immunisation or health facility visits (at 10 weeks, 14 weeks, 6 months, and 9 months), within a setting where the RTS,S/AS01 malaria vaccine became part of the standard of care shortly after study initiation and was administered at 5, 6 and 7 months of age.

Malawi is one of the sub-Saharan African countries which has areas of moderate-high malaria transmission. The clinical study for this PhD work was conducted in the under-five/vaccination clinic at Chikwawa District Hospital in the southern lower-shire region of Malawi. Chikwawa District (Figure 2) is in the Rift Valley, at an elevation of 500 meters above sea level. *Plasmodium falciparum* malaria is endemic in the area, with perennial transmission of the disease.[36] The hospital's catchment area is approximately 20 square kilometres in size, with a population of around 50,000, and is demarcated by natural borders and a declining population migration rate. It is bordered in the northeast by the Rift Valley escarpment, in the west by the Shire River, and in the south by the Nchalo sugar plantation and Lengwe National Park. This

district hospital and research site (Figure 3) were therefore selected because they are in an area of moderate-to-high malaria transmission, and its natural borders aided with participant follow-up.

Coherence of the thesis

The coherence of the thesis is demonstrated by having a single theme: understanding the efficacy, safety, and pharmacokinetics of an antimalarial medication in a complex subpopulation of infants to help inform dosage regimen optimisation in a subpopulation that is often excluded from clinical trials and particularly pharmacokinetic studies yet bears a considerable burden of malaria disease. It begins with describing the challenges associated with antimalarial dose optimisation in this subgroup, the role of pharmacokinetic-pharmacodynamic modelling as a tool to optimise design of clinical studies where only sparse sampling is feasible and analysis of data in this subgroup. A highlight is made for the need for minimal data from well-designed pharmacokinetic-pharmacodynamic studies to inform dosage regimen optimisation (**Chapter 2**). This is built on in subsequent chapters to include a clinical trial methodology that focuses on generating data to help inform dosage regimen optimisation (**Chapter 3**), efficacy and safety findings of a promising antimalarial for malaria preventive treatment in infancy (**Chapter 4**) and exposure profile of a long-acting antimalarial drug, piperaquine, following administration of DP for PMC during routine health facility visits in infants (**Chapters 5**). In **Chapter 6**, additional planned analysis from data generated in the clinical trial is discussed.

I have led all the work described in the thesis. This includes serving as the lead investigator in the design and conduct of the thesis project work (the OPTIMAL clinical trial - Chapter 3), as well as leading authorship on all manuscripts and chapters included in the thesis.

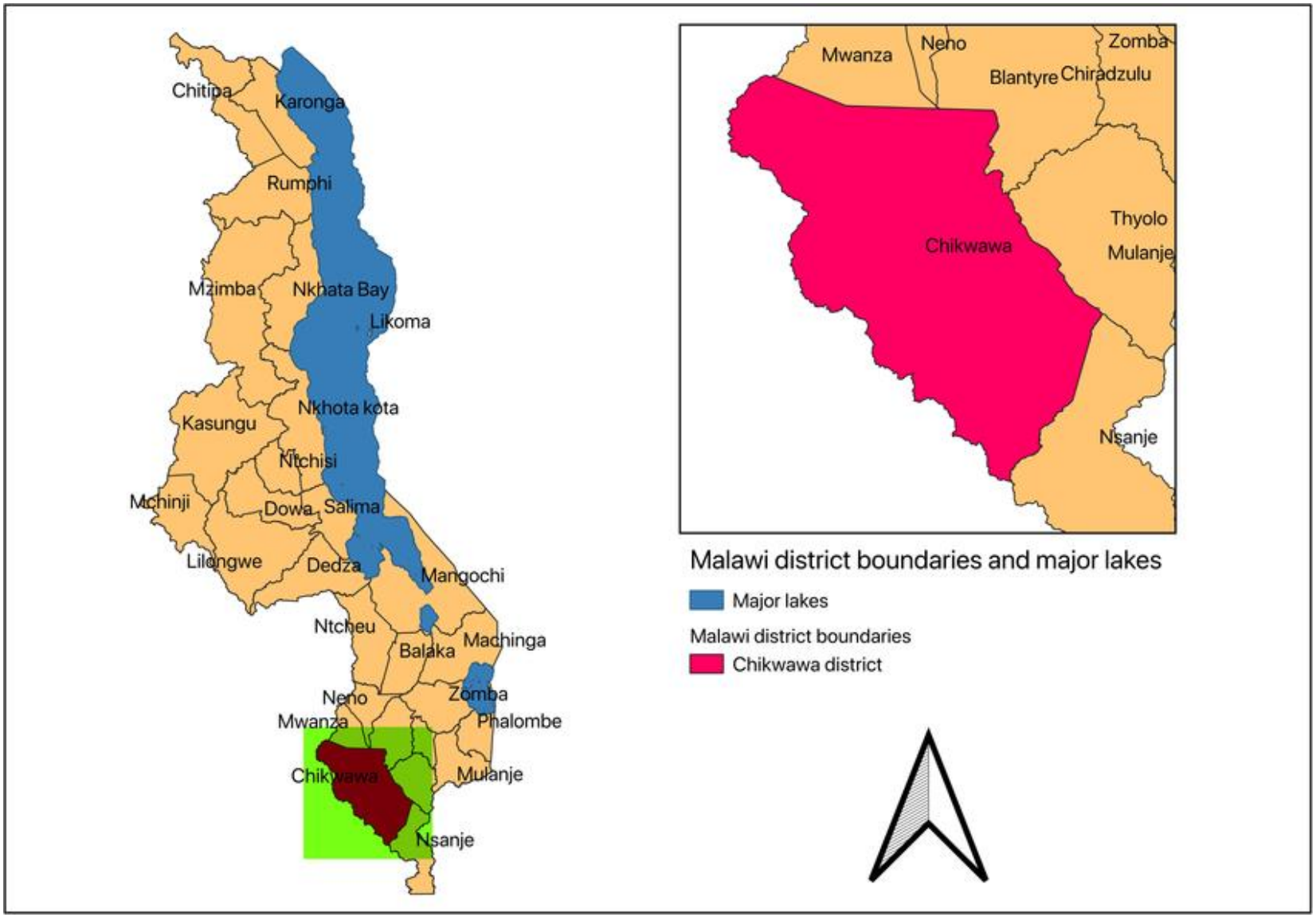

Figure 2: Map of Malawi illustrating the Chikwawa District in the Southern region

Figure 3: Aerial view of the research facility site at Chikwawa District Hospital in Southern Malawi

Summary of the outline of the thesis

Chapters 2 to 6 in the thesis aim to address the key research questions described above.

Chapter 2 provides a review of the application of population pharmacokinetic-pharmacodynamic (PK/PD) modelling for optimising antimalarial doses in infants, should the thesis hypothesis of sub-optimal piperaquine exposure in older infants be confirmed. As there is limited data on antimalarial PK in infants, and conducting traditional antimalarial dose optimisation studies is not ethical or feasible in infants, I review previous studies that have generally applied PK/PD modelling to optimise antimalarial dosing in children. The role of other complementary methodologies such as physiologically-based pharmacokinetic modelling (PBPK) is also discussed. Subsequently, I make a case for applying such methodologies in infants, highlight good practices in using PK/PD modelling in infants, and describe the need for well-designed PK/PD studies to provide minimal data that can be used to support optimal antimalarial medication dosing for malaria preventive or symptomatic treatment in infants. This chapter sets the scene for Chapter 3 on study methods. The review is published in the British Journal of Clinical Pharmacology but presented in a format consistent with the thesis document.

Banda CG, Tarning J, Barnes KI. Use of population pharmacokinetic-pharmacodynamic modelling to inform antimalarial dose optimization in infants. Br J Clin Pharmacol. 2025 Apr;91(4):968-980. doi: 10.1111/bcp.16132. Epub 2024 Jun 10. PMID: 38858224; PMCID: PMC11992656.

In **Chapter 3**, I begin to address the thesis's objectives by describing a protocol for collecting efficacy, safety, and sparse (as opposed to intense) pharmacokinetic data on DP for PMC in infants (the OPTIMAL study). Notably, there are four specific objectives described in the protocol, with a focus on i) age-related changes in piperaquine exposure, ii) efficacy of DP for PMC during routine healthy facility visits, iii) safety and tolerability of DP for PMC, and iv) generating PK/PD data to contribute towards a planned pooled analysis and, if needed,

optimise DP dosage regimen for PMC during routine health facility visits. This thesis addresses specific objectives i) to iii) in Chapters 4 and 5. The fourth specific objective, described in the protocol, will be addressed in future pooled individual patient data meta-analyses as discussed in Chapter 6 of the thesis. The protocol is submitted and published on Wellcome Open Research but presented in a format consistent with the thesis document.

Banda CG, Kantonya MS, Munharo S *et al.* 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: 1 approved, 1 approved with reservations]. Wellcome Open Res 2024, **9**:291 (https://doi.org/10.12688/wellcomeopenres.20355.1)

In **Chapter 4,** I present the efficacy, tolerability and safety results of the OPTIMAL Study.

Chapter 5 assesses piperaquine exposure over time in infancy to test the hypothesis that increasing age in this period is associated with a reduction in piperaquine exposure.

Chapter 6 summarises the findings, conclusions, and future research priorities for optimising DP dosage regimens for PMC in infants, if needed. This chapter consolidates the findings from Chapters 4 and 5. It lays a foundation for future planned work to use all available data in PK/PD (and/or PBPK) models, discussed in Chapter 2, to define how best to align DP administration with routine health facility visits to maximise its feasibility and benefits, including in the context where malaria vaccination is deployed.

CHAPTER 2

Use of population pharmacokinetic-pharmacodynamic modelling to inform antimalarial dose optimisation in infants

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Keywords:

Pharmacokinetic-pharmacodynamics, modelling, infants, antimalarials

Abstract

Infants bear a significant malaria burden but are usually excluded from participating in early dose optimisation studies that inform dosing regimens of antimalarial therapy. Unlike older children, infants' exclusion from early-phase trials has resulted in limited evidence to guide accurate dosing of antimalarial treatment for uncomplicated malaria or malaria preventive treatment in this vulnerable population. Subsequently, doses used in infants are often extrapolated from older children or adults, with the potential for under or overdosing. Population pharmacokinetic-pharmacodynamic (PK-PD) modelling, a quantitative methodology that applies mathematical and statistical techniques, can aid the design of clinical studies in infants that collect sparse pharmacokinetic data as well as support the analysis of such data to derive optimised antimalarial dosing in this complex and at-risk yet understudied subpopulation. In this review, we reflect on what PK-PD modelling can do in programmatic settings of most malaria-endemic areas and how it can be used to inform antimalarial dose optimisation for preventive and curative treatment of uncomplicated malaria in infants. We outline key developmental physiological changes that affect drug exposure in early life, the challenges of conducting dose optimisation studies in infants, and examples of how PK-PD modelling has previously informed antimalarial dose optimisation in this subgroup. Additionally, we have discussed the limitations and gaps of PK-PD modelling when used for dose optimisation in this subgroup. To utilise modelling well, there is a need to generate useful, sparse, PK and PD data in this subpopulation to inform antimalarial optimal dosing in infancy.

Keywords

Pharmacokinetic-pharmacodynamics, modelling, infants, antimalarials

Introduction

Sub-Saharan Africa bears more than 93% of morbidity and mortality due to *Plasmodium falciparum* malaria. In this setting, approximately 36% of the burden is experienced in infants.[2,37] After birth, an infant (a child <1 year of age) is protected from malaria due to the immunity conferred by the mother. Nevertheless, they become vulnerable, as early as three months of age, when this acquired immunity begins to wane.[6] Subsequently, this puts infants at an increased risk of rapid disease progression, severe malaria, and death. To minimise this burden, the World Health Organisation (WHO) initially recommended the use of sulfadoxine-pyrimethamine (SP), administered with routine health facility visits, for intermittent preventive treatment of malaria in infancy (IPTi) in areas of moderate to high malaria transmission in sub-Saharan Africa.[8] Recently, this recommendation has been extended beyond the first year of life and is now termed perennial malaria chemoprevention PMC).[38] However, although recommended by the WHO for over a decade, malaria chemopreventive treatment in infancy has had limited uptake by most malaria-endemic countries, in part due to the reported increasing resistance of malaria parasites to SP. Fortunately, monthly administration of a newer antimalarial drug, dihydroartemisinin-piperaquine (DP), an artemisinin-based combination therapy (ACT), has shown higher protective efficacy than SP.[39,40] Nonetheless, the feasibility of a monthly regimen for malaria chemoprevention is a challenge to implement in programmatic settings.[14,15,41] While acknowledging the potential for ACTs, such as DP, for malaria chemoprevention in young children, the WHO has called for more evidence of their safety, efficacy, and adherence to multi-day regimens, and when administered during routine health facility visits.[38] This highlights an urgent need for evidenced-based antimalarial dosing recommendations in infants, particularly in those under 6 months of age, for both malaria treatment and chemopreventive treatment. This should be done together with other malaria preventive interventions such as vaccines and insecticide-treated bed nets.

However, most therapeutic areas have excluded infants from participating in early dose optimisation clinical trials due to ethical and logistical concerns.[42] This is not different within the malaria field where most clinical trials to optimise the treatment (and prevention) of uncomplicated malaria have excluded infants. This has not been the case with older children, mostly above one year of age, with uncomplicated malaria who are usually studied in Phase III- and severe malaria studies. Consequently, antimalarial doses for the treatment of uncomplicated malaria have been extrapolated from older children as well as adults for use in infants. The challenge with such extrapolation is that antimalarial medications used for symptomatic treatment have the potential to be inaccurately dosed in infants when treating malaria.[43] This may potentially result in safety concerns. For example, the use of the current dispersible formulation of artemether-lumefantrine (Coartem®) in infants weighing <5 kgs resulted in a two- to three-fold increase in artemether and its metabolite dihydroartemisinin compared with children above 5 kgs.[44] To address this challenge, the PAMAfrica consortium was established to develop a new fixed-dose combination of AL for infants weighing < 5 Kg since the current AL dispersible formulation targeted children between 5 to 35 kg.[45] While there are ethical and logistical challenges in conducting dose optimisation studies in infants,[46,47] such as the intense nature of blood sampling that is often required, it is imperative that subgroups that bear the largest burden of disease are included, as early as possible, in clinical trials that inform dosing regimens. Quantitative techniques, such as population pharmacokinetic-pharmacodynamic (PK-PD) modelling, have the advantage of supporting the design and analysis of data from studies that can be conducted to overcome the logistical hurdles associated with conducting dose optimisation studies in infants.

In this review, we will describe how PK-PD modelling can be used to inform antimalarial dose optimisation for preventive and curative treatment of uncomplicated malaria in infants. We will outline key developmental physiological changes that may affect drug exposure in early life, the challenges of conducting dose optimisation studies in infants, the role of PK-PD modelling in informing antimalarial dose optimisation processes and examples of how PK-PD modelling

has previously informed antimalarial dose optimisation in infants. Additionally, we will discuss the limitations and gaps of PK-PD modelling when used for dose optimisation in infants as well as other modelling techniques that can complement population PK-PD methods for dose optimisation in infants.

Developmental physiological changes in early life affect drug exposure

Infancy is characterised by rapid physiological changes that impact the pharmacokinetic exposure profiles of various drugs used during this period.[48] Key pharmacokinetic changes in this subgroup include altered absorption, reduced distribution of drugs to sites of action, and reduction in drug metabolism because of developing metabolic processes (Table 1).[49,50] These changes are driven by differences in body size, body composition, enzyme maturation, and end-organ perfusion. Drugs may be affected by these changes to different extents, hence the need for PK data in infants, albeit sparse, to optimise their dosing. Infants are, therefore, a complex population that requires special attention to achieve optimal dosing of therapeutic agents such as antimalarial medications.

Challenges of dose optimisation studies in infants

The standard dose optimisation studies involve administering a drug and collecting frequent samples thereafter, at prespecified time intervals, to derive individual pharmacokinetic primary parameters such as clearance and volume of distribution as well as secondary parameters such as the area under the concentration-time curve (AUC), peak concentration (C_{max}) and terminal elimination half-life ($t_{1/2}$).[51] Although such optimisation studies provide detailed individual pharmacokinetic parameter estimates, which can be aggregated to provide information about a particular group of participants, they have several challenges when extrapolated to infants.

Table 1: Physiological changes in infancy that affect drug pharmacokinetics

Pharmacokinetic process	Physiological change (s)
Absorption	Increased intestinal transit time Reduced gastric emptying: delayed in infants under 6-8 months and shorter in older infants. Delayed/immature gastric transporter expression.
Distribution	Age-dependent body composition influencing the volume of distribution of drugs (including Increased thickness, perfusion, hydration, and body surface area in infants) Increased body water: fat ratio highest in neonates Plasma protein binding lower in neonates.
Metabolism	Reduced hepatic metabolism in neonates (Cytochrome P-450 and glucuronosyltransferase isoforms) reaching adult levels by 6-12 months
Elimination	Elimination increases non-linearly with weight in infancy. Renal function reaches maturity ~ age 1-2 years

First, the volume of blood and frequency of sampling that is acceptable to be collected from infants and very young children is limited. Guidelines indicate that this should not exceed 1% of total blood volume at any one time (0.8mL/kg) or 3% (2.4mL/kg) within 1 month.[52] Such small volumes may be problematic for most pharmacokinetic assays,[53] including those for antimalarials. However, great advances have been seen in recent years with the development of low-volume LC-MS/MS assays for antimalarial drugs, such as dried blood on filter paper-based assays, showing good sensitivity and accuracy.[54–56] Second, the logistics of collecting multiple capillary samples still entails that they remain longer at the health facility or return frequently. This may not always be acceptable to primary caregivers.[51] Third, there is the ethical concern of the risk-benefit of each infant's study participation. Ethically acceptable studies are those that pose minimal risk or burden to the infant, with the potential for direct benefit.[57] However, the analysis of PK-PD data from these dose optimisation studies at best

only benefits other infants treated in the future, which assumes that the evidence generated is sufficient to inform policy and practice. Furthermore, pharmaceutical manufacturers may need sufficient motivation to develop a formulation, tablet strength and fixed-dose combination ratio suitable for use in infants and very young children.

The role of population PK-PD modelling in antimalarial dose optimisation in infants

Population PK-PD modelling (i.e. nonlinear mixed-effects modelling) has the potential to overcome the challenges presented by standard dose optimisation studies. Population PK-PD models have three components; structural models which describe the time course of a quantified mean response (i.e. fixed effects), in most cases these can be drug concentrations (PK) or treatment response (PD, efficacy/safety); stochastic or statistical models which define the between- and within-patient variability in the observed data (i.e. random effects); and covariate models which quantify the influence of factors that could affect the observed individual response such as age, nutritional status, disease severity.[58] The main strength of population PK-PD modelling is that it fits the model to data both on a population and individual level, which enables the analysis of very sparse, often unbalanced data, where an individual may contribute a small number of samples, and the number of samples/ timings can vary between individuals.[58–60] This makes it particularly relevant for use in the analysis of data from dose optimisation studies in infants, where PK and PD data sampling may be sparse and collected at opportunistic time points. Additionally, PK-PD modelling can be used to inform the design of dose optimisation clinical trials to ensure adequate number and timing of sampling, and the power to detect differences in pharmacokinetic or pharmacodynamic outcomes.[61] Thus, in optimising the dosing of antimalarials in a sub-population that is difficult to study, such as infants, PK-PD modelling could be utilised as a tool for designing as well as analysing data from such studies.[62] This would allow carefully designed and early inclusion of infants in dose optimisation clinical trials, preferably early enough to inform decisions on tablet strength and ratios of fixed-dose combination treatments (i.e., phases IIb or III). Other advantages of applying PK-PD modelling approaches in paediatric PK studies in malaria, include an

improved mechanistic understanding of drug effects and the ability to investigate complex circumstances such as drug-drug interactions, and disease progression effects.[51]

Previous use of PK-PD modelling to inform antimalarial dose optimisation in infants

There is generally a paucity of evidence on the use of PK-PD modelling for antimalarial dose optimisation in infants. Nevertheless, we have utilised evidence on dose optimisation in children to highlight the potential role of PK-PD modelling in antimalarial dose optimisation for use in infants. Table 2 details examples of large individual patient data analyses that have used population PK-PD modelling techniques to optimise the dosing of some of the commonly used first-line antimalarial therapies against *Plasmodium falciparum* in children. These analyses on artesunate-amodiaquine,[63] artemether-lumefantrine,[43] dihydroartemisinin-piperaquine[64] and sulphadoxine-pyrimethamine,[65] underscore the importance of the PK-PD modelling methodologies as a tool for dose optimisation and generation of evidence to inform treatment guidelines. For example, the WHO's recommendation on dosing dihydroartemisinin-piperaquine in very young children was adopted from PK-PD dose optimisation work following concerns that the manufacturers recommended dosing regimen of piperaquine resulted in lower piperaquine concentrations to provide longer-term protection after treatment of uncomplicated malaria.[38,64,66,67] Other examples of studies on antimalarial therapies that have applied PK-PD techniques for malaria (severe and uncomplicated) treatment as well as chemoprevention are further reviewed in Table 3.[68–75]

Notably, there is limited data on antimalarial dosing in infants under 6 months of age when antimalarial therapies are used for malaria chemopreventive treatment. Furthermore, the use of PK-PD methods to optimise antimalarial dosing in this age group is limited (Tables 2 and 3). The lack of evidence on antimalarial dosing in infants under 6 months of age is thought to be due to the perception that malaria in this age group is rare because of maternal antibody protection that is passed on at birth.[2,4] However, this has been shown to not be the case, for example in a large cross-sectional study in Uganda (n= 7,785) the malaria parasitaemia

positivity rate in infants under 6 months of age was 31.6%.[2] This calls for an urgent need for antimalarial dosing recommendations in infants under 6 months for both uncomplicated malaria treatment and chemoprevention. Population PK-PD methodologies have a potential role to support this and ensure accurate and safe dosing regimens in this vulnerable subpopulation.

Additionally, in most analyses reported in Tables 2 and 3, multiple antimalarial exposure parameters such as AUC, C_{max} and Day 7 concentrations were utilised. While this variation reflects the different primary aims of the studies that were included, it could limit comparisons between analyses and interpretations of PK-PD associations. Since Day 7 antimalarial concentrations of long-acting drugs such as lumefantrine are predictive of overall exposure and antimalarial efficacy by day 28,[76] and generally assessed in most antimalarial studies, utilising such a standardised exposure parameter as a proxy for overall exposure could potentially overcome this limitation.

Table 2: Individual patient data analyses using population PK and PD methodologies to optimise antimalarial dosing in children

Antimalarial, year, country	Age range of children included in analysis	Indication for treatment	Total number of participants (all age groups) included in the analysis	No of participants who were children [% of total}	Number of studies included in the analysis	Drug exposure parameter assessed	PD parameter	Summary of key message(s)	Conclusions/ Recommendations
Amodiaquine[63]									
2018, Multicountry	12 months- 5 years	Uncomplicated *P. falciparum* malaria	261	95 [36%]	6	Day 7 plasma concentrations and C_{max}	PD assessment not conducted	Body size and age affected amodiaquine clearance Bioavailability was 22.4% lower at the start of treatment than during convalescence, suggesting malaria disease effect Assuming birth at term, clearance rates for amodiaquine and desethylamodiaquine reached 50% of adult maturation at 2.8 months	Need for optimised dosing regimens to achieve similar drug exposure in all age groups Higher doses of amodiaquine may be needed for some weight ranges (8 kg, 15 to 17 kg)
Lumefantrine[43]									
2018, Multicountry	12 months - 10 years	Uncomplicated *P. falciparum* malaria	3486	1289 [37%]	26	Day 7 plasma concentrations	Malaria recurrence by day 42 post treatment	Currently recommended 6-dose regimen in children weighing <15 kg and 15–24 kg resulted in 24.2% and 13.4% lower predicted median venous lumefantrine concentrations at day 7, respectively, when compared to adult patients	A 5-day regimen of current weight-based standard twice-daily doses for small children is most favourable from a pharmacological perspective
Piperaquine[64]									
2017, Multicountry	6 months - 10 years	Uncomplicated *P. falciparum* malaria	728	448 [62%]	11	Day 7 plasma concentrations	PD assessment not conducted This analysis was informed by previous pooled analysis of 26 studies that showed significant risk of recrudescent malaria in young children (1-5 years) following treatment with dihydroartemisinin-piperaquine	Small children had a substantially lower piperaquine exposure after recommended dosing regimens Derived population pharmacokinetic model was used to develop a revised dose regimen of dihydroartemisinin-piperaquine that is expected to provide equivalent piperaquine exposures safely in all patients, including in small children with malaria	This work was part of the evidence that informed the World Health Organization technical guidelines development group in the development of the 2015 treatment guidelines for malaria
Sulfadoxine and pyrimethamine (SP)[65]									

2018, Multicountry	<2 - 5 years	Uncomplicated *P. falciparum* malaria	801	415 {52%]	4	Day 7 plasma concentrations	PD assessment not conducted	Underweight-for-age children were found to have 15.3% and 26.7% lower bioavailability of sulfadoxine and pyrimethamine. Under current dosing recommendations, the simulation predicted that the median day 7 concentration was below the 25th percentile for a typical adult patient (50 kg) for sulfadoxine for patients in the weight bands of 8 to 9, 19 to 24 kg and 8 to 9, 14 to 24 kg for pyrimethamine	Evidence-based dosing regimen was constructed that would achieve sulfadoxine and pyrimethamine exposures in young children and underweight-for-age young children that were similar to those currently seen in a typical adult

Table 3: Other examples of population pharmacokinetic-pharmacodynamic studies to optimise dosing of antimalarial medications in children for malaria therapeutic and preventive treatment

Antimalarial, year, country	Age range of children included in analysis	Route of administration and Indication for treatment	Number of participants (all age groups) included in the study)	Number of participants who were children [% of total}	Drug exposure parameter assessed	Pharmacodynamic (PD) parameter	Summary of key message(s)	Conclusions/ Recommendations
Quinine								
2005, Cameroon[68]	6 months- 6 years	Oral, uncomplicated *P. falciparum* malaria treatment	30	30 [100%]	Plasma concentrations on days 1 to 3 with calculated clearance and volume of distribution	Parasite load reduction within 72 h Malaria recurrence on days 7 and 14	Clearance and volume of distribution were positively correlated with body weight and increased over time The time to a 4-log reduction of the initial level of parasitaemia was related to the average quinine concentration from 0 to 72 h	Need to evaluate the efficacy of a 5-day treatment course in a larger clinical trial
2001, Ghana[74]	12 months - 10 years	Intramuscular loading and maintenance doses, severe *P.falciparum* malaria treatment	120	120 [100%]	Plasma concentrations from 0 to 12 h post dosing with calculated clearance and volume of distribution	Parasite clearance in 72 h	A two-compartment model with first-order absorption and elimination gave post-hoc estimates for pharmacokinetic parameters that were consistent with those derived from non-population pharmacokinetic studies of clearance and volume of distribution Intramuscular quinine associated with minor, local toxicity (13 of 108; 12%), and one or more episodes of postadmission hypoglycaemia in 11 patients (10%)	A loading dose of intramuscular quinine resulted in predictable population pharmacokinetic profiles in children with severe malaria and may be preferred to the intravenous route of administration in some circumstances
2013, Tanzania[69]	4 months- 8 years	Intramuscular loading and maintenance doses, severe *P.falciparum* malaria treatment	75	75 [100%]	Plasma concentrations from 0 to 24 h post dosing with calculated clearance and volume of distribution	Median time to reach a 50% reduction in hazard (survival over time)	Quinine exposure was reduced at lower body weights after standard weight-based dosing; there was 18% less exposure over 24 h in patients weighing 5 kg than in those weighing 25 kg. Maximum plasma concentrations after the loading dose were unaffected by body weight No evidence of dose-related drug toxicity with the loading dosing regimen	Intramuscular quinine was rapidly and reliably absorbed in children with severe falciparum malaria Based on these pharmacokinetic data, a loading dose of 20 mg salt/kg was recommended, provided that no loading dose was administered within 24 h and no routine dose was administered within 12 h of admission
Pyronaridine								
2015, Multicountry[75]	6 months - 15 years	Oral, uncomplicated *P. falciparum* malaria treatment	349	349 [100%]	$AUC_{0-\infty}$ with derived clearance and volume of distribution	PD assessment not done	Age was identified as a significant predictor of pyronaridine peripheral volume of distribution Formulation was a significant covariate on pyronaridine absorption rate constant	Simulations of pyronaridine concentration-time profiles showed similar exposures across paediatric weight ranges, supporting the proposed labelling for weight-based dosing of pyronaridine granules
Artesunate and metabolite dihydroartemisinin (DHA)								

2006, Multicountry[70]	11 months - 15 years	Intrarectal, severe *P. falciparum* malaria treatment	179	[62%]	AUC_{0-6h} with derived clearance and volume of distribution	Treatment outcome Early rescue treatment Failure of baseline parasitaemia to fall 40% by 12 h Time to clear 50% and 90% of baseline parasitaemia	Gender was associated with increased mean clearance CL/F) of DHA by 1.14 (95% CI: 0.36–1.92) (l/kg/h) for a male compared with a female Weight was positively associated with volume of distribution (V/F). Larger V/Fs were observed for the patients requiring early rescue treatment compared with the remainder, independent of any confounders No associations between the parasitological responses and the posterior individual estimates of V/F, CL/F, and AUC_{0-6h} were observed	Pharmacokinetic properties of DHA were affected only by gender and body weigh Patients with the lowest area under the DHA concentration curve did not have slower parasite clearance, suggesting that rectal artesunate is well absorbed in most patients with moderately severe malaria
Mefloquine								
2006, Thailand[73]	2 - 15 years	Oral, uncomplicated *P. falciparum* malaria treatment	50	Not indicated	$AUC_{0-\infty}$ with derived clearance and volume of distribution	Malaria recurrence by day 63 post dosing	AUC0–∞ was 40% higher than previous estimates for patients given the equivalent conventional-dose regimen (mefloquine given as 15 mg/kg and then 10 mg/kg on the second and third days of treatment) Splitting the 25 mg/kg dose of mefloquine into three doses of 8 mg/kg each resulted in improved oral bioavailability compared to the conventional split-dose regimen results.	New regimen expected to be well tolerated and result in equivalent therapeutic response to conventional split-dose regimen
Piperaquine								
2021, Uganda[71]	2 months - 2 years	Oral, *P. falciparum* malaria weekly versus monthly chemoprevention	280	284 [100%]	Plasma concentration associated with 95% protection from malaria	Cumulative malaria treatment hazard from 2 to 36 months of age	Compared to dihydroartemisinin-piperaquine (DP) every 12 weeks, DP every 4 weeks is associated with 95% protective efficacy (95% CI: 84–99%). Piperaquine plasma concentration of 15.4 ng/mL reduces malaria hazard by 95%. Malnutrition reduces piperaquine exposure.	Simulated regimens showed DP every 4 weeks is optimal across a range of transmission intensities, and age-based dosing improves malaria protection in young or malnourished children.
2019, Burkina Faso[72]	2 months - 5 years	Oral, *P. falciparum* seasonal malaria chemoprevention (SMC)	179	179 [100%]	Minimum inhibitory concentration of piperaquine during SMC with derived parameters PK parameters (clearance and volume of distribution)	Time-to-malaria infection during 4 months of study period (comprising of 3 rounds of chemoprevention and 2 months of passive follow up)	Increasing the DP dosage and extending the dose schedule to four monthly doses result in a predicted relative reduction in malaria incidence of up to 58% during the high transmission season	The higher and extended dosing schedule to cover the high transmission period for seasonal malaria chemoprevention could improve the preventive efficacy substantially

Limitations and gaps of population PK-PD modelling for dose optimisation in infants

Despite being a robust technique to use in analysing drug exposure-response data, even when such data is sparse, population PK-PD modelling has some limitations. First, the rapidly changing physiological states, such as those related to age in infancy or disease conditions, may render the assumptions of the structural model inaccurate or overly simplistic.[77] Second, since infants experience age or weight-related changes, there is always a need to apply scaling techniques to adjust for these varying physiological states when estimating primary parameters of drug exposure as well as optimising dosing regimens.[78,79] Third, when applied to data that is collected from clinical trials or observation studies, the assessment of covariates that may affect drug exposure is limited by the data that was collected and studies are often underpowered to identify significant covariates.[80,81] For example, data on the age of infants may be routinely collected during follow-up visits, but other covariates such as time of food intake before or after drug administration, might be excluded. Additionally, the model may not comprehensively account for all possible programmatic scenarios in which the optimised dosing regimens would be deployed, such as dosing with routine health facility visits (e.g. for vaccination), dosing with food or sub-optimal adherence with doses to be taken at home (e.g. ACT dosing on day 2 and 3). Fourth, although population PK-PD models allow for a better understanding of the processes of drug exposure, including an assessment of compartmental pharmacokinetics, developing models that best fit the observed data can be a lengthy process,[82] and often requires substantial skills that are only present in a few malaria-endemic countries. This may preclude the timely availability of optimised dosing regimens for use in programmatic settings. Fifth, the development of PK-PD models rely on accurate and sensitive drug measurements in small blood volumes,[59] which require expensive equipment (mass spectroscopy) and experienced staff to operate these machines. These assays also come at a high cost of 20-100 USD per sample, which is often prohibitive. Furthermore, the complexity of population PK-PD models may not always be easily understood by stakeholders, such as national malarial control programs.[83] This may further limit the utility of

population PK-PD tools in malaria-endemic areas to inform local optimal dosing of antimalarials.

Other modelling techniques for dose optimisation in infants

Physiologically-based PK (PBPK) modelling is another tool that can be used to optimise the dosing of antimalarial therapy in infants.[84–86] PBPK models account for the development of organs and the ontogeny of specific enzymes, such as cytochrome P450, and transporters that determine the age-related pharmacokinetics of various therapies.[87] This enables them to provide a clear mechanistic understanding of the processes behind altered drug exposure. As a result, they have been previously applied to provide a mechanistic understanding of drug disposition in infants and guide optimal dosing in various complex scenarios of both antimicrobial therapy and other therapeutic agents.[79,88–91] Additionally, they have been applied in drug regulatory reviews[92], and are being used to inform the design of paediatric drug development studies.[93] Nevertheless, these PBPK models, unlike population PK-PD models, often describe average persons in populations and are not able to dissect inter-individual or unexplained variability. Furthermore, PBPK models apply assumptions of rates of individual processes of absorption, distribution, metabolism, and excretion, and these may not always be known for each antimalarial medication of interest.[94] This makes it challenging to easily apply them in practice. Nonetheless, the mechanistic insights that they offer could be capitalised on when combined with PKPD models to optimise dosing in infants. However, their inherent complexity makes them computationally costly and difficult to use.[95]

Recently, machine learning (ML) algorithms have been identified as potential platforms to identify doses for antimicrobial agents. Indeed, ML PK-PD models have been shown to accurately predict concentrations of rifampicin in the treatment of tuberculosis,[96] and beta-lactams when managing hospital-acquired and ventilator-associated pneumonia.[97] Nonetheless, the main limitation of ML PK-PD modelling is that it may not offer a

mechanistic understanding of underlying assumptions of drug exposure. Their application for use in infants and other complex subpopulations needs further exploration.

Conclusion

Pharmacokinetic-pharmacodynamic modelling has a potentially unique advantage of using sparse unbalanced data to inform antimalarial dose optimisation in infants, who bear a disproportionately high burden of malaria but are commonly excluded from clinical trials on antimalarials. Nevertheless, it may not exhaustively account for all physiological, clinical, and programmatic scenarios in which these preventive treatments and uncomplicated malaria treatments would be used. Thus, other modelling tools, such as physiologically-based pharmacokinetic modelling techniques, could further strengthen the ability of pharmacokinetic-pharmacodynamic models to provide a quicker understanding of mechanistic processes related to drug exposure in infants. To utilise modelling well, there is a need to generate reasonably minimal, but useful, PK and PD data in this subpopulation, especially in children under 6 months of age. This should be done as early as ethically possible in antimalarial drug development to inform their optimal dosing.

Addendum: Key knowledge gaps identified from the literature review and addressed in this thesis

- **Limited data on antimalarial drug exposure in children under 6 months of age and on the impact of physiological changes in infancy on antimalarial drug exposure**: There are limited studies that have investigated the impact of physiological changes in infancy on the pharmacokinetics of antimalarials used for malaria treatment or prevention, particularly in children under 6 months of age.

- **Lack of evidence on the efficacy and safety of antimalarials for PMC when given during routine health facility visits:** Few studies have been designed to collect data on the efficacy and safety of antimalarials for malaria prevention in this subgroup, especially when dosing is aligned with routine health facility visits.

These two key knowledge gaps will be addressed in Chapters 3 to 5 of this thesis.

CHAPTER 3

A pharmacokinetic randomised interventional study to optimise dihydroartemisinin piperaquine dosing for malaria preventive treatment in Malawian infants: A protocol for the OPTIMAL study

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

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 understand piperaquine exposure in infancy to inform the development of optimised DP dosing strategies for malaria preventive treatment in infants.

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. In the DP arm, compartmental pharmacokinetic analyses will be conducted to characterise piperaquine exposure over time in infancy,

informing the development of feasible, optimised, efficacious, and safe DP dosing strategies for malaria preventive treatment in this age group.

Conclusions

The findings will provide much-needed evidence and data to inform the development of a dihydroartemisinin-piperaquine dosing regimen for malaria preventive treatment in infants when administered during routine health facility visits.

Introduction

Plasmodium falciparum malaria is estimated to affect up to 36% of infants in high-transmission settings.[98] These very young children are at an increased risk of rapid disease progression, severe malaria and death.[99] 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.[8] Recently, this recommendation has been extended beyond the first year of life and is now termed perennial malaria chemoprevention (PMC).[38] However, there is increasing evidence of widespread SP resistance and reduced protective efficacy, including in Malawi, where IPTi-SP has thus not been implemented.[100] 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.[39,40] 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 the optimal dosing of DP in infants. In a previous effort to optimise DP dosing for malaria prevention, a randomised clinical trial in Uganda suggested that monthly DP is better than three-monthly dosing in children under two years of age.[101] 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.[14,15,41] Optimising DP dosing in infants as part of PMC to enable administration during routine health facility visits 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 and characterise changes in piperaquine concentration at different time points in infancy, and, where necessary, determine the optimal dosing regimen for DP in this important subpopulation when administration is aligned with routine health facility visits. Additionally, a piperaquine minimum concentration target of 15.4 ng/mL has been proposed as a protective threshold of piperaquine when DP is dosed monthly for malaria prevention in children.[71] We will evaluate whether this target is achieved when DP is administered to infants during routine health facility visits for PMC. We hypothesise that age-related physiological changes during infancy (such as the maturation of CYP3A4 enzymes involved in piperaquine metabolism and an increase in fat-to-water body ratio that expands the volume of distribution for lipophilic drugs in early infants)[48–50] lead to enhanced piperaquine clearance and distribution with increasing age. As a result, the currently recommended weight-based dosing regimen of DP may lead to progressively lower piperaquine exposure in older infants (i.e., those greater than 6 months of age). This reduction in systemic exposure could fall below a protective threshold, thereby compromising the effectiveness of DP for PMC in infants. Understanding the variation in piperaquine exposure within the first year of life is essential to inform dose optimisation of DP for malaria preventive (and symptomatic) treatment in this age group. 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%.[102] 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 routine health facility visits in infancy.

Specific objectives

The specific objectives of the study are to:

1. To describe age-related changes in the exposure profile of piperaquine following the administration of DP for PMC at four different routine health facility visits in infancy (i.e., at 10 weeks, 14 weeks, six months, and nine months of age).
2. To evaluate the efficacy of DP for PMC compared with placebo when administration is aligned with routine health facility visits in infancy.
3. To compare the safety and tolerability of DP for PMC with DP-placebo in infants during routine health facility visits.
4. To contribute data generated from this study towards future population pharmacokinetic-pharmacodynamic modelling and simulations, if needed, 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-related changes in exposure profile of piperaquine (area under the concentration time-curve (AUC), day 7 and trough (pre-dose and day 28) concentrations of

piperaquine in infants receiving DP for PMC at four different routine health facility visit time points in infancy (i.e., at 10 weeks, 14 weeks, six months, and nine months of age).

- Incidence of malaria from 2.5 months to 12 months of age in infants receiving DP for PMC or DP-placebo at routine health facility visits. Incidence will be defined as symptomatic infections (fever with parasitaemia confirmed by microscopy and/or qPCR) or asymptomatic parasitaemia (confirmed by microscopy and/or qPCR) at scheduled or unscheduled assessments, reflecting the total burden of infection, and occurring during person-time under follow-up.
- 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.
- Generated pharmacokinetic and pharmacodynamic data contributed towards a pooled analysis aimed at optimising the dosing of DP for PMC during routine health facility visits.

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 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. 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, 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.[103]
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 screening for prior antimalarial use through medical history, 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.[40,101,104]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).[105]

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.

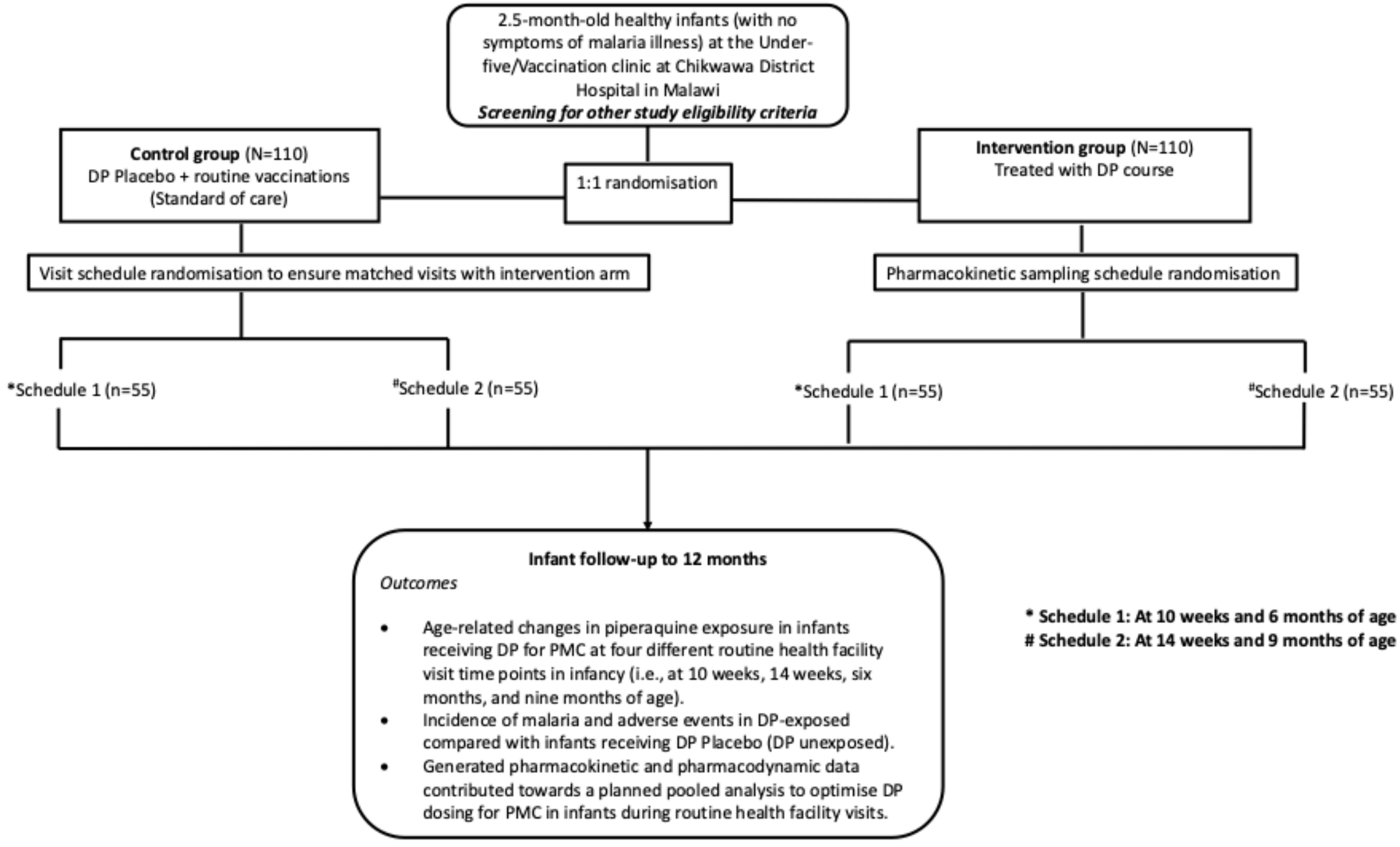


Figure 1: Outline of the study design; a single-blind, placebo-controlled, randomised interventional study

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 and information on use of malaria preventive tools such as insecticide treated bednets	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	
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 to contribute samples at 10 weeks and 6 months (Schedule 1) or 14 weeks and 9 months (Schedule 2). All other study procedures will be the same in both groups

** 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

[§] Anthropometric measures: weight-for-height (WHZ), weight-for-age (WAZ), height-for-age (HAZ) and mid-upper arm circumference (MUAC)

[*#] Clinical adverse event monitoring based on symptoms and signs. Appropriate laboratory investigations will be symptom or sign directed.

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

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 schedule 1 sampling visits 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. Infants will be self-matched at 10 weeks and 6 months or 14 weeks and 9 months to minimise any intra-individual variability. 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 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. The number of visits will be matched in the placebo arm to ensure a similar number of visits in both arms and maintain single-blinding. On days of follow-up visits where blood samples are not collected in the placebo arm, only a clinical review will be conducted.

A sparse pharmacokinetic sampling design, described above, rather than an intensive pharmacokinetic approach, has been opted for in this study to minimise the number of blood draws in this vulnerable population, in line with ethical guidelines for research involving infants.[106–108]

Piperaquine sample processing, storage, and analysis

The blood volume of 200 μL, collected using calibrated micro-collection 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 (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.[109] Internal standards, as part of the assay's quality control (QC), will be used and tested at varying concentrations (low to high) in triplicate within each batch of samples to ensure the assay's accuracy and precision. The corresponding per cent relative standard deviations (% RSD) will be reported.

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)[110] to overcome the microscopy limitation. However, qPCR results will only be available at the end of the study to assess efficacy and cannot 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. Real-time PCR (qPCR) targeting the 18S rRNA gene will be used to detect *Plasmodium falciparum*. [111] For recurring infections in infants, further genotyping will be conducted to distinguish between reinfections and recrudescence.

An additional 100 μL of blood will be used to prepare thin and thick peripheral blood smears for malaria microscopy, prepared using the 10% Giemsa stain method to identify and count malaria parasites at the study site.[112,113] Two microscopists will read each blood smear in a blinded fashion (i.e., 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 malaria infection (symptomatic or asymptomatic 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 infection 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[114]), 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 parasitaemia 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. To avoid the risk of overdosing, no additional tablets will be provided to the caregiver to administer in cases where vomiting occurs at home on day 2 of treatment.

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 judgment 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 the 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 rollout happen while the study is underway, infants in the study would receive the RTS, S/AS01 malaria vaccine at 5, 6, and 7 months of age, with DP or placebo administered at 10 weeks, 14 weeks, 6 months and 9 months of age. Although the addition of the malaria vaccine could potentially result in an underestimation of the true efficacy of DP for PMC in infancy, especially in relation to preventing severe illness, the advantage of combined DP with the RTS, S/AS01 is that it 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 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 and safety and tolerability of DP

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, assuming a baseline malaria infection incidence of 0.5 episodes per person-year in the control group based on

local, unpublished, historical facility data (Health Management Information System (HMIS) surveillance, 2015-2017). 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.[36,115]

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[116] 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).[117] 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 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; https://pactr.samrc.ac.za (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 [35,36,115]. Nevertheless, the DSMB will review any such new information, and appropriate recommendations made. Participants will be insured against any research-related harm.

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

Objective	**Outcome (s)**	**Analysis plan**
1. To describe age-related changes in the exposure profile of piperaquine following the administration of DP for PMC at four different routine health facility visits in infancy (i.e., at 10 weeks, 14 weeks, 6 months, and 9 months of age).	• Overall piperaquine exposure parameter, the area under the concentration time curve from 0 to 28 days ($AUC_{0\text{-}28\ days}$) following DP treatment at 10 weeks, 14 weeks, 6 months and 9 months of age. • Observed piperaquine concentrations on days 7, 28 and pre-dose following DP treatment at 10 weeks, 14 weeks, 6 months and 9 months of age to further describe piperaquine exposure. • The proportion of participants attaining piperaquine trough concentrations (pre-dose and day 28) below a previously proposed minimum protective threshold of 15.4 ng/mL following DP dosing at 10 weeks, 14 weeks, 6 months or 9 months of age.	• The goal of this analysis is to address the hypothesis that increasing age in infancy would result in lower piperaquine exposure due to associated rapid physiological changes (mainly apparent clearance and volume of distribution) • Individual $AUC_{0\text{-}28\ days}$ and observed exposure parameters (pre-dose, $C_{day\ 7}$, and $C_{day\ 28}$) will be log-transformed to calculate their geometric means (GM) with 90% confidence intervals (CIs) at 10 weeks, 14 weeks, 6 months, and 9 months of age. GM ratios (GMRs) will be calculated to compare exposure at 10 weeks with the rest of the dosing time points. The log-transformed parameters will be back-transformed to absolute ng/mL concentrations for reporting. Changes in pharmacokinetic parameters between the dosing time points will be considered statistically significant when the 90% CI of the GMR does not cross the value of 1.[119] • $AUC_{0\text{-}28days}$ will be derived from compartmental analysis using non-linear mixed effects modelling. First a structural distribution model (one, two or three compartments) that best describes the data will be evaluated. In each model, the primary pharmacokinetic parameters (volume of distribution and clearance) will be allometrically scaled for bodyweight by

		normalising the infant's weight to the median weight of the study cohort. A model with the lowest objective function among the three structural models will be selected as the best fit for the data. Given the hypothesis of impact of increasing age on piperaquine exposure, only age will be assessed as a fixed covariate, at this stage, on clearance and volume of distribution. Individual $AUC_{0\text{-}28days,}$ will be derived from the final fitted model by integrating the concentration-time data after each dosing time point. • Furthermore, the proportion of participants attaining trough concentrations (pre-dose and day 28) below a previously proposed piperaquine minimum protective threshold of 15.4 ng/mL[71] will be computed and compared between 10 weeks and 14 weeks, 6 months or 9 months of age.
2. To evaluate the efficacy of DP for PMC compared with placebo when administration is aligned with routine health facility visits in infancy	• Incidence of malaria from 2.5 months to 12 months of age in infants receiving DP for PMC or DP-placebo at routine health facility visits	• Cox proportional hazard models for cumulative malaria hazard, adjusted for repeated malaria events, will be used to estimate overall malaria infection incidence (symptomatic and asymptomatic)
3. To compare the safety and tolerability of DP for PMC with DP-placebo in infants during routine health facility visits	• Adverse events in infants receiving DP for PMC compared with those receiving placebo at routine health facility visits	• 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 placebo at routine health facility visits
4. To contribute data generated from this study towards future population pharmacokinetic-pharmacodynamic modelling and simulations, if needed, to optimise the dosage of piperaquine when administered as DP for PMC	• Generated pharmacokinetic (PK) and pharmacodynamic (PD) data from this study contributed towards a planned pooled analysis to	• Data from this study will be pooled with existing datasets on DP used for preventive treatment in children to robustly quantify age-related changes in

	optimise DP dosing for PMC during routine health facility visits • Optimised DP dosing regimen for PMC aligned with routine health facility visits.	clearance and volume of distribution during infancy. PK/PD modelling and simulation will subsequently be employed, if required, to refine and define an optimised DP dosing regimen for PMC aligned with routine health facility visits (refer to Supplementary Text 1 for further details on the planned pooled analysis) • All PK/PD modelling will be conducted using a nonlinear mixed-effects modelling approach as previously described.[64,67]

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.[106–108]

Quality control and study oversight

The study will be conducted according to the Declaration of Helsinki.[120] 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.[121]

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 malaria infection (symptomatic and asymptomatic) as well as its safety and tolerability. As a subsequent step, in a planned pooled analysis, we will combine data from this study with other existing piperaquine data in infants to robustly 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.[122] 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.[123] 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 a dose optimisation clinical trial in this complex subgroup, providing the much-needed evidence and data to inform the development of a dihydroartemisinin-piperaquine dosing regimen for malaria preventive treatment in infants when administered during routine health facility visits. Furthermore, such a paradigm shift sets a precedent 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.

Supplementary material

Supplementary Text 1: Proposed plan for future pooled analysis to define optimal dosing of DP for PMC during routine health facility visits

- This analysis, as part of the fourth specific objective, extends the analysis from the first specific objective. In this analysis, a previously described three-compartment model of piperaquine exposure in children will be employed as an a priori model,[72] to characterise developmental changes in key PK parameters in infancy (e.g., clearance, volume of distribution), with allometric scaling on body weight, using pooled PK data from this study and other existing infant data (i.e. other preventive studies in children that enrolled infants). The influence of covariates such as nutritional status (time-varying weight-for-height, weight-for-age, height-for-age or mid-upper arm circumference), sex and maternal socioeconomic status will be assessed.

- Likelihood ratio tests will guide the model building to determine statistical significance, diagnostic plots, and validation techniques, including visual predictive checks during covariate assessment. Due to the sparseness of the collected data, a frequentist prior approach may be necessary to stabilise the structural model parameters in the commonly used 3-compartment distribution model following a transit-absorption profile.[72]

- AUC derived from the final model structural model and observed trough piperaquine concentrations will be the modelled pharmacokinetic parameter, while the time interval to malaria infection will be the primary pharmacodynamic outcome. This will allow determination of the protective piperaquine threshold and the time spent above the threshold during PMC. An interval-censoring time-to-event pharmacodynamic model will be explored, as it would provide a more mechanistic understanding of recurrent malaria infections[124] and would be suitable for estimating this in the context of PMC.

- The protective 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.

- A 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)

- Given a growing interest in harmonising weight-based dosing across therapeutic areas,[125] an exploratory analysis will be conducted to evaluate how the proposed DP dosing regimen for PMC during routine health facility visits aligns with the harmonised WHO paediatric antibiotic weight bands.

CHAPTER 4

Efficacy and safety of dihydroartemisinin-piperaquine for perennial malaria chemoprevention aligned with routine health facility visits in Malawian infants (OPTIMAL study): a randomised, single-blind, placebo-controlled, clinical trial

Abstract

Background

Monthly dihydroartemisinin-piperaquine (DP) prevents *Plasmodium falciparum* malaria in infants more effectively than three-monthly treatment. However, aligning perennial malaria chemoprevention (PMC) administration with routine health facility visits has the potential to improve its feasibility and adherence. We investigated the efficacy and safety of DP for PMC when administered with routine health facility visits in infants in Malawi, where routine RTS, S/AS01 malaria vaccination was introduced at 5, 6, and 7 months of age, six months after study initiation.

Methods

In a single-blind, placebo-controlled study conducted from February 2023 to February 2025, 220 eligible infants were randomly assigned to receive a 3-day DP treatment course (n = 110) or placebo (n = 110) at 10 weeks, 14 weeks, 6 months and 9 months of age. Malaria parasitaemia was measured pre-dose and on day 28 after each treatment course using microscopy and quantitative PCR. The incidence of malaria was defined as the occurrence of symptomatic infections (fever with parasitaemia confirmed by microscopy and/or qPCR) or asymptomatic parasitaemia (confirmed by microscopy and/or qPCR) at scheduled or unscheduled assessments from 2.5 to 12 months of age. The limit of detection of the qPCR assay was 2 parasites/μL. The occurrence of any adverse events was monitored until the infants reached 12 months of age.

Results

Malaria incidence was 1 versus 4 cases per 1,000 person-years at risk (0.001 and 0.004 episodes per person-year) in the DP and placebo arms, respectively, with DP associated with a 77% non-statistically significant reduction in malaria incidence (aIRR 0.23, 95% CI

0.03–2.11, p = 0.196). All observed malaria episodes occurred by 7 months of age, when nearly 50% of the infants in both arms had received at least one malaria vaccine dose. After adjusting for the number of malaria vaccine doses received, DP was associated with a 31% reduction in moderate–to–severe anaemia prevalence (18.3% and 12.5% in the DP and placebo arms, respectively; adjusted risk ratio 0.69, 95% CI 0.52– 0.93, p = 0.013). A total of 149 adverse events occurred in the DP arm and 134 in the placebo arm.

Conclusions

Administering DP for PMC during routine health facility visits was safe and associated with a decrease in anaemia and a non-statistically significant decrease in malaria incidence in a setting where the malaria vaccination was introduced shortly after study initiation. As RTS, S/AS01 vaccine administration commences at five months, these findings indicate the potential of DP for PMC in infants up to 7 months of age to complement the protection offered by the RTS, S/AS01 malaria vaccine in the second half of infancy.

Keywords

Efficacy, safety, dihydroartemisinin-piperaquine, malaria, antimalarials, perennial malaria chemoprevention.

Background

The World Health Organisation (WHO) recommends administering a full treatment course of antimalarial therapy at different time points to prevent malaria in the at-risk subpopulation of young children, typically in the first two years of life in areas of moderate-to-high malaria transmission intensity.[126] This strategy is termed perennial malaria chemoprevention (PMC), an extension of a previous focus on infants, known as intermittent preventive malaria treatment in infants (IPTi).[16] In line with this recommendation, dihydroartemisinin-piperaquine, an artemisinin-based combination therapy (ACT), has been shown to prevent *Plasmodium falciparum* malaria in children under 2 years of age more effectively when administered monthly compared with three-monthly intervals, and more effectively than sulphadoxine-pyrimethamine (SP), to which malaria parasites have developed widespread resistance.[39,40,71,101]

However, aligning administration of antimalarials for PMC with routine health facility visits is considered more acceptable and feasible in programmatic settings, than monthly dosing, with the potential to improve adherence.[14,15,41] Nevertheless, there is limited evidence on the efficacy and safety of ACTs for PMC when administration is aligned with routine health facility visits. Given the promising evidence of ACTs for PMC, the WHO has called for more evidence on the efficacy and safety of ACT multi-day treatment courses for PMC to inform malaria preventive treatment guidelines in young children. Additionally, the impact of PMC with ACTs in settings with routine malaria vaccination, such as the RTS, S/AS01 vaccine, has not been evaluated.

We investigated the efficacy and safety of the promising ACT, dihydroartemisinin-piperaquine (DP), for PMC in infants when dosing was aligned with routine health facility

visits in a setting where vaccination with the RTS, S/AS01 malaria vaccine was introduced as the standard of care, six months after study initiation.

Methods

This efficacy and safety study was part of a pharmacokinetic, randomised, interventional clinical trial aimed at optimising dihydroartemisinin-piperaquine dosing for malaria preventive treatment in Malawian infants (OPTIMAL Study), as described previously.[127] Once routine RTS, S/AS01 malaria vaccination was introduced, infants in both groups were scheduled to receive the RTS, S/AS01 malaria vaccine at 5-, 6- and 7 months of age.

Study design, randomisation, allocation concealment and blinding

In a randomised, single-blind, placebo-controlled clinical trial conducted from February 2023 to February 2025, infants were randomly allocated to receive a three-day treatment course of dihydroartemisinin-piperaquine or matched placebo from 10 weeks of age. They received three further treatment courses at scheduled time points aligned with routine health facility visits at 14 weeks, 6 months, and 9 months of age, and were followed up until 12 months of age. As PMC was not the standard of care in Malawi, the use of a placebo was considered ethical and provided an accurate estimate of the background malaria and adverse event incidence in the population.

Pre-determined randomisation codes were concealed in opaque envelopes and used to allocate infants into either study group. Study staff assigning participants to a treatment arm were not aware of the treatment arm to which an enrolled infant would be allocated until the assignment was completed. Throughout the study period, guardians of the participants were blinded to the treatment arm to minimise bias in the reporting of adverse events. However, blinding of study staff was not possible given that pharmacokinetic sampling was only conducted in the intervention arm for ethical reasons.

Study setting and malaria disease burden

The study was conducted at the under-five/vaccination clinic of Chikwawa District Hospital in southern Malawi. Chikwawa is situated within a floodplain of the large Shire River, which fuels local malaria transmission. *Plasmodium falciparum* malaria is endemic in the area, with a prevalence rate of 21-40%.[102] Malaria vaccination details were obtained from each infant's health passport.

Inclusion and exclusion criteria

Infants were enrolled in the study at age 2.5 months (10 weeks), with no malaria symptoms, and with their parents' or guardians' informed consent and willingness to adhere to all study procedures throughout follow-up until age 12 months. Infants were excluded if they had known DP allergies or contraindications, were HIV-exposed, had a pre-existing medical history of significant comorbidities that could influence drug exposure, such as renal, liver, gastrointestinal or cardiac diseases, had severe anaemia (haemoglobin <7 g/dL), the infant (or breastfeeding mother) was on medications that known to have clinically significant DP interactions or they were participating in another clinical trial.

Dihydroartemisinin-piperaquine and placebo administration

WHO-prequalified dispersible dihydroartemisinin-piperaquine (D'Artepp®, Fosun Pharma, Shanghai, China) was administered following the weight-based dosing WHO recommendations.[105] Similarly, matched Fosun-manufactured placebo dispersible tablets were administered to infants according to body weight. D'Artepp® or placebo were dispersed in water and administered without food, according to the manufacturer's instructions. Although the manufacturer recommends that food should not be taken at least 3 hours before and after administration, breastfeeding was not restricted in this study;

caregivers were, however, requested not to provide any other food to the infant at least one hour before and after drug administration. Administration of DP or placebo was observed on days 1 and 3. On day 2, treatment was administered at home by the caregiver, and self-reported adherence was documented on day 3.

Efficacy assessment

Both light microscopy and quantitative PCR (qPCR) were used to assess for malaria parasites in capillary blood samples. Light microscopy guided treatment decisions where needed, as more sensitive qPCR results (targeting the 18S rRNA gene[111]) were only available at study completion. These capillary samples were collected before DP/placebo treatment and 28 days post-treatment at routine health facility visits at 10 weeks, 14 weeks, 6 months and 9 months, and at any unscheduled visit. All symptomatic malaria diagnoses and treatments occurring outside the study clinic were captured. Infants who became unwell outside routine study clinic operating hours were permitted to seek care at the local health facility on any day of the week. As part of standard clinical assessment for febrile illnesses in children, malaria microscopy was performed at these visits. Following each unscheduled presentation, study staff reviewed the documentation recorded in the infant's health passport and transcribed relevant clinical information, including malaria test results and treatments provided. Any additional assessments required by the study protocol were completed at the next study interaction. This approach ensured that malaria episodes diagnosed and managed outside the study clinic were systematically captured and incorporated into the study dataset

Symptomatic or asymptomatic patent malaria parasitaemia, after receiving DP or placebo, was classified as a breakthrough infection and treated with the current standard of care, artemether-lumefantrine (AL), after excluding any features of severe malaria, for which infants would be admitted and treated with injectable artesunate. The incidence of malaria

was defined as the occurrence of symptomatic infections (fever with parasitaemia confirmed by microscopy and/or qPCR) or asymptomatic parasitaemia (confirmed by microscopy and/or qPCR) at scheduled or unscheduled assessments from 2.5 to 12 months of age, reflecting the total burden of infection, and occurring during person-time under follow-up. The limit of detection for qPCR was 2 parasites/μL. Cox proportional hazard models for cumulative malaria hazard, adjusted for repeated malaria events, were used to determine the overall malaria incidence.

Capillary haemoglobin (Hb) concentrations were measured using the HemoCue® Hb 301 System (HemoCue, Sweden) before each DP or placebo treatment course, 28 days post-treatment and at every unscheduled visit.[128,129] The prevalence of mild-moderate anaemia was assessed as Haemoglobin of <11 g/dL at any time of routine or unscheduled health facility visits before each treatment course, stratified by grade of anaemia, mild (<11 - 10 g/dL), moderate (7 - <10 g/dL) and severe (<7 g/dL). This was compared between the placebo and DP arms while accounting for repeated measures using mixed effects regression models.

Safety assessment

Adverse events (AEs), defined according to ICH E2A and ICH E6(R3) as any untoward medical occurrence in a participant following administration of a medicinal product, which does not necessarily have to have a causal relationship with this treatment,[130] were assessed and graded as mild, moderate or severe (serious) using the DAIDS Table for Grading the Severity of Adult and Paediatric Adverse Events.[131] A serious adverse event was any untoward medical occurrence (AE) that results in death, is life-threatening, requires inpatient hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect.[130] Participants' caregivers were routinely asked, using standardised wording at each visit, about any

symptoms experienced by the infant since the previous visit. This was followed by symptom-directed physical examination and any necessary investigations. Two physician investigators independently reviewed all AEs detected at scheduled or unscheduled visits to assess their relationship to the study drug based on whether they were definitely related, probably related, possibly related, unlikely related or not related to the administration of DP or placebo, in line with ICH guidelines,[132] and to determine any action(s) to be taken. A data and safety monitoring board (DSMB) regularly reviewed all adverse events.

Tolerability was defined by vomiting 60 minutes of administration of DP or placebo. If a child vomited within 30 minutes of taking DP or placebo on the days of observed treatment (days 1 and 3), the whole dose was re-administered. If vomiting occurred within 30 to 60 minutes of drug administration, half the dose was administered. No further treatment was administered if the repeat dose was vomited. To avoid the risk of overdosing, no additional tablets were provided to the caregiver to administer in case vomiting occurred at home on day 2 of treatment.

Sample size calculation

Ninety-eight infants in each treatment arm would provide more than 80% power to detect an absolute difference of at least 20% in malaria incidence between the control and intervention groups, assuming a baseline incidence of 0.5 episodes per person-year in the control group based on historical local facility data. After adjusting for a 10% loss to follow-up, 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, assuming an incidence rate of 0.51 per person-year in the control arm as previously reported by a study in Tororo, Uganda, which has a similar malaria transmission intensity as Chikwawa in Malawi.[36,115]

Ethics and regulatory approval

The study received prior approval from the local College of Medicine Research Ethics Committee (P.06/22/3663) in Malawi, the University of Cape Town Human Research Ethics Committee (361/2022), and the Liverpool School of Tropical Medicine Research Ethics Committee (22-038). Additionally, prior regulatory approval was received from the Malawi Pharmacy Medicines Regulatory Authority (PMRA/CTRC/IV/22112022140).

Results

Study profile and participant characteristics

Of 227 infants screened, 220 were randomised, 110 in each of the DP arm and placebo arms. Of these, 102 and 100 infants completed follow-up in the DP arm and placebo arms, respectively (Figure 1). There were more female participants in both arms of the study, and most infants (88% and 90% in the DP and placebo arms, respectively) were reported to always sleep under a mosquito bednet (Table 1). None of the infants received the vaccine at all three scheduled time points (ages 5, 6, and 7 months), but at least one dose of the malaria vaccine was administered to 24 (21.8%) and 20 (18.2%) and two doses were received in 80 (72.7%) and 88 (80%) infants in the DP and placebo arms, respectively (Table S2).

Efficacy of DP for PMC in the context of malaria RTS, S/AS01 vaccine implementation

The incidence of malaria was 0.001 and 0.004 episodes per person-year at risk in infants in the DP and placebo arms, respectively (Table 2). All observed malaria episodes occurred within the first 6 months of follow-up, with no episodes seen from 7 months of age (Figure 2, Table S1). There were no symptomatic malaria cases in the DP arm, while two symptomatic cases were observed in the placebo arm (Table 2). In both cases, malaria parasitaemia, as detected by PCR, was initially submicroscopic on a scheduled visit. Within two weeks of the initial submicroscopic detection, the infants presented with symptomatic infections, confirmed by light microscopy (Table S1). After adjusting for the number of malaria vaccine

doses received by each infant, DP administration was associated with a non-statistically significant reduction in malaria incidence of 77% (adjusted incidence rate ratio, 95% CI: 0.23, 0.03 – 2.11, p= 0.196) (Table 2, Figure 2).

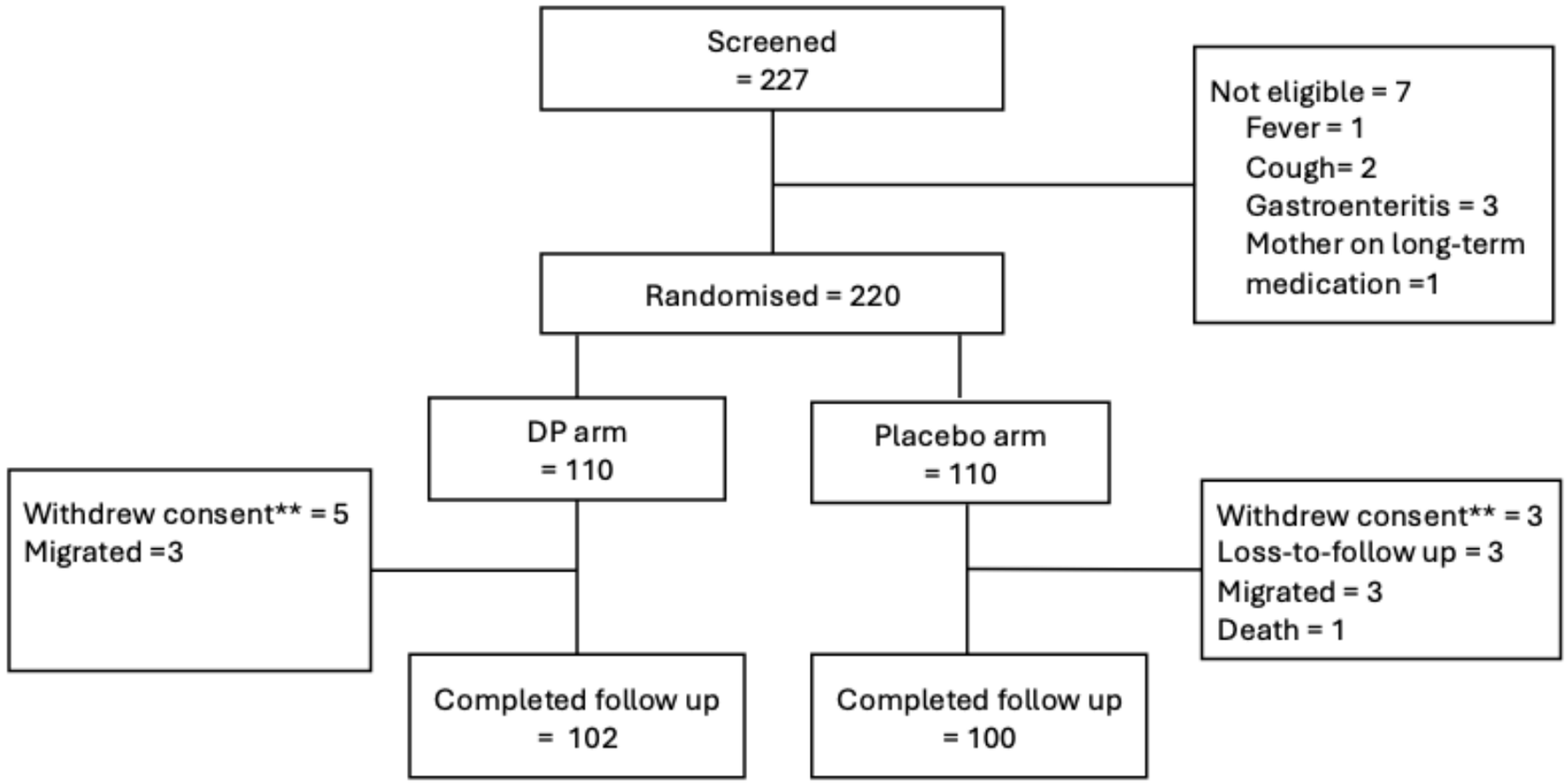


Figure 1. Trial profile.

*Pre-screening of potentially eligible participants included confirming the infant's age from a health passport and checking whether the caregiver was willing to hear more about the clinical trial as part of a consenting process

Table 1. Baseline characteristics of randomised participants (N= 220)

	DP arm (n=110)	Placebo arm (n=110)
Female sex, n [%]	60 [54.6]	66 [60.0]
Age in months, median [IQR]	2.6 [2.5-2.7]	2.6 [2.5-2.7]
Haemoglobin in g/dL, median [IQR]	11.3 [10.4-12.2]	11.2 [10.4-12.0]
Weight in kg, median [IQR]	5.5 [5.0-6.0]	5.4 [5.0-5.8]
Asymptomatic parasite prevalence n [%}	0 [0]	1 [0.9]
Height in cm, median [IQR]	56.8 [55.5-57.8]	56.4 [55.0-57.4]
Reported bednet use per week, n [%]		
Always	97 [88.2]	100 [90.9]
Most times (4-6 days)	3 [2.7]	3 [2.7]
Sometimes (1-3 day)	2 [1.8]	3 [2.7]
Never	8 [7.3]	4 [3.7]
Socioeconomic status*, n [%]		
Lowest (Bottom 25%)	36 [32.7]	30 [27.3]
Lower-middle (25-50%)	23 [20.9]	22 [20.0]
Upper-middle (50-75%)	30 [27.3]	24 [21.8]
Highest (Top 25%)	28 [25.5]	27 [24.6]

* Socioeconomic status computed as a percentile using combined estimate derived from data on availability of electricity in dwelling house, living in an iron-sheet roofed house, adequacy of clothing, household worries of food for meals, available of electronic appliances in the home for entertainment, member of household owning a bank account or mobile phone.

Table 2. Protective efficacy of perennial malaria chemoprevention following DP or placebo administered during routine health facility visits at 10 weeks, 14 weeks, 6- and 9 months of age

Outcome	Episodes (incidence[a]) or prevalence (%)		Unadjusted		Adjusted[b]	
	DP n=110	Placebo n=110	IRR or RR* (95% CI)	p-value	IRR or RR* (95% CI)	p-value
Overall Incidence of malaria[c]	1 (0.001)	4 (0.004)	0.25 (0.03-2.20)	0.209	0.23 (0.03-2.11)	0.196
Symptomatic malaria[d]	0 (0.0)	2 (0.002)	-	-	-	-
Malaria related hospitalisation or death	0 (0.0)	0 (0.0)	-	-	-	-
Anaemia prevalence[e]						
Mild anaemia (10 - 11 g/dL)	159/536 (29.7%)	159/545 (29.2%)	1.02 (0.84 - 1.22)	0.860	1.00 (0.83 - 1.20)	0.978
Moderate - severe anaemia (<10 g/dL)	67/536 (12.5%)	100/545 (18.3%)	0.68 (0.51 - 0.91)	0.008	0.69 (0.52 - 0.93)	0.013

[a]Per person year at risk

[b]Adjusted for number of malaria vaccine doses received as part of the RTS, S/AS01 vaccine administration schedule at 5, 6 and 7 months of age

[c]Symptomatic and asymptomatic infections at scheduled or unscheduled visits determined using microsocopy or rapid diagnostic test or quantitative PCR

[d]Diagnoses by microscopy or RDT with malaria symptoms and confirmed by quantitative PCR

[e]Haemoglobin <11 g/dL at any time of routine or unscheduled health facility visits before each treatment course of DP or placebo, and grouped as mild (<11 - 10 g/dL), moderate (7 - <10 g/dL) and severe (<7 g/dL)

*IRR; incidence rate ratio, RR; relative risk (prevalence ratio)

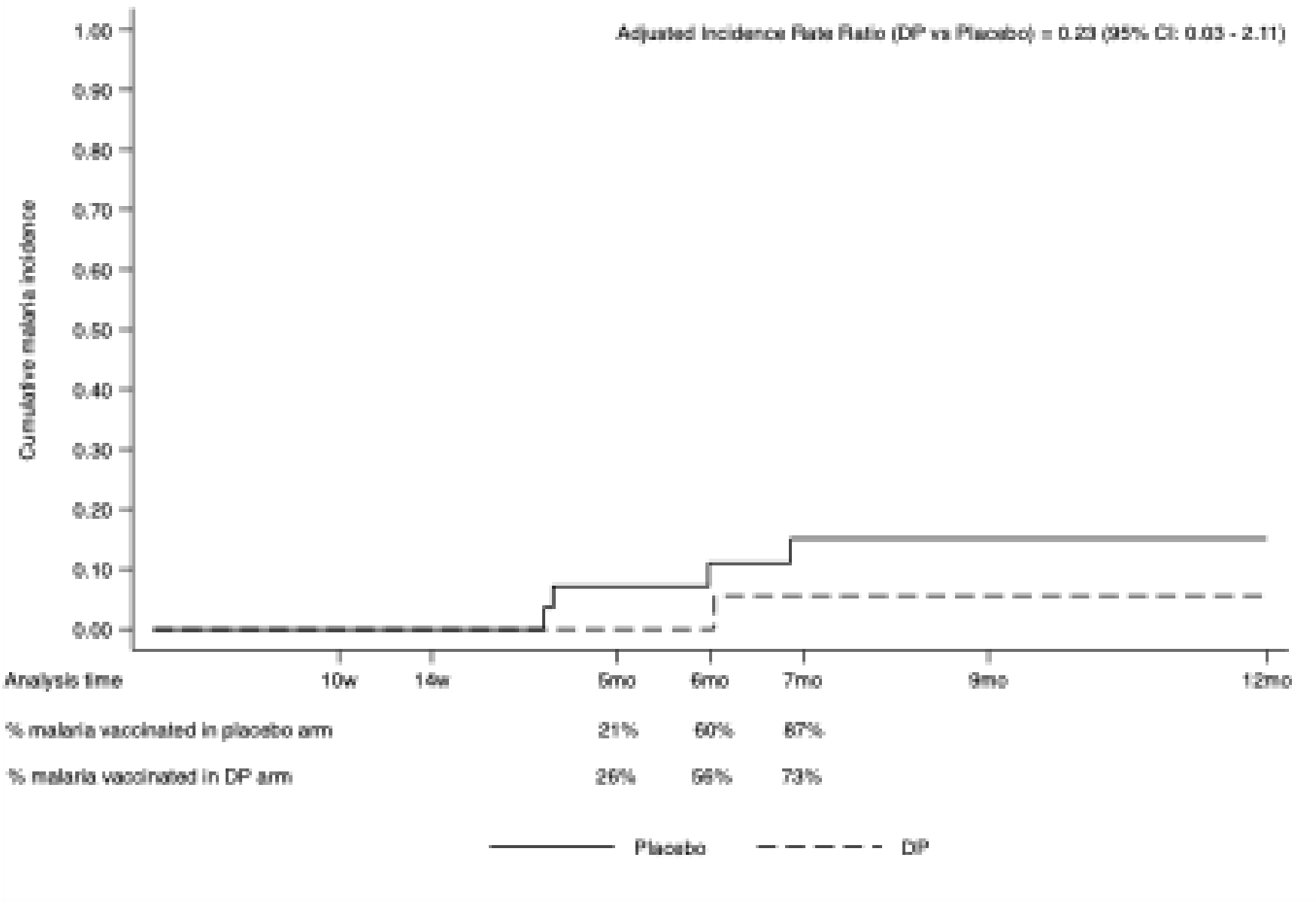


Figure 2. Time to malaria following initiation of PMC intervention in infancy (DP vs Placebo) aligned with routine health facility visits (at 10 weeks, 14 weeks, 6- and 9 months of age) and the malaria RTS,S/AS01 vaccination (at 5, 6 and 7 months of age). The malaria vaccination rates at each time point are presented. The analysis time is from randomisation at 10 weeks to 12 months of age. DP = Dihydroartemisinin-piperaquine**,** PMC= Perennial malaria chemoprevention, w = weeks, mo = months.

Impact of dihydroartemisinin-piperaquine on anaemia prevalence

The prevalence of moderate-severe anaemia (Hb <10 g/dL) was 12.5% in the DP arm and 18.3% in the placebo arm. After adjusting for the number of malaria vaccine doses received, DP was associated with a 31% reduction in the prevalence of moderate–to–severe anaemia (adjusted risk ratio, 95% CI: 0.69, 0.52– 0.93, p = 0.013) (Table 2).

Tolerability and safety outcomes

Fifty-six infants in the DP arm and 48 in the placebo arm experienced at least one adverse event throughout the study, with 149 adverse events in the DP arm and 134 in the placebo arm (Table 3, Figure S1). Of these events, twenty-six were assessed to be possibly related to the administration of the investigational product: 23 in the DP group and 3 in the placebo arm. These 26 events, all of which involved vomiting within 60 minutes of dosing, were classified as mild, as repeat dosing was tolerated (Table 3 and S3). They primarily occurred during the first and second quarters of the clinical trial (Table S4). Following retraining of the study team and caregivers on the optimal technique for administering the investigational product, only 5 and 1 vomiting events were reported in the DP and placebo arms, respectively, in the last two quarters of the study (Table S4).

Eleven serious adverse events were observed: seven in the placebo arm and four in the DP arm (Table 3 and Table S5). None of the serious adverse events were considered related to the administration of the investigational product. One serious adverse event of sepsis with severe anaemia in a 10-month-old infant in the placebo arm resulted in death (Table S5).

Table 3. Tolerability and safety of DP versus Placebo stratified by study arm and severity (N=283 events)

	DP arm (n=149 events in 56 infants)				Placebo arm (n=134 events in 48 infants)			
AE description	Mild	Moderate	Serious/ Severe*	Total	Mild	Moderate	Serious/ Severe*	Total
Tolerability								
Observed and reported vomiting after dosing (within 30 minutes)	23			23	3			3
Safety outcomes								
Upper respiratory tract infection	32	10		42	37	8		45
Skin abscess	1	1		2	2	1		3
Otitis media		1		1	2	1		3
Dysentery	2	3		5		4		4
Sepsis		9		9		2	1[#]	3
Conjunctivitis	6			6	2	2		4
Bronchiolitis				0	1	1	3	5
Bullous impetigo		1		1	2	3		5
Cutaneous larva migrans	1			1	3			3
Gastroenteritis	18	8	1	27	17	4	1	22
Eczema	1			1	2			2
Fever	5			5	5			5
Herpes labialis				0		1		1
Pneumonia	7	14	3	24	2	14	2	18
Mumps				0	1			1
Pruritic pupular eruption				0	2			2
Tinea	1			1	3			3
Urticaria	1			1	1			1
Varicella				0		1		1

*Severe cases classified and managed as serious adverse events

[#]Sepsis with severe anaemia, resulting in death of infant

Discussion

This study aimed to investigate the efficacy and safety of the promising antimalarial, dihydroartemisinin-piperaquine (DP), for perennial malaria chemoprevention (PMC) in infants when dosing was aligned with routine health facility visits in a setting where malaria vaccination with the RTS, S/AS01 vaccine became the standard of care six months after study initiation. DP for PMC in infancy at 10 weeks, 14 weeks, 6, and 9 months was safe and associated with a non-statistically significant reduction in malaria incidence in the first year of life. Notably, no malaria episodes were observed among infants from seven months of age, by which time nearly half had received at least one malaria vaccine dose. Additionally, there was a reduction in the prevalence of moderate to severe anaemia in infants who received DP compared with those who received placebo.

We observed a non-significant reduction in malaria incidence over the follow-up period, potentially due to a lower baseline incidence in the general population than expected and lower than previously reported in infants in settings with similar transmission intensity.[115,133,101] This may be explained by >85% of the infants in our study reportedly always sleeping under a treated mosquito bednet (Table 1). Additionally, the recent introduction of the malaria RTS, S/AS01 vaccine from 5 months of age could have begun to contribute to partial malaria protection in older infants, especially from 7 months of age, when nearly half of the infants had received at least one vaccine dose (Figure 2). However, unlike two or three RTS, S/AS01 vaccine doses in infancy, there is no evidence of malaria protection from a single isolated vaccine dose.[134–136] Nevertheless, these findings suggest a potential role for PMC in areas of moderate-to-high malaria transmission intensity, in early infancy to complement malaria vaccination in older infants receiving additional doses, thereby ensuring continuous protection throughout infancy, particularly in the context of rising insecticide resistance, which undermines the efficacy of widely used bed nets, and the high cost that limits access to more effective dual insecticide-treated nets.

In a programmatic setting, the use of ACTs such as DP for PMC could focus on the first 6 months of life, with dosing aligned with routine under-five health facility visits. DP could be administered at 10 weeks, 14 weeks and 6 months of age in line with standard immunisation visits. Our findings suggest additional doses of DP at 5 months, when RTS, S/AS01 malaria vaccination is commenced, and at 7 months of age to correspond with the third RTS, S/AS01 vaccine dose. With this proposed PMC schedule, a child would be protected throughout the period when maternal malaria antibodies passed on to the infant at birth are waning, and the infant is beginning to develop their own immunity.[6] However, considering that malaria vaccine coverage was low in our cohort, with none of the infants receiving all three recommended doses (Table S2), further studies are needed to confirm how best to align PMC dosing in infants with the RTS, S/AS01 vaccination schedule. Our future work will utilise pharmacokinetic blood samples from this cohort to characterise DP exposure in infancy and, if needed, propose an optimal DP dosing schedule for PMC when administered with routine health facility visits.

The use of ACTs for malaria prevention is known to reduce the overall burden of malaria-associated anaemia in children by reducing parasite load that could cause haemolysis.[39,40,101] In our infant cohort, we observed a reduction in the prevalence of moderate-severe anaemia following DP administration for PMC. This was unexpected given the low malaria incidence observed in our cohort and may be spurious. Alternatively, any impact of DP on anaemia reduction could be mediated through mechanisms other than malaria prevention, for example, through attenuation of inflammatory processes associated with other parasitic or infectious exposures.[137] Importantly, artemisinin derivatives have been shown in vitro to exert immunomodulatory effects (e.g. suppression of pro-inflammatory cytokines, modulation of T- and B-cell responses, promotion of regulatory T-cells).[138] However, the extent to which such immunomodulatory properties contribute to anaemia

reduction in settings with low malaria transmission remains unclear and warrants further investigation.

Generally, DP was safe with the proportion of adverse events that were similar between the two treatment groups (Table 3). However, there were more episodes of post-dose vomiting in the DP arm than in the placebo arm. Notably, these incidents occurred in the early days of the study when the study team and caregivers were not fully conversant with optimal administration techniques for DP. Additionally, in most vomiting cases, the mother reported breastfeeding the infant soon after DP administration. The frequency of vomiting episodes improved over time during the trial following retraining of study staff and caregivers on drug administration technique (Table S4). Nevertheless, the overall observed safety profile of DP in our study is similar to that in the placebo arm and as previously reported in other malaria chemopreventive studies in young children.[40,101]

Our study is not without limitations. First, complete blinding of the participant's guardians could not be perfectly achieved. This was due to an increased number of sampling occasions in the DP- than in the placebo arm, which could have potentially influenced the guardian's behaviour and biased safety assessments. However, this was not a major concern since adverse event reporting by caregivers was similar between the two study arms. Second, our pre-defined time points for parasitaemia assessments may have potentially underestimated the true malaria incidence if self-limiting infections occurred in between visits, as has been previously suggested in an observational infant study in Ghana.[139] Nevertheless, any malaria infections occurring between visits, with the potential to cause clinical symptoms, would have been quantified since parasitaemia assessments were conducted as part of all unscheduled visits, regardless of presenting symptoms. Third, there has been a decline in reported malaria incidence in Malawi over the last two decades, largely due to the scale-up of control interventions, such as insecticide-treated bednets.[140,141]

This would have significantly impacted the number of malaria episodes observed in our study.

Conclusion

The administration of dihydroartemisinin-piperaquine for perennial malaria chemoprevention during routine health facility visits, compared with a placebo, was safe and associated with a decrease in moderate to severe anaemia and a non-statistically significant reduction in malaria incidence in the first six months of infancy in a setting where the RTS, S/AS01 malaria vaccine was introduced shortly after study initiation. Considering that the administration of the RTS, S/AS01 vaccine commences at five months of age, these findings highlight the potential role of malaria chemoprevention in the first six months of life to complement the protection offered by the malaria vaccine in the latter half of infancy.

Supplementary material

Table S1. Details of participants with incident malaria episodes

Participant ID	111248*		111628*		112402	112485	112667
Age in days at occurrence of malaria episode	182	186	128	141	184	131	209
Diagnosis of malaria	PCR	Microscopy & PCR	PCR	Microscopy & PCR	PCR	PCR	PCR
Treatment arm	Placebo	Placebo	Placebo	Placebo	DP	Placebo	Placebo
Sex	Female	Female	Female	Female	Female	Female	Male
Protocol follow up time at occurrence of malaria episode	ST-6 months Pre-dose	Unscheduled visit post ST-6 months	28 days post ST-14 weeks	Unscheduled Visit post ST-14 weeks	ST-6 months Pre-dose	28 days post ST-14 weeks	28 days post ST-6 months
Number of DP or Placebo doses received by the time of malaria episode	2	3	2	2	2	2	3
Socioeconomic status	Upper-middle (50-75%)	Upper-middle (50-75%)	Highest (Top 25%)	Highest (Top 25%)	Lower-middle (25-50%)	Lower-middle (25-50%)	Highest (Top 25%)
Reported bednet use per week	Always	Always	Always	Always	Most times (4-6 days)	Always	Always
Reported exclusive breastfeeding	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Number of malaria vaccine doses received	2	2	0	0	1	0	2

* Malaria episodes diagnosed first on PCR as asymptomatic parasitaemia and then presented within 14 days as a breakthrough symptomatic infection.

ST: Scheduled Treatment

Table S2. **Number of vaccine doses received by randomised infants**

	Treatment arm	
	DP (n = 110)	Placebo (n=110)
Number of vaccine doses		
None	6 (5.5%)	2 (1.8%)
One dose	24 (21.8%)	20 (18.2%)
Two doses	80 (72.7%)	88 (80.0%)
Three doses	0 (0.0%)	0 (0.0%)

Table S3. Relationship between adverse event severity and administration of investigational product (N=283 events)

	DP arm (n=149 events in 56 infants)				Placebo arm (n=134 events in 48 infants)			
	Mild	Moderate	Serious/ Severe*	Total [%]	Mild	Moderate	Serious/ Severe*	Total [%]
Possibly related to investigational product, n [%]	23	0	0	23 [15.4]	3	0	0	3 [2.2]
Unlikely related to investigational product, n [%]	75	47	4	126 [84.6]	81	43	7	131 [97.8]

*Severe cases classified as serious adverse events

Table S4. Occurrence of possibly related adverse events over the study period (N=26 events)

	DP arm (n = 23 events)					Placebo arm (n = 3 events)				
	Q1	Q2	Q3	Q4	Total	Q1	Q2	Q3	Q4	Total
Possibly related to investigational product[#]	12	6	2	3	23	1	1	0	1	3

Q: Study quarter, defined as a period of 180 days calculated from date of study start to end of follow up

[#] Possibly related events were all due to vomiting within an hour of drug administration

Table S5. Details of serious adverse events

No	PID	AE description	Arm	Start date	Summary of SAE	Outcome
1	111065	Bronchiolitis	Placebo	01 September 2023	An 8-month-old male enrolled into the study on 27 February 2023 He was admitted in hospital on 4 September 2023 with working diagnoses of bronchiolitis/severe pneumonia following a three-day history of worsening cough, irritability, vomiting and poor feeding. The infant was treated with standard antibiotics (benzylpenicillin and gentamycin,)and discharged 2 days later following improvement of symptoms. His recovery was reported by the mother on 25 September 2023 following completion of oral antibiotics.	Resolved
2	111263	Severe pneumonia	Placebo	01 July 2023	A 6-month female participant who was enrolled into the study on 18th April 2023. She was admitted in hospital on 03 July 2023 with a working diagnosis of severe pneumonia following a 2-day history of cough, fever, and poor feeding. The infant was treated with benzylpenicillin, gentamycin, dexamethasone, and paracetamol, and was discharged 2 days later, on oral amoxicillin and paracetamol. The mother reported all medications were completed with full recovery within a week from the date of discharge	Resolved
3	111271	Severe pneumonia	DP	22 September 2023	An 8-month-old male participant who was enrolled into the intervention (DP) arm on 19th April 2023. He was admitted in hospital on 23 September 2023 with a working diagnosis of severe pneumonia following a one-day history of cough and shortness of breath. He was treated with ceftriaxone and paracetamol syrup and discharged on 25 September 2023 on oral antibiotics (erythromycin). The mother reported at the next visit that antibiotics were completed within one week of discharge from the hospital, and the child had been well	Resolved
4	111297	Sepsis with severe anaemia	Placebo	06 December 2023	A 10-month-old female who was enrolled into the placebo arm of the study on 24 April 2023. She had all four treatment course schedules. Her initial serious adverse event was reported as hospitalisation due to a working diagnosis of typhoidal sepsis with severe anaemia, on 11 December 2023. This event was preceded by a 5-day history of fever, irritability, and passage of loose stools In hospital, the child received blood transfusion and clinically improved. She was discharged on 14 December 2023 on oral augmentin, metronidazole, paracetamol and continued zinc and oral rehydration salts (ORS). On 22 December 2023, after the study team noticed that the child had missed her planned review date of 21 December 2023, the study team members conducted a follow up visit at home where the mother was found attending a social-cash transfer event. The child was observed to not be well, and the study team agreed with the mother to take the child to the hospital. She did not, however, accept a hospital admission on this occasion and insisted to go home. A day later, the child was brought to the hospital by the mother due to worsening diarrhoea and very high fevers. The child also had an episode of a convulsion while in the ward. On this admission the baby was started on intravenous gentamycin and benzylpenicillin for 48 hours before being switched to intravenous ceftriaxone; she was also started on	Death

					oxygen therapy. The baby remained febrile throughout the admission with temperatures ranging from 38.2 to 40 degrees Celsius. The child deteriorated with persistent fevers and a decreasing level of consciousness. On 26 December 2023, at around 16:00 hours the child was observed to be gasping, resuscitation was unsuccessful, and she died at 16:45 hours.	
5	111370	Gastroenteritis with severe dehydration	Placebo	04 November 2023	An 8-month-old male participant who was enrolled into the placebo arm of the study on 10th May 2023. He was admitted in hospital on 6 November 2023 with differential diagnoses of acute gastroenteritis with severe dehydration / suspected typhoid/non-typhoidal salmonella sepsis. He was treated with intravenous antibiotics (ceftriaxone) and he made steady recovery in the ward. He was discharged on 8 November on oral antibiotics (ciprofloxacin), iron tablets, ORS, and zinc. At the next follow up, the mother reported the child completed oral medications and recovered well.	Resolved
6	111867	Gastroenteritis	DP	15 January 2024	A 7-month-old female participant who was enrolled in the DP arm of the study on August 22, 2023. He was admitted on 16 January 2024 with a working diagnosis of gastroenteritis following a day's history of vomiting and loose stools. In hospital, the child was treated with intravenous fluids and ORS. He made steady recovery and was discharged two days later, on ORS. At the next follow up visit, the mother reported that the child had recovered with no other complaints	Resolved
7	112089	Bronchiolitis	Placebo	30 November 2023	A 4-month-old female participant who got enrolled into the placebo arm of the study on the 10th of October 2023. She was admitted in hospital on 4 December 2023 with a working diagnosis of bronchiolitis following a five-day history of shortness of breath and cough which had not improved. The mother had previously presented with the child to the hospital with similar symptoms three days prior to this visit. At this initial visit, the child was treated with oral antibiotics (amoxycillin). In hospital, the child was treated with oxygen therapy and made steady recovery. The baby was discharged two days later, and at a follow-up telephonic visit (two days after discharge), the mother reported the child was well.	Resolved
8	112683	Bronchiolitis	Placebo	27 March 2024	An 11-week-old male participant who was enrolled into the placebo arm of the study on 26 March 2024. He had developed a cough, fever and poor feeding started. This was on day 2 of receiving the study drug, DP, he had received 2 doses out of 3 when symptoms started. On examination at the hospital, the baby was febrile, breathless, with nasal flaring and bilateral lung crepitations. He was admitted in the hospital on 27 March 2024 with a working of diagnosis of bronchiolitis and a differential diagnosis of pneumonia. The participant was started on empirical intravenous antibiotics for pneumonia (benzylpenicillin and gentamicin) and clinically improved by day 3 of admission (29 March 2024, day of discharge). The baby had recovered by the time of follow-up (a week later)	Resolved
9	112683	Severe pneumonia	Placebo	22 July 2024	This was a second serious adverse event in this participant. The first was an event of bronchiolitis as detailed above	Resolved

					At the time of this second event, the participant was 7 months old and had received three treatment courses of DP. He was admitted in hospital on the 23rd of July, with a diagnosis of pneumonia and a differential diagnosis of bronchiolitis, following a one-day history of cough, fever and worsening shortness of breath. He received intravenous benzylpenicillin, dexamethasone, gentamicin, and oral paracetamol. He was discharged three days later after making remarkable clinical improvement. However, he continued to have residual cough for a week, without shortness of breath or poor feeding. Three weeks post discharge, the mother reported that the child's symptoms had resolved, and the child had made full recovery	
10	112626	Severe pneumonia	DP	5 November 2024	A 10-month-old male infant who was enrolled into the DP arm of the study on 20 March 2024 He was admitted in hospital on 7 November 2024 with a working diagnosis of severe pneumonia and gastroenteritis with no dehydration, and a differential diagnosis of bronchiolitis. This was preceded by a two-day history of fever, cough, difficulties in breathing and non-bloody diarrhoea. He received oxygen therapy and was later put on continuous positive airway pressure (CPAP) before being transitioned back to oxygen via nasal prongs . Additionally, he received intravenous ceftriaxone and was discharged from the hospital on 11 November having made clinical improvement and after being weaned off oxygen therapy. He was continued on oral metronidazole syrup, paracetamol, oral zinc supplement and ORS. The mother reported full recovery of the infant on 3 December 2024.	Resolved
11	112691	Severe pneumonia	DP	1 November 2024	A 9-month-old male infant who was enrolled into DP arm of the study on 26 March 2024 He was admitted in hospital on 1 November 2024 with a one-day history of laboured breathing, cough, fever and poor feeding. The working diagnosis was severe pneumonia with a differential diagnosis of bronchiolitis. He received intravenous antibiotics: benzyl penicillin and gentamicin and paracetamol while in hospital and was discharged three days later having made clinical improvement. Oral antibiotics (amoxicillin) and paracetamol were continued at home, where he was still reported to have residual cough and mild shortness of breath. On follow up, two weeks post hospital discharge, the mother reported the child had made full recovery by 16 November 2024	Resolved

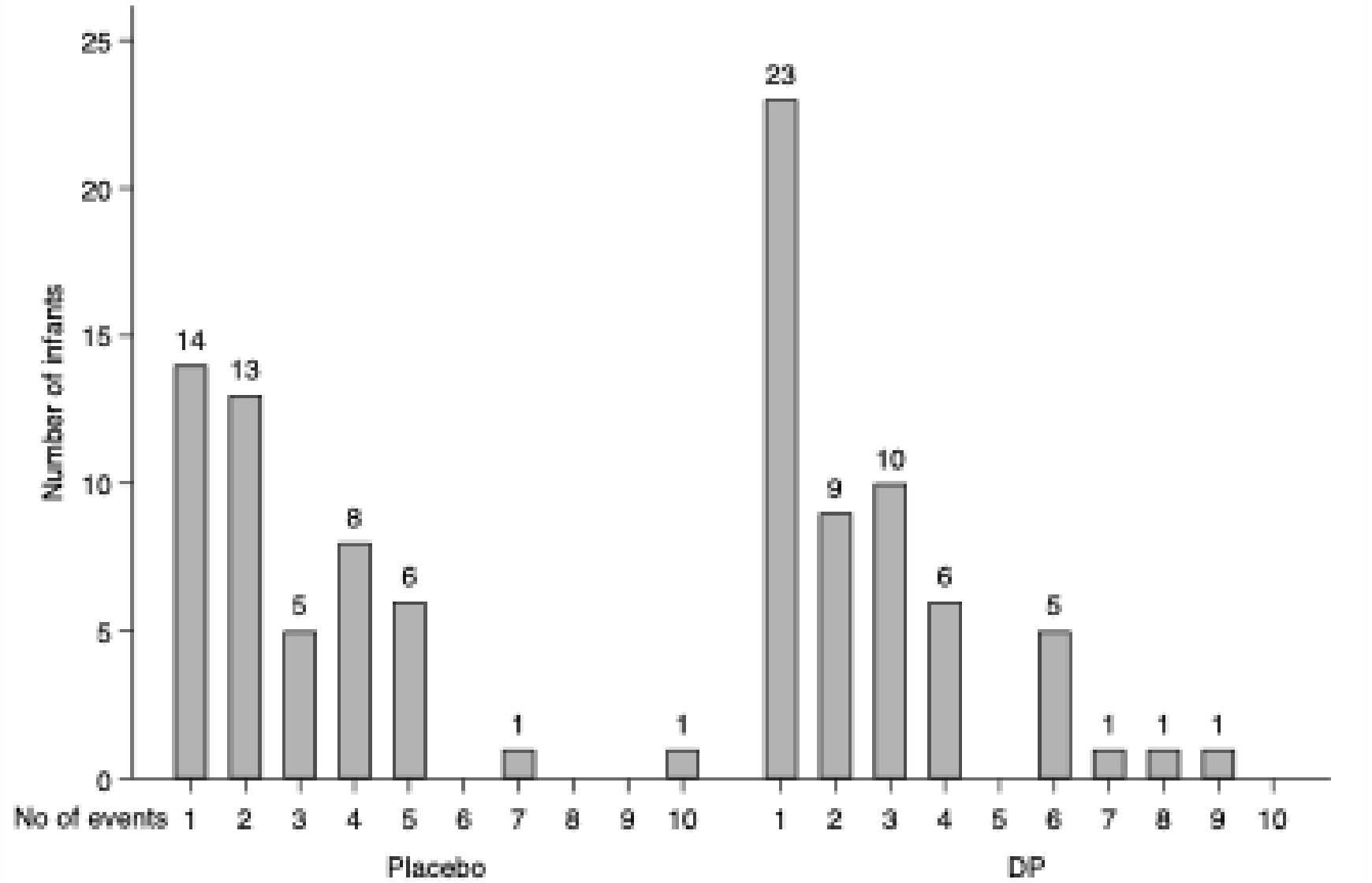


Figure S1. Number of infants experiencing one or more adverse events stratified by treatment arm. The number above each bar represents the infants experiencing events, with the frequency of events represented on the x-axis and ranging from 1 to 10 events.

CHAPTER 5

Piperaquine pharmacokinetic profile in Malawian infants given dihydroartemisinin-piperaquine for perennial malaria chemoprevention during routine health facility visits

Abstract

Background

There is a limited understanding of the pharmacokinetic profile of the long-acting antimalarial piperaquine when given as dihydroartemisinin-piperaquine (DP) for perennial malaria chemoprevention during routine health facility visits in infancy, a strategy expected to enhance feasibility and adherence. We aimed to characterise the effect of increasing age in infancy on piperaquine exposure and trough concentrations following DP dosing for PMC during routine health facility visits at 10 weeks, 14 weeks, 6 months, and 9 months of age.

Methods

Infants randomised to the intervention group (n = 110) of a two-arm, placebo-controlled clinical trial in Malawi received a standard three-day treatment course of DP at routine health facility visits at 10 weeks, 14 weeks, and 6 and 9 months of age. Capillary blood samples were collected pre-dose, and then on days 3, 7, 14, and 28, and at any unscheduled visit. To minimise PK sampling, infants were randomised to be sampled at either 10 weeks and 6 months or 14 weeks and 9 months of age. Nonlinear mixed-effects modelling was used to derive the secondary parameter of piperaquine exposure, $AUC_{0\text{-}28\ days}$, while trough concentrations (pre-dose) were directly observed. Geometric means (90% CI) were calculated to compare these parameters across dosing timepoints.

Results

A two-compartment structural model with first-order absorption provided the best data fit. Piperaquine exposures, $AUC_{0\text{-}28\ days}$, $C_{day\ 28}$, and $C_{day\ 7}$, were 58% (GMR 0.42; 90% CI 0.36 – 0.49), 44% (GMR 0.56; 90% CI 0.43 – 0.74) and 59% (GMR 0.41; 90% CI 0.33 – 0.51) lower, respectively, at 9 months compared with 10 weeks of age. The proportion of infants with pre-dose piperaquine venous plasma concentrations below a previously proposed

protective threshold of 15.4 ng/mL was 2.1%, 33.3%, and 88.9% at 14 weeks, 6 months, and 9 months of age, respectively (p-trend, <0.001).

Conclusions

An increase in infant age from 10 weeks to 9 months was associated with a decrease in piperaquine exposure and a higher proportion of infants with concentrations below the predefined protective concentration threshold. These results underscore the need to optimise the dihydroartemisinin-piperaquine dosing regimen in infants to ensure improved perennial malaria chemoprevention aligned with routine health facility visits.

Keywords

Pharmacokinetics, dihydroartemisinin-piperaquine, malaria, antimalarials, perennial malaria chemoprevention, infants.

Background

In areas of moderate-to-high malaria transmission, previous work has suggested that monthly dosing of the artemisinin-based combination therapy, dihydroartemisinin-piperaquine (DP), would achieve the venous plasma threshold of at least 15.4 ng/mL needed for perennial malaria chemoprevention (PMC) in the first two years of life.[71,101] However, administering a monthly, three-day antimalarial treatment course would present challenges to feasibility and adherence in programmatic settings. Therefore, there is a need to align DP dosing for PMC with routine health facility visits[14,15,41], such as immunisation clinic visits. [38]

Additionally, rapid physiological changes in infants, such as age-dependent alterations in body composition and maturation of metabolising CYP450 enzymes, are known to impact drug exposure.[50,142,143] However, there is limited evidence on the extent to which these changes affect piperaquine exposure, as well as sustaining piperaquine concentrations above the purported protective threshold of 15.4 ng/mL, when DP is administered for PMC during routine health facility visits.

We hypothesised that increasing age in infancy would be associated with a reduction in overall piperaquine exposure due to physiological non-linear changes that increase piperaquine clearance. Thus, we aimed to characterise the impact of increasing age in infancy on the secondary pharmacokinetic parameter (overall exposure, AUC) of the longer-acting partner drug of DP, piperaquine, as well as day 7 concentrations ($C_{day\ 7}$) that are well known to correlate with AUC. In addition, the effect of age on piperaquine trough concentrations (pre-dose and day 28 (C_{28})) falling below the proposed protective threshold of 15.4 ng/mL was assessed to inform the optimal dosing regimen of DP for PMC in infants during routine health facility visits.

Methods

This pharmacokinetic study was part of a randomised, placebo-controlled clinical trial aimed at optimising DP dosing regimen for malaria preventive treatment in Malawian infants (OPTIMAL Study), PACTR202211575727659. The detailed protocol for the clinical trial has been described previously.[127]

Study design

In a single-blind, two-arm, placebo-controlled clinical trial, infants randomised to the interventional arm received a three-day treatment course of DP at routine health facility visits at 10 weeks, 14 weeks, 6- and 9 months of age. They were further randomised to two pharmacokinetic blood sampling schedules to minimise the number of sampling occasions per infant (Figure 1). In Schedule 1, capillary blood samples were collected at pre-dose, then on days 3, 7, 14, and 28, and at any unscheduled visit after the 10-week and 6-month treatment courses. In Schedule 2, capillary blood samples were collected pre-dose and at the same time points after the 14-week and 9-month treatment courses. All infants were followed up until 12 months of age. The study was conducted at the under-five/vaccination clinic of Chikwawa District Hospital in southern Malawi, with pre-screening conducted at selected clinics in the hospital's catchment area.

Inclusion and exclusion criteria

Infants included in the study were 2.5 months (10 weeks) old, had no symptoms of malaria at the time of recruitment, their parents or guardians had given written informed consent to their participation, and were willing to adhere to all study procedures throughout follow-up until 12 months of age. Participants were excluded if they had known allergies or contraindications to the study medication, were HIV-exposed, had a medical history of pre-existing significant comorbidities that could influence drug exposure, such as renal, liver, gastrointestinal or cardiac diseases, had severe anaemia (haemoglobin <7 g/dL), the mother

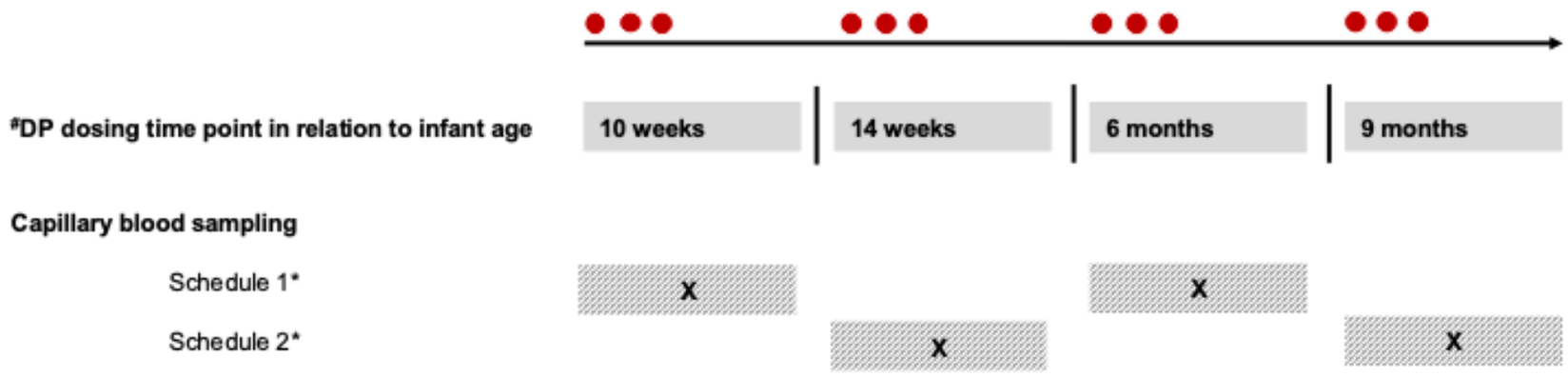
or infant was on medications that are known to have clinically significant interactions with dihydroartemisinin-piperaquine or they were taking part in another clinical trial.

Dihydroartemisinin-piperaquine administration

A WHO-prequalified dispersible fixed-dose combination tablet of dihydroartemisinin-piperaquine (D'Artepp®, Fosun Pharma, Shanghai, China) was administered according to the infant's weight in line with the WHO recommended doses as follows: 160/20 mg for 5 – <8 kg, 240/30 mg for 8 - < 11 kg, 320/40 mg if weighing 11 - <17 kg and 480/60 mg for weight between 17 and <25 kg.[126] D'Artepp® was mixed with water and administered without food, as per the manufacturer's instructions. Although the manufacturer recommends that food should not be consumed at least 3 hours before and after administration, breastfeeding was not restricted in this study. Caregivers were, however, requested not to provide any other food to the infant for at least one hour before and after drug administration. Administration of DP or placebo was observed on days 1 and 3. On day 2, treatment was administered at home by the caregiver, and self-reported adherence was documented on day 3.

Blood sample collection and piperaquine assay

Capillary blood samples were collected onto a 50 µL dry blood spot on Whatman 3MM paper and left to dry at room temperature. Thereafter, they were stored with desiccant in sealed zippered plastic bags. Two blood spots were shipped to the Mahidol-Oxford Tropical Medicine Research Unit (MORU) in Bangkok, Thailand, for piperaquine assay using liquid chromatography coupled with tandem mass spectrometry (LC-MS), as previously described.[109] Quality control (QC) samples at 9, 40, 350, and 800 ng/mL were analysed in triplicate within each batch of samples to ensure the assay's accuracy and precision. The corresponding percent relative standard deviations (% RSD) were 3.55%, 3.76%, 3.79%, and 3.17%, respectively. The lower limit of quantification (LLOQ) was 3 ng/mL.



- #DP = Dihydroartemisinin-piperaquine (represented by red dots at the top of the arrow) administered as a three-day, once a day, treatment course at 10 weeks, 14 weeks, 6 months and 9 months in all infants
- *Details on capillary blood sampling time points in Schedules 1 and 2
    1. Schedule 1: Pre-dose then on days 3, 7, 14 and 28, and any unscheduled visit after the 10-week and 6-month treatment course
    2. Schedule 2: Pre-dose then on days 3, 7, 14 and 28, and any unscheduled visit after the 14-week and 9-month treatment course

Figure 1. Dosing time points and pharmacokinetic sampling schedules

Tolerability assessment

Tolerability of DP was defined by vomiting within 30 or 60 minutes of administration. If a child vomited within 30 minutes of taking DP or placebo on the days of observed treatment at the clinic (days 1 and 3), the whole dose was re-administered. If vomiting occurred within 30 to 60 minutes of drug administration, half the dose was re-administered. No further treatment was administered if the repeat dose was vomited. All episodes of vomiting, including vomiting on day 2 when treatment was administered at home by the guardian, were recorded. To avoid the risk of overdosing, no additional tablets were provided to the caregiver to administer in case vomiting occurred at home on day 2 of treatment.

Malaria parasitaemia assessment

To determine a piperaquine protective concentration threshold, additional capillary blood spots were collected before each treatment course of DP and 28 days after, at 10 weeks, 14 weeks, 6 months, and 9 months, to assess malaria parasitaemia using quantitative PCR (qPCR). These malaria samples corresponded with time points for pharmacokinetic sample collection. Additionally, qPCR samples were collected at all unscheduled visits.

Sample size calculation

The sample size calculation was based on estimating the precision of the impact of increasing age in infancy on piperaquine overall exposure (AUC) and trough concentrations. Using a stimulation re-estimation approach in NONMEM software (ICON Development Solutions, Hanover, MD, USA), a sample size of 100 infants was shown to provide at least 28% precision in estimating this impact. After adjusting for a 10% loss to follow-up, 110 participants were planned for recruitment.

Population pharmacokinetic model

Compartmental pharmacokinetic analyses were conducted using non-linear mixed-effects modelling in Monolix version 2024R1 (Lixoft SAS, Antony, France) to derive individual area under the concentration-time curve (AUC) over 28 days (from 0 to 672 hours) after each dosing time points (10 weeks, 14 weeks, 6 and 9 months). Structural distribution models with 1-, 2-, and 3-compartment distribution and first-order absorption were fitted. More complicated absorption models, such as lag-time and transit-compartment absorption models, were not evaluated as there was no observed data in the absorption phase. In each model, the primary pharmacokinetic parameters (volume of distribution and clearance) were allometrically scaled for bodyweight by normalising the infant's weight to the median weight of the study cohort. These were raised to the power of 0.75 for clearance parameters and 1 for volume of distribution parameters. A change in objective function value (OFV) of 3.84 ($p < 0.05$) was considered statistically significant when comparing two hierarchical models with a one-degree-of-freedom difference. Visual predictive checks were also utilised to determine the model fit.[144] Given the hypothesis that increasing age may impact piperaquine exposure, age in months was evaluated as a fixed covariate in the final pharmacokinetic model. To avoid overparameterization of the final model, given the sparseness of the data, no other covariates were evaluated at this stage.

Statistical analyses

Individual $AUC_{0\text{-}28\ days}$ derived from the final model and observed exposure parameters (pre-dose, $C_{day\ 7}$, and $C_{day\ 28}$) were log-transformed to calculate their geometric mean ratio (GMR) with 90% confidence intervals (CIs) at 10 weeks, 14 weeks, 6 months, and 9 months of age. The log-transformed parameters were compared between these time points using *t* tests and back-transformed to absolute ng/mL concentrations for reporting. Changes in pharmacokinetic parameters between the dosing time points were considered statistically significant when the 90% CI of the GMR did not cross the value of 1.[119]

To determine whether additional dosing was required following vomiting within 60 minutes after a DP dose, the geometric mean (with 90% CI) of $AUC_{0\text{-}28\ days}$, $C_{day\ 28}$ and $C_{day\ 7}$ were compared between infants who vomited and were subsequently redosed and those who did not vomit. The proportion of participants unable to attain trough piperaquine concentrations (pre-dose and $C_{day\ 28}$) above the purported protective threshold of 15.4 ng/mL was calculated and compared across each dosing period.

In participants experiencing a malaria episode, piperaquine concentrations at the time of malaria occurrence were assessed in relation to the time of the last dose of DP. All statistical analyses were performed in Stata version 18.0 (StataCorp LLC, Texas, USA).

Ethics and regulatory approvals and the informed consent process

The study received ethics approval from the local College of Medicine Research Ethics Committee (P.06/22/3663) in Malawi, the University of Cape Town Human Research Ethics Committee (361/2022), and the Liverpool School of Tropical Medicine Research Ethics Committee (22-038). Additionally, regulatory approval was received from the Pharmacy Medicines Regulatory Authority in Malawi (PMRA/CTRC/IV/22112022140). During the informed consent process, the literacy level of the participant's caregiver or legally acceptable representative was assessed by asking whether they were willing to read and explain a brief statement from the consent form. To minimise undue bias of socioeconomic status within the study population, infants of caregivers who were unable to read or write were still considered for participation in the study as long as the caregiver understood the participant information sheet and there was an impartial witness to ensure that the caregiver was provided with the correct and complete information. An impartial witness was someone who was not directly involved with the study e.g. a companion of the caregiver or another health worker from the government health facility or who was literate. The caregiver was asked if they were comfortable with such an arrangement. Consenting took place in a private space, and every effort was made to explain the process and procedures of the study in a

language understandable by the participant. The caregiver was provided with the PIS to take home to read or discuss the information with any other person (if they wished), including members of their family, friends or a healthcare worker that they see routinely. In this case, they were requested to allow the study team to contact them the following day, either through a mobile phone or home visit, for the caregiver to ask any further questions and to communicate their decision. If after providing information, the caregiver agreed to have their child participate in the study, they were asked to sign the consent form in presence of the study personnel, and an impartial witness if the participant is illiterate. In cases where caregiver displayed a clear lack of understanding of the aim of the study, procedures, time commitment, risks and benefits, assessed through question and answers session, and after several attempts to clarify them, the study personnel was allowed to make a judgement to not continue with the consent process.

RESULTS

Study profile and participant characteristics

Among the 110 infants randomised to the DP arm, 60 participants were allocated to sampling Schedule 1 and 50 to sampling Schedule 2. Of the 110 enrolled infants, 102 (92%) completed follow-up (57/60 in Schedule 1 and 45/50 in Schedule 2), as shown in Figure 2. The participant characteristics were similar between the two sampling schedules (Table 1). There were 1,037 scheduled piperaquine concentrations assayed, 577 in Schedule 1 and 460 in Schedule 2. The distribution of piperaquine concentrations is illustrated in Figure 3 and summarised in Table 1.

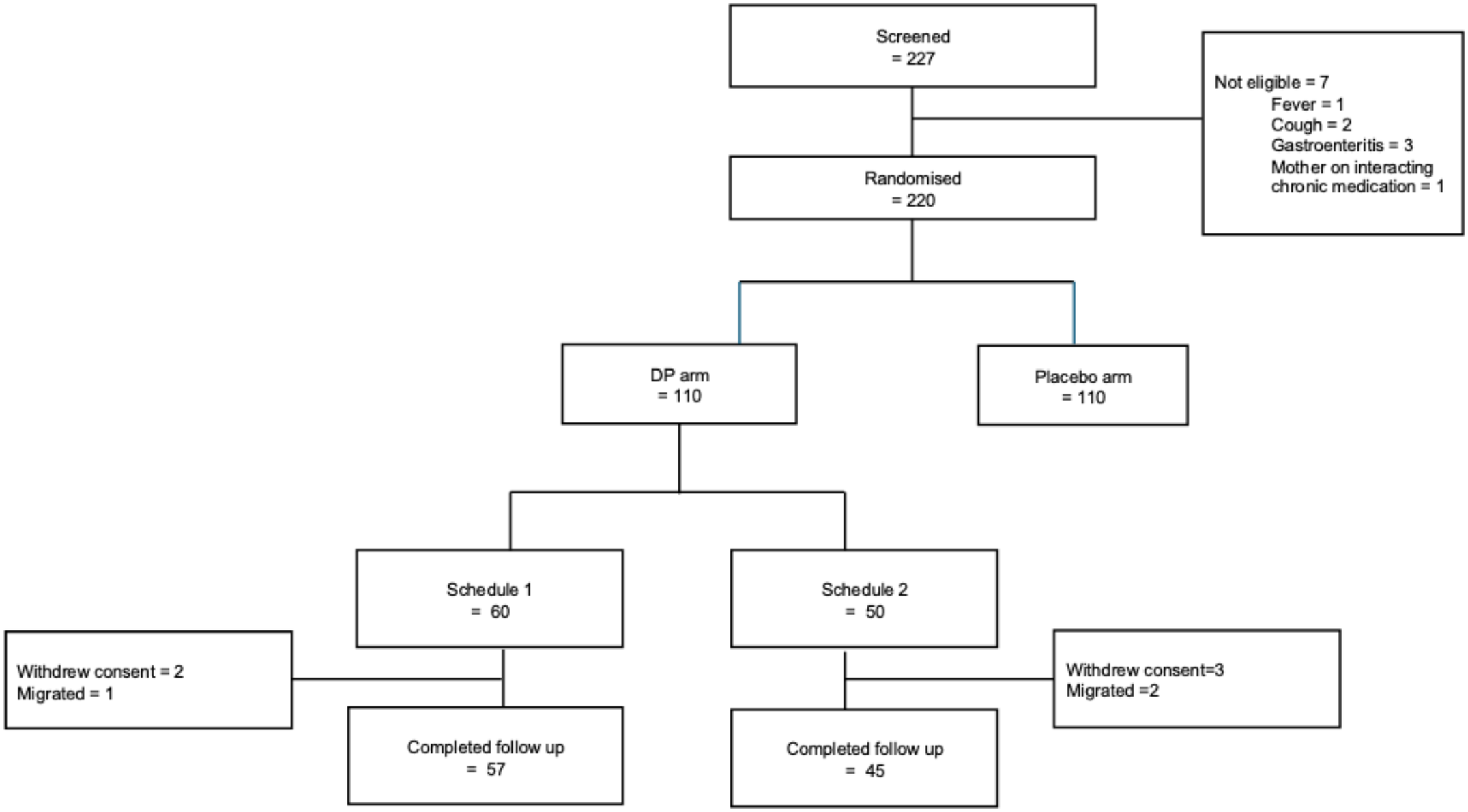


Figure 2. Study profile

Table 1. Baseline characteristics of participants stratified by sampling schedule

	Schedule 1	Schedule 2
Number randomised	60	50
Female sex, n [%]	35 [58.3]	25 [50.0]
Age at enrolment in months, median [IQR]	2.6 [2.5-2.7]	2.6 [2.5-2.7]
Post-menstrual age at enrolment[#] in weeks, median [IQR]	49.2 [49.0 - 49.6]	49.2 [48.9 - 49.5]
Weight in kg, median [IQR]		
10 weeks	5.3 [4.9 - 5.9]	5.6 [5.1 - 6.1]
14 weeks	5.9 [5.4 - 6.7]	6.3 [5.6 - 6.6]
6 months	6.9 [6.4 - 7.7]	7.4 [6.6 - 7.9]
9 months	7.6 [6.9 - 8.3]	7.8 [7.3 - 8.5]
Weight-for-age z score, median [IQR]		
10 weeks	-0.6 [-1.1 to -0.1]	-0.4 [-1.0 to 0.3]
14 weeks	-0.5 [-1.2 to 0.4]	-0.1 [-0.8 to 0.4]
6 months	-0.7 [-1.5 to 0.1]	-0.4 [-1.0 to 0.1]
9 months	-1.0 [-1.6 to -0.2]	-0.8 [-1.2 to -0.4]
Height-for-age z score, median [IQR]		
10 weeks	-1.4 [-2.1 to -0.9]	-1.3 [-2.1 to -0.9]
14 weeks	-1.3 [-2.2 to -0.7]	-1.1 [-2.0 to -0.5]
6 months	-1.4 [-2.2 to -0.9]	-1.1 [-1.7 to -0.7]
9 months	-1.6 [-2.4 to -1.1]	-1.5 [-2.0 to -0.7]
Weight-for-height z score, median [IQR]		
10 weeks	1.1 [0.3 to 1.8]	1.2 [0.4 to 1.9]
14 weeks	1.0 [0.2 to 1.9]	1.1 [0.3 to 2.1]
6 months	0.6 [-0.2 to 1.3]	0.4 [-0.2 to 1.4]
9 months	0.05 [-1.0 to 0.9]	0.03 [-0.6 to 0.6]
Mid-upper arm circumference, median [IQR]		
10 weeks	13.2 [12.7 - 13.8]	13 [12.5 - 13.5]
14 weeks	13.5 [12.8 - 14.2]	13.2 [12.6 - 14.1]
6 months	13.6 [13.1 - 14.3]	13.7 [13.3 - 14.5]
9 months	13.7 [13.2 - 14.3]	13.8 [13.3 - 14.5]
Dose of piperaquine (mg/kg), median [IQR]		
10 weeks	30.2 [28.1 - 32.7]	28.6 [26.2 - 31.4]
14 weeks	27.1 [23.9 - 29.6]	25.6 [24.2 - 28.6]
6 months	23.9 [21.6 - 26.1]	23.5 [21.3 - 25.4]
9 months	23.5 [21.6 - 27.5]	24.2 [21.3 - 28.2]
Scheduled piperaquine concentration samples [number of infants]		
10 weeks	288 [59]	NA
14 weeks	NA	232 [47]
6 months	289 [57]	NA
9 months	NA	228 [45]
Baseline caregiver socioeconomic status*, n [%]		
Lowest (Bottom 25%)	20 [33.3]	16 [32.0]
Lower-middle (25-50%)	11 [18.3]	12 [24.0]
Upper-middle (50-75%)	13 [21.7]	11 [22.0]
Highest (Top 25%)	16 [26.7]	11 [22.0]

[#] Sum of infant's gestational age at birth and chronological age at time of enrolment

NA, piperaquine concentrations not collected in the sampling schedule at the corresponding infant age

* Socioeconomic status computed as a percentile of combined estimate derived from data on availability of electricity in dwelling house, living in an iron-sheet roofed house, adequacy of clothing, household worries of food for meals, available of electronic appliances in the home for entertainment, member of household owning a bank account or mobile phone.[145]

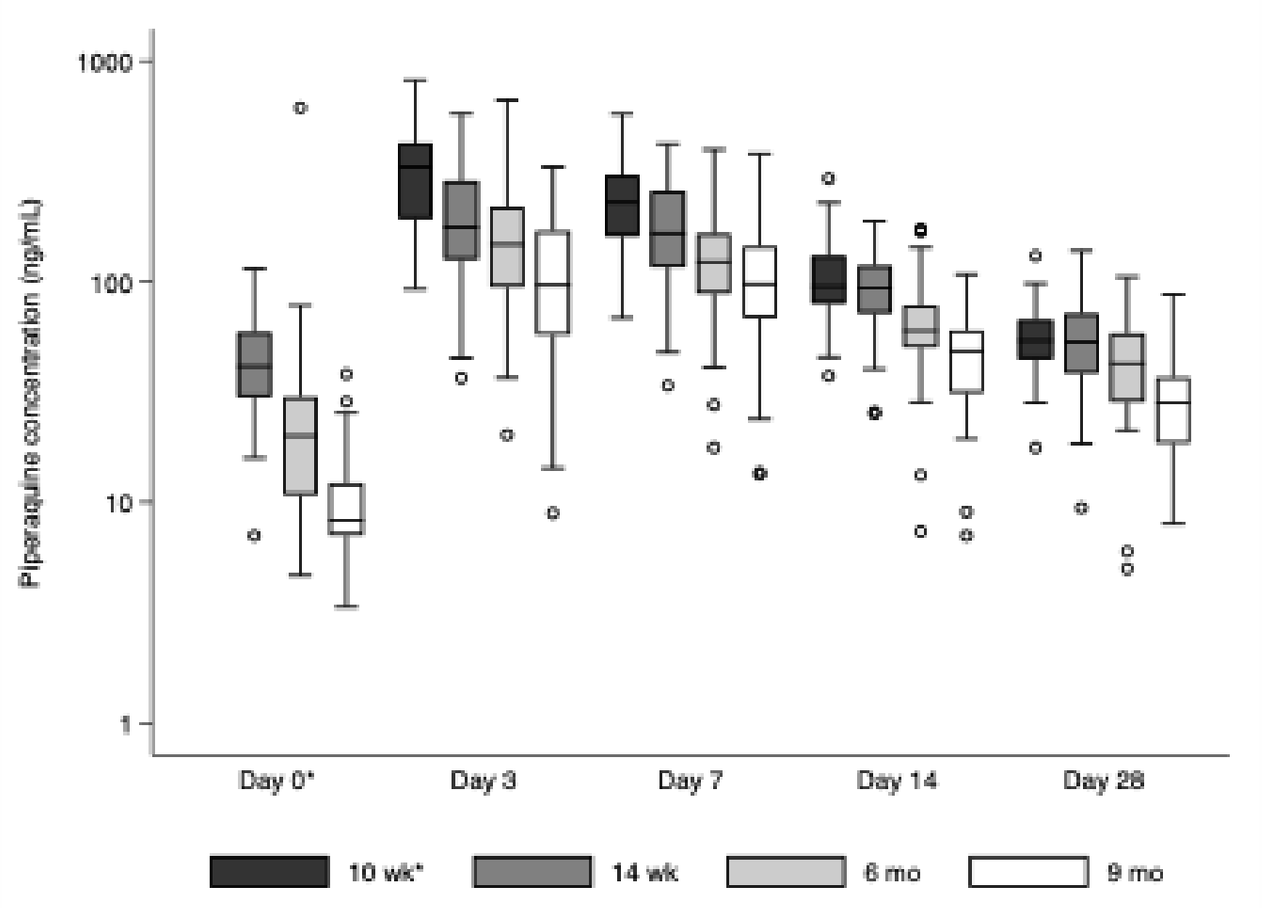


Figure 3. Piperaquine concentrations over a 28-day follow-up period, stratified by infant age. Boxes indicate the median piperaquine concentration with an interquartile range. * Day 0 (pre-dose) concentrations at 10 weeks were below the limit of quantification due to no prior DP exposure before enrolment; therefore, they are not included in this semi-logarithmic plot.

Pharmacokinetic model

A two-compartment model with first-order absorption (OFV = 9728) best described the data, as determined by a visual predictive check, the precision of parameter estimation, and lack of a statistically significant change when compared with a one- or three-compartment model (OFV = 10031 and 9726, respectively) (Table S1 and Figure S1). Inclusion of age as a fixed linear covariate in the two-compartment model, centred on the median age of 9 months, was a significant covariate when added in a stepwise manner on volume of distribution (ΔOFV = -13), with an additional improvement in model fit when adding it also on clearance (ΔOFV = -17). Both covariates were retained in the backward elimination process. In the final two compartment model, with age included on both clearance and volume of distribution, a unit decrease in age, below 9 months, was associated with an 8.3% (95% CI: 2.5% – 13.5%) and 9.2 % (95% CI: 5.3 – 13.1%) decrease in apparent clearance and volume of distribution of piperaquine, respectively (Figure S2 and Table S2). Individual AUCs and primary parameters (CL/F and volume of distribution) derived from this final model were used in the statistical analyses.

Impact of increasing age on piperaquine exposure profile

Piperaquine exposures, $AUC_{0\text{-}28\ days}$, $C_{day\ 28}$, and $C_{day\ 7}$, were 58% (GMR 0.42; 90% CI 0.36 – 0.49), 44% (GMR 0.56; 90% CI 0.43 – 0.74) and 59% (GMR 0.41; 90% CI 0.33 – 0.51) lower, respectively, at 9 months compared with 10 weeks of age (Table 2 and Figures 4). CL/F and volume of distribution (Vd) increased with increasing age, with (CL/F) and (Vd) higher by 2.6 times (GMR 2.61; 90% CI 2.52 – 2.70) and 3.2 times (GMR 3.24; 90% CI 3.01 – 3.49), respectively, following DP treatment at 9 months compared with 10 weeks of age (Table 2 and Figure S3).

Effect of vomiting and redosing on piperaquine exposure

Twenty-three infants vomited within 60 minutes of dosing, 11 on day 0 and 12 on day 3. There were no reported episodes of vomiting on day 2 when DP was administered at home.

A full dose was administered if vomiting occurred within 30 minutes of administration (6 infants) and half the dose if vomiting occurred between 30-60 minutes (17 infants), as specified by the manufacturer. Overall, piperaquine exposures, $AUC_{0\text{-}28\ days}$, $C_{day\ 28}$ and $C_{day\ 7}$, were 31% (GMR 1.31; 90% CI 1.05 – 1.62), 19% (GMR 1.19; 90% CI 1.01 – 1.41), and 38% (GMR 1.38; 90% CI 1.12 – 1.71) higher in infants who had vomited and were redosed compared with those who had not vomited.

Protective piperaquine concentration threshold and malaria occurrence

All pre-dose (day 0) concentrations at 10 weeks, prior to any DP exposure, were below the limit of quantification. The proportion of infants with pre-dose piperaquine concentrations below the purported protective threshold of 15.4 ng/mL was 2.1%, 37.9% and 87% after DP treatment at 14 weeks, 6 months and 9 months of age, respectively (p-trend, <0.001). Similarly, the proportion of participants with day 28 concentrations below the protective threshold was 0%, 2.1%, 3.4%, and 17.4% after the DP treatment course at 10 weeks, 14 weeks, 6 months, and 9 months of age, respectively (p-trend, 0.001) (Figure 5, Table S3).

One infant had a malaria episode at 6 months of age, detected on quantitative PCR before the third treatment course of DP. The pre-dose piperaquine concentration at this visit was not available, as the infant was on the PK sampling schedule in which samples were collected after the second and fourth doses of DP treatment. Pre-treatment piperaquine concentration following the first treatment course (at day 28 post-treatment first DP course, at 14 weeks) was 17.9 ng/mL. Following this infant's second DP treatment course, the overall piperaquine exposure ($AUC_{0\text{-}28\ days}$) was 30,188 ng · hr/mL, with a concentration of 18.4 ng/mL at day 28. The infant had no observed concentration of piperaquine below 15.4 ng/mL, slept under a bed net 4 to 6 times a week, and had received one malaria RTS, S/AS01 vaccine dose. The participant’s caregiver withdrew consent for participation in the study before the infant was 9 months old, prior to the fourth treatment course of piperaquine, as they were relocating to another district.

Table 2. Comparative pharmacokinetic parameters for piperaquine following administration of DP at 10 weeks, 14 weeks, 6- and 9 months

	GM (90% CI)									
Pharmacokinetic parameter	**10 weeks (N=59)**	**14 weeks (N=47)**	**6 months (N=57)**	**9 months (N=45)**	**GM Ratio (90% CI) (14 weeks / 10 weeks)**	**P-value#**	**GM Ratio (90% CI) (6 months / 10 weeks)**	**P-value***	**GM Ratio (90% CI) (9 months / 10 weeks)**	**P-value#**
$AUC_{0\text{-}28\ days}$ (ng.hr/mL)	98,563 (89,960 - 107,990)	72,604 (64,342 - 81,926)	54,793 (49,409 - 60,765)	41,433 (36,768 - 46,689)	0.74 (0.63 - 0.86)	0.001	0.56 (0.48 - 0.64)	<0.001	0.42 (0.36 - 0.49)	<0.001
C_{28} (ng/mL)	54 (50 -58)	51 (46 - 58)	40 (35 - 45)	30 (24 - 39)	0.95 (0.82 - 1.10)	0.591	0.74 (0.64 - 0.85)	0.001	0.56 (0.43 - 0.74)	0.001
C_7 (ng/mL)	218 (196 - 242)	166 (144 - 190)	121 (105 -138)	90 (75 - 107)	0.76 (0.64 - 0.91)	0.011	0.55 (0.47 - 0.66)	<0.001	0.41 (0.33 - 0.51)	<0.001
$C_{pre\text{-}dose}$ (ng/mL)	NA§	39 (35 - 45)	20 (17 - 24)	9 (8 - 11)	NA	NA	NA	NA	NA	NA
CL/F (Litres/hr)	3.43 (3.36 - 3.51)	4.01 (3.93 - 4.09)	6.86 (6.71 - 7.03)	8.96 (8.72 - 9.21)	1.17 (1.13 - 1.20)	<0.001	2.00 (1.94 - 2.06)	<0.001	2.61 (2.52 - 2.70)	<0.001
Vd/F (Litres)	727 (696 - 759)	1,026 (979 - 1076)	1,680 (1,586 - 1779)	2,353 (2,218 - 2,497)	1.41 (1.32 - 1.51)	<0.001	2.31 (2.15 - 2.48)	<0.001	3.24 (3.01 - 3.49)	<0.001

*Paired t-test

Independent t-test

§ Pre-dose concentration at 10 weeks was below limit of quantification for all infants, geometric mean (GM) calculation and comparison across the age groups not applicable (NA)

Abbreviations*: DP* dihydroartemisinin-piperaquin*e, $AUC_{0\text{-}28\ days}$* area under the capillary concentration-time curve from 0 to 28 days, $C_{pre\text{-}dose}$ pre-dose concentration before each three-day treatment course, C_7 day 7 concentrations, C_{28} day 28 concentrations, CL/F apparent clearance, Vd volume of distribution

Note: Geometric means and confidence intervals calculated using log-transformed data (natural log, ln)

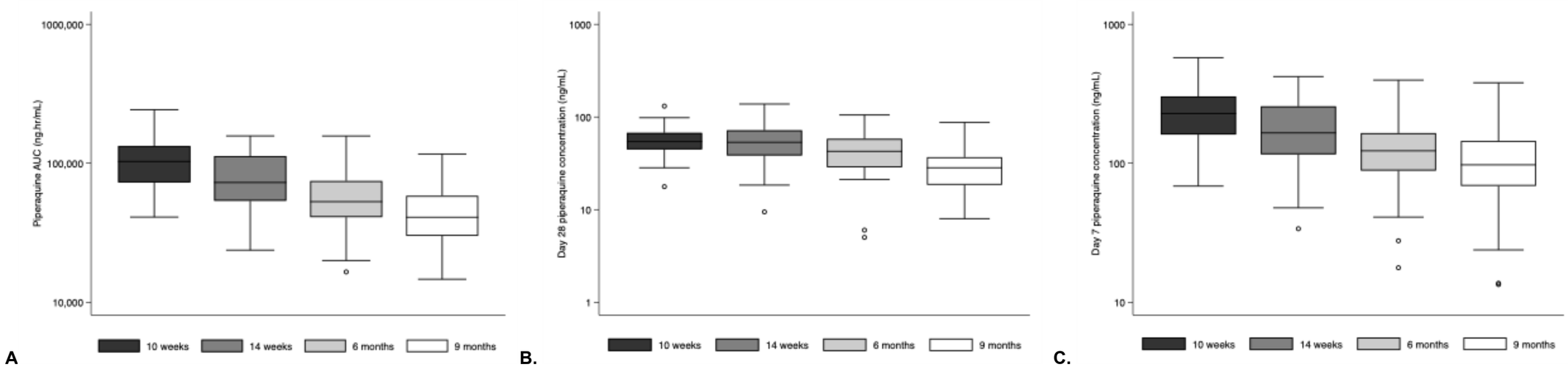


Figure 4. Change in piperaquine exposure with increasing age. (A) Piperaquine overall exposure (area under the concentration-time curve, AUC) (B) Day 28 piperaquine concentration (C) Day 7 piperaquine concentration plotted against different dosing time points at 10 weeks, 14 weeks, 6 months and 9 months of age. The exposure parameters are presented as medians and interquartile ranges on a semi-logarithmic plot.

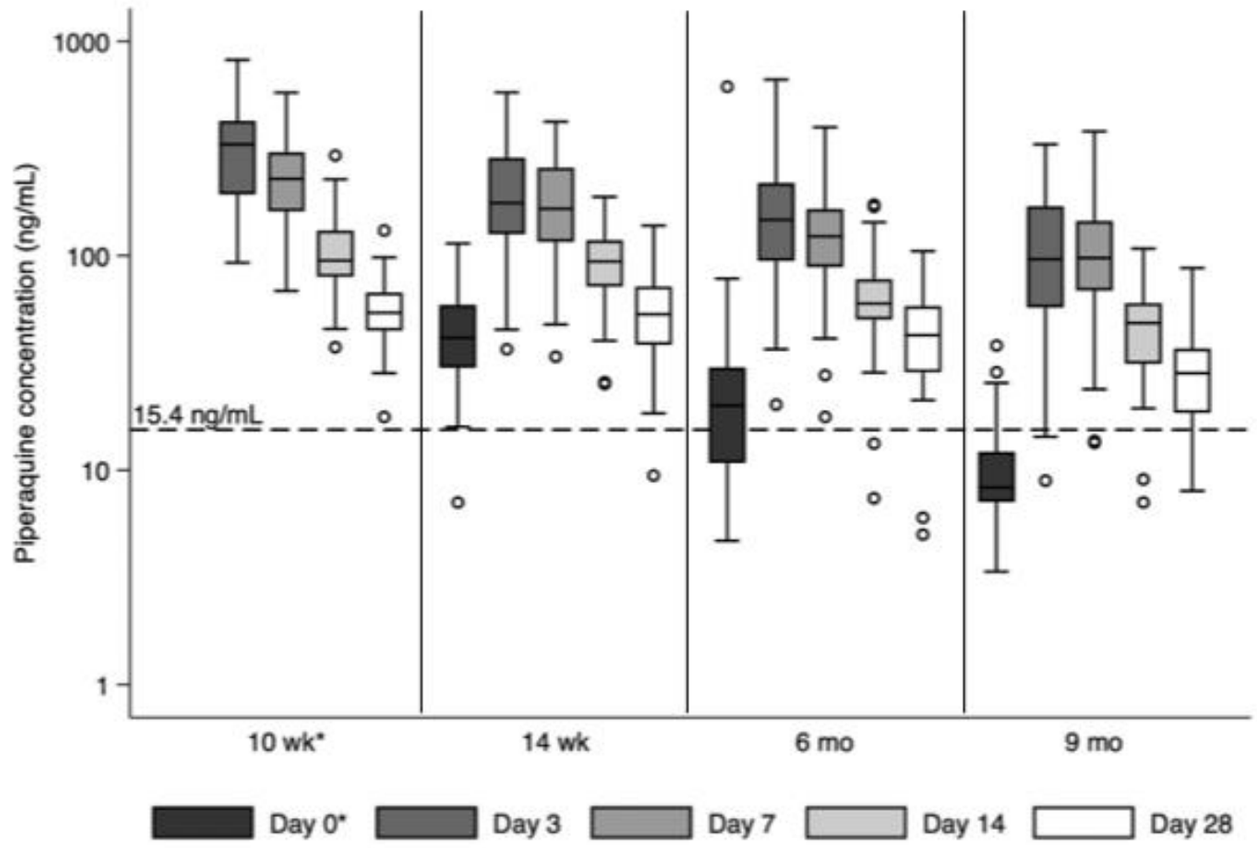


Figure 5. Protective piperaquine threshold and piperaquine concentrations over the follow-up period stratified by age at which dosing occurred (10 weeks, 14 weeks, 6 months and 9 months). *Day 0 (pre-dose) concentrations at 10 weeks were below the limit of quantification due to no prior DP exposure before enrolment; therefore, they are not included in this semi-logarithmic plot. The dashed horizontal line represents a piperaquine protective threshold of 15.4 ng/mL. +-*/-The solid vertical lines highlight the different dosing time points.

DISCUSSION

We investigated the impact of increasing age during infancy on the exposure of the long-acting antimalarial piperaquine following dihydroartemisinin-piperaquine (DP) dosing for perennial malaria chemoprevention during routine health facility visits at 10 weeks, 14 weeks, 6 months, and 9 months of age. Our findings indicate that advancing age was associated with lower overall piperaquine concentrations and a higher proportion of infants with trough concentrations falling below a previously described protective threshold of 15.4 ng/mL after a standard three-day treatment course of DP.

In our cohort, older infants exhibited lower overall piperaquine exposure ($AUC_{0\text{-}28\ days}$) and reduced piperaquine concentrations on days 7 and 28, which was associated with higher piperaquine clearance, compared to those at 10 weeks of age. Additionally, older infants had a larger volume of distribution consistent with age-related changes in body composition. These findings are biologically plausible, as the first year of life is marked by the maturation of hepatic enzymes such as CYP3A4, which is involved in piperaquine metabolism, and by shifts in body composition that influence the distribution of lipophilic drugs like piperaquine. This trend continues until two years of age, as shown in a prior pharmacokinetic study from Uganda where infants aged 8 months (32 weeks) had approximately 26% higher piperaquine exposure than children aged 26 months (104 weeks).[118] Together, the Ugandan study and our findings emphasise the significance of age-related physiological changes (enzyme maturation and evolving body composition) in influencing piperaquine pharmacokinetics. They underscore that weight-based dosing may not adequately compensate for the rapid physiological changes in infancy, and that there is a need to apply additional age-based dosing in optimising DP regimens for PMC in this age group.[125]

Similarly, the proportion of infants with pre-dose piperaquine concentrations below a previously proposed protective threshold of 15.4 ng/mL increased from 14 weeks to 9

months of age (2.1%, 33.3%, and 88.9% at 14 weeks, 6 months, and 9 months of age, respectively) - Figure 5, Table S3. In a context where DP dosing for PMC aligns with routine health facility visits at 10 weeks, 14 weeks, 6 months, and 9 months, our findings suggest that most infants would have subtherapeutic concentrations by 6 months. This supports the potential benefit of introducing an additional DP dose between 14 weeks and 6 months, i.e., at 5 months as well as at 7 months, given the high proportion of infants with piperaquine concentrations below the protective threshold by 9 months of age. Notably, the recent introduction of the RTS,S/AS01 malaria vaccine, administered at 5, 6, and 7 months of age, presents a timely opportunity to integrate additional doses of DP at the 5- and 7-month vaccination visits in addition to the 6-month time point.

In our cohort, only one infant had an asymptomatic infection, and no piperaquine concentration was available at the time of diagnosis. As such, we were unable to define a protective threshold specific to the DP regimen used in our study. Future analyses will aim to pool our data with piperaquine and malaria infection data from similar transmission settings to model a reliable protective threshold and inform an optimised DP dosing regimen for PMC during routine health facility visits.

Redosing DP after vomiting was associated with higher overall exposure, as well as day 7 and trough (day 28) concentrations, than in infants who had not vomited. However, we were unable to stratify the impact of vomiting by setting (following home versus health facility-administered doses) due to a lack of reported vomiting events when administration occurred at home. In our cohort, caregivers may not have detected or reported vomiting. In a programmatic setting, DP for PMC would mostly be administered at home rather than at a health facility. Further investigation is, therefore, needed to determine whether the absence of re-dosing in home settings would significantly impact piperaquine exposure. Additionally, in the context of PMC, there is a need to examine if home-based dosing would result in equivalent piperaquine exposure as seen following health facility dosing.

Our study has some limitations. First, the sparse sampling methodology may have reduced the precision in capturing the absorption phase of piperaquine, potentially leading to underestimation or increased variability of pharmacokinetic parameters. Although a three-compartment model with transit absorption is commonly used when dense data are available,[64,67,71,72] our sparse data were better described using a two-compartment model with first-order absorption. The resulting secondary pharmacokinetic estimate of overall exposure (AUC) was comparable to those reported in previous studies that utilised compartmental analyses (Table S4).[67,72,146,147] Second, while the sequential design, tracking the same infants across four time points, reduced inter-individual variability, we were not adequately powered to investigate the impact of other covariates such as sex, maturation (post-menstrual age) and nutritional indices (weight-for-age, weight-for-height, height-for-age) on primary pharmacokinetic parameters, as well as the subsequent effect on secondary exposure parameters. This was due to the sparseness of data in our cohort, especially in the absorption phase. Notably, with our data, we aimed first to address the hypothesis that exposure to DP for PMC would decrease with increasing age when dosing is administered during routine health facility visits. The effect of covariates on this exposure will be investigated in our next planned step, which will include pooling data from similar infant populations to evaluate the influence of covariates better and refine dosing regimen recommendations for DP in the context of PMC at routine health visits.

CONCLUSION

An increase in infant age from 10 weeks to 9 months was associated with lower overall piperaquine exposure and a greater proportion of infants with concentrations below the protective concentration threshold. These results, observed when dihydroartemisinin-piperaquine was administered over three days in alignment with routine health facility visits (at 10 weeks, 14 weeks, 6 months, and 9 months), underscore the need to optimise the

dosing regimen of dihydroartemisinin-piperaquine to ensure improved perennial malaria chemoprevention in infants.

Supplementary material

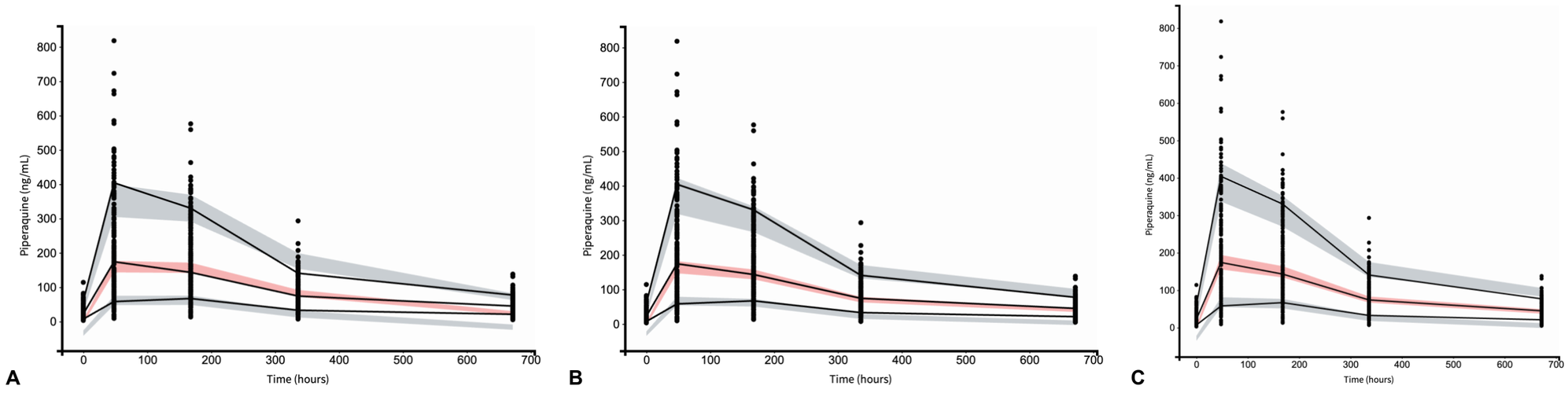


Figure S1: Visual predictive checks of fitted structural pharmacokinetic models for piperaquine. (A) One-compartment model (B) Two-compartment model (C) Three-compartment model**.** Black dots indicate observed data, and lines indicate median (central), 5% (lower) and 95% (upper) of the infant population. The central shaded purple area represents 50%, while the grey lower and upper shaded areas represent the 5% and 95% ranges of the simulated data, respectively**.**

Table S1. Compartmental pharmacokinetic model parameters for capillary piperaquine concentrations in infants

Paramaters	**Population estimate[a]**	**95% CI Lower[b]**	**95% CI Upper[b]**	**%RSE[b]**	**OFV**
One-compartment model					10031
Pharmacokinetics					
CL/F (L/hr)	3.4	3.2	3.7	3.1	
V_c/F (L)	707	653	760	3.9	
Inter-individual variability (%CV)[c]					
CL/F (L /hr)	0.11 (11.2)	0.05	0.17	28.4	
V_C/F (L)	0.28 (28.4)	0.19	0.37	15.8	
Two-compartment model					**9728**
Pharmacokinetics					
CL/F (L/hr)	2.7	2.6	2.9	2.7	
V_c/F (L)	421	370	472	6.2	
Q_1/F (L/hr)	3.9	3.5	4.3	5.1	
V_{P1}/F (L)	779	691	866	5.7	
Inter-individual variability (%CV)[c]					
CL/F (L /hr)	0.10 (9.7)	0.05	0.15	26	
V_C/F (L)	0.31 (31.5)	0.22	0.40	14.9	
Three-compartment model					9726
Pharmacokinetics					
CL/F (L/day)	2.4	2.3	2.9	6.4	
V_c/F (L)	417	352	481	7.9	
Q_1/F (L/hr)	10.1	-40.8	60.9	258	
V_{P1}/F (L)	22.2	-25.5	69.8	110	
Q_2/F (L/hr)	3.1	2.7	3.5	7	
V_{P2}/F (L)	778	607	948	11.2	
Inter-individual variability (%CV)[c]					
CL/F (L /day)	0.11 (10.9)	-0.04	0.26	69	
V_C/F (L)	0.29 (29.4)	0.18	0.4	19.8	

[a] Computed population mean parameter estimates from Monolix calculated for a typical infant of 7 kg body weight and estimated using the Stochastic Approximation of the Expectation-Maximization (SAEM) algorithm

[b] Confidence intervals (CIs) computed from Fisher Information Matrix method of the final model and using the % relative standard errors (RSE)

[e] The coefficient of variation (%CV) for inter-individual variability was calculated as 100 x $\sqrt{e^{\omega^2}}$ - 1, where $\boldsymbol{\omega}$ was the standard deviation of the random effect

CL elimination clearance, V_c central volume of distribution, *Q* intercompartmental clearance, V_P peripheral volume of distribution, OFV objective function value

Bolded compartment highlights the structural compartment that best described the data

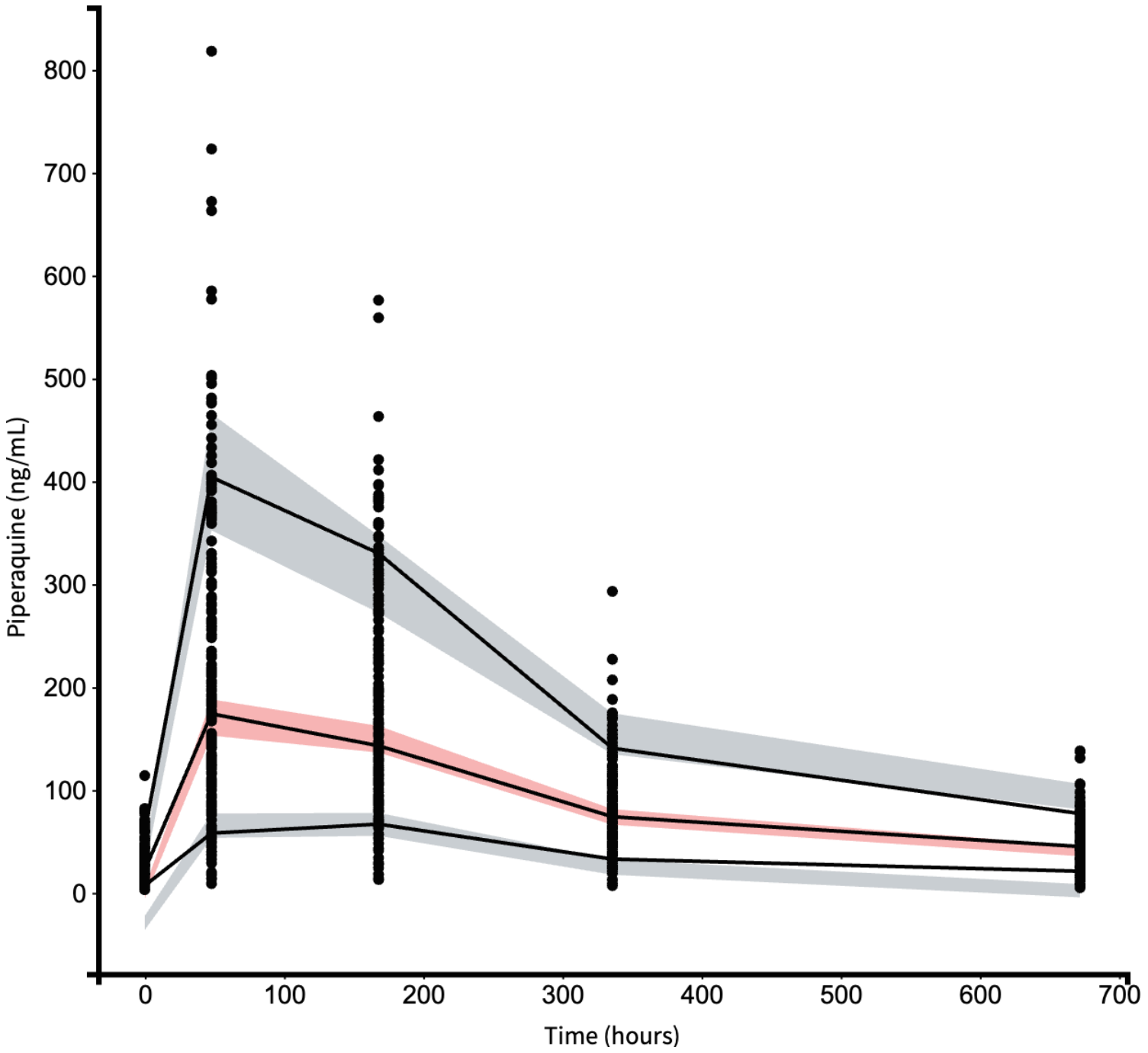


Figure S2. Visual predictive check of the final two-compartment pharmacokinetic model with age (centred, in months) fitted as a covariate on apparent clearance and volume of distribution. Black dots indicate observed data, and lines indicate median (central), 5% (lower) and 95% (upper) of the infant population. The central shaded purple area represents 50%, while the grey lower and upper shaded areas represent the 5% and 95% ranges of the simulated data, respectively.

Table S2. Final two-compartment pharmacokinetic model parameters for capillary piperaquine concentrations in infants

Paramaters	Population estimate[a]	95% CI Lower[b]	95% CI Upper[b]	%RSE[b]	OFV
Two-compartment model with effect of age on CL/F & V_c					**9698**
Pharmacokinetics					
CL/F (L/hr)	3.0	2.3	3.7	12.3	
V_c/F (L)	582	510	655	6.4	
Q_1/F (L/hr)	2.8	2.2	3.3	10.6	
V_{P1}/F (L)	681	514	848	12.5	
Effect of age (centred, in months)[#]					
CL/F (L/hr)	0.08	0.03	0.14	35.5	
Vc/F (L)	0.088	0.05	0.12	20.6	
Inter-individual variability (%CV)[c]					
CL/F (L /hr)	0.10 (9.9)	0.02	0.18	40.4	
V_C/F (L)	0.30 (31.2)	0.22	0.38	13.6	

[a] Computed population mean parameter estimates from Monolix calculated for a typical infant of 7 kg body weight and estimated using the Stochastic Approximation of the Expectation-Maximization (SAEM) algorithm

[b] Confidence intervals (CIs) computed from Fisher Information Matrix method of the final model and using the % relative standard errors (RSE)

[e] The coefficient of variation (%CV) for inter-individual variability was calculated as 100 x $\sqrt{e^{\omega^2}}$ - 1, where $\boldsymbol{\omega}$ was the standard deviation of the random effect

CL elimination clearance, V_c central volume of distribution, *Q* intercompartmental clearance, V_P peripheral volume of distribution, OFV objective function value

[#]Age (in months), centred on the median age of 9 months. Presented parameter is a regression coefficient of the impact of age (in months) on apparent clearance or volume of distribution

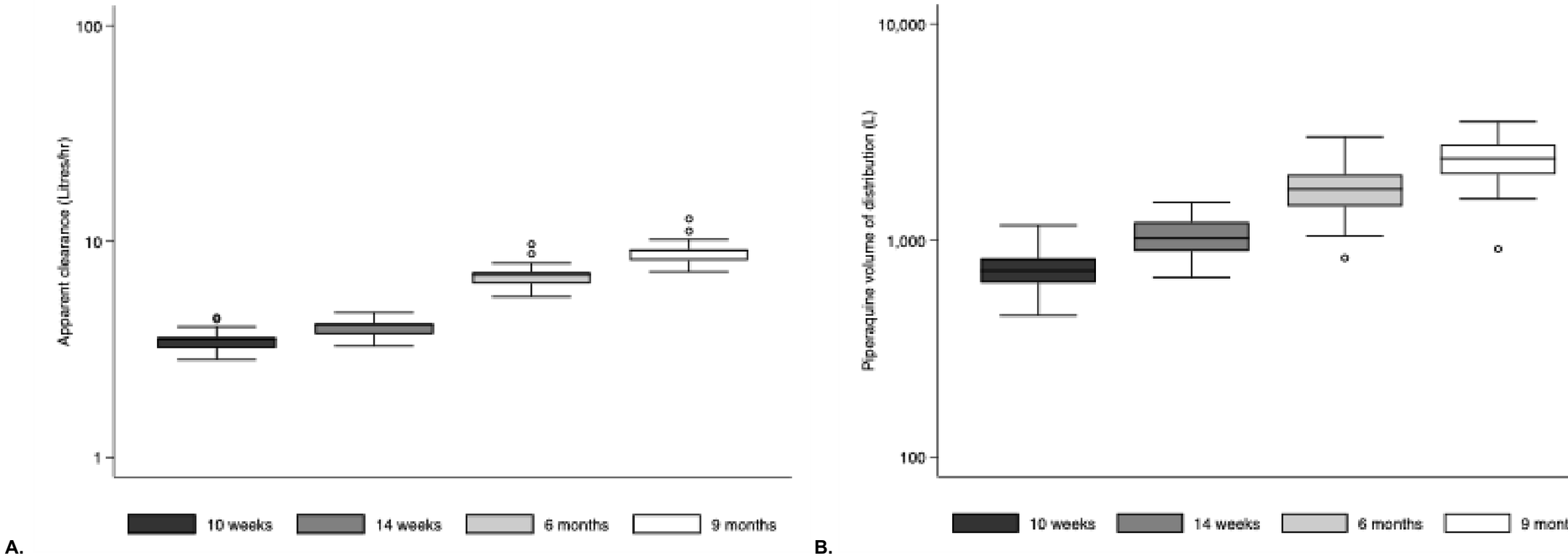


Figure S3. Change in piperaquine apparent clearance and volume of distribution with increasing age. (A) Piperaquine apparent clearance (B) Volume of distribution plotted against different dosing time points at 10 weeks, 14 weeks, 6 months and 9 months of age. The exposure parameters are presented as medians and interquartile ranges on a semi-logarithmic plot.

Table S3. Trough (pre-dose and day 28) concentrations below a protective threshold of 15.4 ng/mL over the follow-up period

	Day 0* (Pre-dose) Number of infants with pre-dose piperaquine concentrations below protective threshold	Day 28* Number of infants with day 28 piperaquine concentrations below protective threshold	Total number of infants treated with DP#	% of infants with predose concentrations below protective thresholds	% of infants with day 28 concentrations below protective thresholds
Age of infant at start of DP treatment					
10 weeks	NA*	0	60	NA	0
14 weeks	1	1	47	2.1	2.1
6 months	22	2	58	37.9	3.4
9 months	40	8	46	87	17.4

*NA: Not applicable, at 10 weeks, pre-dose (day 0) concentrations were below limit of quantification

*Total available scheduled piperaquine concentrations following DP treatment course

Total number of infants treated with DP at each of the age time points

Table S4. Overview of published data on the secondary pharmacokinetic parameter of piperaquine exposure (AUC) in children derived from compartmental pharmacokinetic models

Study	Year	Number of participants	Indication	Age	Pharmacokinetic parameter AUC (ng.hr/mL)
Banda CG *et al* (present study)	2025	110	Malaria prevention - PMC at routine hospital visits	10 weeks 14 weeks 6 months 9 months	98,563 (89,960 - 107,990)* 72,604 (64,342 - 81,926)* 54,793 (49,409 - 60,765)* 41,433 (36,768 - 46,689)*
Tarning J *et al*	2012	236	Uncomplicated malaria treatment	2 - 5 years	36,400 (9,610 - 93,000)[#]
Salman S *et al*	2013	34	Uncomplicated malaria treatment comparison of two formulations	5.5 - 8.6 years	49,451 (40,507 - 52,438)[##] 44,556 (33,215 - 51,873)[##]
Sambol NC *et al*	2013	60	Uncomplicated malaria treatment	6 months - 2 years	71,300 (67,200 - 72,600)[§]
Chotsiri P *et al*	2017	179	Malaria prevention - SMC	2.3 months - 4.8 years	31,200 (24,900-53,900)**

* Geometric mean (90% CI). AUC is $AUC_{0\text{-}28\ days}$

[#] Median (range). AUC is $AUC_{0\text{-}45\ days}$

[##] Mean (IQRI). AUC is $AUC_{0\text{-}infinity}$

[§] Mean (95% CI). $AUC_{0\text{-}infinity}$

** Median (95% CI). AUC is $AUC_{0\text{-}30\ days}$

PMC perennial malaria chemoprevention, *SMC* seasonal malaria chemoprevention

CHAPTER 6

Discussion and conclusions

Infants are a twice-vulnerable subgroup as they are at an increased risk of complicated malaria disease compared to older age groups and yet are excluded from participation in dose optimisation studies.[148,149] The latter is, in part, driven by the challenge of frequent blood sampling needed in pharmacokinetic dose optimisation studies, which may not always be ethically justified in an infant population. Acknowledging this limitation in infants, a minimal, sparse sampling strategy was used to generate data to inform the optimal dosing regimen of a promising antimalarial, DP, for perennial malaria chemoprevention (PMC) in infants when dosing was aligned with routine health facility visits. Such timing has the potential to enhance coverage and adherence when compared to previously recommended monthly dosing.[14,15,41] This evidence is needed to inform WHO's guidelines on PMC in infants in areas of moderate-to-high malaria transmission.[38] We hypothesised that increasing age in infancy was associated with a reduction in piperaquine exposure due to age-related maturation of metabolising enzymes (CYP450) and an increase in volume of distribution;[50,150,151] with potential to impact DP's efficacy for PMC when dosing is given with routine health facility visits at 10 weeks, 14 weeks, 6 months and 9 months of age.

In this study, DP was associated with a 77% non-statistically significant reduction in malaria incidence in the first year of life (adjusted incidence rate ratio of 0.23, 95% CI 0.03–2.11). There was also a 31% reduction in moderate-to-severe anaemia prevalence (adjusted relative risk of 0.69, 95% CI 0.52–0.93, p 0.013) in infants who received DP compared with those who received placebo. Additionally, an increase in infant age from 10 weeks to 9 months was associated with lower piperaquine concentrations. Specifically, piperaquine exposures, $AUC_{0\text{-}28\ days,}$ $C_{day\ 28,}$ and $C_{day\ 7}$, were 58% (GMR 0.42; 90% CI 0.36 – 0.49), 44% (GMR 0.56; 90% CI 0.43 – 0.74) and 59% (GMR 0.41; 90% CI 0.33 – 0.51) lower, respectively, at 9 months compared with 10 weeks of age. Furthermore, the proportion of infants with pre-dose piperaquine concentrations below a previously proposed protective threshold of 15.4 ng/mL increased with age from 14 weeks to 9 months (2.1%, to 33.3%,

and 88.9% from 14 weeks to 6 months, and 9 months of age, respectively). As far as we are aware, this is the first description of DP efficacy and piperaquine exposure when administered for PMC and aligned with routine health facility visits. These thesis findings highlight the promise of DP for PMC but also underscore the need to optimise its dosing regimen when given with routine health facility visits.

Furthermore, although we observed a declining trend in piperaquine exposure from 10 weeks to 9 months of age, piperaquine concentrations in this study were still higher at the different dosing time points than those previously described in older children and adults when DP was administered for PMC or treatment of uncomplicated malaria (Table S1).[64,67,118,146] This provides assurance that, although the improved and currently recommended WHO doses for DP[126] result in subtherapeutic concentrations with increasing age when PMC is administered with routine health facility visits (at 10 weeks, 14 weeks, 6 months and 9 months), the attained piperaquine exposure is sufficient to treat existing asymptomatic parasitaemia or uncomplicated malaria at different ages in infancy.

The first key strength of this thesis is the choice of the study design; a pharmacokinetic, placebo-controlled, single-blind, clinical trial (described in Chapter 3),[127] which allowed us to incorporate two clinical sub-studies; first, a longitudinal assessment of drug exposure in infancy (Chapter 5), and second, an evaluation of efficacy and safety outcomes of DP compared to placebo for PMC during routine health facility visits (Chapter 4). Notably, each infant contributed blood samples at sparse intervals and within ethically permissible volumes. The latter aspect (minimal, sparse, sampling) was vital considering the constraints of including infants in dose optimisation studies, both from the perspective of their caregivers and ethical guidance (as highlighted in Chapter 2).

The inclusion of a placebo arm in the study was ethically permissible in Malawi, at the time of the study's conduct, where infants were not routinely given PMC. It innovatively enabled

the evaluation of baseline malaria incidence in the population, given the general lack of precise estimates of the malaria burden in infants.[2] The placebo arm also contributed to a better understanding of the safety and tolerability of DP. Thus, the design of the study and this thesis aimed to contribute to a paradigm shift in conducting clinical pharmacology studies in vulnerable groups (such as infants) when critical knowledge gaps exist.

Second, this thesis has generated evidence to help inform dose regimen optimisation of DP for PMC. In programmatic settings, aligning dosing of malaria chemopreventive treatment with routine health facility visits is a key priority to improve adherence and feasibility.[38,41] Considering the promising efficacy and safety findings of DP for PMC described in this thesis, a pooled analysis of all available efficacy, safety and pharmacokinetic data of DP for PMC in infants and older age groups would allow refined estimation of the impact of age on piperaquine primary pharmacokinetic parameters (clearance and volume of distribution) as well as its overall exposure and inform an optimised DP dosing regimen for PMC in infants.

In addition to the 10-week, 14-week, and 9-month visits available in the routine immunisation program,[152] the 5-month health facility visit, when malaria vaccination (RTS, S/AS01 or R21/Matrix-M) is expected to commence, would provide an opportunity for additional DP dosing. In our next planned step, building on the work of this PhD thesis, we will utilise pharmacokinetic-pharmacodynamic modelling and physiologically based pharmacokinetic models (discussed in Chapter 2 of this thesis) to conduct a pooled analysis of all existing efficacy and pharmacokinetic data of DP in infants and older age groups (derived from our clinical trial and other malaria prevention or treatment studies) in areas of moderate-to-high malaria transmission. The goal of this exercise will be to inform an optimal DP dosing regimen for PMC, aligned with routine health facility visits. This could also help inform the target piperaquine exposure for therapeutic efficacy in uncomplicated malaria, enabling DP treatment dose optimisation in infants with uncomplicated (or asymptomatic) malaria.

While the clinical trial reported in Chapter 4 was conducted in a perennial-transmission context in Malawi, the findings may still inform malaria prevention strategies in highly seasonal settings such as Sahelian West Africa, though with important contextual considerations. Seasonal malaria chemoprevention (SMC) programmes use monthly administration of antimalarials (typically sulfadoxine–pyrimethamine plus amodiaquine, SP–AQ) during the short rainy season and have demonstrated substantial real-world impact. For example, an observational scale-up study across several countries showed high effectiveness of SMC, with marked reductions in malaria incidence and low rates of serious adverse events [154] Importantly, DP has also been evaluated as an SMC alternative in these settings; in Burkina Faso, a randomised non-inferiority trial comparing DP to SP–AQ over three monthly rounds reported comparable protective efficacy and good tolerability.[155] The recommendations arising from our trial, DP administration at 10 and 14 weeks, then 5, 6, and 7 months of age, aligned with routine infant health facility visits, are best suited to perennial transmission settings. Nonetheless, in seasonal transmission areas, DP remains a viable option for malaria prevention when administered for SMC. Thus, even in regions with marked seasonality, a similar protective benefit of DP to that observed in our study would be expected, although the optimal delivery schedule would differ from that used in perennial settings.

Furthermore, the timing of DP administration in Chapters 3 and 4 (at 10 weeks, 14 weeks, and later at 6 and 9 months) aligns with the period when passive maternal immunity wanes and infants' exposure risk increases (as discussed in Chapter 1). This supports the rationale for evaluating changes in DP exposure over the first year of life to inform optimisation of dosing regimens. Evidence from previous studies indicates that chemoprevention initiated in early infancy, after the decline of maternally derived antibodies, is immunologically safe and unlikely to impair the development of long-term naturally acquired immunity. Infants continue to mount antibody responses despite reduced malaria exposure, and such chemoprevention

complements, rather than interferes with, natural immune maturation. Moreover, it may act synergistically with concurrent or future malaria vaccines (Table S2).[7,27,28,156,157] By anchoring the design of the intervention on these epidemiological and immunological principles, this thesis builds on a robust scientific foundation to evaluate DP-based PMC during the critical first year of life.

Nonetheless, the studies in this thesis had some limitations. In the efficacy and safety component of the clinical trial, complete single blinding may not have been achieved, as infants in the DP arm contributed more blood samples than those in the placebo arm, despite having the same number of study visits in both arms. This could have biased the reporting of adverse events by caregivers. Nevertheless, except for vomiting, which was observed at the study clinic, there were no differences in the reporting of adverse events by caregivers in the two arms. Additionally, the pharmacokinetic component of the clinical trial aimed to illustrate the impact of increasing age on piperaquine exposure, due to the expected increase in drug clearance and volume of distribution, and the subsequent effect on post-treatment prophylactic threshold as previously defined as a trough concentration of 15.4ng/mL.[71] Therefore, the sparse sampling time points in the study focused on capturing the elimination phase of piperaquine, rather than the absorption phase. It would have been challenging for caregivers to consent to frequent blood sampling, as would be required in an intensive pharmacokinetic study. Consequently, we had insufficient data points from this study alone to adequately power the assessment of the effect of other covariates (such as sex and time-varying nutritional indices) on parameter estimates, which would inform the optimisation of DP dosing regimens for PMC (or treatment of uncomplicated malaria). Furthermore, the introduction of a malaria vaccine and the high reported bed net usage in our cohort likely resulted in a low number of observed malaria cases over the follow-up period. Thus, these limitations further justify the investment in a pooled analysis of all available data, which we have planned, to generate robust evidence that would inform the development of a PMC guideline.

Overall, the work presented in this thesis contributes knowledge and data on a) the need to adjust the dosing regimen of DP for PMC between 10 weeks and 9 months of age, and b) how to further advance the efforts to optimise the use of this promising antimalarial for PMC in the twice vulnerable population of infants, particularly in settings with moderate-to-high malaria transmission, and potentially for the treatment of uncomplicated malaria.

Table S1. Overview of some of the published data on piperaquine exposure in infants compared with older children and adults when administered for preventive or uncomplicated *Plasmodium falciparum* malaria treatment

Study	Year	Indication	Age	Pharmacokinetic parameter		
				AUC (ng.hr/mL)	Day 7 (ng/mL)	Day 28 or Day 21 (ng/mL)
Banda CG *et al* (present study)	2025	Malaria prevention - PMC treatment course at routine hospital visits	10 weeks	98,563 (89,960 - 107,990)*	218 (196 - 242)[a]	54 (50 -58)[1]
			14 weeks	72,604 (64,342 - 81,926)*	166 (144 - 190)[a]	51 (46 - 58)[1]
			6 months	54,793 (49,409 - 60,765)*	121 (105 -138)[a]	40 (35 - 45)[1]
			9 months	41,433 (36,768 - 46,689)*	90 (75 - 107)[a]	30 (24 - 39)[1]
Whalen ME	2020	Malaria prevention - PMC treatment course	32 weeks	11,400 (9,770 -13,200)*#	22.6 (18.8 - 27.2)[b]	9.42 (8.18 - 10.8)[2]
			104 weeks	8,240 (7,280 - 9,330)*#	16.3 (13.6 - 19.6)[b]	7.09 (6.03 - 8.34)[2]
			Adults (19 -32 years)	17,600 (15,100 - 20,700)*#	39.0 (32.3 - 47.2)[b]	14.5 (12.2 - 17.1)[2]
Tarning J *et al*	2012	Uncomplicated malaria treatment	2 - 5 years	36,400 (9,610 - 93,000)#	64.0 (16.8 - 130)[c]	NA
Sambol NC *et al*	2013	Uncomplicated malaria treatment	6 months - 2 years	71,300 (67,200 - 72,600)[§]	42.5 (39.4 - 45.9)[d]	NA
Hoglund RM *et al*	2017	Uncomplicated malaria (pooled individual data analysis)	6 months - 55 years			
			Smaller children (<25 kg)	NA	29.4 (19.3 - 44.3)[e]	NA
			Older children and adults (≥25 kg)	NA	38.1 (25.8 - 56.3)[e]	NA

* Geometric mean (90% CI). AUC is $AUC_{0\text{-}28\ days}$
*# Geometric mean (95% CI). AUC is $AUC_{0\text{-}21\ days}$
Median (range). AUC is $AUC_{0\text{-}45\ days}$
Mean (interquartile range). AUC is $AUC_{0\text{-}infinity}$
§ Mean (95% CI). $AUC_{0\text{-}infinity}$
** Median (95% CI). AUC is $AUC_{0\text{-}30\ days}$
[1] Day 28 concentrations, geometric mean (90% CI)
[2] Day 21 concentrations
NA Not applicable for the referenced study
[a] Geometric mean (90% CI)
[b] Geometric mean (95% CI)
[c] Median (range)
[d] Geometric mean (95% CI)
[e] Median (interquartile range)
PMC, perennial malaria chemoprevention

Table S2. Evidence informing chemoprevention timing in infancy in relation to immunity development

Study	Intervention & Timing	Population	Findings (Immunity / Clinical)	Relevance to this Thesis
Guinovart et al, Mozambique, 2012[28]	Monthly sulfadoxine-pyrimethamine (SP) + artesunate at different windows (early vs later in first year)	Infants, followed into second year of life	No major rebound in clinical malaria. No evidence was found that the age of first exposure to malaria affects the rate of acquisition of naturally acquired immunity	Supports that early chemoprevention does not prevent development of immunity; justifies DP timing in early infancy
Nhabomba et al, Mozambique, 2014[27]	Impact of age on first exposure to *Plasmodium falciparum* on antibody response	Infants in randomised trial	Age of exposure did not critically change acquisition of immunity	Underlines flexibility of intervention timing in infancy
Barua et al., Malawi, 2019[156]	Observational study of natural infection	Children up to 18 months	Antibodies to surface proteins (MSP1 19kD, MSP-2,) erythrocyte binding antigen 175 (EBA175), and variant surface antigen (VSA) was significantly higher in exposed vs unexposed; the timing of first infection (before vs after 6 months) did not strongly influence final antibody levels.	Suggests early exposure is not strictly necessary for antibody acquisition; meaning suppressing early exposure may not undermine immunity
Natama et al, Burkina Faso, 2023[157]	Cohort measuring maternal IgG at birth	Infants (first year)	Specific maternal IgG subclasses associated with clinical malaria risk in infancy	Highlights the role of passively transferred immunity and its decline, which supports rationale for prophylaxis once maternal IgG declines
Kobbe et al, Ghana, 2007[158]	IPTi-SP at expanded programme of immunisation (EPI) contacts	Infants across Africa	Protective efficacy differs by age; lower malaria incidence in those receiving IPTi; immunity effects modest	Reinforces age-dependent benefit, and potential for integration with EPI schedule

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APPENDICES

1. Appendix 1: UCT Ethics approval and renewal until 2025 (University of Cape Town Human Research Ethics Committee - UCT HREC)
2. Appendix 2: Local (Malawi) ethics approval and renewal until 2025 (College of Medicine Research Ethics Committee - COMREC)
3. Appendix 3: Local regulatory authority approval for the clinical trial in Malawi (Pharmacy and Medicines Regulatory Authority – PMRA)
4. Appendix 4: Data collection tools (Case Report Forms)

APPENDIX 1: UCT Ethics approval and renewal until 2025 (University of Cape Town Human Research Ethics Committee - UCT HREC)

UNIVERSITY OF CAPE TOWN
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11th October 2022

HREC REF: 361/2022

Prof Karen Barnes
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Dear Prof Barnes

PROJECT TITLE: A PHARMACOKINETIC RANDOMISED INTERVENTIONAL STUDY TO OPTIMISE DIHYDROARTEMISININ-PIPERAQUINE DOSING FOR MALARIA PREVENTIVE TREATMENT IN MALAWIAN INFANTS (OPTIMAL STUDY).(PHD DEGREE - DR CLIFFORD GEORGE BANDA)

Thank you for submitting your response to the Faculty of Health Sciences Human Research Ethics Committee dated 28th September 2022.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

The following documents are noted and approved for the above study

- new protocol application form
- Support letter from local institution where study will be held in Malawi
- (Chikhwawa District Hospital)
- Letter of commitment for trial sponsorship
- Investigator's brochure
- Copy of Material Transfer Agreements signed by Investigators (pending
- review by UCT Contracts Officer)
- Curricula vitae and GCP certificates of Investigators
- PI Synopsis- tracked and clean versions
- Study protocol: OPTIMAL_Study_Protocol_(v3.0_01September2022)
- Informed consent forms in English and Chichewa (both v3.0_01September2022)
- Copy of certificate of trial insurance
- Responses to comments by LSTM REC
- Responses to comments made by COMREC (the local ethics committee)
- Approval letter of v2.0_1August2022 of the protocol by COMREC

Approval is granted for one year until the 11th October 2023.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

(Forms can be found on our website: https://health.uct.ac.za/home/human-research-ethics)

HREC REF NO. 548/2022

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

The HREC acknowledges that the following staff member will be involved in the study: Dr Clifford George Banda.

Please also note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF number 361/2022 in all your correspondence.

Yours sincerely

PROFESSOR MARC BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCE HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) **This serves as notification of annual approval, including any documentation described below.**			
☑ Approved	Annual progress report	Approved until/next renewal date	11.10.2025
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	10/10/2024

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC
Thank you for the comprehensive update

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	09/10/2024		
HREC REF Number	361/2022	Current Ethics Approval was granted until	11/10/2024
Protocol title	A pharmacokinetic randomised interventional study to optimise dihydroartemisinin-piperaquine dosing for malaria preventive treatment in Malawian infants (OPTIMAL Study)- PhD Degree: Dr Clifford George Banda)		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?		☐ Yes	☒ No
If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Prof Karen I Barnes		
Department / Office Internal Mail Address	Department of Medicine, Division of Clinical Pharmacology, K47, Old Main Building, Groote Schuur Hospital, Observatory, 7925		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

HUMAN RESEARCH ETHICS COMMITTEE
10 OCT 2024
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

APPENDIX 2: Local (Malawi) ethics approval and renewal until 2025 (College of Medicine Research Ethics Committee - COMREC)

CERTIFICATE OF ETHICS APPROVAL

This is to certify that the College of Medicine Research and Ethics Committee (COMREC) has reviewed and approved a study entitled:

P.06/22/3663 - A pharmacokinetic randomised interventional study to optimise dihydroartemisinin-piperaquine dosing for malaria preventive treatment in Malawian infants (OPTIMAL Study) version 2.0 by Dr Clifford George Banda

On 08-Sep-22

As you proceed with the implementation of your study, we would like you to adhere to international ethical guidelines, national guidelines and all requirements by COMREC some of which are indicated on the next page for your study

08-Sep-22

Prof. E. Umar -Chairperson (COMREC)

Date

Approved by College of Medicine
08-Sep-2022
(COMREC)
Research and Ethics Committee

KAMUZU UNIVERSITY
OF HEALTH SCIENCES

Vice Chancellor
Prof. M. Mallewa (BMedSc, MBBS, MRCP, MRCPCH, DRM&H, PhD)
Our Ref: **P.06/22/3663**
Your Ref: : **P.06/22/3663**

14/08/2024

CRSU MLW

Malawi Liverpool Wellcome Trust

Submission Type: Annual Renewal

Re: A pharmacokinetic randomised interventional study to optimise dihydroartemisinin-piperaquine dosing \r\nfor malaria preventive treatment in Malawian infants (OPTIMAL Study)

I am pleased to inform you that **COMREC** reviewed the progress report to the above study reference number **P 06/22/3663** and found it satisfactory. In this respect, annual renewal of the study is granted. The study was initially approved on **16/02/2023** and this new approval will expire on **08/09/2025**.

Yours Sincerely,

Prof. Eric Umar (Comrec Chairperson)
COMREC

Approved by
College of Medicine
14/08/2024
(COMREC)
Research and Ethics Committee

APPENDIX 3: Local regulatory authority approval for the clinical trial in Malawi (Pharmacy and Medicines Regulatory Authority – PMRA)

PHARMACY AND MEDICINES REGULATORY AUTHORITY
Quality Medicines for Malawi

Ref. No. **PMRA/CTRC/IV/22112022140** | 15 December 2022

Orig. REG08

Malawi-Liverpool Wellcome Trust
Clinical Research Programme
P.O. Box 30096
Chichiri
BLANTYRE 3
Email: cgbanda@mlw.mw

Attention: Dr. Clifford George Banda

Dear Sir,

A PHARMACOKINETIC RANDOMISED INTERVENTIONAL STUDY TO OPTIMISE DIHYDROARTEMISININ-PIPERAQUINE DOSING FOR MALARIA PREVENTIVE TREATMENT IN MALAWIAN INFANTS (OPTIMAL STUDY) v.3.1 29 SEPTEMBER 2022

Refer to your application to register the above mentioned Clinical Trial Application (CTA) with the Pharmacy and Medicines Regulatory Authority (PMRA) as required under section 75 of PMRA Act 2019.

The PMRA, having reviewed the CTA, grants you permission to proceed with the implementation of the study.

Note:

1 The Clinical Trial Review Committee may provide further advise at any stage of the trial.
2 While the Clinical Trial Certificate will be issued in due course, the renewal date for the trial is 16th December of every year.

Yours faithfully,

Mphatso Kawaye
DIRECTOR GENERAL

PHARMACY & MEDICINES REGULATORY AUTHORITY
15 DEC 2022
DIRECTOR GENERAL
P.O. BOX 30241, LILONGWE 3

All correspondence should be addressed to the Director General
Off Paul Kagame • Chilambula Road. P.O. Box 30241, Capital City, Lilongwe, Malawi • Phone: +265 (0) 1 755 165 • Fax: +265 (0) 1 755 204 • Email: info@pmra.mw • Web: www.pmra.mw

PHARMACY AND MEDICINES REGULATORY AUTHORITY
Quality Medicines for Malawi

Ref. No. **PMRA/CTRC/ IV/22112022140** 5 February 2024

Orig. REG03

Malawi-Liverpool- Welcome Trust
Clinical Research Programme
P.O Box 30096
Chichiri
BLANTYRE 3

Attention: Dr. Clifford George Banda

Dear Sir,

A PHARMACOKINETIC RANDOMISED INTERVENTIONAL STUDY TO OPTIMISE DIHYDROARTEMISININ-PIPERAQUINE DOSING FOR MALARIA PREVENTIVE TREATMENT IN MALAWI INFANTS (OPTIMAL STUDY) Version 3.1

Reference is made to the letter dated 2nd January 2024. Pharmacy and Medicines Regulatory Authority (PMRA) acknowledges receipt of application for renewal documents for the study.

Included in the submission are:

1. Cover Letter
2. A completed progress report form.
3. Study protocol stamped by COMREC.
4. DSMB report
5. Adverse event summary table.
6. Stamped Informed Consent Form.
7. Stamped Assent form.
8. All three protocol deviations that have occurred in the study including appropriate corrective action plans.
9. External monitoring visit reports and finding log.
10. COMREC audit visit report and fonding log.
11. Clinical trial insurance certificate

All correspondence should be addressed to the Director General
Off Paul Kagame • Chilambula Road, P.O. Box 30241, Capital City, Lilongwe, Malawi • Phone: +265 (0) 1 755 165 • Fax: +265 (0) 1 755 204 • Email: info@pmra.mw • Web: www.pmra.mw

12. Clinical trial indemnity cover
13. Proof of renewal payment

Renewal license will be issued in due course and you may proceed with the study.

Yours faithfully,

Mphatso Kawaye
DIRECTOR GENERAL

PHARMACY & MEDICINES REGULATORY AUTHORITY
05 FEB 2024
DIRECTOR GENERAL
P.O. BOX 30241, LILONGWE 3

APPENDIX 4: Data collection tools (Case Report Forms)

First name, middle name, surname _____

National Identity no of parent/guardian. | | | | | | | | | | | | | | | |

Physical address, including _____

directions (landmarks etc.) _____

_____

_____

Parent/guardian/close contact's telephone nos. (Cell) _____

Emergency contact name _____

Emergency telephone nos. (Cell) _____

Usual clinic name/address _____

Clinical folder no. | | | | | | | | | | | | | | | |

Transport pick-up point when needed _____

COMMENTS

Staff initials_____ Checked by _____ on _____

OPTIMAL STUDY

STUDY ID	OPT___ ___ ___	PARTICIPANT'S INITIALS	___ ___ ___	FORM NUMBER	___ ___

ADVERSE EVENT LOG								
AE line number (e.g. 01)	Description of Adverse Event	Date of onset DD-MMM-YYYY	Severity (Please refer to key text below)	Relation ship to study medication	Action taken	Outcome	Date of resolution DD-MMM-YYYY	Serious Adverse Event?
		_ _/_ _ _/_ _ _ _	☐	☐	☐	☐	_ _/_ _ _/_ _ _ _	☐ *Yes* ☐ *No*
		_ _/_ _ _/_ _ _ _	☐	☐	☐	☐	_ _/_ _ _/_ _ _ _	☐ *Yes* ☐ *No*
		_ _/_ _ _/_ _ _ _	☐	☐	☐	☐	_ _/_ _ _/_ _ _ _	☐ *Yes* ☐ *No*
		_ _/_ _ _/_ _ _ _	☐	☐	☐	☐	_ _/_ _ _/_ _ _ _	☐ *Yes* ☐ *No*
		_ _/_ _ _/_ _ _ _	☐	☐	☐	☐	_ _/_ _ _/_ _ _ _	☐ *Yes* ☐ *No*

Severity: 1=mild, 2=moderate, 3=severe, 4=life threatening.

Relationship to study medication: 1=definitely unrelated, 2=unlikely related, 3=possibly related, 4=probably related 5=definitely related

Action taken: 1=no action, 2=dosing interrupted, 3=dosing discontinued

Outcome: 1=recovered/resolved, 2=recovered/resolved with sequelae, 3=recovering/resolving, 4=not recovered/resolved, 5=died, 6=Not known

Serious Adverse Event: 1=death, 2=life threatening, 3=hospitalisation, 4=persistent/significant disability, 5=medically important, 6=congenital anomaly.

In case of an SAE, please complete the SAE form.

Checked by (initials):__ __ __ on __ __/__ __ __/__ __ __ __

OPTIMAL STUDY SAE Form (*To be submitted with corresponding AE report form)*

Serious Adverse Events **SAE No.** |__|__| Participant ID: | O | P | T |__|__|__| Participant initials: |__|__|__|

1. Report Status

○	Initial report	**Date of Report**|__|__|-|__|__|__|-20|__|__| *(eg. 01-Jan-2013)*
○	Follow-up report	**Date of Report**|__|__|-|__|__|__|-20|__|__| *(eg. 01-Jan-2013)*
○	Follow-up report	**Date of Report**|__|__|-|__|__|__|-20|__|__| *(eg. 01-Jan-2013)*
○	Follow-up report	**Date of Report**|__|__|-|__|__|__|-20|__|__| *(eg. 01-Jan-2013)*
○	Final report	**Date of Report**|__|__|-|__|__|__|-20|__|__| *(eg. 01-Jan-2013)*

Subject Information

2a. Date of Birth |__|__|-|__|__|__|-|__|__|__|__| *(eg. 01-Jan-2013)* **OR** 2b. Age |__|__| months

3. Gender Female

Event Classification

4. Serious Adverse Event (provide key word or cause of death, **NB term should match corresponding AE page**):

5. Describe the serious adverse event ***(include all relevant laboratory results and add any further notes in the last section of the form as additional SAE notes)***

6. Relationship to study drug and action taken (To be completed by study doctor)

AE term						
Severity	☐ Mild	☐ Moderate	☐ Severe	☐ Life threatening		
Outcome	☐ Recovered/resolved	☐ Recovered/resolved with sequalae	☐ Recovering/resolving	☐ Not recovered /resolved	☐ Died	☐ Not known
Relationship to DP	☐ Definitely unrelated	☐ Unlikely related	☐ Possibly related	☐ Probably related	☐ Definitely related	
Alternative cause						
Therapy? Check all that apply	☐ None	☐ Prescribed drug/self-medication (complete concomitant med form)		☐ Other _____ _____		
Was AE serious?	☐ Yes* ☐ No * complete remaining fields this section	☐ Hospitalisation ☐ Prolonged hospitalisation Date of admission: _____/_____/_____ Date of discharge: _____/_____/_____				
	☐ Fatal	☐ Life-threatening				
	☐ Persistent disability / incapacity	☐ Medically significant	☐ Congenital anomaly/ defect			
Action taken w.r.t. DP	☐ No action	☐ Dosing interrupted	☐ Dosing discontinued			

Study Drug Detail

7. Study drug start date: |__|__|-|__|__|__|-20|__|__| *(eg. 01-Jan-2013)*

7a. Date of last study drug taken |__|__|-|__|__|__|-20|__|__| *(eg. 01-Jan-2013)*

7b. Time of last study drug taken |__|__|:|__|__| *(eg. 14:00)*

Therapy

8. Medication given to treat current event ○ Yes (if yes, please fill in below table) ○ No

Medication (Generic name)	Dose	Frequency	Route of administration	Start Date *(eg. 01-Jan-2013)*	End Date *(eg. 01-Jan-2013)*	Indication

9. Serious adverse event clinical notes

Date/time	Clinical notes (SAE as described by volunteer, assessment and investigations, description of action taken with regard to participation, study drug and concomitant medications given)	Initials

Investigator/Study Physician (print full name): _____

Signature: _____ Date: _____

OPTIMAL STUDY

STUDY ID	OPT_____

ADVERSE EVENTS (AE)								
Any AEs?	☐ Yes	☐ No	**What is the AE term?**				**AE number**	
Start date/time	|__|__|-|__|__|__|-|__|__|__|__| |__|__|:|__|__|			**End date/time**	|__|__|-|__|__|__|-|__|__|__|__| |__|__|:|__|__|		**Ongoing?** ☐	
Toxicity grade	☐ Mild		☐ Moderate	☐ Severe	☐ Life threatening[1]	☐ Fatal		
Outcome	☐ Recovered/ Resolved		☐ Recovering**/** Resolving with sequelae	☐ Recovering/ Resolving	☐ Not recovered/ Not resolved	☐ Fatal	☐ Unknown	
Relationship to study treatment	☐ Not related		☐ Unlikely related	☐ Possibly related	☐ Probably related	☐ Definitely related	**If AE related to study treatment, specify investigational product**	
Action taken with study treatment	☐ Drug withdrawn		☐ Dose reduced	☐ Dose not changed	☐ Unknown	☐ NA	☐ Other	**If other, specify**
Other action taken[2]	☐ Yes	☐ No	**Describe other action taken**					

[1] Complete SAE CRF
[2] If the AE resulted in a treatment administered, please record in the concomitant medication section

Staff initials_____ Checked by _____ on _____

OPTIMAL STUDY

STUDY ID	OPT_____

Is the AE serious	☐ Yes[3]	☐ No	**Is this a special interest AE? I**	☐ Yes	☐ No	**Was the AE expected?**	☐ Yes	☐ No
A description of the AE and supporting hospital documents (where applicable) should be provided								

[3] If classified as serious, please complete a SAE CRF

Staff initials_____ Checked by _____ on _____

OPTIMAL STUDY

STUDY ID	OPT_____

CAPILLARY HAEMOGLOBIN DURING SCHEDULED VISITS

Scheduled treatment	Sample time in relation to DP dose	Haemoglobin value	Haemoglobin sample No.	Initials
Treatment at 10 weeks of age (at enrolment)	Pre-dose	|__|__|.__| g/dL	ST1Hbd1	
Treatment at 14 weeks of age	Pre-dose	|__|__|.__| g/dL	ST2Hbd1	
Treatment at 6 months of age	Pre-dose	|__|__|.__| g/dL	ST3Hbd1	
Treatment at 9 months of age	Pre-dose	|__|__|.__| g/dL	ST4Hbd1	

OPTIMAL STUDY

STUDY ID	OPT_____

CAPILLARY HAEMOGLOBIN DURING UNSCHEDULED VISITS

Scheduled treatment	Unscheduled visit in relation to relation to DP dose	Haemoglobin value	Haemoglobin sample No.	Initials
Unscheduled visit 1	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	|__|__|.__| g/dL	UVHbd1	
Unscheduled visit 2	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	|__|__|.__| g/dL	UVHbd2	
Unscheduled visit 3	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	|__|__|.__| g/dL	UVHbd3	
Unscheduled visit 4	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	|__|__|.__| g/dL	UVHbd4	
Unscheduled visit 5	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	|__|__|.__| g/dL	UVHbd5	

OPTIMAL STUDY

STUDY ID	OPT_____

Unscheduled visit 6	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	**|__|__|.__| g/dL**	**UVHbd6**	
Unscheduled visit 7	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	**|__|__|.__| g/dL**	**UVHbd7**	
Unscheduled visit 8	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	**|__|__|.__| g/dL**	**UVHbd8**	
Unscheduled visit 9	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	**|__|__|.__| g/dL**	**UVHbd9**	
Unscheduled visit 10	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	**|__|__|.__| g/dL**	**UVHbd10**	

OPTIMAL STUDY

STUDY ID	OPT_____

ENROLMENT

PHYSICAL EXAMINATION

Anthropometric measurements	Results	Units
Weight	|__|__|__|.|__|	kg
Height	|__|__|__|.|__|	cm
Mid-upper arm circumference	|__|__|.|__|	cm

PHYSICAL EXAMINATION

Body system examined	Results
General appearance (including jaundice, anaemia, clubbing, cyanosis, oedema, lymphadenopathy)	☐ Normal ☐ Abnormal
Head, eyes, ears, nose, throat	☐ Normal ☐ Abnormal
Respiratory	☐ Normal ☐ Abnormal
Cardiovascular	☐ Normal ☐ Abnormal
Gastrointestinal	☐ Normal ☐ Abnormal
Neurological	☐ Normal ☐ Abnormal
Joints	☐ Normal ☐ Abnormal
Skin	☐ Normal ☐ Abnormal

TREATMENT GROUP DETAILS

Treatment group allocated	Intervention group ☐ Control group ☐

OPTIMAL STUDY

STUDY ID	OPT_____

CHECKLIST FOR INTERVENTION GROUP PROCEDURES AT ENROLMENT

Procedure description	Yes	No	Reference
PK sampling			
Pre-dose PK capillary sample	☐	☐	PK sampling form
Efficacy assessment			
Malaria slide	☐	☐	Efficacy assessment form
Quantitative PCR sample on filter paper	☐	☐	
Safety assessment			
Adverse event monitoring	☐	☐	AE log, AE form, SAE form

CHECKLIST FOR CONTROL GROUP PROCEDURES AT ENROLMENT

Procedure description	Yes	No	Reference
Malaria incidence assessment			
Malaria slide	☐	☐	Malaria incidence assessment form
Quantitative PCR sample on filter paper	☐	☐	
Safety assessment			
Adverse event monitoring	☐	☐	AE log, AE form, SAE form

OPTIMAL STUDY EXIT FORM

STUDY ID	OPT_____

1	How did the participant leave the study? *(Note: "completed" includes treatment failures and adequate responses)*	☐ Completed ☐ Withdrawn consent ☐ Withdrawn for safety reasons ☐ Lost to follow up ☐ Screening failure ☐ Death
2	Date of last contact	☐☐ ☐☐☐ ☐☐☐☐
3	Last contact visit aligned with	10-week-old visits, Day ☐ 14-week-old visits, Day ☐ 6-month-old visits, Day ☐ 9§-month-old visits, Day ☐
4	Are all adverse event forms signed off?	Yes ☐ No ☐
5	Are all concomitant forms signed off?	Yes ☐ No ☐
Notes		

Study Staff Initials ☐☐☐	**Signature**_____	☐☐ D D	☐☐☐ M M M ☐☐☐☐ Y Y Y Y
Checked by ☐☐☐	**Signature**_____	☐☐ D D	☐☐☐ M M M ☐☐☐☐ Y Y Y Y

OPTIMAL STUDY CONCOMITANT MEDICATIONS FORM

STUDY ID	OPT__________

Any study, prescription/non-prescription/traditional meds, vitamins, herbal/dietary supplements, or vaccinations **from 4 weeks before screening (ALL OTHER)** and throughout the trial. **PLEASE CHECK ANY OTHER EXISTING HEALTH PASSPORTS AND CROSS-CHECK AGAINST THE ELIGIBILITY CRITERIA/PROHIBITED MEDICINES LIST**						Use additional forms as required if medications stop and re-start	
Medication name	**Start date** *(dd/mm/yyyy)*	**Stop date** *(dd/mm/yyyy)*	**Route of administration**	**Dose and dose unit (e.g. 1 tablet or 500mg)**	**Frequency**	**Indication(s)**	**Initials**
			☐ Oral ☐ Respiratory ☐ Intravenous ☐ Intramuscular ☐ Nasal ☐ Vaginal ☐ Rectal ☐ Topical Other: ____________	______________ ☐ Tablet/capsule ☐ g ☐ mg ☐ µg Other:	☐ Once daily ☐ BD ☐ TID ☐ QID ☐ PRN Other:		
			☐ Oral ☐ Respiratory ☐ Intravenous ☐ Intramuscular ☐ Nasal ☐ Vaginal ☐ Rectal ☐ Topical Other: ____________	______________ ☐ Tablet/capsule ☐ g ☐ mg ☐ µg Other:	☐ Once daily ☐ BD ☐ TID ☐ QID ☐ PRN Other:		
			☐ Oral ☐ Respiratory ☐ Intravenous ☐ Intramuscular ☐ Nasal ☐ Vaginal ☐ Rectal ☐ Topical Other: ____________	______________ ☐ Tablet/capsule ☐ g ☐ mg ☐ µg Other:	☐ Once daily ☐ BD ☐ TID ☐ QID ☐ PRN Other:		
			☐ Oral ☐ Respiratory ☐ Intravenous ☐ Intramuscular ☐ Nasal ☐ Vaginal ☐ Rectal ☐ Topical Other: ____________	______________ ☐ Tablet/capsule ☐ g ☐ mg ☐ µg Other:	☐ Once daily ☐ BD ☐ TID ☐ QID ☐ PRN Other:		

Staff initials________ Checked by ________ on ______________

OPTIMAL STUDY

STUDY ID	OPT_____

Pharmacokinetic sampling and DP dosing

Scheduled treatment	Sample time in relation to DP dose	Protocol time (HH:MM)	Dosing Actual time (HH:MM)	Bloods		Comment (e.g. problems sampling, observations post-dose)	Initials
				Actual time (HH:MM)	Piperaquine PK sample No.		
Treatment at 10 weeks of age (at enrolment)	Pre-dose sample			__:__	**ST1d1**		
	Day 3	__:__	__:__	__:__	**ST1d3**		
	Day 7 ± 1	__:__		__:__	**ST1d7**		
	Day 14 ± 2	__:__		__:__	**ST1d14**		
	Day 28 ± 5	__:__		__:__	**ST1d28**		

OPTIMAL STUDY

STUDY ID	OPT_____

Pharmacokinetic sampling and DP dosing

Scheduled treatment	Sample time in relation to DP dose	Protocol time (HH:MM)	Dosing Actual time (HH:MM)	Bloods		Comment (e.g. problems sampling, observations post-dose)	Initials
				Actual time (HH:MM)	Piperaquine PK sample No.		
Treatment at 14 weeks of age (at enrolment)	Pre-dose sample			__:__	**ST2d1**		
	Day 3	__:__	__:__	__:__	**ST2d3**		
	Day 7 ± 1	__:__		__:__	**ST2d7**		
	Day 14 ± 2	__:__		__:__	**ST2d14**		
	Day 28 ± 5	__:__		__:__	**ST2d28**		

OPTIMAL STUDY

STUDY ID	OPT_____

Pharmacokinetic sampling and DP dosing

Scheduled treatment	Sample time in relation to DP dose	Protocol time (HH:MM)	Dosing Actual time (HH:MM)	Bloods		Comment (e.g. problems sampling, observations post-dose)	Initials
				Actual time (HH:MM)	Piperaquine PK sample No.		
Treatment at 6 months of age (at enrolment)	Pre-dose sample			:	**ST3d1**		
	Day 3	:	:	:	**ST3d3**		
	Day 7 ± 1	:		:	**ST3d7**		
	Day 14 ± 2	:		:	**ST3d14**		
	Day 28 ± 5	:		:	**ST3d28**		

OPTIMAL STUDY

STUDY ID	OPT_____

Pharmacokinetic sampling and DP dosing

Scheduled treatment	Sample time in relation to DP dose	Protocol time (HH:MM)	Dosing Actual time (HH:MM)	Bloods		Comment (e.g. problems sampling, observations post-dose)	Initials
				Actual time (HH:MM)	Piperaquine PK sample No.		
Treatment at 9 months of age (at enrolment)	Pre-dose sample			:	**ST4d1**		
	Day 3	:	:	:	**ST4d3**		
	Day 7 ± 1	:		:	**ST4d7**		
	Day 14 ± 2	:		:	**ST4d14**		
	Day 28 ± 5	:		:	**ST4d28**		

OPTIMAL STUDY

STUDY ID	OPT_____

Pharmacokinetic sampling and DP dosing

Scheduled treatment	Sample time in relation to DP dose	Protocol time (HH:MM)	Dosing Actual time (HH:MM)	Bloods		Comment	Initials
				Actual time (HH:MM)	Piperaquine PK sample No.	Scheduled treatment (ST) No	
Unscheduled visits	Visit 1	:		:	**UVd1**	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	
	Visit 2	:		:	**UVd2**	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	
	Visit 3	:		:	**UVd3**	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	
	Visit 4	:		:	**UVd4**	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	
	Visit 5	:		:	**UVd5**	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	

OPTIMAL STUDY

STUDY ID	OPT_____

Pharmacokinetic sampling and DP dosing

Scheduled treatment	Sample time in relation to DP dose	Protocol time (HH:MM)	Dosing Actual time (HH:MM)	Bloods		Comment	Initials
				Actual time (HH:MM)	Piperaquine PK sample No.	Scheduled treatment (ST) No	
Unscheduled visits	Visit 6	:		:	**UVd6**	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	
	Visit 7	:		:	**UVd7**	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	
	Visit 8	:		:	**UVd8**	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	
	Visit 9	:		:	**UVd9**	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	
	Visit 10	:		:	**UVd10**	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	

OPTIMAL STUDY

STUDY ID	OPT_____

STUDY DRUG ADMINISTRATION IN THE INTERVENTIO GROUP

Scheduled treatment	Scheduled dose number	Treatment name	Number of tablets	Date of dose Time of dose	OR Missed dose	Administration observed	Given with fat/food	Did the participant vomit within 30 minutes of the dose	Time of vomit	Dose/Re-treatment
Treatment at 10 weeks of age (at enrolment)	1*	DP	I__I.I__I	I__I__I-I__I__I__I-I__I__I__I__I I__I__I:I__I__I	☐	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	I__I__I:I__I__I	☐ Dose ☐ Redose
	2#	DP	I__I.I__I	I__I__I-I__I__I__I-I__I__I__I__I I__I__I:I__I__I	☐	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	I__I__I:I__I__I	☐ Dose ☐ Redose
	3*	DP	I__I.I__I	I__I__I-I__I__I__I-I__I__I__I__I I__I__I:I__I__I	☐	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	I__I__I:I__I__I	☐ Dose ☐ Redose
Treatment at 14 weeks of age	1*	DP	I__I.I__I	I__I__I-I__I__I__I-I__I__I__I__I I__I__I:I__I__I	☐	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	I__I__I:I__I__I	☐ Dose ☐ Redose
	2#	DP	I__I.I__I	I__I__I-I__I__I__I-I__I__I__I__I I__I__I:I__I__I	☐	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	I__I__I:I__I__I	☐ Dose ☐ Redose
	3*	DP	I__I.I__I	I__I__I-I__I__I__I-I__I__I__I__I I__I__I:I__I__I	☐	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	I__I__I:I__I__I	☐ Dose ☐ Redose

* Observed doses at the study clinic

Dose administered at home by the guardian

OPTIMAL STUDY

STUDY ID	OPT_____

STUDY DRUG ADMINISTRATION

Scheduled treatment	Scheduled dose number	Treatment name	Number of tablets	Date of dose Time of dose	OR Missed dose	Administration observed	Given with fat/food	Did the participant vomit within 30 minutes of the dose	Time of vomit	Dose/Re-treatment
Treatment at 6 months of age	1*	DP	__|__.|__	__|__|-|__|__|__|-|__|__|__|__| __|__:|__|__	☐	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	__|__:|__|__	☐ Dose ☐ Redose
	2#	DP	__|__.|__	__|__|-|__|__|__|-|__|__|__|__| __|__:|__|__	☐	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	__|__:|__|__	☐ Dose ☐ Redose
	3*	DP	__|__.|__	__|__|-|__|__|__|-|__|__|__|__| __|__:|__|__	☐	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	__|__:|__|__	☐ Dose ☐ Redose
Treatment at 9 months of age	1*	DP	__|__.|__	__|__|-|__|__|__|-|__|__|__|__| __|__:|__|__	☐	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	__|__:|__|__	☐ Dose ☐ Redose
	2#	DP	__|__.|__	__|__|-|__|__|__|-|__|__|__|__| __|__:|__|__	☐	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	__|__:|__|__	☐ Dose ☐ Redose
	3*	DP	__|__.|__	__|__|-|__|__|__|-|__|__|__|__| __|__:|__|__	☐	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	__|__:|__|__	☐ Dose ☐ Redose

* Observed doses at the study clinic

Dose administered at home by the guardian

OPTIMAL STUDY

STUDY ID	OPT_____

DAY 1 OF EACH TREATMENT SCHEDULE IN THE INTERVENTION ARM

SCHEDULED VISIT DAY IN RELATION TO DP TREATMENT

☐ ST1 ☐ ST2

☐ ST3. ☐ ST4

PHYSICAL EXAMINATION

Anthropometric measurements	Results	Units
Weight	|__|__|__|.|__|	kg
Height	|__|__|__|.|__|	cm
Mid-upper arm circumference	|__|__|.|__|	cm

OPTIMAL STUDY

STUDY ID	OPT_____

PHYSICAL EXAMINATION	
Body system examined	**Results**
General appearance (including jaundice, anaemia, clubbing, cyanosis, oedema, lymphadenopathy)	☐ Normal ☐ Abnormal
Head, eyes, ears, nose, throat	☐ Normal ☐ Abnormal
Respiratory	☐ Normal ☐ Abnormal
Cardiovascular	☐ Normal ☐ Abnormal
Gastrointestinal	☐ Normal ☐ Abnormal
Neurological	☐ Normal ☐ Abnormal
Joints	☐ Normal ☐ Abnormal
Skin	☐ Normal ☐ Abnormal

** **Provide details of abnormal findings on AE form. Include any hospital records**

CHECKLIST FOR SCHEDULED PROCEDURES

Procedure description	Yes	No	NA	Reference
Capillary haemoglobin				
Capillary Hb sample	☐	☐	☐	Capillary Hb form
PK sampling				
Pre-dose PK capillary sample	☐	☐	☐	PK sampling form
Efficacy assessment				
Malaria slide	☐	☐	☐	Efficacy assessment form
Quantitative PCR sample on filter paper	☐	☐	☐	
Safety assessment				
Adverse event monitoring	☐	☐	☐	AE log, AE form, SAE form
Concomitant medication assessment				
Concomitant medication being used by infant and mother who is breastfeeding	☐	☐	☐	Concomitant medication form

Staff initials_____ Checked by _____ on _____

OPTIMAL STUDY

STUDY ID	OPT_____

DAY 3 INTERVENTION ARM

SCHEDULED VISIT DAY IN RELATION TO DP TREATMENT

☐ ST1 ☐ ST2

☐ ST3. ☐ ST4

PHYSICAL EXAMINATION

Anthropometric measurements	Results	Units
Weight	|__|__|__|.|__|	kg
Height	|__|__|__|.|__|	cm
Mid-upper arm circumference	|__|__|.|__|	cm

PHYSICAL EXAMINATION

Body system examined	Results
General appearance (including jaundice, anaemia, clubbing, cyanosis, oedema, lymphadenopathy)	☐ Normal ☐ Abnormal
Head, eyes, ears, nose, throat	☐ Normal ☐ Abnormal
Respiratory	☐ Normal ☐ Abnormal
Cardiovascular	☐ Normal ☐ Abnormal
Gastrointestinal	☐ Normal ☐ Abnormal
Neurological	☐ Normal ☐ Abnormal
Joints	☐ Normal ☐ Abnormal
Skin	☐ Normal ☐ Abnormal

** **Provide details of abnormal findings on AE form. Include any hospital records**

Staff initials_____ Checked by _____ on _____

OPTIMAL STUDY

STUDY ID	OPT_____

CHECKLIST FOR INTERVENTION GROUP PROCEDURES AT ENROLMENT

Procedure description	Yes	No	NA	Reference
PK sampling				
Pre-dose PK capillary sample	☐	☐	☐	PK sampling form
Efficacy assessment				
Malaria slide	☐	☐	☐	Efficacy assessment form
Quantitative PCR sample on filter paper	☐	☐	☐	
Safety assessment				
Adverse event monitoring	☐	☐	☐	AE log, AE form, SAE form
Concomitant medication assessment				
Concomitant medication being used by infant and mother who is breastfeeding	☐	☐	☐	Concomitant medication form

OPTIMAL STUDY

STUDY ID	OPT_____

DAY 7 INTERVENTION ARM

SCHEDULED VISIT DAY IN RELATION TO DP TREATMENT

☐ ST1 ☐ ST2

☐ ST3. ☐ ST4

PHYSICAL EXAMINATION

Anthropometric measurements	Results	Units
Weight	|__|__|__|.|__|	kg
Height	|__|__|__|.|__|	cm
Mid-upper arm circumference	|__|__|.|__|	cm

PHYSICAL EXAMINATION

Body system examined	Results
General appearance (including jaundice, anaemia, clubbing, cyanosis, oedema, lymphadenopathy)	☐ Normal ☐ Abnormal
Head, eyes, ears, nose, throat	☐ Normal ☐ Abnormal
Respiratory	☐ Normal ☐ Abnormal
Cardiovascular	☐ Normal ☐ Abnormal
Gastrointestinal	☐ Normal ☐ Abnormal
Neurological	☐ Normal ☐ Abnormal
Joints	☐ Normal ☐ Abnormal
Skin	☐ Normal ☐ Abnormal

** **Provide details of abnormal findings on AE form. Include any hospital records**

Staff initials_____ Checked by _____ on _____

OPTIMAL STUDY

STUDY ID	OPT_____

CHECKLIST FOR INTERVENTION GROUP PROCEDURES AT ENROLMENT

Procedure description	Yes	No	NA	Reference
PK sampling				
Pre-dose PK capillary sample	☐	☐	☐	PK sampling form
Efficacy assessment				
Malaria slide	☐	☐	☐	Efficacy assessment form
Quantitative PCR sample on filter paper	☐	☐	☐	
Safety assessment				
Adverse event monitoring	☐	☐	☐	AE log, AE form, SAE form
Concomitant medication assessment				
Concomitant medication being used by infant and mother who is breastfeeding	☐	☐	☐	Concomitant medication form

OPTIMAL STUDY

STUDY ID	OPT_____

DAY 14 INTERVENTION ARM

SCHEDULED VISIT DAY IN RELATION TO DP TREATMENT

☐ ST1 ☐ ST2

☐ ST3. ☐ ST4

PHYSICAL EXAMINATION

Anthropometric measurements	Results	Units
Weight	|__|__|__|.|__|	kg
Height	|__|__|__|.|__|	cm
Mid-upper arm circumference	|__|__|.|__|	cm

PHYSICAL EXAMINATION

Body system examined	Results
General appearance (including jaundice, anaemia, clubbing, cyanosis, oedema, lymphadenopathy)	☐ Normal ☐ Abnormal
Head, eyes, ears, nose, throat	☐ Normal ☐ Abnormal
Respiratory	☐ Normal ☐ Abnormal
Cardiovascular	☐ Normal ☐ Abnormal
Gastrointestinal	☐ Normal ☐ Abnormal
Neurological	☐ Normal ☐ Abnormal
Joints	☐ Normal ☐ Abnormal
Skin	☐ Normal ☐ Abnormal

** **Provide details of abnormal findings on AE form. Include any hospital records**

Staff initials_____ Checked by _____ on _____

OPTIMAL STUDY

STUDY ID	OPT_____

CHECKLIST FOR INTERVENTION GROUP PROCEDURES AT ENROLMENT

Procedure description	Yes	No	NA	Reference
PK sampling				
Pre-dose PK capillary sample	☐	☐	☐	PK sampling form
Efficacy assessment				
Malaria slide	☐	☐	☐	Efficacy assessment form
Quantitative PCR sample on filter paper	☐	☐	☐	
Safety assessment				
Adverse event monitoring	☐	☐	☐	AE log, AE form, SAE form
Concomitant medication assessment				
Concomitant medication being used by infant and mother who is breastfeeding	☐	☐	☐	Concomitant medication form

Staff initials_____ Checked by _____ on _____

OPTIMAL STUDY

STUDY ID	OPT_____

DAY 28 INTERVENTION ARM

SCHEDULED VISIT DAY IN RELATION TO DP TREATMENT

☐ ST1 ☐ ST2

☐ ST3. ☐ ST4

PHYSICAL EXAMINATION

Anthropometric measurements	Results	Units
Weight	|__|__|__|.|__|	kg
Height	|__|__|__|.|__|	cm
Mid-upper arm circumference	|__|__|.|__|	cm

PHYSICAL EXAMINATION

Body system examined	Results
General appearance (including jaundice, anaemia, clubbing, cyanosis, oedema, lymphadenopathy)	☐ Normal ☐ Abnormal
Head, eyes, ears, nose, throat	☐ Normal ☐ Abnormal
Respiratory	☐ Normal ☐ Abnormal
Cardiovascular	☐ Normal ☐ Abnormal
Gastrointestinal	☐ Normal ☐ Abnormal
Neurological	☐ Normal ☐ Abnormal
Joints	☐ Normal ☐ Abnormal
Skin	☐ Normal ☐ Abnormal

** **Provide details of abnormal findings on AE form. Include any hospital records**

Staff initials_____ Checked by _____ on _____

OPTIMAL STUDY

STUDY ID	OPT_____

CHECKLIST FOR INTERVENTION GROUP PROCEDURES AT ENROLMENT

Procedure description	Yes	No	NA	Reference
PK sampling				
Pre-dose PK capillary sample	☐	☐	☐	PK sampling form
Efficacy assessment				
Malaria slide	☐	☐	☐	Efficacy assessment form
Quantitative PCR sample on filter paper	☐	☐	☐	
Safety assessment				
Adverse event monitoring	☐	☐	☐	AE log, AE form, SAE form
Concomitant medication assessment				
Concomitant medication being used by infant and mother who is breastfeeding	☐	☐	☐	Concomitant medication form

Staff initials_____ Checked by _____ on _____

OPTIMAL STUDY

STUDY ID	OPT_____

MICROSCOPY OF MALARIA SLIDE FOR PLASMODIUM FALCIPARUM DETECTION DURING SCHEDULED VISITS

Scheduled treatment	Sample time in relation to DP dose	Slide quality	Smear type	Parasite type	Parasite count	Parasite count units			Slide No.	Initials
Treatment at 10 weeks of age (at enrolment)	Pre-dose	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**ST1SLd1**	
Treatment at 10 weeks of age[1]*	28 days post-DP dose	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**ST1SLd28**	
Treatment at 14 weeks of age	28 days post-DP dose	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**ST2SLd28**	

[1]* This sample will also be a pre-dose of the 14 weeks treatment. Therefore, no pre-dose treatment sample is indicated

OPTIMAL STUDY

STUDY ID	OPT_____

Treatment at 6 months of age	Pre-dose	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**ST3SLd1**	
Treatment at 6 months of age	28 days post-DP dose	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**ST3SLd28**	
Treatment at 9 months of age	Pre-dose	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**ST4SLd1**	
Treatment at 9 months of age	28 days post-DP dose	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**ST4SLd28**	

OPTIMAL STUDY

STUDY ID	OPT_____

MICROSCOPY OF MALARIA SLIDE FOR PLASMODIUM FALCIPARUM DETECTION DURING UNSCHEDULED VISITS

Unscheduled treatment	Unscheduled visit in relation to relation to DP dose	Slide quality	Smear type	Parasite type	Parasite count	Parasite count units			Slide No.	Initials
Unscheduled visit 1	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd1**	
Unscheduled visit 2	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd2**	
Unscheduled visit 3	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd3**	
Unscheduled visit 4	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd4**	
Unscheduled visit 5	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd5**	

OPTIMAL STUDY

STUDY ID	OPT_____

Unscheduled visit 6	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd6**	
Unscheduled visit 7	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd7**	
Unscheduled visit 8	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd8**	
Unscheduled visit 9	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd9**	
Unscheduled visit 10	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd10**	

OPTIMAL STUDY

STUDY ID	OPT_____

FILTER PAPER FOR QUANTITATIVE PLASMODIUM FALCIPARUM PCR DURING SCHEDULED VISITS

Scheduled treatment	Sample time in relation to DP dose	Filter paper sample collected	Filter paper sample No.	Initials
Treatment at 10 weeks of age (at enrolment)	Pre-dose	☐ Yes ☐ No	**ST1FPd1**	
Treatment at 10 weeks of age[2]*	28 days post-DP dose	☐ Yes ☐ No	**ST1FPd28**	
Treatment at 14 weeks of age	28 days post-DP dose	☐ Yes ☐ No	**ST2FPd28**	
Treatment at 6 months of age	Pre-dose	☐ Yes ☐ No	**ST3FPd1**	
Treatment at 6 months of age	28 days post-DP dose	☐ Yes ☐ No	**ST3FPd28**	
Treatment at 9 months of age	Pre-dose	☐ Yes ☐ No	**ST4FPd1**	
Treatment at 9 months of age	28 days post-DP dose	☐ Yes ☐ No	**ST4FPd28**	

[2]* This sample will also be a pre-dose of the 14 weeks treatment. Therefore, no pre-dose treatment sample is indicated

OPTIMAL STUDY

STUDY ID	OPT_____

FILTER PAPER FOR QUANTITATIVE PLASMODIUM FALCIPARUM PCR DURING UNSCHEDULED VISITS

Scheduled treatment	Scheduled treatment (ST) No	Sample time in relation to any treatment dose	Filter paper sample collected	Filter paper sample No.	Initials
Unscheduled visit 1	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd1**	
Unscheduled visit 2	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd2**	
Unscheduled visit 3	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd3**	
Unscheduled visit 4	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd4**	
Unscheduled visit 5	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd5**	
Unscheduled visit 6	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd6**	

OPTIMAL STUDY

STUDY ID	OPT_____

Unscheduled visit 7	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd7**	
Unscheduled visit 8	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd8**	
Unscheduled visit 9	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd9**	
Unscheduled visit 10	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd10**	

OPTIMAL STUDY

STUDY ID	OPT_____

UNSCHEDULED VISIT IN THE INTERVENTION ARM

UNSCHEDULED VISIT IN RELATION TO SCHEDULED VISIT

☐ 10 weeks of age ☐ 14 weeks of age

☐ 6 months of age ☐ 9 months of age

UNSCHEDULED VISIT NUMBER: |__|__|

PHYSICAL EXAMINATION

Anthropometric measurements	Results	Units
Weight	|__|__|__|.|__|	kg
Height	|__|__|__|.|__|	cm
Mid-upper arm circumference	|__|__|.|__|	cm

OPTIMAL STUDY

STUDY ID	OPT_____

PHYSICAL EXAMINATION

Body system examined	Results
General appearance (including jaundice, anaemia, clubbing, cyanosis, oedema, lymphadenopathy)	☐ Normal ☐ Abnormal
Head, eyes, ears, nose, throat	☐ Normal ☐ Abnormal
Respiratory	☐ Normal ☐ Abnormal
Cardiovascular	☐ Normal ☐ Abnormal
Gastrointestinal	☐ Normal ☐ Abnormal
Neurological	☐ Normal ☐ Abnormal
Joints	☐ Normal ☐ Abnormal
Skin	☐ Normal ☐ Abnormal

** **Provide details of abnormal findings on AE form. Include any hospital records**

CHECKLIST FOR UNSCHEDULED VISIT PROCEDURES

Procedure description	Yes	No	NA	Reference
Capillary haemoglobin				
Capillary Hb sample	☐	☐	☐	Capillary Hb form
Efficacy assessment				
Malaria slide	☐	☐	☐	Efficacy assessment form
Quantitative PCR sample on filter paper	☐	☐	☐	
PK sampling				
Pre-dose PK capillary sample	☐	☐	☐	PK sampling form
Safety assessment				
Adverse event monitoring	☐	☐	☐	AE log, AE form, SAE form
Concomitant medication assessment				
Concomitant medication being used by infant and mother who is breastfeeding	☐	☐	☐	Concomitant medication form

Staff initials_____ Checked by _____ on _____

OPTIMAL STUDY

STUDY ID	OPT_____

DAY 1 SCHEDULED VISIT IN THE CONTROL ARM

VISIT DAY IN RELATION TO SCHEDULED VISIT

☐ 10 weeks of age ☐ 14 weeks of age

☐ 6 months of age ☐ 9 months of age

PHYSICAL EXAMINATION

Anthropometric measurements	Results	Units
Weight	|__|__|__|.|__|	kg
Height	|__|__|__|.|__|	cm
Mid-upper arm circumference	|__|__|.|__|	cm

OPTIMAL STUDY

STUDY ID	OPT_____

PHYSICAL EXAMINATION

Body system examined	Results
General appearance (including jaundice, anaemia, clubbing, cyanosis, oedema, lymphadenopathy)	☐ Normal ☐ Abnormal
Head, eyes, ears, nose, throat	☐ Normal ☐ Abnormal
Respiratory	☐ Normal ☐ Abnormal
Cardiovascular	☐ Normal ☐ Abnormal
Gastrointestinal	☐ Normal ☐ Abnormal
Neurological	☐ Normal ☐ Abnormal
Joints	☐ Normal ☐ Abnormal
Skin	☐ Normal ☐ Abnormal

** **Provide details of abnormal findings on AE form. Include any hospital records**

CHECKLIST FOR SCHEDULED PROCEDURES

Procedure description	Yes	No	NA	Reference
Capillary haemoglobin				
Capillary Hb sample	☐	☐	☐	Capillary Hb form
Malaria incidence assessment				
Malaria slide	☐	☐	☐	Malaria incidence assessment form
Quantitative PCR sample on filter paper	☐	☐	☐	
Safety assessment				
Adverse event monitoring	☐	☐	☐	AE log, AE form, SAE form
Concomitant medication assessment				
Concomitant medication being used by infant and mother who is breastfeeding	☐	☐	☐	Concomitant medication form

OPTIMAL STUDY

STUDY ID	OPT_____

DAY 28 SCHEDULED VISIT IN THE CONTROL ARM

VISIT DAY IN RELATION TO SCHEDULED VISIT

☐ 10 weeks of age ☐ 14 weeks of age

☐ 6 months of age ☐ 9 months of age

PHYSICAL EXAMINATION

Anthropometric measurements	Results	Units
Weight	|__|__|__|.|__|	kg
Height	|__|__|__|.|__|	cm
Mid-upper arm circumference	|__|__|.|__|	cm

OPTIMAL STUDY

STUDY ID	OPT_____

PHYSICAL EXAMINATION	
Body system examined	**Results**
General appearance (including jaundice, anaemia, clubbing, cyanosis, oedema, lymphadenopathy)	☐ Normal ☐ Abnormal
Head, eyes, ears, nose, throat	☐ Normal ☐ Abnormal
Respiratory	☐ Normal ☐ Abnormal
Cardiovascular	☐ Normal ☐ Abnormal
Gastrointestinal	☐ Normal ☐ Abnormal
Neurological	☐ Normal ☐ Abnormal
Joints	☐ Normal ☐ Abnormal
Skin	☐ Normal ☐ Abnormal

** **Provide details of abnormal findings on AE form. Include any hospital records**

CHECKLIST FOR SCHEDULED PROCEDURES

Procedure description	Yes	No	NA	Reference
Capillary haemoglobin				
Capillary Hb sample	☐	☐	☐	Capillary Hb form
Malaria incidence assessment				
Malaria slide	☐	☐	☐	Malaria incidence assessment form
Quantitative PCR sample on filter paper	☐	☐	☐	
Safety assessment				
Adverse event monitoring	☐	☐	☐	AE log, AE form, SAE form
Concomitant medication assessment				
Concomitant medication being used by infant and mother who is breastfeeding	☐	☐	☐	Concomitant medication form

Staff initials_____ Checked by _____ on _____

OPTIMAL STUDY

STUDY ID	OPT_____

MICROSCOPY OF MALARIA SLIDE FOR PLASMODIUM FALCIPARUM DETECTION DURING SCHEDULED VISITS

Scheduled visit	Sample time in relation to follow up time	Slide quality	Smear type	Parasite type	Parasite count	Parasite count units			Slide No.	Initials
10 weeks of age (baseline)	Day 1	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**SV1SLd1**	
10 weeks of age[1]*	Day 28	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**SV1SLd28**	
14 weeks of age	Day 28	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**SV2SLd28**	

[1]* This sample will also be a day 1 sample of the 14 weeks visit. Therefore, no day 1 sample is indicated

OPTIMAL STUDY

STUDY ID	OPT_____

6 months of age	Day 1	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**SV3SLd1**	
6 months of age	Day 28	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**SV3SLd28**	
9 months of age	Day 1	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**SV4SLd1**	
9 months of age	Day 28	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**SV4SLd28**	

OPTIMAL STUDY

STUDY ID	OPT_____

MICROSCOPY OF MALARIA SLIDE FOR PLASMODIUM FALCIPARUM DETECTION DURING UNSCHEDULED VISITS

Unscheduled treatment	Unscheduled visit in relation to relation to DP dose	Slide quality	Smear type	Parasite type	Parasite count	Parasite count units			Slide No.	Initials
Unscheduled visit 1	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd1**	
Unscheduled visit 2	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd2**	
Unscheduled visit 3	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd3**	
Unscheduled visit 4	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd4**	
Unscheduled visit 5	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd5**	

OPTIMAL STUDY

STUDY ID	OPT_____

Unscheduled visit 6	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd6**	
Unscheduled visit 7	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd7**	
Unscheduled visit 8	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd8**	
Unscheduled visit 9	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd9**	
Unscheduled visit 10	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	☐ Good ☐ Poor ☐ Missing	☐ Thick smear ☐ Thin smear	☐ Asexual ☐ Sexual	I__I__I__I__I	☐ /_____WBC	☐ /_____HPF	☐ /_____RBC	**UVSLd10**	

OPTIMAL STUDY

STUDY ID	OPT_____

FILTER PAPER FOR QUANTITATIVE PLASMODIUM FALCIPARUM PCR DURING SCHEDULED VISITS

Scheduled treatment	Sample time in relation to DP dose	Filter paper sample collected	Filter paper sample No.	Initials
10 weeks of age (baseline)	Day 1	☐ Yes ☐ No	**SVFPd1**	
10 weeks of age[2]*	Day 28	☐ Yes ☐ No	**SVFPd28**	
14 weeks of age	Day 28	☐ Yes ☐ No	**SVFPd28**	
6 months of age	Day 1	☐ Yes ☐ No	**SVFPd1**	
6 months of age	Day 28	☐ Yes ☐ No	**SVFPd28**	
9 months of age	Day 1	☐ Yes ☐ No	**SVFPd1**	
9 months of age	Day 28	☐ Yes ☐ No	**SVFPd28**	

[2]* This sample will also be a day 1 sample of the 14 weeks visit. Therefore, no day 1 sample is indicated

OPTIMAL STUDY

STUDY ID	OPT_____

FILTER PAPER FOR QUANTITATIVE PLASMODIUM FALCIPARUM PCR DURING UNSCHEDULED VISITS

Scheduled treatment	Scheduled treatment (ST) No	Sample time in relation to any treatment dose	Filter paper sample collected	Filter paper sample No.	Initials
Unscheduled visit 1	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd1**	
Unscheduled visit 2	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd2**	
Unscheduled visit 3	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd3**	
Unscheduled visit 4	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd4**	
Unscheduled visit 5	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd5**	
Unscheduled visit 6	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd6**	

OPTIMAL STUDY

STUDY ID	OPT_____

Unscheduled visit 7	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd7**	
Unscheduled visit 8	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd8**	
Unscheduled visit 9	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd9**	
Unscheduled visit 10	☐ ST1 ☐ ST2 ☐ ST3. ☐ ST4	Pre-dose	☐ Yes ☐ No	**UVFPd10**	

OPTIMAL STUDY

STUDY ID	OPT_____

UNSCHEDULED VISIT IN THE CONTROL ARM

UNSCHEDULED VISIT IN RELATION TO SCHEDULED VISIT

☐ 10 weeks of age ☐ 14 weeks of age

☐ 6 months of age ☐ 9 months of age

UNSCHEDULED VISIT NUMBER: |__|__|

PHYSICAL EXAMINATION

Anthropometric measurements	Results	Units
Weight	|__|__|__|.|__|	kg
Height	|__|__|__|.|__|	cm
Mid-upper arm circumference	|__|__|.|__|	cm

OPTIMAL STUDY

STUDY ID	OPT_____

PHYSICAL EXAMINATION	
Body system examined	**Results**
General appearance (including jaundice, anaemia, clubbing, cyanosis, oedema, lymphadenopathy)	☐ Normal ☐ Abnormal
Head, eyes, ears, nose, throat	☐ Normal ☐ Abnormal
Respiratory	☐ Normal ☐ Abnormal
Cardiovascular	☐ Normal ☐ Abnormal
Gastrointestinal	☐ Normal ☐ Abnormal
Neurological	☐ Normal ☐ Abnormal
Joints	☐ Normal ☐ Abnormal
Skin	☐ Normal ☐ Abnormal

** **Provide details of abnormal findings on AE form. Include any hospital records**

CHECKLIST FOR UNSCHEDULED VISIT PROCEDURES

Procedure description	Yes	No	NA	Reference
Capillary haemoglobin				
Capillary Hb sample	☐	☐	☐	Capillary Hb form
Malaria incidence assessment				
Malaria slide	☐	☐	☐	Malaria incidence assessment form
Quantitative PCR sample on filter paper	☐	☐	☐	
Safety assessment				
Adverse event monitoring	☐	☐	☐	AE log, AE form, SAE form
Concomitant medication assessment				
Concomitant medication being used by infant and mother who is breastfeeding	☐	☐	☐	Concomitant medication form

Staff initials_____ Checked by _____ on _____