BMJ Open

Evaluation of the safety and efficacy of dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in HIV-infected pregnant women: protocol of a multi-centre, two-arm, randomized, placebo-controlled, superiority clinical trial (MAMAH project)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-053197
Article Type:	Protocol
Date Submitted by the Author:	10-May-2021
Complete List of Authors:	González, Raquel; Barcelona Institute for Global Health, ; CIBERESP, Nhampossa, Tacilta; Centro de Investigacao em Saude de Manhica Mombo-Ngoma, Ghyslain; CERMEL, Clinical Operations Mischlinger, Johannes; Bernhard Nocht Institute of Tropical Medicine Esen, Meral; University of Tübingen, Tchouatieu, André-Marie; Medecine for Malaria Venture Pons-Duran, Clara; Barcelona Institute for Global Health, Maternal, Child and Reproductive Health Initiative Dimessa, Lia ; Centre de Recherches Médicales de Lambaréné Lell, Bertrand; Medical Research Centre of Lambarene Lagler, Heimo; Medical University of Vienna Garcia-Otero, Laura; Barcelona Institute for Global Health Zoleko Manego, Rella; Centre de Recherches Médicales de Lambaréné El Gaaloul, Myriam; Medicines for Malaria Venture Sanz, Sergi; Barcelona Institute for Global Health, Biostatistics and Data Management Unit; CIBERESP, Piqueras, Mireia; Barcelona Institute for Global Health Sevene, Esperanca; Centro de Investigacao em Saude de Manhica; Universidade Eduardo Mondlane Ramharter, Michael; Bernhard-Nocht-Institut fur Tropenmedizin Saute, Francisco; Centro de Investigação em Saúde de Manhiça Menendez, Clara; Barcelona Institute for Global Health
Keywords:	HIV & AIDS < INFECTIOUS DISEASES, Maternal medicine < OBSTETRICS, Clinical trials < THERAPEUTICS, TROPICAL MEDICINE, Epidemiology < TROPICAL MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Title: Evaluation of the safety and efficacy of dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in HIV-infected pregnant women: protocol of a multi-centre, two-arm, randomized, placebo-controlled, superiority clinical trial (MAMAH project)

Names of protocol contributors: Raquel González [1, 2, 3], Tacilta Nhampossa[2,4], Ghyslain Mombo-Ngoma[5], Johannes Mischlinger[6,7], Meral Esen[8], André-Marie Tchouatieu[9], Clara Pons-Duran[1, 3], Lia Betty Dimessa[5], Bertrand Lell[5,10], Heimo Lagler[10], Laura Garcia-Otero[1,2], Rella Zoleko-Manego[5], Myriam El Gaaloul[9], Sergi Sanz[1,3,11], Mireia Piqueras[1], Esperança Sevene[2], Michael Ramharter[6,7], Francisco Saúte[2], Clara Menendez[1, 2, 3].

Corresponding author: Raquel González

Corresponding author email address: raquel.gonzalez@isglobal.org

Affiliations:

1. Barcelona Institute for Global Health (ISGlobal), Hospital Clínic- Universitat de Barcelona, Spain
2. Centro de Investigação em Saúde de Manhiça (CISM), Manhiça, Mozambique
3. Consorcio de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP)
4. Instituto Nacional de Saúde, Ministério de Saúde, Mozambique
5. Centre de Recherches Médicales de Lambaréné (CERMEL), Lambaréné, Gabon
6. Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine & I Dept. of Medicine University Medical Center Hamburg-Eppendorf, Hamburg, Germany
7. German Center for Infection Research, partner site Hamburg-Lübeck-Borstel-Riems
8. Institut für Tropenmedizin, Eberhard Karls University of Tübingen (EKUT), Tübingen, Germany
9. Medicines for Malaria Venture (MMV), Geneva, Switzerland

10. Department of Medicine I, Division of Infectious Diseases and Tropical Medicine, Medical University of Vienna, Vienna, Austria.
11. Department of Basic Clinical Practice, Faculty of Medicine, Universitat de Barcelona, Barcelona, Spain

Email addresses:

Raquel González	raquel.gonzalez@isglobal.org
Tacilta Nhampossa	tacilta.nhampossa@manhica.net
Ghyslain Mombo-Ngoma	ghyslain.mombongoma@gmail.com
Johannes Mischlinger	mischlinger@bnitm.de
Meral Esen	meral.esen@uni-tuebingen.de
André-MarieTchouatieu	tchouatieua@mmv.org
Clara Pons-Duran	clara.pons@isglobal.org
Lia Betty Dimessa Mbadinga	dimessalia@gmail.com
Bertrand Lell	Bertrand.lell@meduniwien.ac.at
Heimo Lagler	heimo.lagler@meduniwien.ac.at
Laura Garcia-Otero	laura.garcia@isglobal.org
Rella Zoleko-Manego	manegorella@yahoo.fr
Myriam El Gaaloul	elgaaloulm@mmv.org
Sergi Sanz	sergi.sanz@isglobal.org
Mireia Piqueras	mireia.piqueras@isglobal.org
Esperança Sevene	esperanca.sevene@manhica.net
Michael Ramharter	ramharter@bnitm.de

Francisco Saúte	francisco.saute@manhica.net
Clara Menéndez	clara.menendez@isglobal.org

Word count: 3286

Abstract

Introduction: Malaria infection during pregnancy is an important driver of maternal and neonatal health especially among HIV-infected women. Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine is recommended for malaria prevention in HIV-uninfected women but it is contraindicated in those HIV-infected on cotrimoxazole prophylaxis (CTXp) due to potential adverse effects. Consequently, the women most vulnerable to malaria are currently the least protected. Dihydroartemisinin-piperaquine (DHA-PPQ), because of its long half-life and good tolerability has been shown to improve antimalarial protection in HIV-uninfected pregnant women, constituting the most promising candidate for IPTp in HIV-infected pregnant women. The objective of the trial is to determine if monthly three-day IPTp courses of DHA-PPQ added to daily CTXp are safe and superior to CTXp alone in decreasing the proportion of peripheral malaria parasitaemia at the end of pregnancy in HIV-infected women.

Methods and analysis: This is a multi-centre, two-arm, placebo-controlled, individually randomized trial in HIV-infected women receiving CTXp and antiretroviral (ARV) treatment. A total of 664 women will be enrolled at the first antenatal care clinic visit in sites from Gabon and Mozambique where malaria and HIV infection are moderate to highly prevalent. Participants will receive an insecticide-treated net and they will be administered monthly IPTp with DHA-PPQ or placebo (1:1 ratio) as directly observed therapy from the second trimester of pregnancy. Participants will be followed until six weeks after the end of pregnancy and their infants until one year of age to also evaluate the impact of DHA-PPQ on mother to child transmission of HIV.

Ethics and dissemination: The project was reviewed and approved by the institutional and national ethics committees of Gabon (042/2018/PR/SG/CNER) and Mozambique (504/CBNS/18) and the Hospital Clinic of Barcelona (HCB/2018/0650,Spain). Project results will be presented to all stakeholders and published in open access journals.

Trial registration: ClinicalTrials.gov, NCT03671109. Registered on 14th September 2018.

Strengths and limitations of this study

- A major strength of this trial is its double blind placebo-controlled design which will allow to yield conclusive results about the efficacy of the study intervention.
- The inclusion of pregnant women from different sub-Saharan countries will provide a wide representation of different malaria endemicity areas and HIV subgroups.
- The study is also adequately powered to test the superiority hypothesis.
- On the other hand, the study sample size will not be sufficient to test the superiority hypothesis in secondary infant outcomes (only differences between arms will be analysed).

Keywords

Malaria, HIV, pregnancy, prevention, treatment.

1. Background

Malaria accounted for 229 million cases and 409 000 deaths in 2019 worldwide. The burden of disease concentrates in sub-Saharan Africa (SSA) where 94% of malaria cases and 95% of malaria deaths occurred in 2019[1]. For reasons not well understood, pregnant women are at increased risk of the infection and of severe clinical episodes [2-4]. Especially in SSA, malaria in pregnancy constitutes an important driver of maternal and infant health and a significant cause of maternal and infant mortality and morbidity [4-6]. An estimated 20 million HIV-infected individuals in SSA live in malaria endemic areas and over 12 million are women of reproductive age[7]. In addition, approximately one million pregnancies each year are complicated by co-infection with malaria and HIV in this region [8].

Both malaria and HIV infections are among the leading causes of mortality and morbidity in African pregnant women and their children. Thus, modest effects of one infection on the other could lead to a substantial negative impact on the health of pregnant women and their infants [9]. The interaction between the two infections is particularly deleterious in pregnancy leading to increased risk and severity of both malaria infection and disease, as well as to increased HIV viral load, which may raise the frequency of mother to child transmission of HIV (MTCT-HIV) [10].

Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) given monthly at each antenatal care (ANC) clinic visit from the second trimester, along with long lasting insecticide treated nets (LLITNs), are the cornerstone for malaria prevention recommended by the WHO in stable transmission areas in HIV-uninfected pregnant women [11]. In HIV-infected pregnant women living in areas with limited health resources and high HIV prevalence, universal cotrimoxazole prophylaxis (CTXp) is recommended to prevent opportunistic infections [12]. However, SP is contraindicated in women on CTXp due to potential adverse effects. Thus, even though IPTp-SP is a life-saving and highly cost-effective intervention

it cannot be administered to the most vulnerable group, HIV-infected women [13-16]. Consequently and paradoxically the most susceptible women to malaria are currently the least protected [17].

A placebo-controlled trial conducted in three sub-Saharan countries between 2010 and 2013 showed that an effective antimalarial added to CTXp and long-lasting ITNs (LLITNs) in HIV-infected pregnant women has a significant impact in improving malaria prevention and maternal health through reductions in hospital admissions [18]. However, the antimalarial used (mefloquine) was not well tolerated, and most importantly, it was associated with a two-fold increase in the frequency of mother to child transmission of HIV (MTCT-HIV), limiting its potential for IPTp. These findings indicate the need to find drug alternatives with better tolerability and safety profile to reduce malaria in this vulnerable group [18].

Dihydroartemisinin-piperaquine (DHA-PPQ) is an artemisinin-based combination therapy (ACT) recommended by the WHO for treatment of uncomplicated malaria in adults and children aged ≥ six months[19]. Among the available antimalarial drugs, DHA-PPQ constitutes one of the best candidates for IPTp because it is not used as first-line malaria treatment, it is well tolerated, and it is recommended for treatment of clinical malaria in the second and third trimesters of pregnancy [19].

DHA-PPQ has been safely used for case management in HIV-uninfected pregnant women in Thailand and in a multicentre trial in sub-Saharan Africa [20, 21]. Moreover, studies comparing IPTp-SP with IPTp-DHA-PPQ in HIV-uninfected pregnant women from Kenya and Uganda indicate that the drug could be an alternative to SP in terms of antimalarial efficacy and safety in pregnancy [22, 23]. A recent meta-analysis of the safety and efficacy of repeated doses of DHA-PPQ for prevention and treatment of malaria including 11 studies (among adults, children and pregnant women) has concluded that monthly DHA-PPQ is well tolerated and effective for IPT and that additional data are needed in pregnancy and to further explore the cardiac safety

with monthly dosing [24]. Scientific evidence shows that efficacy and safety findings from malaria control strategies evaluated in HIV-uninfected pregnant women cannot be directly extrapolated to HIV-infected women [18]. A trial comparing monthly IPTp with DHA-PPQ to CTXp among 200 HIV-infected Ugandan women did not find differences in the risk of histopathologically detected placental malarial infection and other outcomes between groups [25]. However, authors acknowledge the limitations of their results due to the low prevalence of malaria in the study area at the time of the trial [25]. Therefore, it is of highest public health priority to provide conclusive evidence as to whether the most vulnerable population (HIV-infected pregnant women) will benefit from the use of the currently most promising and available alternative drug for IPTp, DHA-PPQ [17].

The objectives of the trial are (1) to evaluate the safety, tolerability and efficacy of DHA-PPQ as IPTp for malaria prevention in HIV-infected pregnant women receiving daily CTXp and ARV drugs, (2) to assess the effect of DHA-PPQ as IPTp on mother to child transmission of HIV and (3) to evaluate the effectiveness of CTXp in clearing malaria parasites in HIV-infected pregnant women.

2. Methods

This is multi-centre, two-arm, placebo-controlled, individually randomized superiority clinical trial with two study arms including HIV-infected pregnant women. The study will be carried out and reported according to Consolidated Standards of Reporting Trials guidelines [26].

Study settings

The trial will be conducted in two countries from Central and South Eastern sub-Saharan Africa (Gabon and Mozambique), where HIV prevalence among pregnant women ranges from 6 to 29% [27, 28]. Malaria epidemiologic indicators and HIV prevalence in pregnancy in study sites are

shown in Table 1. The trial sites have been selected to provide representation of different malaria endemicity and HIV subgroups (HIV-1 subtype C in Mozambique and HIV-1 CRF02_AG in Gabon, where HIV-2 also circulates [29, 30]).

Study population

All pregnant women attending the study ANC services for the first time and/or who have not received IPTp during their current pregnancy will be screened for participation in the trial. Inclusion criteria are (1) permanent resident in the study area, (2) gestational age at the first antenatal visit ≤ 28 weeks, (3) HIV seropositive status and (4) agreement to deliver in the study site's maternity(ies) wards. Exclusion criteria are (1) residence outside the study area or planning to move out in the following 10 months from enrolment, (2) gestational age at the first antenatal visit > 28 weeks of pregnancy, (3) known history of allergy to CTX, (4) known history of allergy or contraindications to DHA-PPQ and (5) participating in other intervention studies.

Informed consent

All participants will receive information about study procedures, including knowledge about malaria and HIV infection in pregnancy. A signed informed consent (or thumb-printed with a witness whenever the woman is illiterate) will be obtained before any study tests or evaluations are carried out. If the participant is under the legal age of maturity, she will sign the assent form and her legal guardian will sign the informed consent according to national ethics local policies. The informed consent will cover the woman and the new born infant.

Recruitment and randomization

After the study details are explained and informed consent is signed, pregnant women will be given a study number and automatically randomized to one of the study arms: 1) Daily CTX + monthly IPTp-DHA-PPQ or 2) Daily CTX + Monthly IPTp-placebo. Each participant will be uniquely

identified in the study by a combination of her site code and participant number. Allocation of participants to study arms will be done centrally by the trial's Sponsor (the Barcelona Institute for Global Health, ISGlobal) by block randomization and stratified by country. This method will ensure balanced allocation to both arms during different malaria seasons in the two study countries. Each subject number will be related to a treatment number which assigns them to one of the IPT arms. Study number allocation for each study participant will be concealed in opaque sealed envelopes that will be opened only after recruitment. At each site the first participant will be assigned a patient number, and consecutive numbers will be assigned to subsequent women. A study identification card containing the individual study number and basic demographic information will be given to the participant in order to facilitate identification at all study contacts.

Figure 1 displays the study design.

Blinding

Study tablets (IPTp-DHA-PPQ and IPTp-Placebo) will be identically packaged. Study personnel will prepare and deliver the medication to the participant. All study personnel, investigators, outcome assessors, data analysts and the participants will remain blinded throughout the trial.

Interventions

a) IPTp administration

Administration of the three-day IPTp course will always be done under fasting conditions and direct observation by study personnel. The number of daily IPTp tablets to be administered will be based on bodyweight and according to the treatment guidelines set by WHO [target dose (range) of 4 (2–10) mg/kg/ day of DHA and 18 (16–27) mg/kg/day of PPQ given once a day for three days for adults]. Following physical examination, recruited women of gestational age≥13

weeks will receive the assigned IPTp drug. In case of gestational age< 13 weeks, first IPTp administration will be scheduled one month later. If participants report malaria treatment in the preceding four weeks, first IPTp administration will also be delayed one month. Administration of the second and third day treatment course will be done by study personnel either at the study health facility or household level. Women will be observed for 60 minutes after administration of the IPTp dose. Those women vomiting within the first 30 minutes of IPTp administration will be given a second full IPTp dose; women vomiting after 30-60 minutes of IPTp administration will be given an additional half dose of the drug. Subsequent doses of IPTp will be given coinciding with the next scheduled monthly ANC clinic visit, at least one month apart from the previous dose.

b) CTX administration

Cotrimoxazole (fixed combination drug containing 800 mg of trimethoprim and 160 mg of sulfamethoxazole) will be taken daily from enrolment until delivery as per national guidelines for prevention of opportunistic infections. Women will be asked to visit the ANC clinic monthly to receive the amount of CTX tablets for the whole month. At each ANC clinic visit, adherence to CTX prophylaxis will be assessed.

c) ARV therapy and concomitant medications

Treatment for prevention of HIV MTCT (Option B+) will be given by the study health personnel, according to national HIV/AIDS control guidelines[31]. Any other concomitant treatment received by the study participants will be recorded in the study questionnaires.

d) Long lasting insecticide treated nets

Regardless of gestational age at the time of recruitment, all women will receive a LLITN and details about its use will be explained.

Study Outcomes

The primary outcome of the trial will be the prevalence of maternal parasitaemia at delivery defined by the presence of *Plasmodium falciparum (P. falciparum)* asexual parasites of any density in peripheral blood (determined by microscopy). The secondary maternal and infant endpoints can be found in Table 2.

Sample size

Based on previous estimations at the study sites and assuming a prevalence of peripheral parasitaemia at delivery of 7.5% with CTXp, it is estimated that 298 women per arm will be required to detect with 80% power a significant ($p<0.05$) decrease of 5% or more in the prevalence of peripheral parasitaemia in the CTXp+IPTp-DHA-PPQ group [18]. In order to allow for 10% losses to follow up, it is calculated that 332 women/study arm will need to be recruited (total n=664). Furthermore, assuming a 5% MTCT-HIV in the control group, this sample size will have an 80% power to detect at the 5% level of significance, 2.2 times difference in the risk of MTCT-HIV. Considering the prevalence of HIV infection among pregnant women in both sites (please refer to Table 1), a total of 444 women are planned to be recruited in Mozambique and 220 women in Gabon to allow for a recruitment rate of 33 women/month on average. These estimations are based on recruitment rates of previous IPTp clinical trials conducted among pregnant women in the two study sites [18, 32, 33].

Follow up and measurements of outcomes

At baseline, the woman's demographic and obstetric information will be recorded in study specific case report forms (CRF).

a) Physical and clinical examination at enrolment

The physical examination of the woman will include the following assessments: weight, height, gestational age by bimanual palpation and measurement of middle-upper arm circumference (MUAC). Ultrasound (US) will be performed to determine gestational age at enrolment and confirm pregnancy viability.

b) Baseline biological samples

At enrolment, a venous blood sample will be collected for analysis of haemoglobin level, CD4 cell counts, HIV viral load and malaria PCR.

c) Follow-up and household visits

Women will be given an appointment to attend the subsequent ANC clinic visit one month after the first one. The subsequent IPTp doses will be given at least four weeks apart from the previous one. Study participants will be asked to visit the study facilities in case of any illness. Women will be visited at home the day after recruitment to confirm residence status, assess drug tolerability and the correct use of the net. Adherence to CTX prophylaxis, ARV therapy and compliance with the LLITNs use will be assessed monthly at the ANC attendance.

d) Adverse events monitoring and reporting

Active safety monitoring will consist in household visits to study participants two days after each IPTp administration to assess drug tolerability and record all Adverse Events (AEs). In addition, a health facility-based passive surveillance system will be established to capture unscheduled visits of participants during follow-up. Information on unsolicited adverse events will be collected at each scheduled and unscheduled visit. Any participant passively reporting being sick during the study visits, will be referred to the clinical services as per routine system in place. A blood smear will be collected in those presenting with malaria related signs/symptoms (fever (≥37,5° C) or having history of fever in the past 24 hours, arthromyalgias or headache). In case

of malaria parasitaemia or anaemia, they will be treated following national guidelines. Serious Adverse Events (SAEs) will be reported by the site investigator within 24 hours of being made aware to the trial's safety monitor and the Data Safety Monitoring Board (DSMB).

e) End of pregnancy and infant assessments

At the end of pregnancy, maternal blood, placental and cord blood samples will be collected for haematological and parasitological examination. The new-born will be examined, weighed and measured and his/her gestational age assessed by the modified Ballard method [34].

f) Post-partum visit

Participants will be visited approximately after six weeks of end of pregnancy at the study health facility where a blood smear for malaria screening will be collected. A summary of study procedures is displayed in Table 3.

g) Infants follow up

Infants born to study participants will be followed up until one year of age. Mothers will be asked to bring their child to the study facilities at weeks 4-6 of age and at 6, 9 and 12 months after birth. At each scheduled visit, study infants will be physically examined and their psychomotor and neurological development will be assessed following a standard protocol for African settings [35-37]. Weight, height and axillary temperature will be measured and recorded. A capillary blood sample will be taken from infants with fever (axillary temperature ≥37.5°C) or history of fever in the last 24 hours, or appearing pale, for malaria parasitaemia examination and haematological determination. In case of malaria parasitaemia or anaemia, they will be treated following national guidelines. At each scheduled visit, HIV DNA will be evaluated in the infant from dried blood spot on filter paper (for PCR). As per routine healthcare national programs HIV-exposed infants will receive CTX prophylaxis starting four to six weeks after birth. Infants will

also receive ARV, according to national guidelines. Infant's follow up visits and study procedures are described in Table 4.

Data management

Data are collected using case report forms (CRFs) developed for the study. Data is double entered into the study database using the OpenClinica open source software (version 3.14) for clinical data management (www.OpenClinica.com) at each research site. Automatic quality checks are performed to ensure CRF completeness. Concomitant medications registered into the database are coded using the WHO Drug Reference List, which employs the Anatomical Therapeutic Chemical classification system. Medical history/current medical conditions and adverse events are coded using the Medical dictionary for regulatory activities (MedDRA) terminology.

Analysis plan

The following populations of analysis have been defined: a) Intention to treat (ITT): it includes all randomized pregnant women (target population for the efficacy analysis); b) According to protocol (ATP): it includes participants who fulfil all the inclusion-exclusion criteria, took monthly IPTp-DHA-PPQ/placebo study doses, received a LLITN, received CTXp and ARV drugs and from whom data is available for the analysis; c) Safety: it includes participants who received at least one dose of IPTp DHA-PPQ/placebo and had at least one post- baseline safety assessment. The primary analysis of the trial will be the comparison of the proportion of pregnant women with peripheral parasitaemia at delivery in the ITT and ATP cohorts, adjusted by gravidity, country, seasonality and other variables associated with the prevalence of malaria. Proportions will be compared between groups using the Fisher exact test and presented as relative risk ratio (RR) or reduction of the RR (1- RR *100%) if RR lower than 1. Adjustment for co-variates and possible confounders will be done using Poisson regression with a log link and robust estimate of the covariance (Huber method), using the method proposed by Zou [38, 39]. Continuous variables

will be compared between groups using Wilcoxon rank sum test and the effect presented as Mean Difference. Adjustment for co-variates and possible cofounders will be done using ordinary least square regression. Incidence of clinical malaria, overall admissions and outpatient attendances will be estimated as the number of episodes over the time at risk. Time at risk will be estimated as the time from the start of follow up until the end of follow-up (visit one month after end of pregnancy for mothers) or withdrawal due to censoring or death, whatever occurs first. The total number of events will be compared between groups using Negative Binomial regression models which takes into account a possible extra Poisson variation due to different frailty of the subjects. The comparison will be expressed as relative rate ratio (RRate).

Patient and public involvement

Patients will not be directly involved in the design, conduct, reporting, or dissemination plans of the study. However, a Community Advisory Board (CAB) has been set at each study site to strengthen communication and interaction between the local study community and research teams. The CAB also oversees and guides the study team on key issues such as potential risks and burdens for participants or host communities that may be hidden from researchers, and how to minimize them [40].

Ethics and dissemination

The study is conducted in accordance with the EMA/ICH Guideline on Good Clinical Practice and in total agreement with the applicable international, EU and national law of all the participating countries[41]. The study protocol and the informed consent forms have been reviewed and approved by the institutional and national ethics committees of Gabon (042/2018/PR/SG/CNER) and Mozambique (504/CBNS/18) and the Hospital Clinic of Barcelona (HCB/2018/0650,Spain). An independent DSMB monitors regularly the safety of

study participants. The trial is registered on clinicaltrials.gov (https://clinicaltrials.gov/ct2/show/NCT03671109).

A project communication has been developed in order to ensure timely, accurate, and effective dissemination of project results. After concluding the trial's data analysis, findings will be made available to all partners, key stakeholders and Ministries of Health. The project members will actively disseminate information to the scientific community through reports, presentations at scientific forums and publications in international open access journals. Trial results will also be shared with the WHO Global Malaria Program and Malaria Policy Advisory Groups.

Authors' contributions

RG and CM conceived the concept of the study. RG and CPD wrote the first draft of the manuscript. RG and SS wrote sections on sample size, data management and analysis plan. All authors participated in the design of the study, reviewed and approved the manuscript.

Funding statement

This project is part of the EDCTP2 programme supported by the European Union (grant number RIA2016MC-1613-MAMAH) and the Medicines for Malaria Venture partnership (funding agreement PO19/00554). The trial is sponsored by the Barcelona Institute for Global Health (ISGlobal). ISGlobal is supported by the Spanish Ministry of Science and Innovation through the "Centro de Excelencia Severo Ochoa 2019-2023" Program [CEX2018-000806-S] and it is a member of the CERCA Programme, Generalitat de Catalunya. CISM is supported by the Government of Mozambique and the Agency for International Development (AECID).

Competing interests

None declared.

References

1. WHO. World Malaria report 2020. WHO. 2020;Available at https://www.who.int/publications/i/item/9789240015791 [Accessed March 2021].
2. Mombo-Ngoma G, Mackanga JR, Gonzalez R, Ouedraogo S, Kakolwa MA, Manego RZ, et al. Young adolescent girls are at high risk for adverse pregnancy outcomes in sub-Saharan Africa: an observational multicountry study. BMJ open. 2016;6(6):e011783. Epub 2016/07/01. doi: 10.1136/bmjopen-2016-011783. PubMed PMID: 27357200.
3. Group WAbCTAABS. Clinical determinants of early parasitological response to ACTs in African patients with uncomplicated falciparum malaria: a literature review and meta-analysis of individual patient data. BMC Med. 2015;13:212. Epub 2015/09/08. doi: 10.1186/s12916-015-0445-x. PubMed PMID: 26343145; PubMed Central PMCID: PMCPMC4561425.
4. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoa K, Brabin B, et al. Epidemiology and burden of malaria in pregnancy. Lancet Infect Dis. 2007;7(2):93-104. Epub 2007/01/26. doi: S1473-3099(07)70021-X [pii]

10.1016/S1473-3099(07)70021-X [doi]. PubMed PMID: 17251080.
5. Menendez C, D'Alessandro U, ter Kuile FO. Reducing the burden of malaria in pregnancy by preventive strategies. Lancet Infect Dis. 2007;7(2):126-35. Epub 2007/01/26. doi: S1473-3099(07)70024-5 [pii]

10.1016/S1473-3099(07)70024-5 [doi]. PubMed PMID: 17251083.
6. Menendez C, Ordi J, Ismail MR, Ventura PJ, Aponte JJ, Kahigwa E, et al. The impact of placental malaria on gestational age and birth weight. J Infect Dis. 2000;181(5):1740-5. Epub 2000/05/24. doi: 10.1086/315449. PubMed PMID: 10823776.
7. UNAIDS/WHO. Global report: UNAIDS report on the global AIDS Epidemic 2013. WHO Library Cataloguing-in-Publication Data 2013;UNAIDS(JC2502/1/E).
8. WHO. Malaria and HIV interactions and their implications for public health policy WHO. 2005;ISBN 92 4 1593350.
9. ter Kuile FO, Parise ME, Verhoeff FH, Udhayakumar V, Newman RD, van Eijk AM, et al. The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant women in sub-saharan Africa. Am J Trop Med Hyg. 2004;71(2 Suppl):41-54. PubMed PMID: 15331818.
10. Gonzalez R, Ataide R, Naniche D, Menendez C, Mayor A. HIV and malaria interactions: where do we stand? Expert Rev Anti Infect Ther. 2012;10(2):153-65. doi: 10.1586/eri.11.167. PubMed PMID: 22339190.
11. WHO. Intermittent Preventive Treatment of malaria in pregnancy using Sulfadoxine-Pyrimethamine (IPTp-SP). Updated WHO Policy Recommendation. WHO. 2012;http://www.who.int/malaria/iptp_sp_updated_policy_recommendation_en_102012.pdf .
12. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection Recommendations for a public health approach June 2013. 2013.
13. Sevene E, Gonzalez R, Menendez C. Current knowledge and challenges of antimalarial drugs for treatment and prevention in pregnancy. Expert Opin Pharmacother. 2010;11(8):1277-93. PubMed PMID: 20408744.
14. Ward SA, Sevene EJ, Hastings IM, Nosten F, McGready R. Antimalarial drugs and pregnancy: safety, pharmacokinetics, and pharmacovigilance. Lancet Infect Dis. 2007;7(2):136-44. Epub 2007/01/26. doi: S1473-3099(07)70025-7 [pii]

10.1016/S1473-3099(07)70025-7 [doi]. PubMed PMID: 17251084.

15. Menendez C, Bardaji A, Sigauque B, Sanz S, Aponte JJ, Mabunda S, et al. Malaria prevention with IPTp during pregnancy reduces neonatal mortality. PloS one. 2010;5(2):e9438. PubMed PMID: 20195472.
16. Eisele TP, Larsen DA, Anglewicz PA, Keating J, Yukich J, Bennett A, et al. Malaria prevention in pregnancy, birthweight, and neonatal mortality: a meta-analysis of 32 national cross-sectional datasets in Africa. Lancet Infect Dis. 2012;12(12):942-9. Epub 2012/09/22. doi: 10.1016/s1473-3099(12)70222-0. PubMed PMID: 22995852.
17. Gonzalez R, Sevene E, Jagoe G, Slutsker L, Menendez C. A Public Health Paradox: The Women Most Vulnerable to Malaria Are the Least Protected. PLoS Med. 2016;13(5):e1002014. Epub 2016/05/04. doi: 10.1371/journal.pmed.1002014. PubMed PMID: 27139032.
18. Gonzalez R, Desai M, Macete E, Ouma P, Kakolwa MA, Abdulla S, et al. Intermittent Preventive Treatment of Malaria in Pregnancy with Mefloquine in HIV-Infected Women Receiving Cotrimoxazole Prophylaxis: A Multicenter Randomized Placebo-Controlled Trial. PLoS Med. 2014;11(9):e1001735. Epub 2014/09/24. doi: 10.1371/journal.pmed.1001735. PubMed PMID: 25247995.
19. WHO. Guidelines for the treatment of malaria. Second edition. WHO. 2010;ISBN 9789241547925.
20. Rijken MJ, McGready R, Boel ME, Barends M, Proux S, Pimanpanarak M, et al. Dihydroartemisinin-piperaquine rescue treatment of multidrug-resistant Plasmodium falciparum malaria in pregnancy: a preliminary report. Am J Trop Med Hyg. 2008;78(4):543-5. Epub 2008/04/04. PubMed PMID: 18385345.
21. Pekyi D, Ampromfi AA, Tinto H, Traore-Coulibaly M, Tahita MC, Valea I, et al. Four Artemisinin-Based Treatments in African Pregnant Women with Malaria. N Engl J Med. 2016;374(10):913-27. Epub 2016/03/11. doi: 10.1056/NEJMoa1508606. PubMed PMID: 26962727.
22. Desai M, Gutman J, L'Lanziva A, Otieno K, Juma E, Kariuki S, et al. Intermittent screening and treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine versus intermittent preventive treatment with sulfadoxine-pyrimethamine for the control of malaria during pregnancy in western Kenya: an open-label, three-group, randomised controlled superiority trial. Lancet. 2015. Epub 2015/10/03. doi: 10.1016/s0140-6736(15)00310-4. PubMed PMID: 26429700.
23. Kakuru A, Jagannathan P, Muhindo MK, Natureeba P, Awori P, Nakalembe M, et al. Dihydroartemisinin-Piperaquine for the Prevention of Malaria in Pregnancy. N Engl J Med. 2016;374(10):928-39. Epub 2016/03/11. doi: 10.1056/NEJMoa1509150. PubMed PMID: 26962728; PubMed Central PMCID: PMCPMC4847718.
24. Gutman J, Kovacs S, Dorsey G, Stergachis A, Ter Kuile FO. Safety, tolerability, and efficacy of repeated doses of dihydroartemisinin-piperaquine for prevention and treatment of malaria: a systematic review and meta-analysis. Lancet Infect Dis. 2017;17(2):184-93. Epub 2016/11/21. doi: 10.1016/s1473-3099(16)30378-4. PubMed PMID: 27865890; PubMed Central PMCID: PMCPMC5266794.
25. Natureeba P, Kakuru A, Muhindo M, Ochieng T, Ategeka J, Koss CA, et al. Intermittent Preventive Treatment With Dihydroartemisinin-Piperaquine for the Prevention of Malaria Among HIV-Infected Pregnant Women. J Infect Dis. 2017;216(1):29-35. Epub 2017/03/23. doi: 10.1093/infdis/jix110. PubMed PMID: 28329368; PubMed Central PMCID: PMCPMC5853208.
26. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. Annals of internal medicine. 2010;152(11):726-32. Epub 2010/03/26. doi: 10.7326/0003-4819-152-11-201006010-00232. PubMed PMID: 20335313.
27. Gonzalez R, Munguambe K, Aponte J, Bavo C, Nhalungo D, Macete E, et al. High HIV prevalence in a southern semi-rural area of Mozambique: a community-based survey. HIV Med. 2012. PubMed PMID: 22500780.

28. Manego RZ, Mombo-Ngoma G, Witte M, Held J, Gmeiner M, Gebru T, et al. Demography, maternal health and the epidemiology of malaria and other major infectious diseases in the rural department Tsamba-Magotsi, Ngounie Province, in central African Gabon. BMC Public Health. 2017;17(1):130. Epub 2017/01/29. doi: 10.1186/s12889-017-4045-x. PubMed PMID: 28129759; PubMed Central PMCID: PMCPMC5273856.

29. Caron M, Lekana-Douki SE, Makuwa M, Obiang-Ndong GP, Biba O, Nkoghe D, et al. Prevalence, genetic diversity and antiretroviral drugs resistance-associated mutations among untreated HIV-1-infected pregnant women in Gabon, central Africa. BMC infectious diseases. 2012;12:64. Epub 2012/03/22. doi: 10.1186/1471-2334-12-64. PubMed PMID: 22433277; PubMed Central PMCID: PMCPMC3359209.

30. Lahuerta M, Aparicio E, Bardaji A, Marco S, Sacarlal J, Mandomando I, et al. Rapid spread and genetic diversification of HIV type 1 subtype C in a rural area of southern Mozambique. AIDS Res Hum Retroviruses. 2008;24(2):327-35. PubMed PMID: 18271719.

31. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016. Reccomendations for a public health approach. Second edition.: World Health Organization; 2016.

32. Gonzalez R, Mombo-Ngoma G, Ouedraogo S, Kakolwa MA, Abdulla S, Accrombessi M, et al. Intermittent Preventive Treatment of Malaria in Pregnancy with Mefloquine in HIV-Negative Women: A Multicentre Randomized Controlled Trial. PLoS Med. 2014;11(9):e1001733. Epub 2014/09/24. doi: 10.1371/journal.pmed.1001733. PubMed PMID: 25247709.

33. Menendez C, Bardaji A, Sigauque B, Romagosa C, Sanz S, Serra-Casas E, et al. A randomized placebo-controlled trial of intermittent preventive treatment in pregnant women in the context of insecticide treated nets delivered through the antenatal clinic. PloS one. 2008;3(4):e1934. Epub 2008/04/10. doi: 10.1371/journal.pone.0001934 [doi]. PubMed PMID: 18398460; PubMed Central PMCID: PMC2277457.

34. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R. New Ballard Score, expanded to include extremely premature infants. The Journal of pediatrics. 1991;119(3):417-23. Epub 1991/09/01. PubMed PMID: 1880657.

35. Gladstone M, Lancaster GA, Umar E, Nyirenda M, Kayira E, van den Broek NR, et al. The Malawi Developmental Assessment Tool (MDAT): the creation, validation, and reliability of a tool to assess child development in rural African settings. PLoS Med. 2010;7(5):e1000273. Epub 2010/06/04. doi: 10.1371/journal.pmed.1000273. PubMed PMID: 20520849; PubMed Central PMCID: PMCPMC2876049.

36. Koura KG, Boivin MJ, Davidson LL, Ouedraogo S, Zoumenou R, Alao MJ, et al. Usefulness of child development assessments for low-resource settings in francophone Africa. Journal of developmental and behavioral pediatrics : JDBP. 2013;34(7):486-93. Epub 2013/08/01. doi: 10.1097/DBP.0b013e31829d211c. PubMed PMID: 23899660; PubMed Central PMCID: PMCPMC3821168.

37. Prado EL, Abubakar AA, Abbeddou S, Jimenez EY, Some JW, Ouedraogo JB. Extending the Developmental Milestones Checklist for use in a different context in Sub-Saharan Africa. Acta paediatrica (Oslo, Norway : 1992). 2014;103(4):447-54. Epub 2013/12/21. doi: 10.1111/apa.12540. PubMed PMID: 24354938.

38. Zou G. A modified poisson regression approach to prospective studies with binary data. American journal of epidemiology. 2004;159(7):702-6. Epub 2004/03/23. PubMed PMID: 15033648.

39. Zou GY, Donner A. Extension of the modified Poisson regression model to prospective studies with correlated binary data. Statistical methods in medical research. 2013;22(6):661-70. Epub 2011/11/11. doi: 10.1177/0962280211427759. PubMed PMID: 22072596.

40. NHREC. Guidelines for Community Advisory Groups. NHREC. 2012;Available at : http://research.ukzn.ac.za/Files/National%20Guidelines%20for%20Community%20Advisory%20Groups%20for%20Research%20(2012).pdf [Accessed April 2021].

41. EMA. Guideline for Good Clinical Practice E6 (R2). EMA. 2015;Available at https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-good-clinical-practice-e6r2-4-step-2b_en.pdf [Accessed April 2021].
42. UNAIDS. Mozambique country Fact sheet. 2015:Available at http://www.unaids.org/sites/default/files/media/documents/UNAIDS_GlobalplanCountryfactsheet_mozambique_en.pdf [accessed February 2017].
43. Ministère-de-la-Santé-et-de-la-Prévoyance-sociale-Gabon. Rapport National sur la Réponse au VIH/SIDA 2014. 2015:Available at http://www.unaids.org/sites/default/files/country/documents/GAB_narrative_report_2015.pdf [accessed February 7].

Figure Legends:

Figure 1. MAMAH trial design

519 **Tables**

520 **Table 1. Malaria and HIV epidemiology in the study countries**

Site/ Country	Malaria Transmission	High season	EIR*	*P. falciparum* infection prevalence in women at delivery†	HIV prevalence in pregnant women	Frequency of MTCT of HIV
Manhiça/ Mozambique	Hypoendemic	Sep-Mar	21-50	6%	29%	**6%**[42]
Lambaréné/ Gabon	Mesoendemic	Oct-May	21-50	11%	6%	**12%**[43]
Libreville/ Gabon	Mesoendemic	Oct-May	21-50	NI	**6%**	**12%**[43]

521 *EIR: Entomological Inoculation Rate

522 †Data from 2010-2012 in women receiving either two IPTp doses of mefloquine or SP

523 (Tuikue-Ndam et al, unpublished). NI: No information

524

Table 2. Study outcomes

Primary endpoint
Prevalence of maternal parasitaemia at delivery (defined by the presence of *P. falciparum* asexual parasites of any density in peripheral blood determined by microscopy)
Secondary endpoints
Maternal
Incidence of clinical malaria during pregnancy
Incidence of all-cause admissions
Incidence of all-cause outpatient attendances
Frequency and severity of adverse events (including cardiotoxic signals)
Mean haemoglobin concentration at delivery
Prevalence of submicroscopic *P. falciparum* peripheral parasitaemia at delivery
Prevalence of anaemia at delivery (Hb < 11 g/dL)
Prevalence of severe anaemia at delivery (Hb < 7 g/dL)
Mean CD4 + T cell counts levels at delivery
Proportion of women with detectable HIV viral load at delivery
Prevalence of placental *P. falciparum* infection (defined by the presence of parasites and/or pigment in the histological examination, or microscopic or sub-microscopic in the impression smear from placental blood)
Prevalence of *P. falciparum* peripheral parasitaemia at the post-partum visit
Maternal mortality rate
Infant
Prevalence of *P. falciparum* parasitaemia in cord blood (microscopic and sub-microscopic)
Prevalence of neonatal anaemia (Hb < 12,5 g/dL in cord blood, and Hb < 13g/dL in neonatal blood)
Mean birth weight
Prevalence of low birth weight (<2500 g)
Mean gestational age at birth
Prevalence of prematurity
Prevalence of embryo and foetal losses (miscarriages and stillbirths)
Prevalence of small for gestational age
Frequency of congenital malformations
Incidence of clinical malaria
Neonatal mortality rate
Frequency of mother to child transmission of HIV at one and at 12 months of age.
Infant mortality rate

Table 3. Schedule of enrolment, interventions and maternal assessments

	STUDY PERIOD							
	Pre-enrolment	**Allocation**	**First ANC clinic visit**	**Household visits**	**Monthly ANC visits**	**End of pregnancy**	**One month after end of pregnancy**	**Unscheduled visits**
TIMEPOINT	**0**	**0**	**1**	**+2 days**	**+1 month and then monthly**			
SCREENING AND ENROLMENT:								
Eligibility screen	X							
Informed consent	X							
Randomization		X						
INTERVENTIONS:								
IPTp administration			X	X	X			
CTX administration			X	*	*	*	*	*
ARV administration			X	*	*	*	*	*
LLITN distribution			X					
MATERNAL ASSESSMENTS:								
Demographics, medical history			X					X
Socio-economic characteristics				#		X		
Record of concomitant medication			X	X	X	X	X	X
Record of adverse			X	X	X	X	X	X

events						
Physical/clinical examination	X			X		X
Gestational age by ultrasound	X		X	X		X
Temperature					X	X
Blood pressure	X			X	X	X
Weight	X		X		X	X
Height	X					
MUAC	X				X	
RPR test	X					
CD4 count and HIV viral load	X			X		
Blood smear (malaria)	†		†	X	X	†
Haemoglobin test	X			X	X	
Intrapartum samples (cord blood, placenta)				X		
Drug tolerability assessment	X	X	X			
Compliance with LLITNs check		X	X	X	X	X

Only in the first household visit after the ANC visit of first IPTp administration.

* CTX and ARV adherence should be assessed at each scheduled visit.

† Only in women passively reporting sick AND presenting with malaria related signs/symptoms (fever (≥37,5° C) or having history of fever in the past 24 hours, arthromyalgia or headache), as per national management guidelines

Table 4. Schedule of infant visits and procedures

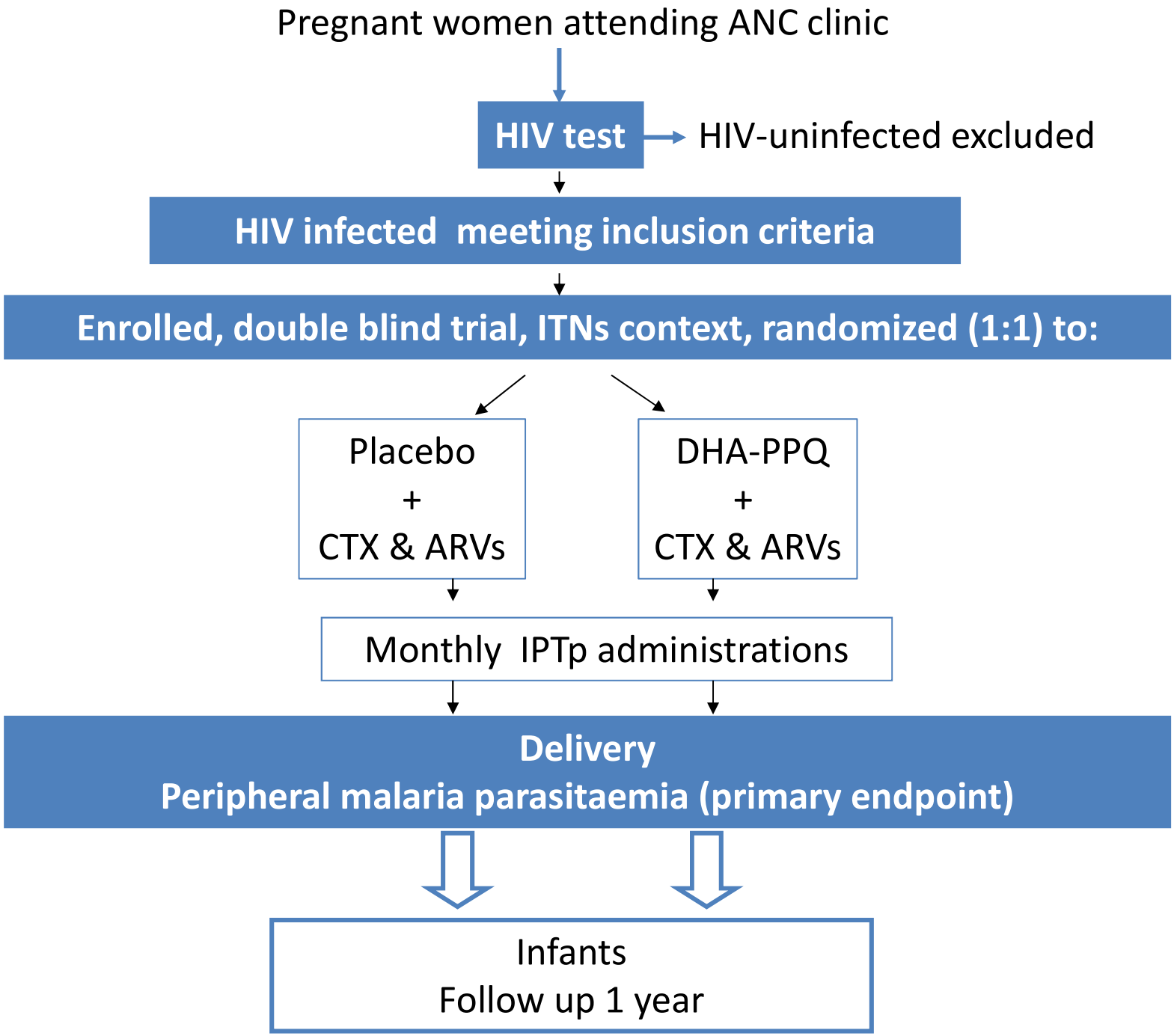
	TIMEPOINTS					
	Birth	**1 month***	**6 months**	**9 months**	**12 months**	**Unscheduled visits**
PROCEDURES:						
Medical history	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X
Psychomotor development assessment	X	X	X	X	X	
Weight	X	X	X	X	X	X
Height	X	X	X	X	X	X
Temperature	X	X	X	X	X	X
Blood smear	X	†	†	†	†	†
Haemoglobin test	X	†	†	†	†	†
HIV PCR ±		X	X	X	X	
Malaria PCR (filter paper)	X					
HIV prophylaxis adherence	#	#	#	#	#	#
HIV treatment adherence	#	#	#	#	#	#

* First visit will be scheduled 1 month after birth or coinciding with first EPI visit

† Only if fever (≥37,5° C) or history of fever in the past 24 hours or signs suggestive of malaria.

± HIV PCR test should also be repeated at month 18 after birth.

Adherence should be assessed at each visit.



CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	4
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	6
	2b	Specific objectives or hypotheses	8
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	8
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	9
	4b	Settings and locations where the data were collected	8-9
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	10-11
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	12, Table 2
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	12
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	10
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	10
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	10
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	10
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	10

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	15
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	16
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	5
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	5
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
Other information			
Registration	23	Registration number and name of trial registry	4
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	22

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ Open

Evaluation of the safety and efficacy of dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in HIV-infected pregnant women: protocol of a multi-centre, two-arm, randomized, placebo-controlled, superiority clinical trial (MAMAH project)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-053197.R1
Article Type:	Protocol
Date Submitted by the Author:	04-Aug-2021
Complete List of Authors:	González, Raquel; Barcelona Institute for Global Health, ; CIBERESP, Nhampossa, Tacilta; Centro de Investigacao em Saude de Manhica Mombo-Ngoma, Ghyslain; CERMEL, Clinical Operations Mischlinger, Johannes; Bernhard Nocht Institute of Tropical Medicine Esen, Meral; University of Tübingen, Tchouatieu, André-Marie; Medecine for Malaria Venture Pons-Duran, Clara; Barcelona Institute for Global Health, Maternal, Child and Reproductive Health Initiative Dimessa, Lia ; Centre de Recherches Médicales de Lambaréné Lell, Bertrand; Medical Research Centre of Lambarene Lagler, Heimo; Medical University of Vienna Garcia-Otero, Laura; Barcelona Institute for Global Health Zoleko Manego, Rella; Centre de Recherches Médicales de Lambaréné El Gaaloul, Myriam; Medicines for Malaria Venture Sanz, Sergi; Barcelona Institute for Global Health, Biostatistics and Data Management Unit; CIBERESP, Piqueras, Mireia; Barcelona Institute for Global Health Sevene, Esperanca; Centro de Investigacao em Saude de Manhica; Universidade Eduardo Mondlane Ramharter, Michael; Bernhard-Nocht-Institut fur Tropenmedizin Saute, Francisco; Centro de Investigação em Saúde de Manhiça Menendez, Clara; Barcelona Institute for Global Health
Primary Subject Heading:	Global health
Secondary Subject Heading:	Epidemiology, Infectious diseases, HIV/AIDS, Obstetrics and gynaecology, Public health
Keywords:	HIV & AIDS < INFECTIOUS DISEASES, Maternal medicine < OBSTETRICS, Clinical trials < THERAPEUTICS, TROPICAL MEDICINE, Epidemiology < TROPICAL MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Title: Evaluation of the safety and efficacy of dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in HIV-infected pregnant women: protocol of a multi-centre, two-arm, randomized, placebo-controlled, superiority clinical trial (MAMAH project)

Names of protocol contributors: Raquel González [1, 2, 3], Tacilta Nhampossa[2,4], Ghyslain Mombo-Ngoma[5], Johannes Mischlinger[6,7], Meral Esen[8], André-Marie Tchouatieu[9], Clara Pons-Duran[1, 3], Lia Betty Dimessa[5], Bertrand Lell[5,10], Heimo Lagler[10], Laura Garcia-Otero[1,2], Rella Zoleko-Manego[5], Myriam El Gaaloul[9], Sergi Sanz[1,3,11], Mireia Piqueras[1], Esperança Sevene[2], Michael Ramharter[6,7], Francisco Saúte[2], Clara Menendez[1, 2, 3].

Corresponding author: Raquel González

Corresponding author email address: raquel.gonzalez@isglobal.org

Affiliations:

1. Barcelona Institute for Global Health (ISGlobal), Hospital Clínic- Universitat de Barcelona, Spain
2. Centro de Investigação em Saúde de Manhiça (CISM), Manhiça, Mozambique
3. Consorcio de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP)
4. Instituto Nacional de Saúde, Ministério de Saúde, Mozambique
5. Centre de Recherches Médicales de Lambaréné (CERMEL), Lambaréné, Gabon
6. Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine & I Dept. of Medicine University Medical Center Hamburg-Eppendorf, Hamburg, Germany
7. German Center for Infection Research, partner site Hamburg-Lübeck-Borstel-Riems
8. Institut für Tropenmedizin, Eberhard Karls University of Tübingen (EKUT), Tübingen, Germany
9. Medicines for Malaria Venture (MMV), Geneva, Switzerland

10. Department of Medicine I, Division of Infectious Diseases and Tropical Medicine, Medical University of Vienna, Vienna, Austria.
11. Department of Basic Clinical Practice, Faculty of Medicine, Universitat de Barcelona, Barcelona, Spain

Email addresses:

Raquel González	raquel.gonzalez@isglobal.org
Tacilta Nhampossa	tacilta.nhampossa@manhica.net
Ghyslain Mombo-Ngoma	ghyslain.mombongoma@gmail.com
Johannes Mischlinger	mischlinger@bnitm.de
Meral Esen	meral.esen@uni-tuebingen.de
André-MarieTchouatieu	tchouatieua@mmv.org
Clara Pons-Duran	clara.pons@isglobal.org
Lia Betty Dimessa Mbadinga	dimessalia@gmail.com
Bertrand Lell	Bertrand.lell@meduniwien.ac.at
Heimo Lagler	heimo.lagler@meduniwien.ac.at
Laura Garcia-Otero	laura.garcia@isglobal.org
Rella Zoleko-Manego	manegorella@yahoo.fr
Myriam El Gaaloul	elgaaloulm@mmv.org
Sergi Sanz	sergi.sanz@isglobal.org
Mireia Piqueras	mireia.piqueras@isglobal.org
Esperança Sevene	esperanca.sevene@manhica.net
Michael Ramharter	ramharter@bnitm.de

Francisco Saúte	francisco.saute@manhica.net
Clara Menéndez	clara.menendez@isglobal.org

Word count: 3837

Abstract

Introduction: Malaria infection during pregnancy is an important driver of maternal and neonatal health especially among HIV-infected women. Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine is recommended for malaria prevention in HIV-uninfected women but it is contraindicated in those HIV-infected on cotrimoxazole prophylaxis (CTXp) due to potential adverse effects. Dihydroartemisinin-piperaquine (DHA-PPQ), has been shown to improve antimalarial protection in HIV-uninfected pregnant women, constituting a promising candidate for IPTp. The objective of this trial is to determine if monthly three-day IPTp courses of DHA-PPQ added to daily CTXp are safe and superior to CTXp alone in decreasing the proportion of peripheral malaria parasitaemia at the end of pregnancy.

Methods and analysis: This is a multi-centre, two-arm, placebo-controlled, individually randomized trial in HIV-infected pregnant women receiving CTXp and antiretroviral treatment. A total of 664 women will be enrolled at the first antenatal care clinic visit in sites from Gabon and Mozambique. Participants will receive an insecticide-treated net and they will be administered monthly IPTp with DHA-PPQ or placebo (1:1 ratio) as directly observed therapy from the second trimester of pregnancy. Primary study outcome is the prevalence of maternal parasitaemia at delivery. Secondary outcomes include prevalence of malaria-related maternal and infant outcomes and proportion of adverse perinatal outcomes. Participants will be followed until six weeks after the end of pregnancy and their infants until one year of age to also evaluate the impact of DHA-PPQ on mother to child transmission of HIV. The analysis will be done in the Intention to Treat and According to Protocol cohorts, adjusted by gravidity, country, seasonality and other variables associated with malaria.

Ethics and dissemination: The protocol (version 1.0, 2nd May 2018) was reviewed and approved by the institutional and national ethics committees of Gabon (042/2018/PR/SG/CNER) and Mozambique (504/CBNS/18) and the Hospital Clinic of Barcelona (HCB/2018/0650,Spain).

Strengths and limitations of this study

- A major strength of this trial is its double blind placebo-controlled design which will allow to yield conclusive results about the efficacy of the study intervention.
- The inclusion of pregnant women from different sub-Saharan countries will provide a wide representation of different malaria endemicity areas and HIV subgroups.
- The study is also adequately powered to test the superiority hypothesis.

Keywords

Malaria, HIV, pregnancy, prevention, treatment.

Trial registration

ClinicalTrials.gov, NCT03671109. Registered on 14th September 2018. Recruitment is currently ongoing.

1. Background

Malaria accounted for 229 million cases and 409 000 deaths in 2019 worldwide. The burden of disease concentrates in sub-Saharan Africa (SSA) where 94% of malaria cases and 95% of malaria deaths occurred in 2019 [1]. For reasons not well understood, pregnant women are at increased risk of the infection and of severe clinical episodes [2-4]. Especially in SSA, malaria in pregnancy constitutes an important driver of maternal and infant health and a significant cause of maternal and infant mortality and morbidity [4-8]. An estimated 20 million HIV-infected individuals in SSA live in malaria endemic areas and over 12 million are women of reproductive age [9]. In addition, approximately one million pregnancies each year are complicated by co-infection with malaria and HIV in this region [10]. Prevalence of malaria and HIV co-infection in pregnant women from SSA has been estimated to range between 0.94% to 37% in a recent review [11].

Both malaria and HIV infections are among the leading causes of mortality and morbidity in African pregnant women and their children. Thus, modest effects of one infection on the other could lead to a substantial negative impact on the health of pregnant women and their infants [12]. The interaction between the two infections is particularly deleterious in pregnancy leading to increased risk and severity of both malaria infection and disease, as well as to increased HIV viral load, which may raise the frequency of mother to child transmission of HIV (MTCT-HIV) [13].

Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) given monthly at each antenatal care (ANC) clinic visit from the second trimester, along with long lasting insecticide treated nets (LLITNs), are the cornerstone for malaria prevention recommended by the WHO in stable transmission areas in HIV-uninfected pregnant women [14]. In HIV-infected pregnant women living in areas with limited health resources and high HIV prevalence, universal cotrimoxazole prophylaxis (CTXp) is recommended to prevent opportunistic infections [15]. However, SP is contraindicated in women on CTXp due to potential

adverse effects. Thus, even though IPTp-SP is a life-saving and highly cost-effective intervention it cannot be administered to the most vulnerable group, HIV-infected women [16-19]. Consequently and paradoxically the most susceptible women to malaria are currently the least protected [20].

A placebo-controlled trial conducted in three sub-Saharan countries between 2010 and 2013 showed that an effective antimalarial added to CTXp and long-lasting ITNs (LLITNs) in HIV-infected pregnant women has a significant impact in improving malaria prevention and maternal health through reductions in hospital admissions [21]. However, the antimalarial used (mefloquine) was not well tolerated, and most importantly, it was associated with a two-fold increase in the frequency of mother to child transmission of HIV (MTCT-HIV), limiting its potential for IPTp. These findings indicate the need to find drug alternatives with better tolerability and safety profile to reduce malaria in this vulnerable group [21].

Dihydroartemisinin-piperaquine (DHA-PPQ) is an artemisinin-based combination therapy (ACT) recommended by the WHO for treatment of uncomplicated malaria in adults and children aged ≥ six months[22]. Among the available antimalarial drugs, DHA-PPQ constitutes one of the best candidates for IPTp because it is not used as first-line malaria treatment, it is well tolerated, and it is recommended for treatment of clinical malaria in the second and third trimesters of pregnancy [22].

DHA-PPQ has been safely used for case management in HIV-uninfected pregnant women in Thailand and in a multicentre trial in sub-Saharan Africa [23, 24]. Moreover, studies comparing IPTp-SP with IPTp-DHA-PPQ in HIV-uninfected pregnant women from Kenya and Uganda indicate that the drug could be an alternative to SP in terms of antimalarial efficacy and safety in pregnancy [25, 26]. A recent meta-analysis of the safety and efficacy of repeated doses of DHA-PPQ for prevention and treatment of malaria including 11 studies (among adults, children and pregnant women) has concluded that monthly DHA-PPQ is well tolerated and effective for

IPT and that additional data are needed in pregnancy and to further explore the cardiac safety with monthly dosing [27]. Scientific evidence shows that efficacy and safety findings from malaria control strategies evaluated in HIV-uninfected pregnant women cannot be directly extrapolated to HIV-infected women [21]. A trial comparing monthly IPTp with DHA-PPQ to CTXp among 200 HIV-infected Ugandan women did not find differences in the risk of histopathologically detected placental malarial infection and other outcomes between groups [28]. However, authors acknowledge the limitations of their results due to the low prevalence of malaria in the study area at the time of the trial [28]. Therefore, it is of highest public health priority to provide conclusive evidence as to whether the most vulnerable population (HIV-infected pregnant women) will benefit from the use of the currently most promising and available alternative drug for IPTp, DHA-PPQ [20].

The objectives of the MAMAH (Improving **M**aternal he**A**lth by reducing **M**alaria in **A**frican **H**iV women) trial are (1) to evaluate the safety, tolerability and efficacy of DHA-PPQ as IPTp for malaria prevention in HIV-infected pregnant women receiving daily CTXp and ARV drugs, (2) to assess the effect of DHA-PPQ as IPTp on mother to child transmission of HIV and (3) to evaluate the effectiveness of CTXp in clearing malaria parasites in HIV-infected pregnant women.

2. Methods

This is multi-centre, two-arm, placebo-controlled, individually randomized superiority clinical trial with two study arms including HIV-infected pregnant women. The study will be carried out and reported according to Consolidated Standards of Reporting Trials guidelines [29].

Study settings

The trial will be conducted in two countries from Central and South Eastern sub-Saharan Africa (Gabon and Mozambique), where HIV prevalence among pregnant women ranges from 6 to 29%

[30, 31]. Malaria epidemiologic indicators and HIV prevalence in pregnancy in study sites are shown in Table 1. The trial sites have been selected to provide representation of different malaria endemicity and HIV subgroups (HIV-1 subtype C in Mozambique and HIV-1 CRF02_AG in Gabon, where HIV-2 also circulates [32, 33]).

Study population

All pregnant women attending the study ANC services for the first time and/or who have not received IPTp during their current pregnancy will be screened for participation in the trial. Inclusion criteria are (1) permanent resident in the study area, (2) gestational age at the first antenatal visit ≤ 28 weeks, (3) HIV seropositive status and (4) agreement to deliver in the study site's maternity(ies) wards. Exclusion criteria are (1) planning to move out in the following 10 months from enrolment, (2) gestational age at the first antenatal visit > 28 weeks of pregnancy, (3) known history of allergy to CTX, (4) known history of allergy or contraindications to DHA-PPQ and (5) participating in other intervention studies.

Informed consent

All participants will receive information about study procedures, including knowledge about malaria and HIV infection in pregnancy. A signed informed consent (or thumb-printed with a witness whenever the woman is illiterate) will be obtained before any study tests or evaluations are carried out. If the participant is under the legal age of maturity, she will sign the assent form and her legal guardian will sign the informed consent according to national ethics local policies. The informed consent will cover the woman and the new born infant.

Recruitment and randomization

After the study details are explained and informed consent is signed, pregnant women will be given a study number and automatically randomized to one of the study arms: 1) Daily CTX +

monthly IPTp-DHA-PPQ or 2) Daily CTX + Monthly IPTp-placebo. Each participant will be uniquely identified in the study by a combination of her site code and participant number. Allocation of participants to study arms will be done centrally by the trial's Sponsor (the Barcelona Institute for Global Health, ISGlobal) by block randomization and stratified by country. This method will ensure balanced allocation to both arms during different malaria seasons in the two study countries. Each subject number will be related to a treatment number which assigns them to one of the IPT arms. Study number allocation for each study participant will be concealed in opaque sealed envelopes that will be opened only after recruitment. At each site the first participant will be assigned a patient number, and consecutive numbers will be assigned to subsequent women. A study identification card containing the individual study number and basic demographic information will be given to the participant in order to facilitate identification at all study contacts.

Figure 1 displays the study design.

Blinding

Study tablets (IPTp-DHA-PPQ and IPTp-Placebo) will be identically packaged. Study personnel will prepare and deliver the medication to the participant. All study personnel, investigators, outcome assessors, data analysts and the participants will remain blinded throughout the trial. Unblinding is only envisaged in case of a medical emergency (in such case, the investigator on site will have justify to the Sponsor and the DSMB the need for unblinding).

Interventions

a) IPTp administration

Administration of the three-day IPTp course will always be done under fasting conditions (following DHA-PPQ administration recommendations) and direct observation by study

personnel. The number of daily IPTp tablets to be administered will be based on bodyweight and according to the treatment guidelines set by WHO [target dose (range) of 4 (2–10) mg/kg/ day of DHA and 18 (16–27) mg/kg/day of PPQ given once a day for three days for adults]. Following physical examination, recruited women of gestational age≥13 weeks will receive the assigned IPTp drug. In case of gestational age< 13 weeks, first IPTp administration will be scheduled one month later. If participants report malaria treatment in the preceding four weeks, first IPTp administration will also be delayed one month. Administration of the second and third day treatment course will be done by study personnel either at the study health facility or household level. Women will be observed for 60 minutes after administration of the IPTp dose. Those women vomiting within the first 30 minutes of IPTp administration will be given a second full IPTp dose; women vomiting after 30-60 minutes of IPTp administration will be given an additional half dose of the drug. Subsequent doses of IPTp will be given coinciding with the next scheduled monthly ANC clinic visit, at least one month apart from the previous dose.

b) CTX administration

Cotrimoxazole (fixed combination drug containing 800 mg of trimethoprim and 160 mg of sulfamethoxazole) will be taken daily from enrolment until delivery as per national guidelines for prevention of opportunistic infections. Women will be asked to visit the ANC clinic monthly to receive the amount of CTX tablets for the whole month. At each ANC clinic visit, adherence to CTX prophylaxis will be assessed.

c) ARV therapy and concomitant medications

Treatment for prevention of HIV MTCT (Option B+) will be given by the study health personnel, according to national HIV/AIDS control guidelines[34]. Any other concomitant treatment received by the study participants will be recorded in the study questionnaires.

d) Long lasting insecticide treated nets

Regardless of gestational age at the time of recruitment, all women will receive a LLITN and details about its use will be explained.

Study Outcomes

The primary outcome of the trial will be the prevalence of maternal parasitaemia at delivery defined by the presence of *Plasmodium falciparum (P. falciparum)* asexual parasites of any density in peripheral blood (determined by microscopy). The secondary maternal and infant endpoints can be found in Table 2.

Sample size

Based on previous estimations at the study sites and assuming a prevalence of peripheral parasitaemia at delivery of 7.5% with CTXp, it is estimated that 298 women per arm will be required to detect with 80% power a significant ($p<0.05$) decrease of 5% or more in the prevalence of peripheral parasitaemia in the CTXp+IPTp-DHA-PPQ group [21]. In order to allow for 10% losses to follow up, it is calculated that 332 women/study arm will need to be recruited (total n=664). Considering the prevalence of HIV infection among pregnant women in both sites (please refer to Table 1), a total of 444 women are planned to be recruited in Mozambique and 220 women in Gabon to allow for a recruitment rate of 33 women/month on average. These estimations are based on recruitment rates of previous IPTp clinical trials conducted among pregnant women in the two study sites [21, 35, 36].

Follow up and measurements of outcomes

At baseline, the woman's demographic and obstetric information will be recorded in study specific case report forms (CRF).

a) Physical and clinical examination at enrolment

The physical examination of the woman will include the following assessments: weight, height, gestational age by bimanual palpation and measurement of middle-upper arm circumference (MUAC). Ultrasound (US) will be performed to determine gestational age at enrolment and confirm pregnancy viability.

b) Baseline biological samples

At enrolment, a venous blood sample (5 mL) will be collected for analysis of haemoglobin level, CD4 cell counts, HIV viral load and malaria PCR.

c) Follow-up and household visits

Women will be given an appointment to attend the subsequent ANC clinic visit one month after the first one. Participants will receive the standard ANC package of interventions, which includes iron and folate supplementation, following national guidelines. The subsequent IPTp doses will be given at least four weeks apart from the previous one. MAMAH participants will be asked to visit the study facilities in case of any illness. A malaria blood smear will be collected in those participants passively reporting sick and presenting with malaria related signs/symptoms (fever (≥37,5º C) or having history of fever in the past 24 hours, arthromyalgias or headache), as per national management guidelines. Women will be visited at home the day after recruitment to confirm residence status, assess drug tolerability and the correct use of the net. Adherence to CTX prophylaxis, ARV therapy and compliance with the LLITNs use will be assessed monthly at the ANC attendance.

d) Adverse events monitoring and reporting

Active safety monitoring will consist in household visits to study participants two days after each IPTp administration to assess drug tolerability and record all Adverse Events (AEs). In addition, a health facility-based passive surveillance system will be established to capture unscheduled

visits of participants during follow-up. Information on unsolicited adverse events will be collected at each scheduled and unscheduled visit. Any participant passively reporting being sick during the study visits, will be referred to the clinical services as per routine system in place. A blood smear will be collected in those presenting with malaria related signs/symptoms (fever (≥37,5° C) or having history of fever in the past 24 hours, arthromyalgias or headache). In case of malaria parasitaemia or anaemia, they will be treated following national guidelines. Serious Adverse Events (SAEs) will be reported by the site investigator within 24 hours of being made aware to the trial's safety monitor and the Data Safety Monitoring Board (DSMB).

e) End of pregnancy and infant assessments

At the end of pregnancy, maternal blood (5 mL), placental and cord blood samples (5 mL) will be collected for haematological and parasitological examination. The new-born will be examined, weighed and measured and his/her gestational age assessed by the modified Ballard method [37].

f) Post-partum visit

Participants will be visited approximately after six weeks of end of pregnancy at the study health facility where a blood smear for malaria screening will be collected. A summary of study procedures is displayed in Table 3.

g) Infants follow up

Infants born to study participants will be followed up until one year of age. Mothers will be asked to bring their child to the study facilities at weeks 4-6 of age and at 6, 9 and 12 months after birth. At each scheduled visit, study infants will be physically examined and their psychomotor and neurological development will be assessed following a standard protocol for African settings [38-40]. Weight, height and axillary temperature will be measured and recorded. A capillary

blood sample will be taken from infants with fever (axillary temperature ≥37.5°C) or history of fever in the last 24 hours, or appearing pale, for malaria parasitaemia examination and haematological determination. In case of malaria parasitaemia or anaemia, they will be treated following national guidelines. At each scheduled visit, HIV DNA will be evaluated in the infant from dried blood spot on filter paper (for PCR). As per routine healthcare national programs HIV-exposed infants will receive CTX prophylaxis starting four to six weeks after birth. Infants will also receive ARV, according to national guidelines. Infant's follow up visits and study procedures are described in Table 4.

Laboratory tests

a) Parasitological and haematological determinations

Thick and thin blood smears will be stained with Giemsa's stain and examined for *Plasmodium spp*. following standard procedures. Also, blood haemoglobin will be determined following local SOPs.

b) Detection of HIV and quantitative determination of viral load

Quantitative PCR HIV viral load will be determined from the venous blood samples drawn at enrolment and delivery. Additionally, vertical transmission of HIV will be determined by qualitative DNA PCR performed on samples drawn from infants at one month and 12 months of age.

c) Immunological determinations related to HIV status

CD4+T cell count will be determined by flow cytometry after staining of whole blood with CD3, CD8 and CD4 fluorochrometolabelled antibodies and acquisition using FACSCalibur (BD Biosciences) and TruCOUNT tubes (Becton Dickinson, San Jose, CA; USA) *or MiniVIdas device*. HIV viral load will be determined from plasma cryopreserved at -80ºC using the devices in place *in the study sites (such as* COBAS® AMPLICOR , AmpliPrep [Roche Diagnostics] *or GeneXpert).*

d) Placental samples analysis

A tissue sample (approximately 2 cm^3) will be collected from the maternal surface of the placenta and will be immediately placed in 25 mL of 10% neutral buffered formalin and kept at 4ºC until processed and embedded in paraffin wax by standard techniques. Paraffin sections 4um thick from the placental tissue, will be stained with hematoxylin and eosin, Giemsa's stain and the periodic acid-Schiff technique. Placentas will be classified histologically as: i) not infected, ii) active infection and iii) past infection, depending on the presence or absence of parasites, pigment or both [41]. Impression smears will be prepared from the placental blood for parasitological examination. Blood from the placenta will also be collected onto filter paper for PCR determination of malaria parasites.

Data management

Data will be collected using paper case report forms (CRFs) developed for the trial by study personnel at each scheduled and unscheduled visit. The quality of the data recorded in the study source documents and CRFs will be monitored regularly following the principles of Good Clinical Practices by the trials' clinical monitor [42]. Data will be double entered into the study database using the OpenClinica open source software (version 3.14) for clinical data management (www.OpenClinica.com) at each study site. Automatic quality checks will be performed to ensure CRF completeness. Concomitant medications registered into the database are coded using the WHO Drug Reference List, which employs the Anatomical Therapeutic Chemical classification system. Medical history/current medical conditions and adverse events are coded using the Medical dictionary for regulatory activities (MedDRA) terminology.

Analysis plan

The following populations of analysis have been defined: a) Intention to treat (ITT): it includes all randomized pregnant women who have data on outcome (target population for the efficacy analysis); b) According to protocol (ATP): it includes participants who fulfil all the inclusion-exclusion criteria, took monthly IPTp-DHA-PPQ/placebo study doses, received a LLITN, received CTXp and ARV drugs and from whom data is available for the analysis; c) Safety: it includes participants who received at least one dose of IPTp DHA-PPQ/placebo and had at least one post-baseline safety assessment. No interim analyses of data is envisaged. The primary analysis of the trial will be the comparison of the proportion of pregnant women with peripheral parasitaemia at delivery in the ITT and ATP cohorts, adjusted by gravidity, country, seasonality and other variables associated with the prevalence of malaria. Proportions will be compared between groups using the Fisher exact test and presented as relative risk ratio (RR) or reduction of the RR (1- RR *100%) if RR lower than 1. Adjustment for co-variates and possible confounders will be done using Poisson regression with a log link and robust estimate of the covariance (Huber method), using the method proposed by Zou [43, 44]. Continuous variables will be compared between groups using Wilcoxon rank values. The differences in rank values will be assessed by looking at the expected rank sums in each group under the null hypothesis. Adjustment for co-variates and possible cofounders (such as country, gestational age, gravidity, anaemia, literacy, Mid-Upper Arm Circumference (MUAC), viral load and CD4+T cell count) will be done using ordinary least square regression. Subgroup analysis is not envisaged. Incidence of clinical malaria, overall admissions and outpatient attendances will be estimated as the number of episodes over the time at risk. Time at risk will be estimated as the time from the start of follow up until the end of follow-up (visit one month after end of pregnancy for mothers) or withdrawal due to censoring or death, whatever occurs first. The total number of events will be compared between groups using Negative Binomial regression models which takes into account a possible extra Poisson variation due to different frailty of the subjects. The comparison will be expressed

as relative rate ratio (RRate). Data analysis will be performed using Stata (Stata Corp., College Station, TX, US).

Patient and public involvement

Patients will not be directly involved in the design, conduct, reporting, or dissemination plans of the study. However, a Community Advisory Board (CAB) has been set at each study site to strengthen communication and interaction between the local study community and research teams. The CAB also oversees and guides the study team on key issues such as potential risks and burdens for participants or host communities that may be hidden from researchers, and how to minimize them [45].

Ethics and dissemination

The study is conducted in accordance with the EMA/ICH Guideline on Good Clinical Practice and in total agreement with the applicable international, EU and national law of all the participating countries[46]. The study protocol and the informed consent forms have been reviewed and approved by the institutional and national ethics committees of Gabon (042/2018/PR/SG/CNER) and Mozambique (504/CBNS/18) and the Hospital Clinic of Barcelona (HCB/2018/0650,Spain). An independent DSMB monitors regularly the safety of study participants. The trial is registered on clinicaltrials.gov (https://clinicaltrials.gov/ct2/show/NCT03671109).

The findings of the clinical trial will be submitted for publication in a peer-reviewed journal within 12 months of study completion through an open access mechanism, or otherwise made available publicly in compliance with H2020 open access requirements. Primary project raw data will be published in the project website. At no stage will data containing personal information of research participants be released.

A project communication has been developed in order to ensure timely, accurate, and effective dissemination of project results. After concluding the trial's data analysis, findings will be made available to all partners, key stakeholders and Ministries of Health. The project members will actively disseminate information to the scientific community through reports, presentations at scientific forums and publications in international open access journals. Trial results will also be shared with the WHO Global Malaria Program and Malaria Policy Advisory Groups.

Authors' contributions

Conceived and designed the study: RG and CM. Gave inputs to protocol methodology: RG, TN, GMN, JM, ME, AMT, CPD, LBD, BL, HL, LGO, RZM, MEG, SS, MP, ES, MR, FS, CM. Wrote the first draft of the manuscript: RG and CPD. Wrote sections on sample size, data management and analysis plan: SS and RG. Wrote, reviewed and approved the manuscript: RG, TN, GMN, JM, ME, AMT, CPD, LBD, BL, HL, LGO, RZM, MEG, SS, MP, ES, MR, FS, CM

Funding statement

This project is part of the EDCTP2 programme supported by the European Union (grant number RIA2016MC-1613-MAMAH) and the Medicines for Malaria Venture partnership (funding agreement PO19/00554). The project funders do not have any role on study design, data collection and analysis. The trial is sponsored by the Barcelona Institute for Global Health (ISGlobal). ISGlobal is supported by the Spanish Ministry of Science and Innovation through the "Centro de Excelencia Severo Ochoa 2019-2023" Program [CEX2018-000806-S] and it is a member of the CERCA Programme, Generalitat de Catalunya.

Competing interests

None declared.

References

1. WHO. World Malaria report 2020. WHO. 2020;Available at https://www.who.int/publications/i/item/9789240015791 [Accessed March 2021].
2. Mombo-Ngoma G, Mackanga JR, Gonzalez R, Ouedraogo S, Kakolwa MA, Manego RZ, et al. Young adolescent girls are at high risk for adverse pregnancy outcomes in sub-Saharan Africa: an observational multicountry study. BMJ open. 2016;6(6):e011783. Epub 2016/07/01. doi: 10.1136/bmjopen-2016-011783. PubMed PMID: 27357200.
3. Group WAbCTAABS. Clinical determinants of early parasitological response to ACTs in African patients with uncomplicated falciparum malaria: a literature review and meta-analysis of individual patient data. BMC Med. 2015;13:212. Epub 2015/09/08. doi: 10.1186/s12916-015-0445-x. PubMed PMID: 26343145; PubMed Central PMCID: PMCPMC4561425.
4. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoa K, Brabin B, et al. Epidemiology and burden of malaria in pregnancy. Lancet Infect Dis. 2007;7(2):93-104. Epub 2007/01/26. doi: S1473-3099(07)70021-X [pii]

10.1016/S1473-3099(07)70021-X [doi]. PubMed PMID: 17251080.
5. Menendez C, D'Alessandro U, ter Kuile FO. Reducing the burden of malaria in pregnancy by preventive strategies. Lancet Infect Dis. 2007;7(2):126-35. Epub 2007/01/26. doi: S1473-3099(07)70024-5 [pii]

10.1016/S1473-3099(07)70024-5 [doi]. PubMed PMID: 17251083.
6. Menendez C, Ordi J, Ismail MR, Ventura PJ, Aponte JJ, Kahigwa E, et al. The impact of placental malaria on gestational age and birth weight. J Infect Dis. 2000;181(5):1740-5. Epub 2000/05/24. doi: 10.1086/315449. PubMed PMID: 10823776.
7. Bardají A, Sigauque B, Sanz S, Maixenchs M, Ordi J, Aponte JJ, et al. Impact of malaria at the end of pregnancy on infant mortality and morbidity. J Infect Dis. 2011;203(5):691-9. Epub 2011/01/05. doi: 10.1093/infdis/jiq049. PubMed PMID: 21199881; PubMed Central PMCID: PMCPMC3071276.
8. Desai M, Hill J, Fernandes S, Walker P, Pell C, Gutman J, et al. Prevention of malaria in pregnancy. Lancet Infect Dis. 2018;18(4):e119-e32. Epub 2018/02/06. doi: 10.1016/s1473-3099(18)30064-1. PubMed PMID: 29395997.
9. UNAIDS/WHO. Global report: UNAIDS report on the global AIDS Epidemic 2013. WHO Library Cataloguing-in-Publication Data 2013;UNAIDS(JC2502/1/E).
10. WHO. Malaria and HIV interactions and their implications for public health policy WHO. 2005;ISBN 92 4 1593350.
11. Kwenti TE. Malaria and HIV coinfection in sub-Saharan Africa: prevalence, impact, and treatment strategies. Research and reports in tropical medicine. 2018;9:123-36. Epub 2018/08/14. doi: 10.2147/rrtm.S154501. PubMed PMID: 30100779; PubMed Central PMCID: PMCPMC6067790.
12. ter Kuile FO, Parise ME, Verhoeff FH, Udhayakumar V, Newman RD, van Eijk AM, et al. The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant women in sub-saharan Africa. Am J Trop Med Hyg. 2004;71(2 Suppl):41-54. PubMed PMID: 15331818.
13. Gonzalez R, Ataide R, Naniche D, Menendez C, Mayor A. HIV and malaria interactions: where do we stand? Expert Rev Anti Infect Ther. 2012;10(2):153-65. doi: 10.1586/eri.11.167. PubMed PMID: 22339190.
14. WHO. Intermittent Preventive Treatment of malaria in pregnancy using Sulfadoxine-Pyrimethamine (IPTp-SP). Updated WHO Policy Recommendation. WHO. 2012;http://www.who.int/malaria/iptp_sp_updated_policy_recommendation_en_102012.pdf .

15. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection Recommendations for a public health approach June 2013. 2013.
16. Sevene E, Gonzalez R, Menendez C. Current knowledge and challenges of antimalarial drugs for treatment and prevention in pregnancy. Expert Opin Pharmacother. 2010;11(8):1277-93. PubMed PMID: 20408744.
17. Ward SA, Sevene EJ, Hastings IM, Nosten F, McGready R. Antimalarial drugs and pregnancy: safety, pharmacokinetics, and pharmacovigilance. Lancet Infect Dis. 2007;7(2):136-44. Epub 2007/01/26. doi: S1473-3099(07)70025-7 [pii]

10.1016/S1473-3099(07)70025-7 [doi]. PubMed PMID: 17251084.
18. Menendez C, Bardaji A, Sigauque B, Sanz S, Aponte JJ, Mabunda S, et al. Malaria prevention with IPTp during pregnancy reduces neonatal mortality. PloS one. 2010;5(2):e9438. PubMed PMID: 20195472.
19. Eisele TP, Larsen DA, Anglewicz PA, Keating J, Yukich J, Bennett A, et al. Malaria prevention in pregnancy, birthweight, and neonatal mortality: a meta-analysis of 32 national cross-sectional datasets in Africa. Lancet Infect Dis. 2012;12(12):942-9. Epub 2012/09/22. doi: 10.1016/s1473-3099(12)70222-0. PubMed PMID: 22995852.
20. Gonzalez R, Sevene E, Jagoe G, Slutsker L, Menendez C. A Public Health Paradox: The Women Most Vulnerable to Malaria Are the Least Protected. PLoS Med. 2016;13(5):e1002014. Epub 2016/05/04. doi: 10.1371/journal.pmed.1002014. PubMed PMID: 27139032.
21. Gonzalez R, Desai M, Macete E, Ouma P, Kakolwa MA, Abdulla S, et al. Intermittent Preventive Treatment of Malaria in Pregnancy with Mefloquine in HIV-Infected Women Receiving Cotrimoxazole Prophylaxis: A Multicenter Randomized Placebo-Controlled Trial. PLoS Med. 2014;11(9):e1001735. Epub 2014/09/24. doi: 10.1371/journal.pmed.1001735. PubMed PMID: 25247995.
22. WHO. Guidelines for the treatment of malaria. Second edition. WHO. 2010;ISBN 9789241547925.
23. Rijken MJ, McGready R, Boel ME, Barends M, Proux S, Pimanpanarak M, et al. Dihydroartemisinin-piperaquine rescue treatment of multidrug-resistant Plasmodium falciparum malaria in pregnancy: a preliminary report. Am J Trop Med Hyg. 2008;78(4):543-5. Epub 2008/04/04. PubMed PMID: 18385345.
24. Pekyi D, Ampromfi AA, Tinto H, Traore-Coulibaly M, Tahita MC, Valea I, et al. Four Artemisinin-Based Treatments in African Pregnant Women with Malaria. N Engl J Med. 2016;374(10):913-27. Epub 2016/03/11. doi: 10.1056/NEJMoa1508606. PubMed PMID: 26962727.
25. Desai M, Gutman J, L'Lanziva A, Otieno K, Juma E, Kariuki S, et al. Intermittent screening and treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine versus intermittent preventive treatment with sulfadoxine-pyrimethamine for the control of malaria during pregnancy in western Kenya: an open-label, three-group, randomised controlled superiority trial. Lancet. 2015. Epub 2015/10/03. doi: 10.1016/s0140-6736(15)00310-4. PubMed PMID: 26429700.
26. Kakuru A, Jagannathan P, Muhindo MK, Natureeba P, Awori P, Nakalembe M, et al. Dihydroartemisinin-Piperaquine for the Prevention of Malaria in Pregnancy. N Engl J Med. 2016;374(10):928-39. Epub 2016/03/11. doi: 10.1056/NEJMoa1509150. PubMed PMID: 26962728; PubMed Central PMCID: PMCPMC4847718.
27. Gutman J, Kovacs S, Dorsey G, Stergachis A, Ter Kuile FO. Safety, tolerability, and efficacy of repeated doses of dihydroartemisinin-piperaquine for prevention and treatment of malaria: a systematic review and meta-analysis. Lancet Infect Dis. 2017;17(2):184-93. Epub 2016/11/21. doi: 10.1016/s1473-3099(16)30378-4. PubMed PMID: 27865890; PubMed Central PMCID: PMCPMC5266794.
28. Natureeba P, Kakuru A, Muhindo M, Ochieng T, Ategeka J, Koss CA, et al. Intermittent Preventive Treatment With Dihydroartemisinin-Piperaquine for the Prevention of Malaria

Among HIV-Infected Pregnant Women. J Infect Dis. 2017;216(1):29-35. Epub 2017/03/23. doi: 10.1093/infdis/jix110. PubMed PMID: 28329368; PubMed Central PMCID: PMCPMC5853208.

29. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. Annals of internal medicine. 2010;152(11):726-32. Epub 2010/03/26. doi: 10.7326/0003-4819-152-11-201006010-00232. PubMed PMID: 20335313.

30. Gonzalez R, Munguambe K, Aponte J, Bavo C, Nhalungo D, Macete E, et al. High HIV prevalence in a southern semi-rural area of Mozambique: a community-based survey. HIV Med. 2012. PubMed PMID: 22500780.

31. Manego RZ, Mombo-Ngoma G, Witte M, Held J, Gmeiner M, Gebru T, et al. Demography, maternal health and the epidemiology of malaria and other major infectious diseases in the rural department Tsamba-Magotsi, Ngounie Province, in central African Gabon. BMC Public Health. 2017;17(1):130. Epub 2017/01/29. doi: 10.1186/s12889-017-4045-x. PubMed PMID: 28129759; PubMed Central PMCID: PMCPMC5273856.

32. Caron M, Lekana-Douki SE, Makuwa M, Obiang-Ndong GP, Biba O, Nkoghe D, et al. Prevalence, genetic diversity and antiretroviral drugs resistance-associated mutations among untreated HIV-1-infected pregnant women in Gabon, central Africa. BMC infectious diseases. 2012;12:64. Epub 2012/03/22. doi: 10.1186/1471-2334-12-64. PubMed PMID: 22433277; PubMed Central PMCID: PMCPMC3359209.

33. Lahuerta M, Aparicio E, Bardaji A, Marco S, Sacarlal J, Mandomando I, et al. Rapid spread and genetic diversification of HIV type 1 subtype C in a rural area of southern Mozambique. AIDS Res Hum Retroviruses. 2008;24(2):327-35. PubMed PMID: 18271719.

34. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016. Reccomendations for a public health approach. Second edition.: World Health Organization; 2016.

35. Gonzalez R, Mombo-Ngoma G, Ouedraogo S, Kakolwa MA, Abdulla S, Accrombessi M, et al. Intermittent Preventive Treatment of Malaria in Pregnancy with Mefloquine in HIV-Negative Women: A Multicentre Randomized Controlled Trial. PLoS Med. 2014;11(9):e1001733. Epub 2014/09/24. doi: 10.1371/journal.pmed.1001733. PubMed PMID: 25247709.

36. Menendez C, Bardaji A, Sigauque B, Romagosa C, Sanz S, Serra-Casas E, et al. A randomized placebo-controlled trial of intermittent preventive treatment in pregnant women in the context of insecticide treated nets delivered through the antenatal clinic. PloS one. 2008;3(4):e1934. Epub 2008/04/10. doi: 10.1371/journal.pone.0001934 [doi]. PubMed PMID: 18398460; PubMed Central PMCID: PMC2277457.

37. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R. New Ballard Score, expanded to include extremely premature infants. The Journal of pediatrics. 1991;119(3):417-23. Epub 1991/09/01. PubMed PMID: 1880657.

38. Gladstone M, Lancaster GA, Umar E, Nyirenda M, Kayira E, van den Broek NR, et al. The Malawi Developmental Assessment Tool (MDAT): the creation, validation, and reliability of a tool to assess child development in rural African settings. PLoS Med. 2010;7(5):e1000273. Epub 2010/06/04. doi: 10.1371/journal.pmed.1000273. PubMed PMID: 20520849; PubMed Central PMCID: PMCPMC2876049.

39. Koura KG, Boivin MJ, Davidson LL, Ouedraogo S, Zoumenou R, Alao MJ, et al. Usefulness of child development assessments for low-resource settings in francophone Africa. Journal of developmental and behavioral pediatrics : JDBP. 2013;34(7):486-93. Epub 2013/08/01. doi: 10.1097/DBP.0b013e31829d211c. PubMed PMID: 23899660; PubMed Central PMCID: PMCPMC3821168.

40. Prado EL, Abubakar AA, Abbeddou S, Jimenez EY, Some JW, Ouedraogo JB. Extending the Developmental Milestones Checklist for use in a different context in Sub-Saharan Africa. Acta paediatrica (Oslo, Norway : 1992). 2014;103(4):447-54. Epub 2013/12/21. doi: 10.1111/apa.12540. PubMed PMID: 24354938.

41. Ismail MR, Ordi J, Menendez C, Ventura PJ, Aponte JJ, Kahigwa E, et al. Placental pathology in malaria: a histological, immunohistochemical, and quantitative study. Human pathology. 2000;31(1):85-93. Epub 2000/02/09. PubMed PMID: 10665918.
42. Pharmalys. Pharmalys CRO. 2021;Available at https://pharmalys.com/ [Accessed July 2021].
43. Zou G. A modified poisson regression approach to prospective studies with binary data. American journal of epidemiology. 2004;159(7):702-6. Epub 2004/03/23. PubMed PMID: 15033648.
44. Zou GY, Donner A. Extension of the modified Poisson regression model to prospective studies with correlated binary data. Statistical methods in medical research. 2013;22(6):661-70. Epub 2011/11/11. doi: 10.1177/0962280211427759. PubMed PMID: 22072596.
45. NHREC. Guidelines for Community Advisory Groups. NHREC. 2012;Available at : http://research.ukzn.ac.za/Files/National%20Guidelines%20for%20Community%20Advisory%20Groups%20for%20Research%20(2012).pdf [Accessed April 2021].
46. EMA. Guideline for Good Clinical Practice E6 (R2). EMA. 2015;Available at https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-good-clinical-practice-e6r2-4-step-2b_en.pdf [Accessed April 2021].
47. Alonso PL, Sacarlal J, Aponte JJ, Leach A, Macete E, Milman J, et al. Efficacy of the RTS,S/AS02A vaccine against Plasmodium falciparum infection and disease in young African children: randomised controlled trial. Lancet. 2004;364(9443):1411-20. Epub 2004/10/19. doi: 10.1016/s0140-6736(04)17223-1. PubMed PMID: 15488216.
48. UNAIDS. Mozambique country Fact sheet. 2015:Available at http://www.unaids.org/sites/default/files/media/documents/UNAIDS_GlobalplanCountryfactsheet_mozambique_en.pdf [accessed February 2017].
49. Sylla EH, Kun JF, Kremsner PG. Mosquito distribution and entomological inoculation rates in three malaria-endemic areas in Gabon. Trans R Soc Trop Med Hyg. 2000;94(6):652-6. Epub 2001/02/24. doi: 10.1016/s0035-9203(00)90219-0. PubMed PMID: 11198649.
50. Ministère-de-la-Santé-et-de-la-Prévoyance-sociale-Gabon. Rapport National sur la Réponse au VIH/SIDA 2014. 2015:Available at http://www.unaids.org/sites/default/files/country/documents/GAB_narrative_report_2015.pdf [accessed February 7].
51. Mourou JR, Coffinet T, Jarjaval F, Cotteaux C, Pradines E, Godefroy L, et al. Malaria transmission in Libreville: results of a one year survey. Malar J. 2012;11:40. Epub 2012/02/11. doi: 10.1186/1475-2875-11-40. PubMed PMID: 22321336; PubMed Central PMCID: PMCPMC3310827.

Figure Legends:

Figure 1. MAMAH trial design

Tables

Table 1. Malaria and HIV epidemiology in the study sites

Site/ Country	Malaria Transmission	High season	EIR*	*P. falciparum* infection prevalence in women at delivery#	HIV prevalence in pregnant women	Frequency of MTCT of HIV
Manhiça† / Mozambique	Hypoendemic	Sep-Mar	21-50 [47]	6%	29%	**6%**[48]
Lambaréné‡/ Gabon	Mesoendemic	Oct-May	21-50[49]	11%	6%	**12%**[50]
Libreville/ Gabon**	Mesoendemic	Oct-May	21-50[51]	NI	**6%**	**12%**[50]

*EIR: Entomological Inoculation Rate

Data from 2010-2012 in women receiving either two IPTp doses of mefloquine or SP (Tuikue-Ndam et al, unpublished). NI: No information.

† The trial will be conducted at the ANC services of the Manhiça District Hospital where the *Centro de Investigação em Saúde de Manhiça* (CISM) is situated; the average monthly number of pregnant women attending first ANC clinic visit is 110.

‡ In Lambaréné, the trial will be conducted at the ANC services and maternity of the Albert Schweitzer Hospital by the Centre de Recherches Médicales de Lambaréné (CERMEL); the average monthly number of pregnant women attending first ANC clinic visit is 115.

** In Libreville, the trial will be conducted at the ANC services of *the Centre hospitalier Régional Estuaire de Melen- Unité de Recherche Clinique sur le Paludisme* and the Jeanne Ebori Hospital; the average monthly number of pregnant women attending first ANC clinic visit is 150.

Table 2. Study outcomes

Primary endpoint
Prevalence of maternal parasitaemia at delivery (defined by the presence of *P. falciparum* asexual parasites of any density in peripheral blood determined by microscopy)
Secondary endpoints
Maternal
Incidence of clinical malaria during pregnancy
Incidence of all-cause admissions
Incidence of all-cause outpatient attendances
Frequency and severity of adverse events (including cardiotoxic signals)
Mean haemoglobin concentration at delivery
Prevalence of submicroscopic *P. falciparum* peripheral parasitaemia at delivery
Prevalence of anaemia at delivery (Hb < 11 g/dL)
Prevalence of severe anaemia at delivery (Hb < 7 g/dL)
Mean CD4 + T cell counts levels at delivery
Proportion of women with detectable HIV viral load at delivery
Prevalence of placental *P. falciparum* infection (defined by the presence of parasites and/or pigment in the histological examination, or microscopic or sub-microscopic in the impression smear from placental blood)
Prevalence of *P. falciparum* peripheral parasitaemia at the post-partum visit
Maternal mortality rate
Infant
Prevalence of *P. falciparum* parasitaemia in cord blood (microscopic and sub-microscopic)
Prevalence of neonatal anaemia (Hb < 12,5 g/dL in cord blood, and Hb < 13g/dL in neonatal blood)
Mean birth weight
Prevalence of low birth weight (<2500 g)
Mean gestational age at birth
Prevalence of prematurity
Prevalence of embryo and foetal losses (miscarriages and stillbirths)
Prevalence of small for gestational age
Frequency of congenital malformations
Incidence of clinical malaria
Neonatal mortality rate
Frequency of mother to child transmission of HIV at one and at 12 months of age.
Infant mortality rate

Table 3. Schedule of enrolment, interventions and maternal assessments

	STUDY PERIOD							
	Pre-enrolment	**Allocation**	**First ANC clinic visit**	**Household visits**	**Monthly ANC visits**	**End of pregnancy**	**One month after end of pregnancy**	**Unscheduled visits**
TIMEPOINT	**0**	**0**	**1**	**+2 days**	**+1 month and then monthly**			
SCREENING AND ENROLMENT:								
Eligibility screen	X							
Informed consent	X							
Randomization		X						
INTERVENTIONS:								
IPTp administration			X	X	X			
CTX administration			X	*	*	*	*	*
ARV administration			X	*	*	*	*	*
LLITN distribution			X					
MATERNAL ASSESSMENTS:								
Demographics, medical history			X					X
Socio-economic characteristics				#		X		
Record of concomitant medication			X	X	X	X	X	X
Record of adverse			X	X	X	X	X	X

events						
Physical/clinical examination	X			X		X
Gestational age by ultrasound	X		X	X		X
Temperature					X	X
Blood pressure	X			X	X	X
Weight	X		X		X	X
Height	X					
MUAC	X				X	
RPR test	X					
CD4 count and HIV viral load	X			X		
Blood smear (malaria)	†		†	X	X	†
Haemoglobin test	X			X	X	
Intrapartum samples (cord blood, placenta)				X		
Drug tolerability assessment	X	X	X			
Compliance with LLITNs check		X	X	X	X	X

Only in the first household visit after the ANC visit of first IPTp administration.

* CTX and ARV adherence should be assessed at each scheduled visit.

† Only in women passively reporting sick AND presenting with malaria related signs/symptoms (fever (≥37,5° C) or having history of fever in the past 24 hours, arthromyalgia or headache), as per national management guidelines

623 **Table 4. Schedule of infant visits and procedures**

	TIMEPOINTS					
	Birth	**1 month***	**6 months**	**9 months**	**12 months**	**Unscheduled visits**
PROCEDURES:						
Medical history	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X
Psychomotor development assessment	X	X	X	X	X	
Weight	X	X	X	X	X	X
Height	X	X	X	X	X	X
Temperature	X	X	X	X	X	X
Blood smear	X	†	†	†	†	†
Haemoglobin test	X	†	†	†	†	†
HIV PCR ±		X	X	X	X	
Malaria PCR (filter paper)	X					
HIV prophylaxis adherence	#	#	#	#	#	#
HIV treatment adherence	#	#	#	#	#	#

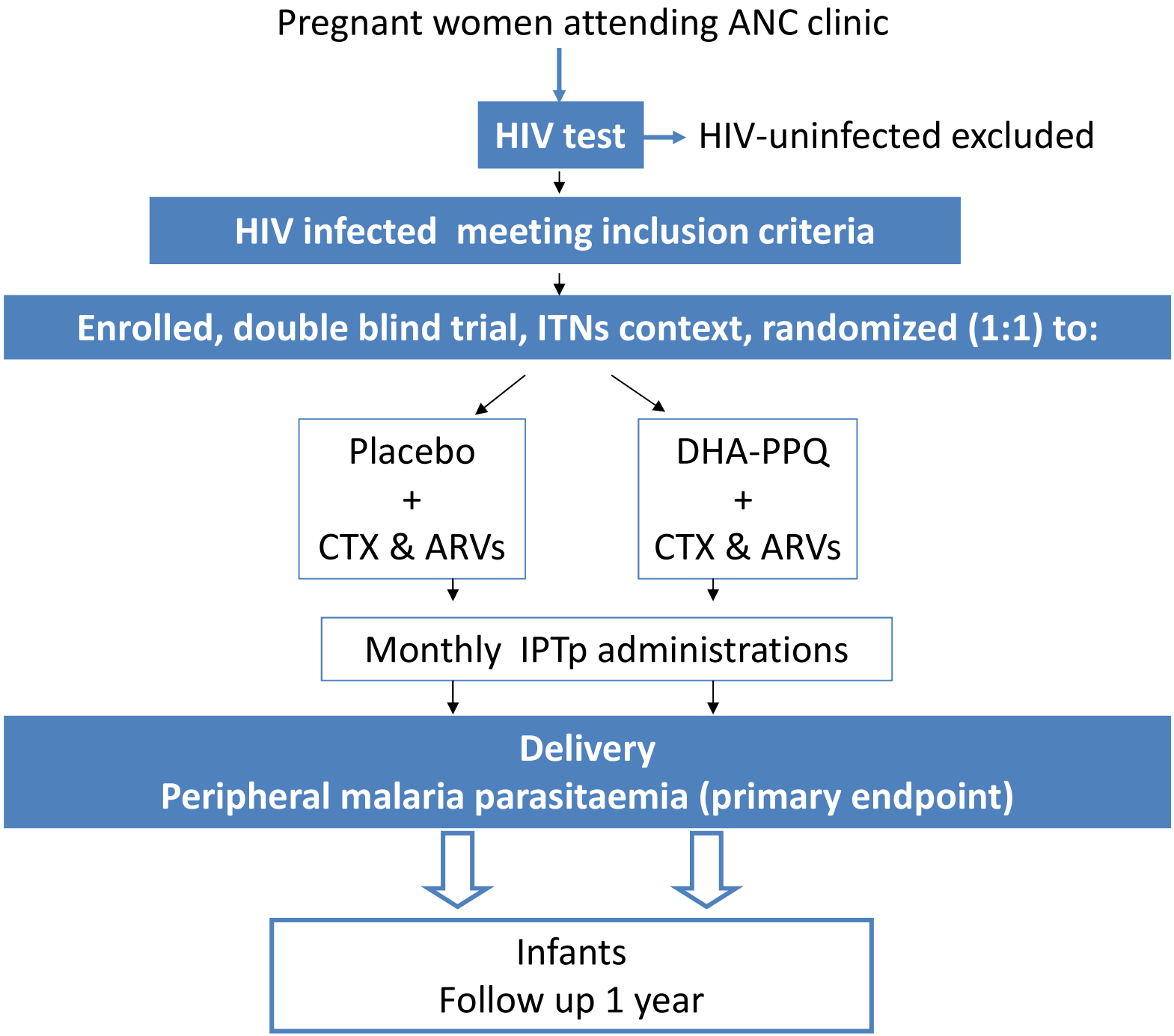
624 * First visit will be scheduled 1 month after birth or coinciding with first EPI visit

625 † Only if fever (≥37,5° C) or history of fever in the past 24 hours or signs suggestive of malaria.

626 ± HIV PCR test should also be repeated at month 18 after birth.

627 # Adherence should be assessed at each visit.

628



SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	5
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	5
Funding	4	Sources and types of financial, material, and other support	18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,2,3, 18
	5b	Name and contact information for the trial sponsor	3
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	18
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	7,8,9
	6b	Explanation for choice of comparators	7,8
Objectives	7	Specific objectives or hypotheses	9
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	9
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9,10
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	10
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11,12
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	n/a
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	14,15
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12-15
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	13, Table 2
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	11,12, Figure 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	13
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	13
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10-11
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10-11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10-11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10-11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	11
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	17-18
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14-15-17

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	17
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	18
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	18
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	18
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	14-15
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	18
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14-15
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	19
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	19

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	17
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	20
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	19
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	19
	31b	Authorship eligibility guidelines and any intended use of professional writers	20
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	19
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	n/a
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Evaluation of the safety and efficacy of dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in HIV-infected pregnant women: protocol of a multi-centre, two-arm, randomized, placebo-controlled, superiority clinical trial (MAMAH project)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-053197.R2
Article Type:	Protocol
Date Submitted by the Author:	16-Sep-2021
Complete List of Authors:	González, Raquel; Barcelona Institute for Global Health, ; CIBERESP, Nhampossa, Tacilta; Centro de Investigacao em Saude de Manhica Mombo-Ngoma, Ghyslain; CERMEL, Clinical Operations Mischlinger, Johannes; Bernhard Nocht Institute of Tropical Medicine Esen, Meral; University of Tübingen, Tchouatieu, André-Marie; Medecine for Malaria Venture Pons-Duran, Clara; Barcelona Institute for Global Health, Maternal, Child and Reproductive Health Initiative Dimessa, Lia ; Centre de Recherches Médicales de Lambaréné Lell, Bertrand; Medical Research Centre of Lambarene Lagler, Heimo; Medical University of Vienna Garcia-Otero, Laura; Barcelona Institute for Global Health Zoleko Manego, Rella; Centre de Recherches Médicales de Lambaréné El Gaaloul, Myriam; Medicines for Malaria Venture Sanz, Sergi; Barcelona Institute for Global Health, Biostatistics and Data Management Unit; CIBERESP, Piqueras, Mireia; Barcelona Institute for Global Health Sevene, Esperanca; Centro de Investigacao em Saude de Manhica; Universidade Eduardo Mondlane Ramharter, Michael; Bernhard-Nocht-Institut fur Tropenmedizin Saute, Francisco; Centro de Investigação em Saúde de Manhiça Menendez, Clara; Barcelona Institute for Global Health
Primary Subject Heading:	Global health
Secondary Subject Heading:	Epidemiology, Infectious diseases, HIV/AIDS, Obstetrics and gynaecology, Public health
Keywords:	HIV & AIDS < INFECTIOUS DISEASES, Maternal medicine < OBSTETRICS, Clinical trials < THERAPEUTICS, TROPICAL MEDICINE, Epidemiology < TROPICAL MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Title: Evaluation of the safety and efficacy of dihydroartemisinin-piperaquine for intermittent preventive treatment of malaria in HIV-infected pregnant women: protocol of a multi-centre, two-arm, randomized, placebo-controlled, superiority clinical trial (MAMAH project)

Names of protocol contributors: Raquel González [1, 2, 3], Tacilta Nhampossa[2,4], Ghyslain Mombo-Ngoma[5], Johannes Mischlinger[6,7], Meral Esen[8], André-Marie Tchouatieu[9], Clara Pons-Duran[1, 3], Lia Betty Dimessa[5], Bertrand Lell[5,10], Heimo Lagler[10], Laura Garcia-Otero[1,2], Rella Zoleko-Manego[5], Myriam El Gaaloul[9], Sergi Sanz[1,3,11], Mireia Piqueras[1], Esperança Sevene[2], Michael Ramharter[6,7], Francisco Saúte[2], Clara Menendez[1, 2, 3].

Corresponding author: Raquel González

Corresponding author email address: raquel.gonzalez@isglobal.org

Affiliations:

1. Barcelona Institute for Global Health (ISGlobal), Hospital Clínic- Universitat de Barcelona, Spain
2. Centro de Investigação em Saúde de Manhiça (CISM), Manhiça, Mozambique
3. Consorcio de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP)
4. Instituto Nacional de Saúde, Ministério de Saúde, Mozambique
5. Centre de Recherches Médicales de Lambaréné (CERMEL), Lambaréné, Gabon
6. Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine & I Dept. of Medicine University Medical Center Hamburg-Eppendorf, Hamburg, Germany
7. German Center for Infection Research, partner site Hamburg-Lübeck-Borstel-Riems
8. Institut für Tropenmedizin, Eberhard Karls University of Tübingen (EKUT), Tübingen, Germany
9. Medicines for Malaria Venture (MMV), Geneva, Switzerland

10. Department of Medicine I, Division of Infectious Diseases and Tropical Medicine, Medical University of Vienna, Vienna, Austria.
11. Department of Basic Clinical Practice, Faculty of Medicine, Universitat de Barcelona, Barcelona, Spain

Email addresses:

Raquel González	raquel.gonzalez@isglobal.org
Tacilta Nhampossa	tacilta.nhampossa@manhica.net
Ghyslain Mombo-Ngoma	ghyslain.mombongoma@gmail.com
Johannes Mischlinger	mischlinger@bnitm.de
Meral Esen	meral.esen@uni-tuebingen.de
André-MarieTchouatieu	tchouatieua@mmv.org
Clara Pons-Duran	clara.pons@isglobal.org
Lia Betty Dimessa Mbadinga	dimessalia@gmail.com
Bertrand Lell	Bertrand.lell@meduniwien.ac.at
Heimo Lagler	heimo.lagler@meduniwien.ac.at
Laura Garcia-Otero	laura.garcia@isglobal.org
Rella Zoleko-Manego	manegorella@yahoo.fr
Myriam El Gaaloul	elgaaloulm@mmv.org
Sergi Sanz	sergi.sanz@isglobal.org
Mireia Piqueras	mireia.piqueras@isglobal.org
Esperança Sevene	esperanca.sevene@manhica.net
Michael Ramharter	ramharter@bnitm.de

Francisco Saúte	francisco.saute@manhica.net
Clara Menéndez	clara.menendez@isglobal.org

Word count: 3907

Abstract

Introduction: Malaria infection during pregnancy is an important driver of maternal and neonatal health especially among HIV-infected women. Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine is recommended for malaria prevention in HIV-uninfected women but it is contraindicated in those HIV-infected on cotrimoxazole prophylaxis (CTXp) due to potential adverse effects. Dihydroartemisinin-piperaquine (DHA-PPQ), has been shown to improve antimalarial protection, constituting a promising IPTp candidate. This trial's objective is to determine if monthly three-day IPTp courses of DHA-PPQ added to daily CTXp are safe and superior to CTXp alone in decreasing the proportion of peripheral malaria parasitaemia at the end of pregnancy.

Methods and analysis: This is a multi-centre, two-arm, placebo-controlled, individually randomized trial in HIV-infected pregnant women receiving CTXp and antiretroviral treatment. A total of 664 women will be enrolled at the first antenatal care clinic visit in sites from Gabon and Mozambique. Participants will receive an insecticide-treated net and they will be administered monthly IPTp with DHA-PPQ or placebo (1:1 ratio) as directly observed therapy from the second trimester of pregnancy. Primary study outcome is the prevalence of maternal parasitaemia at delivery. Secondary outcomes include prevalence of malaria-related maternal and infant outcomes and proportion of adverse perinatal outcomes. Participants will be followed until six weeks after the end of pregnancy and their infants until one year of age to also evaluate the impact of DHA-PPQ on mother to child transmission of HIV. The analysis will be done in the Intention to Treat and According to Protocol cohorts, adjusted by gravidity, country, seasonality and other variables associated with malaria.

Ethics and dissemination: The protocol was reviewed and approved by the institutional and national ethics committees of Gabon and Mozambique and the Hospital Clinic of Barcelona. Project results will be presented to all stakeholders and published in open access journals.

Strengths and limitations of this study

- A major strength of this trial is its double blind placebo-controlled design which will allow to yield conclusive results about the efficacy of the study intervention.
- The inclusion of pregnant women from different sub-Saharan countries will provide a wide representation of different malaria endemicity areas and HIV subgroups.
- The study is also adequately powered to test the superiority hypothesis.

Keywords

Malaria, HIV, pregnancy, prevention, treatment.

Trial registration

ClinicalTrials.gov, NCT03671109. Registered on 14th September 2018. Recruitment is currently ongoing.

1. Background

Malaria accounted for 229 million cases and 409 000 deaths in 2019 worldwide. The burden of disease concentrates in sub-Saharan Africa (SSA) where 94% of malaria cases and 95% of malaria deaths occurred in 2019 [1]. For reasons not well understood, pregnant women are at increased risk of the infection and of severe clinical episodes [2-4]. Especially in SSA, malaria in pregnancy constitutes an important driver of maternal and infant health and a significant cause of maternal and infant mortality and morbidity [4-8]. An estimated 20 million HIV-infected individuals in SSA live in malaria endemic areas and over 12 million are women of reproductive age [9]. In addition, approximately one million pregnancies each year are complicated by co-infection with malaria and HIV in this region [10]. Prevalence of malaria and HIV co-infection in pregnant women from SSA has been estimated to range between 0.94% to 37% in a recent review [11].

Both malaria and HIV infections are among the leading causes of mortality and morbidity in African pregnant women and their children. Thus, modest effects of one infection on the other could lead to a substantial negative impact on the health of pregnant women and their infants [12]. The interaction between the two infections is particularly deleterious in pregnancy leading to increased risk and severity of both malaria infection and disease, as well as to increased HIV viral load, which may raise the frequency of mother to child transmission of HIV (MTCT-HIV) [13].

Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) given monthly at each antenatal care (ANC) clinic visit from the second trimester, along with long lasting insecticide treated nets (LLITNs), are the cornerstone for malaria prevention recommended by the WHO in stable transmission areas in HIV-uninfected pregnant women [14]. In HIV-infected pregnant women living in areas with limited health resources and high HIV prevalence, universal cotrimoxazole prophylaxis (CTXp) is recommended to prevent opportunistic infections [15]. However, SP is contraindicated in women on CTXp due to potential

adverse effects. Thus, even though IPTp-SP is a life-saving and highly cost-effective intervention it cannot be administered to the most vulnerable group, HIV-infected women [16-19]. Consequently and paradoxically the most susceptible women to malaria are currently the least protected [20].

A placebo-controlled trial conducted in three sub-Saharan countries between 2010 and 2013 showed that an effective antimalarial added to CTXp and long-lasting ITNs (LLITNs) in HIV-infected pregnant women has a significant impact in improving malaria prevention and maternal health through reductions in hospital admissions [21]. However, the antimalarial used (mefloquine) was not well tolerated, and most importantly, it was associated with a two-fold increase in the frequency of mother to child transmission of HIV (MTCT-HIV), limiting its potential for IPTp. These findings indicate the need to find drug alternatives with better tolerability and safety profile to reduce malaria in this vulnerable group [21].

Dihydroartemisinin-piperaquine (DHA-PPQ) is an artemisinin-based combination therapy (ACT) recommended by the WHO for treatment of uncomplicated malaria in adults and children aged ≥ six months[22]. Among the available antimalarial drugs, DHA-PPQ constitutes one of the best candidates for IPTp because it is not used as first-line malaria treatment, it is well tolerated, and it is recommended for treatment of clinical malaria in the second and third trimesters of pregnancy [22].

DHA-PPQ has been safely used for case management in HIV-uninfected pregnant women in Thailand and in a multicentre trial in sub-Saharan Africa [23, 24]. Moreover, studies comparing IPTp-SP with IPTp-DHA-PPQ in HIV-uninfected pregnant women from Kenya and Uganda indicate that the drug could be an alternative to SP in terms of antimalarial efficacy and safety in pregnancy [25, 26]. A recent meta-analysis of the safety and efficacy of repeated doses of DHA-PPQ for prevention and treatment of malaria including 11 studies (among adults, children and pregnant women) has concluded that monthly DHA-PPQ is well tolerated and effective for

IPT and that additional data are needed in pregnancy and to further explore the cardiac safety with monthly dosing [27]. Scientific evidence shows that efficacy and safety findings from malaria control strategies evaluated in HIV-uninfected pregnant women cannot be directly extrapolated to HIV-infected women [21]. A trial comparing monthly IPTp with DHA-PPQ to CTXp among 200 HIV-infected Ugandan women did not find differences in the risk of histopathologically detected placental malarial infection and other outcomes between groups [28]. However, authors acknowledge the limitations of their results due to the low prevalence of malaria in the study area at the time of the trial [28]. Therefore, it is of highest public health priority to provide conclusive evidence as to whether the most vulnerable population (HIV-infected pregnant women) will benefit from the use of the currently most promising and available alternative drug for IPTp, DHA-PPQ [20].

The objectives of the MAMAH (Improving **M**aternal he**A**lth by reducing **M**alaria in **A**frican **H**iV women) trial are (1) to evaluate the safety, tolerability and efficacy of DHA-PPQ as IPTp for malaria prevention in HIV-infected pregnant women receiving daily CTXp and ARV drugs, (2) to assess the effect of DHA-PPQ as IPTp on mother to child transmission of HIV and (3) to evaluate the effectiveness of CTXp in clearing malaria parasites in HIV-infected pregnant women.

2. Methods

This is multi-centre, two-arm, placebo-controlled, individually randomized superiority clinical trial with two study arms including HIV-infected pregnant women. The study will be carried out and reported according to Consolidated Standards of Reporting Trials guidelines [29].

Study settings

The trial will be conducted in two countries from Central and South Eastern sub-Saharan Africa (Gabon and Mozambique), where HIV prevalence among pregnant women ranges from 6 to 29%

[30, 31]. Malaria epidemiologic indicators and HIV prevalence in pregnancy in study sites are shown in Table 1. The trial sites have been selected to provide representation of different malaria endemicity and HIV subgroups (HIV-1 subtype C in Mozambique and HIV-1 CRF02_AG in Gabon, where HIV-2 also circulates [32, 33]).

Study population

All pregnant women attending the study ANC services for the first time and/or who have not received IPTp during their current pregnancy will be screened for participation in the trial. Inclusion criteria are (1) permanent resident in the study area, (2) gestational age at the first antenatal visit ≤ 28 weeks, (3) HIV seropositive status and (4) agreement to deliver in the study site's maternity(ies) wards. Exclusion criteria are (1) planning to move out in the following 10 months from enrolment, (2) gestational age at the first antenatal visit > 28 weeks of pregnancy, (3) known history of allergy to CTX, (4) known history of allergy or contraindications to DHA-PPQ and (5) participating in other intervention studies.

Informed consent

All participants will receive information about study procedures, including knowledge about malaria and HIV infection in pregnancy. A signed informed consent form (or thumb-printed with a witness whenever the woman is illiterate) will be obtained before any study tests or evaluations are carried out by study nurses in each site. The trial's informed consent is available as Supplemental Material 1. If the participant is under the legal age of maturity, she will sign the assent form and her legal guardian will sign the informed consent according to national ethics local policies. The informed consent will cover the woman and the new born infant.

Recruitment and randomization

After the study details are explained and informed consent is signed, pregnant women will be given a study number and automatically randomized to one of the study arms: 1) Daily CTX + monthly IPTp-DHA-PPQ or 2) Daily CTX + Monthly IPTp-placebo. Each participant will be uniquely identified in the study by a combination of her site code and participant number. Allocation of participants to study arms will be done centrally by the trial's Sponsor (the Barcelona Institute for Global Health, ISGlobal) by block randomization and stratified by country. This method will ensure balanced allocation to both arms during different malaria seasons in the two study countries. Each subject number will be related to a treatment number which assigns them to one of the IPT arms. Study number allocation for each study participant will be concealed in opaque sealed envelopes that will be opened only after recruitment. At each site the first participant will be assigned a patient number, and consecutive numbers will be assigned to subsequent women. A study identification card containing the individual study number and basic demographic information will be given to the participant in order to facilitate identification at all study contacts.

Figure 1 displays the study design.

Blinding

Study tablets (IPTp-DHA-PPQ and IPTp-Placebo) will be identically packaged. Study personnel will prepare and deliver the medication to the participant. All study personnel, investigators, outcome assessors, data analysts and the participants will remain blinded throughout the trial. Unblinding is only envisaged in case of a medical emergency (in such case, the investigator on site will have to justify to the Sponsor and the DSMB the need for unblinding).

Interventions

a) IPTp administration

Administration of the three-day IPTp course will always be done under fasting conditions (following DHA-PPQ administration recommendations) and direct observation by study personnel. The number of daily IPTp tablets to be administered will be based on bodyweight and according to the treatment guidelines set by WHO [target dose (range) of 4 (2–10) mg/kg/ day of DHA and 18 (16–27) mg/kg/day of PPQ given once a day for three days for adults]. Following physical examination, recruited women of gestational age≥13 weeks will receive the assigned IPTp drug. In case of gestational age< 13 weeks, first IPTp administration will be scheduled one month later. If participants report malaria treatment in the preceding four weeks, first IPTp administration will also be delayed one month. Administration of the second and third day treatment course will be done by study personnel either at the study health facility or household level. Women will be observed for 60 minutes after administration of the IPTp dose. Those women vomiting within the first 30 minutes of IPTp administration will be given a second full IPTp dose; women vomiting after 30-60 minutes of IPTp administration will be given an additional half dose of the drug. Subsequent doses of IPTp will be given coinciding with the next scheduled monthly ANC clinic visit, at least one month apart from the previous dose.

b) CTX administration

Cotrimoxazole (fixed combination drug containing 800 mg of trimethoprim and 160 mg of sulfamethoxazole) will be taken daily from enrolment until delivery as per national guidelines for prevention of opportunistic infections. Women will be asked to visit the ANC clinic monthly to receive the amount of CTX tablets for the whole month. At each ANC clinic visit, adherence to CTX prophylaxis will be assessed.

c) ARV therapy and concomitant medications

Treatment for prevention of HIV MTCT (Option B+) will be given by the study health personnel, according to national HIV/AIDS control guidelines[34]. Any other concomitant treatment received by the study participants will be recorded in the study questionnaires.

d) Long lasting insecticide treated nets

Regardless of gestational age at the time of recruitment, all women will receive a LLITN and details about its use will be explained.

Study Outcomes

The primary outcome of the trial will be the prevalence of maternal parasitaemia at delivery defined by the presence of *Plasmodium falciparum (P. falciparum)* asexual parasites of any density in peripheral blood (determined by microscopy). The secondary maternal and infant endpoints can be found in Table 2.

Sample size

Based on previous estimations at the study sites and assuming a prevalence of peripheral parasitaemia at delivery of 7.5% with CTXp, it is estimated that 298 women per arm will be required to detect with 80% power a significant ($p<0.05$) decrease of 5% or more in the prevalence of peripheral parasitaemia in the CTXp+IPTp-DHA-PPQ group [21]. In order to allow for 10% losses to follow up, it is calculated that 332 women/study arm will need to be recruited (total n=664). Considering the prevalence of HIV infection among pregnant women in both sites (please refer to Table 1), a total of 444 women are planned to be recruited in Mozambique and 220 women in Gabon to allow for a recruitment rate of 33 women/month on average. These estimations are based on recruitment rates of previous IPTp clinical trials conducted among pregnant women in the two study sites [21, 35, 36].

Follow up and measurements of outcomes

At baseline, the woman's demographic and obstetric information will be recorded in study specific case report forms (CRF).

a) Physical and clinical examination at enrolment

The physical examination of the woman will include the following assessments: weight, height, gestational age by bimanual palpation and measurement of middle-upper arm circumference (MUAC). Ultrasound (US) will be performed to determine gestational age at enrolment and confirm pregnancy viability.

b) Baseline biological samples

At enrolment, a venous blood sample (5 mL) will be collected for analysis of haemoglobin level, CD4 cell counts, HIV viral load and malaria PCR.

c) Follow-up and household visits

Women will be given an appointment to attend the subsequent ANC clinic visit one month after the first one. Participants will receive the standard ANC package of interventions, which includes iron and folate supplementation, following national guidelines. The subsequent IPTp doses will be given at least four weeks apart from the previous one. MAMAH participants will be asked to visit the study facilities in case of any illness. A malaria blood smear will be collected in those participants passively reporting sick and presenting with malaria related signs/symptoms (fever (≥37,5º C) or having history of fever in the past 24 hours, arthromyalgias or headache), as per national management guidelines. Women will be visited at home the day after recruitment to confirm residence status, assess drug tolerability and the correct use of the net. Adherence to CTX prophylaxis, ARV therapy and compliance with the LLITNs use will be assessed monthly at the ANC attendance.

d) Adverse events monitoring and reporting

Active safety monitoring will consist in household visits to study participants two days after each IPTp administration to assess drug tolerability and record all Adverse Events (AEs). In addition, a health facility-based passive surveillance system will be established to capture unscheduled visits of participants during follow-up. Information on unsolicited adverse events will be collected at each scheduled and unscheduled visit. Any participant passively reporting being sick during the study visits, will be referred to the clinical services as per routine system in place. A blood smear will be collected in those presenting with malaria related signs/symptoms (fever (≥37,5° C) or having history of fever in the past 24 hours, arthromyalgias or headache). In case of malaria parasitaemia or anaemia, they will be treated following national guidelines. Serious Adverse Events (SAEs) will be reported by the site investigator within 24 hours of being made aware to the trial's safety monitor and the Data Safety Monitoring Board (DSMB).

e) End of pregnancy and infant assessments

At the end of pregnancy, maternal blood (5 mL), placental and cord blood samples (5 mL) will be collected for haematological and parasitological examination. The new-born will be examined, weighed and measured and his/her gestational age assessed by the modified Ballard method [37].

f) Post-partum visit

Participants will be visited approximately after six weeks of end of pregnancy at the study health facility where a blood smear for malaria screening will be collected. A summary of study procedures is displayed in Table 3.

g) Infants follow up

Infants born to study participants will be followed up until one year of age. Mothers will be asked to bring their child to the study facilities at weeks 4-6 of age and at 6, 9 and 12 months after

birth. At each scheduled visit, study infants will be physically examined and their psychomotor and neurological development will be assessed following a standard protocol for African settings [38-40]. Weight, height and axillary temperature will be measured and recorded. A capillary blood sample will be taken from infants with fever (axillary temperature ≥37.5°C) or history of fever in the last 24 hours, or appearing pale, for malaria parasitaemia examination and haematological determination. In case of malaria parasitaemia or anaemia, they will be treated following national guidelines. At each scheduled visit, HIV DNA will be evaluated in the infant from dried blood spot on filter paper (for PCR). As per routine healthcare national programs HIV-exposed infants will receive CTX prophylaxis starting four to six weeks after birth. Infants will also receive ARV, according to national guidelines. Infant's follow up visits and study procedures are described in Table 4.

Laboratory tests

a) Parasitological and haematological determinations

Thick and thin blood smears will be stained with Giemsa's stain and examined for *Plasmodium spp*., following standard procedures. Also, blood haemoglobin will be determined following local SOPs.

b) Detection of HIV and quantitative determination of viral load

Quantitative PCR HIV viral load will be determined from the venous blood samples drawn at enrolment and delivery. Additionally, vertical transmission of HIV will be determined by qualitative DNA PCR performed on samples drawn from infants at one month and 12 months of age.

c) Immunological determinations related to HIV status

CD4+T cell count will be determined by flow cytometry after staining of whole blood with CD3, CD8 and CD4 fluorochrometolabelled antibodies and acquisition using FACSCalibur (BD

Biosciences) and TruCOUNT tubes (Becton Dickinson, San Jose, CA; USA) *or MiniVIdas device*. HIV viral load will be determined from plasma cryopreserved at -80ºC using the devices in place *in the study sites (such as* COBAS® AMPLICOR , AmpliPrep [Roche Diagnostics] *or GeneXpert).*

d) Placental samples analysis

A tissue sample (approximately 2 cm^3) will be collected from the maternal surface of the placenta and will be immediately placed in 25 mL of 10% neutral buffered formalin and kept at 4ºC until processed and embedded in paraffin wax by standard techniques. Paraffin sections 4um thick from the placental tissue, will be stained with hematoxylin and eosin, Giemsa's stain and the periodic acid-Schiff technique. Placentas will be classified histologically as: i) not infected, ii) active infection and iii) past infection, depending on the presence or absence of parasites, pigment or both [41]. Impression smears will be prepared from the placental blood for parasitological examination. Blood from the placenta will also be collected onto filter paper for PCR determination of malaria parasites.

Data management

Data will be collected using paper case report forms (CRFs) developed for the trial by study personnel at each scheduled and unscheduled visit. The quality of the data recorded in the study source documents and CRFs will be monitored regularly following the principles of Good Clinical Practices by the trials' clinical monitor [42]. Data will be double entered into the study database using the OpenClinica open source software (version 3.14) for clinical data management (www.OpenClinica.com) at each study site. Automatic quality checks will be performed to ensure CRF completeness. The database system will be designed to protect the confidentiality (sensitive data will be automatically encrypted) and integrity of the data and will include authorization, authentication, auditing and availability features to safeguard the access and usage of the data. Concomitant medications registered into the database will be coded using

the WHO Drug Reference List, which employs the Anatomical Therapeutic Chemical classification system. Medical history/current medical conditions and adverse events will be coded using the Medical dictionary for regulatory activities (MedDRA) terminology.

Analysis plan

The following populations of analysis have been defined: a) Intention to treat (ITT): it includes all randomized pregnant women who have data on outcome (target population for the efficacy analysis); b) According to protocol (ATP): it includes participants who fulfil all the inclusion-exclusion criteria, took monthly IPTp-DHA-PPQ/placebo study doses, received a LLITN, received CTXp and ARV drugs and from whom data is available for the analysis; c) Safety: it includes participants who received at least one dose of IPTp DHA-PPQ/placebo and had at least one post-baseline safety assessment. No interim analyses of data is envisaged. The primary analysis of the trial will be the comparison of the proportion of pregnant women with peripheral parasitaemia at delivery in the ITT and ATP cohorts, adjusted by gravidity, country, seasonality and other variables associated with the prevalence of malaria. Proportions will be compared between groups using the Fisher exact test. Crude and adjusted analysis will be done using Poisson regression with a log link and robust estimate of the covariance (Huber method), using the method proposed by Zou [43, 44]. Relative risk ratio (RR) or reduction of the RR (1- RR *100%) if RR lower than 1 will be presented. Continuous variables will be compared between groups using T-test and the Wilcoxon rank values according to variables' characteristics. The differences in rank values will be assessed by looking at the expected rank sums in each group under the null hypothesis. Adjustment for co-variates and possible cofounders (such as country, gestational age, gravidity, anaemia, literacy, Mid-Upper Arm Circumference (MUAC), viral load and CD4+T cell count) will be done using ordinary least square regression. Subgroup analysis is not envisaged. Incidence of clinical malaria, overall admissions and outpatient attendances will be estimated as the number of episodes over the time at risk. Time at risk will be estimated as

the time from the start of follow up until the end of follow-up (visit one month after end of pregnancy for mothers) or withdrawal due to censoring or death, whatever occurs first. The total number of events will be compared between groups using Negative Binomial regression models which takes into account a possible extra Poisson variation due to different frailty of the subjects. The comparison will be expressed as relative rate ratio (RRate). Data analysis will be performed using Stata (Stata Corp., College Station, TX, US).

Patient and public involvement

Patients will not be directly involved in the design, conduct, reporting, or dissemination plans of the study. However, a Community Advisory Board (CAB) has been set at each study site to strengthen communication and interaction between the local study community and research teams. The CAB also oversees and guides the study team on key issues such as potential risks and burdens for participants or host communities that may be hidden from researchers, and how to minimize them [45].

Ethics and dissemination

The study is conducted in accordance with the EMA/ICH Guideline on Good Clinical Practice and in total agreement with the applicable international, EU and national law of all the participating countries[46]. The study protocol (version 1.0, 2nd May 2018) and the informed consent forms have been reviewed and approved by the institutional and national ethics committees of Gabon (042/2018/PR/SG/CNER) and Mozambique (504/CBNS/18) and the Hospital Clinic of Barcelona (HCB/2018/0650, Spain). An independent DSMB monitors regularly the safety of study participants. The trial is registered on clinicaltrials.gov (https://clinicaltrials.gov/ct2/show/NCT03671109).

The findings of the clinical trial will be submitted for publication in a peer-reviewed journal within 12 months of study completion through an open access mechanism, or otherwise made

available publicly in compliance with H2020 open access requirements. Primary project raw data will be published in the project website. At no stage will data containing personal information of research participants be released.

A project communication has been developed in order to ensure timely, accurate, and effective dissemination of project results. After concluding the trial's data analysis, findings will be made available to all partners, key stakeholders and Ministries of Health. The project members will actively disseminate information to the scientific community through reports, presentations at scientific forums and publications in international open access journals. Trial results will also be shared with the WHO Global Malaria Program and Malaria Policy Advisory Groups.

Authors' contributions

Conceived and designed the study: RG and CM. Gave inputs to protocol methodology: RG, TN, GMN, JM, ME, AMT, CPD, LBD, BL, HL, LGO, RZM, MEG, SS, MP, ES, MR, FS, CM. Wrote the first draft of the manuscript: RG and CPD. Wrote sections on sample size, data management and analysis plan: SS and RG. Wrote, reviewed and approved the manuscript: RG, TN, GMN, JM, ME, AMT, CPD, LBD, BL, HL, LGO, RZM, MEG, SS, MP, ES, MR, FS, CM

Funding statement

This project is part of the EDCTP2 programme supported by the European Union (grant number RIA2016MC-1613-MAMAH) and the Medicines for Malaria Venture partnership (funding agreement PO19/00554). The project funders do not have any role on study design, data collection and analysis. The trial is sponsored by the Barcelona Institute for Global Health (ISGlobal). ISGlobal is supported by the Spanish Ministry of Science and Innovation through the "Centro de Excelencia Severo Ochoa 2019-2023" Program [CEX2018-000806-S] and it is a member of the CERCA Programme, Generalitat de Catalunya.

Competing interests

None declared.

References

1. WHO. World Malaria report 2020. WHO. 2020;Available at https://www.who.int/publications/i/item/9789240015791 [Accessed March 2021].
2. Mombo-Ngoma G, Mackanga JR, Gonzalez R, Ouedraogo S, Kakolwa MA, Manego RZ, et al. Young adolescent girls are at high risk for adverse pregnancy outcomes in sub-Saharan Africa: an observational multicountry study. BMJ open. 2016;6(6):e011783. Epub 2016/07/01. doi: 10.1136/bmjopen-2016-011783. PubMed PMID: 27357200.
3. Group WAbCTAABS. Clinical determinants of early parasitological response to ACTs in African patients with uncomplicated falciparum malaria: a literature review and meta-analysis of individual patient data. BMC Med. 2015;13:212. Epub 2015/09/08. doi: 10.1186/s12916-015-0445-x. PubMed PMID: 26343145; PubMed Central PMCID: PMCPMC4561425.
4. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoa K, Brabin B, et al. Epidemiology and burden of malaria in pregnancy. Lancet Infect Dis. 2007;7(2):93-104. Epub 2007/01/26. doi: S1473-3099(07)70021-X [pii]

10.1016/S1473-3099(07)70021-X [doi]. PubMed PMID: 17251080.
5. Menendez C, D'Alessandro U, ter Kuile FO. Reducing the burden of malaria in pregnancy by preventive strategies. Lancet Infect Dis. 2007;7(2):126-35. Epub 2007/01/26. doi: S1473-3099(07)70024-5 [pii]

10.1016/S1473-3099(07)70024-5 [doi]. PubMed PMID: 17251083.
6. Menendez C, Ordi J, Ismail MR, Ventura PJ, Aponte JJ, Kahigwa E, et al. The impact of placental malaria on gestational age and birth weight. J Infect Dis. 2000;181(5):1740-5. Epub 2000/05/24. doi: 10.1086/315449. PubMed PMID: 10823776.
7. Bardají A, Sigauque B, Sanz S, Maixenchs M, Ordi J, Aponte JJ, et al. Impact of malaria at the end of pregnancy on infant mortality and morbidity. J Infect Dis. 2011;203(5):691-9. Epub 2011/01/05. doi: 10.1093/infdis/jiq049. PubMed PMID: 21199881; PubMed Central PMCID: PMCPMC3071276.
8. Desai M, Hill J, Fernandes S, Walker P, Pell C, Gutman J, et al. Prevention of malaria in pregnancy. Lancet Infect Dis. 2018;18(4):e119-e32. Epub 2018/02/06. doi: 10.1016/s1473-3099(18)30064-1. PubMed PMID: 29395997.
9. UNAIDS/WHO. Global report: UNAIDS report on the global AIDS Epidemic 2013. WHO Library Cataloguing-in-Publication Data 2013;UNAIDS(JC2502/1/E).
10. WHO. Malaria and HIV interactions and their implications for public health policy WHO. 2005;ISBN 92 4 1593350.
11. Kwenti TE. Malaria and HIV coinfection in sub-Saharan Africa: prevalence, impact, and treatment strategies. Research and reports in tropical medicine. 2018;9:123-36. Epub 2018/08/14. doi: 10.2147/rrtm.S154501. PubMed PMID: 30100779; PubMed Central PMCID: PMCPMC6067790.
12. ter Kuile FO, Parise ME, Verhoeff FH, Udhayakumar V, Newman RD, van Eijk AM, et al. The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant women in sub-saharan Africa. Am J Trop Med Hyg. 2004;71(2 Suppl):41-54. PubMed PMID: 15331818.
13. Gonzalez R, Ataide R, Naniche D, Menendez C, Mayor A. HIV and malaria interactions: where do we stand? Expert Rev Anti Infect Ther. 2012;10(2):153-65. doi: 10.1586/eri.11.167. PubMed PMID: 22339190.
14. WHO. Intermittent Preventive Treatment of malaria in pregnancy using Sulfadoxine-Pyrimethamine (IPTp-SP). Updated WHO Policy Recommendation. WHO. 2012;http://www.who.int/malaria/iptp_sp_updated_policy_recommendation_en_102012.pdf .

15. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection Recommendations for a public health approach June 2013. 2013.
16. Sevene E, Gonzalez R, Menendez C. Current knowledge and challenges of antimalarial drugs for treatment and prevention in pregnancy. Expert Opin Pharmacother. 2010;11(8):1277-93. PubMed PMID: 20408744.
17. Ward SA, Sevene EJ, Hastings IM, Nosten F, McGready R. Antimalarial drugs and pregnancy: safety, pharmacokinetics, and pharmacovigilance. Lancet Infect Dis. 2007;7(2):136-44. Epub 2007/01/26. doi: S1473-3099(07)70025-7 [pii]

10.1016/S1473-3099(07)70025-7 [doi]. PubMed PMID: 17251084.
18. Menendez C, Bardaji A, Sigauque B, Sanz S, Aponte JJ, Mabunda S, et al. Malaria prevention with IPTp during pregnancy reduces neonatal mortality. PloS one. 2010;5(2):e9438. PubMed PMID: 20195472.
19. Eisele TP, Larsen DA, Anglewicz PA, Keating J, Yukich J, Bennett A, et al. Malaria prevention in pregnancy, birthweight, and neonatal mortality: a meta-analysis of 32 national cross-sectional datasets in Africa. Lancet Infect Dis. 2012;12(12):942-9. Epub 2012/09/22. doi: 10.1016/s1473-3099(12)70222-0. PubMed PMID: 22995852.
20. Gonzalez R, Sevene E, Jagoe G, Slutsker L, Menendez C. A Public Health Paradox: The Women Most Vulnerable to Malaria Are the Least Protected. PLoS Med. 2016;13(5):e1002014. Epub 2016/05/04. doi: 10.1371/journal.pmed.1002014. PubMed PMID: 27139032.
21. Gonzalez R, Desai M, Macete E, Ouma P, Kakolwa MA, Abdulla S, et al. Intermittent Preventive Treatment of Malaria in Pregnancy with Mefloquine in HIV-Infected Women Receiving Cotrimoxazole Prophylaxis: A Multicenter Randomized Placebo-Controlled Trial. PLoS Med. 2014;11(9):e1001735. Epub 2014/09/24. doi: 10.1371/journal.pmed.1001735. PubMed PMID: 25247995.
22. WHO. Guidelines for the treatment of malaria. Second edition. WHO. 2010;ISBN 9789241547925.
23. Rijken MJ, McGready R, Boel ME, Barends M, Proux S, Pimanpanarak M, et al. Dihydroartemisinin-piperaquine rescue treatment of multidrug-resistant Plasmodium falciparum malaria in pregnancy: a preliminary report. Am J Trop Med Hyg. 2008;78(4):543-5. Epub 2008/04/04. PubMed PMID: 18385345.
24. Pekyi D, Ampromfi AA, Tinto H, Traore-Coulibaly M, Tahita MC, Valea I, et al. Four Artemisinin-Based Treatments in African Pregnant Women with Malaria. N Engl J Med. 2016;374(10):913-27. Epub 2016/03/11. doi: 10.1056/NEJMoa1508606. PubMed PMID: 26962727.
25. Desai M, Gutman J, L'Lanziva A, Otieno K, Juma E, Kariuki S, et al. Intermittent screening and treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine versus intermittent preventive treatment with sulfadoxine-pyrimethamine for the control of malaria during pregnancy in western Kenya: an open-label, three-group, randomised controlled superiority trial. Lancet. 2015. Epub 2015/10/03. doi: 10.1016/s0140-6736(15)00310-4. PubMed PMID: 26429700.
26. Kakuru A, Jagannathan P, Muhindo MK, Natureeba P, Awori P, Nakalembe M, et al. Dihydroartemisinin-Piperaquine for the Prevention of Malaria in Pregnancy. N Engl J Med. 2016;374(10):928-39. Epub 2016/03/11. doi: 10.1056/NEJMoa1509150. PubMed PMID: 26962728; PubMed Central PMCID: PMCPMC4847718.
27. Gutman J, Kovacs S, Dorsey G, Stergachis A, Ter Kuile FO. Safety, tolerability, and efficacy of repeated doses of dihydroartemisinin-piperaquine for prevention and treatment of malaria: a systematic review and meta-analysis. Lancet Infect Dis. 2017;17(2):184-93. Epub 2016/11/21. doi: 10.1016/s1473-3099(16)30378-4. PubMed PMID: 27865890; PubMed Central PMCID: PMCPMC5266794.
28. Natureeba P, Kakuru A, Muhindo M, Ochieng T, Ategeka J, Koss CA, et al. Intermittent Preventive Treatment With Dihydroartemisinin-Piperaquine for the Prevention of Malaria

Among HIV-Infected Pregnant Women. J Infect Dis. 2017;216(1):29-35. Epub 2017/03/23. doi: 10.1093/infdis/jix110. PubMed PMID: 28329368; PubMed Central PMCID: PMCPMC5853208.

29. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. Annals of internal medicine. 2010;152(11):726-32. Epub 2010/03/26. doi: 10.7326/0003-4819-152-11-201006010-00232. PubMed PMID: 20335313.

30. Gonzalez R, Munguambe K, Aponte J, Bavo C, Nhalungo D, Macete E, et al. High HIV prevalence in a southern semi-rural area of Mozambique: a community-based survey. HIV Med. 2012. PubMed PMID: 22500780.

31. Manego RZ, Mombo-Ngoma G, Witte M, Held J, Gmeiner M, Gebru T, et al. Demography, maternal health and the epidemiology of malaria and other major infectious diseases in the rural department Tsamba-Magotsi, Ngounie Province, in central African Gabon. BMC Public Health. 2017;17(1):130. Epub 2017/01/29. doi: 10.1186/s12889-017-4045-x. PubMed PMID: 28129759; PubMed Central PMCID: PMCPMC5273856.

32. Caron M, Lekana-Douki SE, Makuwa M, Obiang-Ndong GP, Biba O, Nkoghe D, et al. Prevalence, genetic diversity and antiretroviral drugs resistance-associated mutations among untreated HIV-1-infected pregnant women in Gabon, central Africa. BMC infectious diseases. 2012;12:64. Epub 2012/03/22. doi: 10.1186/1471-2334-12-64. PubMed PMID: 22433277; PubMed Central PMCID: PMCPMC3359209.

33. Lahuerta M, Aparicio E, Bardaji A, Marco S, Sacarlal J, Mandomando I, et al. Rapid spread and genetic diversification of HIV type 1 subtype C in a rural area of southern Mozambique. AIDS Res Hum Retroviruses. 2008;24(2):327-35. PubMed PMID: 18271719.

34. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016. Reccomendations for a public health approach. Second edition.: World Health Organization; 2016.

35. Gonzalez R, Mombo-Ngoma G, Ouedraogo S, Kakolwa MA, Abdulla S, Accrombessi M, et al. Intermittent Preventive Treatment of Malaria in Pregnancy with Mefloquine in HIV-Negative Women: A Multicentre Randomized Controlled Trial. PLoS Med. 2014;11(9):e1001733. Epub 2014/09/24. doi: 10.1371/journal.pmed.1001733. PubMed PMID: 25247709.

36. Menendez C, Bardaji A, Sigauque B, Romagosa C, Sanz S, Serra-Casas E, et al. A randomized placebo-controlled trial of intermittent preventive treatment in pregnant women in the context of insecticide treated nets delivered through the antenatal clinic. PloS one. 2008;3(4):e1934. Epub 2008/04/10. doi: 10.1371/journal.pone.0001934 [doi]. PubMed PMID: 18398460; PubMed Central PMCID: PMC2277457.

37. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R. New Ballard Score, expanded to include extremely premature infants. The Journal of pediatrics. 1991;119(3):417-23. Epub 1991/09/01. PubMed PMID: 1880657.

38. Gladstone M, Lancaster GA, Umar E, Nyirenda M, Kayira E, van den Broek NR, et al. The Malawi Developmental Assessment Tool (MDAT): the creation, validation, and reliability of a tool to assess child development in rural African settings. PLoS Med. 2010;7(5):e1000273. Epub 2010/06/04. doi: 10.1371/journal.pmed.1000273. PubMed PMID: 20520849; PubMed Central PMCID: PMCPMC2876049.

39. Koura KG, Boivin MJ, Davidson LL, Ouedraogo S, Zoumenou R, Alao MJ, et al. Usefulness of child development assessments for low-resource settings in francophone Africa. Journal of developmental and behavioral pediatrics : JDBP. 2013;34(7):486-93. Epub 2013/08/01. doi: 10.1097/DBP.0b013e31829d211c. PubMed PMID: 23899660; PubMed Central PMCID: PMCPMC3821168.

40. Prado EL, Abubakar AA, Abbeddou S, Jimenez EY, Some JW, Ouedraogo JB. Extending the Developmental Milestones Checklist for use in a different context in Sub-Saharan Africa. Acta paediatrica (Oslo, Norway : 1992). 2014;103(4):447-54. Epub 2013/12/21. doi: 10.1111/apa.12540. PubMed PMID: 24354938.

41. Ismail MR, Ordi J, Menendez C, Ventura PJ, Aponte JJ, Kahigwa E, et al. Placental pathology in malaria: a histological, immunohistochemical, and quantitative study. Human pathology. 2000;31(1):85-93. Epub 2000/02/09. PubMed PMID: 10665918.

42. Pharmalys. Pharmalys CRO. 2021;Available at https://pharmalys.com/ [Accessed July 2021].

43. Zou G. A modified poisson regression approach to prospective studies with binary data. American journal of epidemiology. 2004;159(7):702-6. Epub 2004/03/23. PubMed PMID: 15033648.

44. Zou GY, Donner A. Extension of the modified Poisson regression model to prospective studies with correlated binary data. Statistical methods in medical research. 2013;22(6):661-70. Epub 2011/11/11. doi: 10.1177/0962280211427759. PubMed PMID: 22072596.

45. NHREC. Guidelines for Community Advisory Groups. NHREC. 2012;Available at : http://research.ukzn.ac.za/Files/National%20Guidelines%20for%20Community%20Advisory%20Groups%20for%20Research%20(2012).pdf [Accessed April 2021].

46. EMA. Guideline for Good Clinical Practice E6 (R2). EMA. 2015;Available at https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-good-clinical-practice-e6r2-4-step-2b_en.pdf [Accessed April 2021].

47. Alonso PL, Sacarlal J, Aponte JJ, Leach A, Macete E, Milman J, et al. Efficacy of the RTS,S/AS02A vaccine against Plasmodium falciparum infection and disease in young African children: randomised controlled trial. Lancet. 2004;364(9443):1411-20. Epub 2004/10/19. doi: 10.1016/s0140-6736(04)17223-1. PubMed PMID: 15488216.

48. UNAIDS. Mozambique country Fact sheet. 2015:Available at http://www.unaids.org/sites/default/files/media/documents/UNAIDS_GlobalplanCountryfactsheet_mozambique_en.pdf [accessed February 2017].

49. Sylla EH, Kun JF, Kremsner PG. Mosquito distribution and entomological inoculation rates in three malaria-endemic areas in Gabon. Trans R Soc Trop Med Hyg. 2000;94(6):652-6. Epub 2001/02/24. doi: 10.1016/s0035-9203(00)90219-0. PubMed PMID: 11198649.

50. Ministère-de-la-Santé-et-de-la-Prévoyance-sociale-Gabon. Rapport National sur la Réponse au VIH/SIDA 2014. 2015:Available at http://www.unaids.org/sites/default/files/country/documents/GAB_narrative_report_2015.pdf [accessed February 7].

51. Mourou JR, Coffinet T, Jarjaval F, Cotteaux C, Pradines E, Godefroy L, et al. Malaria transmission in Libreville: results of a one year survey. Malar J. 2012;11:40. Epub 2012/02/11. doi: 10.1186/1475-2875-11-40. PubMed PMID: 22321336; PubMed Central PMCID: PMCPMC3310827.

Supplemental Material 1. Study Informed consent form

Figure Legends:

Figure 1. MAMAH trial design

Tables

Table 1. Malaria and HIV epidemiology in the study sites

Site/ Country	Malaria Transmission	High season	EIR*	*P. falciparum* infection prevalence in women at delivery#	HIV prevalence in pregnant women	Frequency of MTCT of HIV
Manhiça† / Mozambique	Hypoendemic	Sep-Mar	21-50 [47]	6%	29%	**6%**[48]
Lambaréné‡/ Gabon	Mesoendemic	Oct-May	21-50[49]	11%	6%	**12%**[50]
Libreville/ Gabon**	Mesoendemic	Oct-May	21-50[51]	NI	**6%**	**12%**[50]

*EIR: Entomological Inoculation Rate

Data from 2010-2012 in women receiving either two IPTp doses of mefloquine or SP (Tuikue-Ndam et al, unpublished). NI: No information.

† The trial will be conducted at the ANC services of the Manhiça District Hospital where the *Centro de Investigação em Saúde de Manhiça* (CISM) is situated; the average monthly number of pregnant women attending first ANC clinic visit is 110.

‡ In Lambaréné, the trial will be conducted at the ANC services and maternity of the Albert Schweitzer Hospital by the Centre de Recherches Médicales de Lambaréné (CERMEL); the average monthly number of pregnant women attending first ANC clinic visit is 115.

** In Libreville, the trial will be conducted at the ANC services of *the Centre hospitalier Régional Estuaire de Melen- Unité de Recherche Clinique sur le Paludisme* and the Jeanne Ebori Hospital; the average monthly number of pregnant women attending first ANC clinic visit is 150.

Table 2. Study outcomes

Primary endpoint
Prevalence of maternal parasitaemia at delivery (defined by the presence of *P. falciparum* asexual parasites of any density in peripheral blood determined by microscopy)
Secondary endpoints
Maternal
Incidence of clinical malaria during pregnancy
Incidence of all-cause admissions
Incidence of all-cause outpatient attendances
Frequency and severity of adverse events (including cardiotoxic signals)
Mean haemoglobin concentration at delivery
Prevalence of submicroscopic *P. falciparum* peripheral parasitaemia at delivery
Prevalence of anaemia at delivery (Hb < 11 g/dL)
Prevalence of severe anaemia at delivery (Hb < 7 g/dL)
Mean CD4 + T cell counts levels at delivery
Proportion of women with detectable HIV viral load at delivery
Prevalence of placental *P. falciparum* infection (defined by the presence of parasites and/or pigment in the histological examination, or microscopic or sub-microscopic in the impression smear from placental blood)
Prevalence of *P. falciparum* peripheral parasitaemia at the post-partum visit
Maternal mortality rate
Infant
Prevalence of *P. falciparum* parasitaemia in cord blood (microscopic and sub-microscopic)
Prevalence of neonatal anaemia (Hb < 12,5 g/dL in cord blood, and Hb < 13g/dL in neonatal blood)
Mean birth weight
Prevalence of low birth weight (<2500 g)
Mean gestational age at birth
Prevalence of prematurity
Prevalence of embryo and foetal losses (miscarriages and stillbirths)
Prevalence of small for gestational age
Frequency of congenital malformations
Incidence of clinical malaria
Neonatal mortality rate
Frequency of mother to child transmission of HIV at one and at 12 months of age.
Infant mortality rate

Table 3. Schedule of enrolment, interventions and maternal assessments

	STUDY PERIOD							
	Pre-enrolment	**Allocation**	**First ANC clinic visit**	**Household visits**	**Monthly ANC visits**	**End of pregnancy**	**One month after end of pregnancy**	**Unscheduled visits**
TIMEPOINT	**0**	**0**	**1**	**+2 days**	**+1 month and then monthly**			
SCREENING AND ENROLMENT:								
Eligibility screen	X							
Informed consent	X							
Randomization		X						
INTERVENTIONS:								
IPTp administration			X	X	X			
CTX administration			X	*	*	*	*	*
ARV administration			X	*	*	*	*	*
LLITN distribution			X					
MATERNAL ASSESSMENTS:								
Demographics, medical history			X					X
Socio-economic characteristics				#		X		
Record of concomitant medication			X	X	X	X	X	X
Record of adverse			X	X	X	X	X	X

events						
Physical/clinical examination	X			X		X
Gestational age by ultrasound	X		X	X		X
Temperature					X	X
Blood pressure	X			X	X	X
Weight	X		X		X	X
Height	X					
MUAC	X				X	
RPR test	X					
CD4 count and HIV viral load	X			X		
Blood smear (malaria)	†		†	X	X	†
Haemoglobin test	X			X	X	
Intrapartum samples (cord blood, placenta)				X		
Drug tolerability assessment	X	X	X			
Compliance with LLITNs check		X	X	X	X	X

Only in the first household visit after the ANC visit of first IPTp administration.
* CTX and ARV adherence should be assessed at each scheduled visit.
† Only in women passively reporting sick AND presenting with malaria related signs/symptoms (fever (≥37,5° C) or having history of fever in the past 24 hours, arthromyalgia or headache), as per national management guidelines

Table 4. Schedule of infant visits and procedures

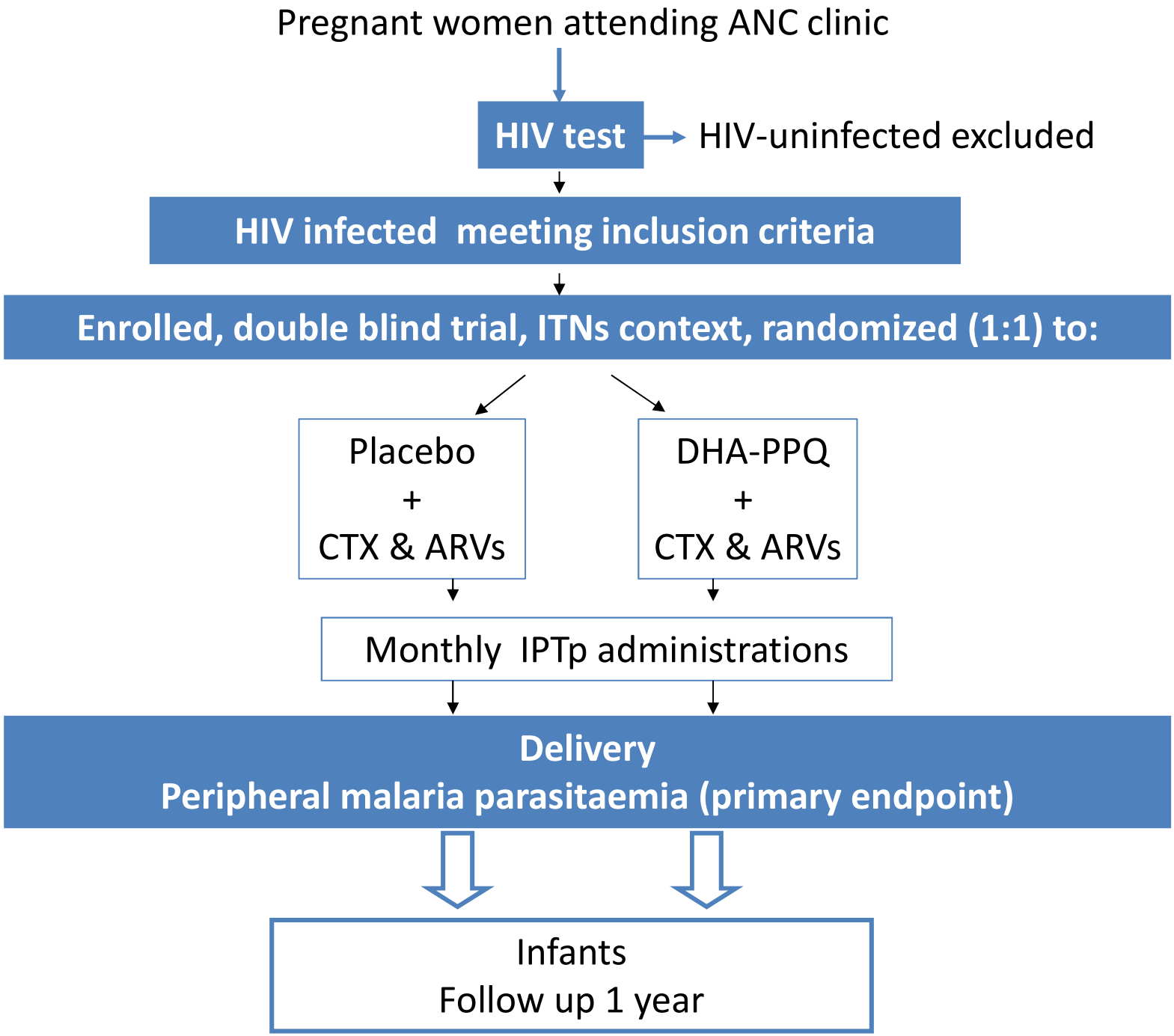
	TIMEPOINTS					
	Birth	**1 month***	**6 months**	**9 months**	**12 months**	**Unscheduled visits**
PROCEDURES:						
Medical history	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X
Psychomotor development assessment	X	X	X	X	X	
Weight	X	X	X	X	X	X
Height	X	X	X	X	X	X
Temperature	X	X	X	X	X	X
Blood smear	X	†	†	†	†	†
Haemoglobin test	X	†	†	†	†	†
HIV PCR ±		X	X	X	X	
Malaria PCR (filter paper)	X					
HIV prophylaxis adherence	#	#	#	#	#	#
HIV treatment adherence	#	#	#	#	#	#

* First visit will be scheduled 1 month after birth or coinciding with first EPI visit

† Only if fever (≥37,5° C) or history of fever in the past 24 hours or signs suggestive of malaria.

± HIV PCR test should also be repeated at month 18 after birth.

Adherence should be assessed at each visit.



MAMAH trial Informed Consent

This form covers the woman and infant. It must be signed by the woman participating in the study (and in some countries by a legal guardian according to national policies).

Title of the study

Evaluation of the safety and efficacy of dihydroartemisinine-piperaquine (DHA-PPQ) for intermittent preventive treatment of malaria in HIV-infected pregnant women.

Introduction

The Barcelona Institute for Global Health (ISGlobal) in Spain is coordinating a study to evaluate drugs to prevent malaria in pregnant women from Gabon and Mozambique.

The study will be testing if a drug called dihydroartemisinine-piperaquine (DHA-PPQ) can prevent pregnant women receiving cotrimoxazole and antiretroviral therapy from malaria while they are using treated mosquito nets. As you know, sometimes you may have malaria without feeling sick. Malaria may be hidden in the placenta and can cause baby to be born small and weak, even if you never feel sick. The baby may also be born too early. If the woman is infected with HIV, this increases even more her chances of getting malaria and makes it difficult to treat.

Right now pregnant women who are infected with the HIV receive cotrimoxazole to prevent infections (including malaria). Also, they must take antiretroviral therapy for controlling the infection and to avoid transmission of the virus to their baby while pregnant. The preventive antimalarial drug that is currently recommended to HIV-uninfected women, cannot be given to HIV-infected women because all the medicines received can have interaction with each other. That is why it is necessary to look for anti-malarial drugs to prevent malaria in pregnant women. Of the current available antimalarial drugs for pregnant women, dihydroartemisinine-piperaquine is the most promising.

You are being asked to participate because the initial screening makes you eligible to join the study. Before you decide if you wish to be in this study, you will be informed about the study and about things that you will be asked to do if you agree to join.

Purpose of the study and study groups

The information coming from this study will help to prevent malaria in African women infected with HIV.

The trial will compare dihydroartemisinine-piperaquine (DHA-PPQ) to placebo (a substance similar to DHA-PPQ but without any effect) as prevention for malaria in pregnancy together with using cotrimoxazole, antiretrovirals and insecticide treated mosquito nets. There will be 664 pregnant women from Mozambique and Gabon enrolled in this study.

Some women in the study will be receiving dihydroartemisinine-piperaquine and other placebo. Also you will be given cotrimoxazole and antiretrovirals to take with you home and administer one tablet every day to prevent any infection. Neither the study team nor you can pick the study group as this could affect the study results. You will be put into one of the two groups by chance.

Participants from both groups will have the same study visits. Before you learn about the study, it is important that you know that your participation in this study is voluntary and you may decide not to participate, not to have the tests, or to withdraw from the study at any time.

Let me explain to you what we mean by placebo. The placebo is a tablet that looks like dihydroartemisinine-piperaquine tablet but it does not have the ingredients that the dihydroartemisinine-piperaquine has and it will not prevent against malaria. You will receive either dihydroartemisinine-piperaquine or placebo by chance.

What happens during the study

If you agree to be in this study, your first visit will continue today, after you read, discuss, and sign or put thumbprint on this form.

You will be asked to come back to the clinic monthly before delivery. In addition, you must agree to deliver your baby at the study facility rather than at home. Because you have HIV virus, you will be offered drugs (antriretrovirals) for your treatment and for the prevention of mother to child transmission of HIV as per routine antenatal care and will be followed up as usual.

If you agree to be in this study:

- We will first ask you some questions about yourself and your health
- We will ask you to give information on where you live and how to keep in contact with you. The study staff will use this information to visit you at home to see how you are feeling and to remind you about your study visits.
- A study clinician will examine you and will check your pregnancy status
- You will also be asked to give a venous blood sample at the first visit for tests of your blood
- At the first study visit at the clinic, in the presence of the study nurse, you will take either dihydroartemisinine-piperaquine or placebo (assigned by chance)
- The following day and the day after you will be visited by study personnel at horme to complete the three day course treatment of DHA-PPQ
- Subsequent doses of DHA-PPQ or placebo will be given to you at the next scheduled monthly ANC clinic visit at least one month apart
- You will also receive cotrimoxazole and ARV drugs to take home and take it once a day as per routine ANC care
- In case you will be unwell with malaria or other infection, you will have additional blood tests done and if needed you will be given medicine and asked to come back here as scheduled by study staff
- At enrolment you will receive a long lasting insecticide treated net and will be told how to use it
- You and your baby will receive a unique identification number (ID) and identification study card, which you will be requested to present to the study staff at every visit
- Even though you will receive drug for malaria prevention (if in dihydroartemisinine-piperaquine group), it is possible that you may still get sick. Therefore you will be asked to come to the clinic whenever you feel unwell, get fever or any other symptoms.
- At delivery you will be visited during in the labour ward and you and your new-born baby will be examined by the study personnel.
- In addition to venous blood being collected from you, also a sample of cord blood will be taken to be tested for malaria
- A piece of placenta will be examined at the study laboratory and also tested for malaria
- Blood sample will be taken from your baby for malaria tests

- You must agree to deliver at the health facility but in case you deliver at home, the study staff will visit you as soon as possible but not later than one week after delivery and will ask you questions about your delivery and about health of your infant. At this visit you and your infant will be examined by the study personnel. Blood sample will be taken from you and your baby for tests of malaria
- When your baby is born, your child will be followed up until he/she is 12 months old
- You will be asked to come back with your new-born to the study clinic around 1, 6 , 9 and 12 months after delivery.
- One month after birth and at 12 months, a blood sample will be collected from your baby for HIV testing
- During these visits we will exam your baby to see if your baby is growing well
- If your child has signs of malaria, blood will be taken for tests and appropriate treatment given
- Study staff will visit you at home a few times after delivery to exam your baby

Unscheduled visits

- Throughout the twelve months of your baby's life she/he will be attended by the study staff when you bring her/him to the clinic. During those visits study nurses will exam your child and only if necessary take a sample of blood and provide treatment.
- You can come to the clinic at any time during this study. If you feel discomfort or are in pain, you should call the study staff or come to the clinic.
- You can also ask any questions at any point during the study, even during time other than your scheduled visit

Alternatives to joining the study

If you choose not to participate in this study or to leave the study after enrolment you are encouraged to come to this ANC for your routine visits and for any questions or concerns you may have related to your pregnancy. You will receive standard ANC care as before. We will refer you to another doctor if necessary.

Risks or discomforts *(mother and infant)*

Risks from blood draws

You will feel slight pain when we take blood from your finger or vein and your baby will feel slight pain if we take blood from the baby's heel. There will be no other risks to your new-born baby. Sometimes you may feel little dizzy or your head may feel light. There may be a small swelling of the skin where the needle went in. Those will go away in short time and the study personnel will examine you and your baby for those symptoms.

Risks from study drugs

Dihydroartemisinine-piperaquine is well tolerated when used to prevent malaria. Sometimes side effects are: headache, anaemia, fever, weakness, palpitations.

Benefits to you and your infant

By participating in the study, you may get better diagnosis of malaria because of increased number of tests for malaria. You and your baby will be regularly seen by clinical staff and in case of any symptoms or abnormal test results you and your baby will be either treated here or referred to another clinic for medical care.

Reasons for taking you out of the study without your consent

You may be removed from the study without your consent for the following reasons:

- You are found to not be eligible for the study

- The research study is stopped or cancelled
- The study staff feels that staying in the study would be harmful to you
- You are not able to attend visits or complete the study tests
- Other administrative reasons that will be made clear to you

Costs to you

There is no cost to you for participating in the study. Treatments available to you from the study for malaria will be given to you free of charge.

Your records will be private

Efforts will be made to keep your personal information as confidential as it is possible and allowed by the law. You and your baby will be identified by a study participant ID number and personal information from you and your baby records will not be released without your written permission. You and your baby will not be personally identified in any publication about this study. Your records may be reviewed by: study monitors, study staff, study auditors, sponsors, and Ethics Committees.

To ensure your medical safety, study staff may verify, from time to time, that you are not enrolled in any other research studies. In addition, if needed, we will request permission to access non-study medical records related to any of your illness. All copies of your records will be kept in a locked file cabinet. Only study staff will have access to this cabinet.

New findings

You will be told of any new information learned during the study that might cause you to change your mind about staying in the study.

Injury because of being in the study

Based upon what we know, it is unlikely that you will be injured as a result of being in this study. It is important that you tell the study staff if you feel that you have been injured because of taking part in this study. If you are injured as a result of being in this study, the study clinic will give you immediate necessary treatment for your injuries. The cost of this treatment will not be charged to you. You will then be told where you may receive additional treatment for injuries.

When you sign this consent form, you do not give up your rights to care for injury caused by being in the study.

Contact information

You will be given a copy of this form to take with you.

If you ever have questions about this study or in case you are injured as a result of participation in this study, you should contact: **Principal Investigator**, ***[each site will provide name and phone number of their site PI]***

Your rights as a study participant

This research has been reviewed and approved by the Ethics Committee of the Hospital Clinic of Barcelona, Spain and the local IRB in your country. These committees have reviewed this study in order to help protect participants.

If you accept to participate in this study please answer to the following questions:

1. What kind of treatments can you receive during the study?
2. In addition to the drugs, what else will you receive to prevent malaria?
3. Can you recall how many times we will ask you to give a blood sample? And how many times your child will be finger-pricked?
4. Can you recall how many times in the presence of study nurse you will take dihydroartemisinine-piperaquine or placebo to prevent malaria?
5. When do you have to bring your child to the study clinic after birth?
6. Can you get malaria even if you participate in the study? And your baby?
7. Where do you have to go if you or baby has fever?

STATEMENT of CONSENT AND SIGNATURE

Participant and new-born approval:
The consent form has been explained to me and I agree to take part in this study. I also agree to let my new-born baby take part in this study. I understand that I am free to choose to be in the study and that saying "No" will not affect the treatment I get in this clinic, now and in future.
NOTE: You are not giving up any of your legal rights by signing this informed consent document.

If you agree circle YES

Volunteer's Name *(print)*	Volunteer's Signature or Thumbprint *(if cannot write)*	Date
Volunteer's Legal Guardian or Representative (as per country policy) *(print)*	Legal Guardian's Signature	Date
Witness's Name (if participant illiterate) *(print)*	Witness's Signature	Date

I have explained the purpose of this study to the volunteer. To the best of my knowledge, she understands the purpose, procedures, risks and benefits of this study.

Investigator/Designee Name *(print)*	Investigator/Designee Signature	Date

NOTE: This consent form with original signatures must be retained on file by the principal investigator. A copy must be given to the volunteer.

If the woman refuses to take her copy of the consent with her, she states so below and signs and dates her decline statement.

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	5
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	5
Funding	4	Sources and types of financial, material, and other support	18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,2,3, 18
	5b	Name and contact information for the trial sponsor	3
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	18
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	7,8,9
	6b	Explanation for choice of comparators	7,8
Objectives	7	Specific objectives or hypotheses	9
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	9
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9,10
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	10
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11,12
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	n/a
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	14,15
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12-15
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	13, Table 2
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	11,12, Figure 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	13
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	13
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10-11
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10-11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10-11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10-11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	11
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	17-18
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14-15-17

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	17
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	18
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	18
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	18
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	14-15
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	18
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14-15
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	19
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	19

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	17
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	20
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	19
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	19
	31b	Authorship eligibility guidelines and any intended use of professional writers	20
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	19
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplemental Material 1
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.