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# 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)**

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

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31 **Word count: 3286**

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3 **33 Abstract**  
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6 **34 Introduction:** Malaria infection during pregnancy is an important driver of maternal and  
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8 **35** neonatal health especially among HIV-infected women. Intermittent preventive treatment in  
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10 **36** pregnancy (IPTp) with sulphadoxine-pyrimethamine is recommended for malaria prevention in  
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12 **37** HIV-uninfected women but it is contraindicated in those HIV-infected on cotrimoxazole  
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14 **38** prophylaxis (CTXp) due to potential adverse effects. Consequently, the women most vulnerable  
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16 **39** to malaria are currently the least protected. Dihydroartemisinin-piperaquine (DHA-PPQ),  
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18 **40** because of its long half-life and good tolerability has been shown to improve antimalarial  
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20 **41** protection in HIV-uninfected pregnant women, constituting the most promising candidate for  
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22 **42** IPTp in HIV-infected pregnant women. The objective of the trial is to determine if monthly three-  
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24 **43** day IPTp courses of DHA-PPQ added to daily CTXp are safe and superior to CTXp alone in  
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26 **44** decreasing the proportion of peripheral malaria parasitaemia at the end of pregnancy in HIV-  
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28 **45** infected women.  
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34 **46 Methods and analysis:** This is a multi-centre, two-arm, placebo-controlled, individually  
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36 **47** randomized trial in HIV-infected women receiving CTXp and antiretroviral (ARV) treatment. A  
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38 **48** total of 664 women will be enrolled at the first antenatal care clinic visit in sites from Gabon and  
39  
40 **49** Mozambique where malaria and HIV infection are moderate to highly prevalent. Participants  
41  
42 **50** will receive an insecticide-treated net and they will be administered monthly IPTp with DHA-  
43  
44 **51** PPQ or placebo (1:1 ratio) as directly observed therapy from the second trimester of pregnancy.  
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46 **52** Participants will be followed until six weeks after the end of pregnancy and their infants until  
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48 **53** one year of age to also evaluate the impact of DHA-PPQ on mother to child transmission of HIV.  
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53 **54 Ethics and dissemination:** The project was reviewed and approved by the institutional and  
54  
55 **55** national ethics committees of Gabon (042/2018/PR/SG/CNER) and Mozambique  
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57 **56** (504/CBNS/18) and the Hospital Clinic of Barcelona (HCB/2018/0650,Spain). Project results will  
58  
59 **57** be presented to all stakeholders and published in open access journals.  
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3 58 **Trial registration:** ClinicalTrials.gov, NCT03671109. Registered on 14<sup>th</sup> September 2018.  
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3 60 **Strengths and limitations of this study**  
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- 6 61 • A major strength of this trial is its double blind placebo-controlled design which will allow to  
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8 62 yield conclusive results about the efficacy of the study intervention.  
9  
10 63 • The inclusion of pregnant women from different sub-Saharan countries will provide a wide  
11  
12 64 representation of different malaria endemicity areas and HIV subgroups.  
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14 65 • The study is also adequately powered to test the superiority hypothesis.  
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16 66 • On the other hand, the study sample size will not be sufficient to test the superiority  
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18 67 hypothesis in secondary infant outcomes (only differences between arms will be analysed).  
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26 69 **Keywords**  
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29 70 Malaria, HIV, pregnancy, prevention, treatment.  
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## 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

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97 it cannot be administered to the most vulnerable group, HIV-infected women [13-16].

98 Consequently and paradoxically the most susceptible women to malaria are currently the least  
99 protected [17].

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

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

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

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3 122 with monthly dosing [24]. Scientific evidence shows that efficacy and safety findings from  
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5 123 malaria control strategies evaluated in HIV-uninfected pregnant women cannot be directly  
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7 124 extrapolated to HIV-infected women [18]. A trial comparing monthly IPTp with DHA-PPQ to CTXp  
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9 125 among 200 HIV-infected Ugandan women did not find differences in the risk of  
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11 126 histopathologically detected placental malarial infection and other outcomes between groups  
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14 127 [25]. However, authors acknowledge the limitations of their results due to the low prevalence  
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16 128 of malaria in the study area at the time of the trial [25]. Therefore, it is of highest public health  
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18 129 priority to provide conclusive evidence as to whether the most vulnerable population (HIV-  
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20 130 infected pregnant women) will benefit from the use of the currently most promising and  
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22 131 available alternative drug for IPTp, DHA-PPQ [17].  
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26 132 The objectives of the trial are (1) to evaluate the safety, tolerability and efficacy of DHA-PPQ as  
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28 133 IPTp for malaria prevention in HIV-infected pregnant women receiving daily CTXp and ARV  
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30 134 drugs, (2) to assess the effect of DHA-PPQ as IPTp on mother to child transmission of HIV and (3)  
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32 135 to evaluate the effectiveness of CTXp in clearing malaria parasites in HIV-infected pregnant  
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34 136 women.  
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## 41 138 **2. Methods**

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45 139 This is multi-centre, two-arm, placebo-controlled, individually randomized superiority clinical  
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47 140 trial with two study arms including HIV-infected pregnant women. The study will be carried out  
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49 141 and reported according to Consolidated Standards of Reporting Trials guidelines [26].  
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### 52 142 ***Study settings***

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55 143 The trial will be conducted in two countries from Central and South Eastern sub-Saharan Africa  
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57 144 (Gabon and Mozambique), where HIV prevalence among pregnant women ranges from 6 to 29%  
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59 145 [27, 28]. Malaria epidemiologic indicators and HIV prevalence in pregnancy in study sites are  
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3 146 shown in Table 1. The trial sites have been selected to provide representation of different  
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5 147 malaria endemicity and HIV subgroups (HIV-1 subtype C in Mozambique and HIV-1 CRF02\_AG in  
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7 148 Gabon, where HIV-2 also circulates [29, 30]).  
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### 10 11 149 ***Study population***

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14 150 All pregnant women attending the study ANC services for the first time and/or who have not  
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16 151 received IPTp during their current pregnancy will be screened for participation in the trial.  
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18 152 Inclusion criteria are (1) permanent resident in the study area, (2) gestational age at the first  
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20 153 antenatal visit  $\leq$  28 weeks, (3) HIV seropositive status and (4) agreement to deliver in the study  
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22 154 site's maternity(ies) wards. Exclusion criteria are (1) residence outside the study area or planning  
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24 155 to move out in the following 10 months from enrolment, (2) gestational age at the first antenatal  
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26 156 visit  $>$  28 weeks of pregnancy, (3) known history of allergy to CTX, (4) known history of allergy  
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28 157 or contraindications to DHA-PPQ and (5) participating in other intervention studies.  
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### 31 32 33 158 ***Informed consent***

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36 159 All participants will receive information about study procedures, including knowledge about  
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38 160 malaria and HIV infection in pregnancy. A signed informed consent (or thumb-printed with a  
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40 161 witness whenever the woman is illiterate) will be obtained before any study tests or evaluations  
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42 162 are carried out. If the participant is under the legal age of maturity, she will sign the assent form  
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44 163 and her legal guardian will sign the informed consent according to national ethics local policies.  
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46 164 The informed consent will cover the woman and the new born infant.  
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### 50 51 165 ***Recruitment and randomization***

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54 166 After the study details are explained and informed consent is signed, pregnant women will be  
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56 167 given a study number and automatically randomized to one of the study arms: 1) Daily CTX +  
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58 168 monthly IPTp-DHA-PPQ or 2) Daily CTX + Monthly IPTp-placebo. Each participant will be uniquely  
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3 169 identified in the study by a combination of her site code and participant number. Allocation of  
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5 170 participants to study arms will be done centrally by the trial's Sponsor (the Barcelona Institute  
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7 171 for Global Health, ISGlobal) by block randomization and stratified by country. This method will  
8  
9 172 ensure balanced allocation to both arms during different malaria seasons in the two study  
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11 173 countries. Each subject number will be related to a treatment number which assigns them to  
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13 174 one of the IPT arms. Study number allocation for each study participant will be concealed in  
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15 175 opaque sealed envelopes that will be opened only after recruitment. At each site the first  
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17 176 participant will be assigned a patient number, and consecutive numbers will be assigned to  
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19 177 subsequent women. A study identification card containing the individual study number and  
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21 178 basic demographic information will be given to the participant in order to facilitate identification  
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23 179 at all study contacts.

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29 180 Figure 1 displays the study design.

### 30 31 32 181 **Blinding**

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35 182 Study tablets (IPTp-DHA-PPQ and IPTp-Placebo) will be identically packaged. Study personnel  
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37 183 will prepare and deliver the medication to the participant. All study personnel, investigators,  
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39 184 outcome assessors, data analysts and the participants will remain blinded throughout the trial.

### 40 41 42 43 185 **Interventions**

#### 44 45 46 186 **a) IPTp administration**

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49 187 Administration of the three-day IPTp course will always be done under fasting conditions and  
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51 188 direct observation by study personnel. The number of daily IPTp tablets to be administered will  
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53 189 be based on bodyweight and according to the treatment guidelines set by WHO [target dose  
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55 190 (range) of 4 (2–10) mg/kg/ day of DHA and 18 (16–27) mg/kg/day of PPQ given once a day for  
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57 191 three days for adults]. Following physical examination, recruited women of gestational age $\geq$ 13  
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3 192 weeks will receive the assigned IPTp drug. In case of gestational age < 13 weeks, first IPTp  
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5 193 administration will be scheduled one month later. If participants report malaria treatment in the  
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7 194 preceding four weeks, first IPTp administration will also be delayed one month. Administration  
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10 195 of the second and third day treatment course will be done by study personnel either at the study  
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12 196 health facility or household level. Women will be observed for 60 minutes after administration  
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14 197 of the IPTp dose. Those women vomiting within the first 30 minutes of IPTp administration will  
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16 198 be given a second full IPTp dose; women vomiting after 30-60 minutes of IPTp administration  
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18 199 will be given an additional half dose of the drug. Subsequent doses of IPTp will be given  
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20 200 coinciding with the next scheduled monthly ANC clinic visit, at least one month apart from the  
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23 201 previous dose.

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26 202 ***b) CTX administration***

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29 203 Cotrimoxazole (fixed combination drug containing 800 mg of trimethoprim and 160 mg of  
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31 204 sulfamethoxazole) will be taken daily from enrolment until delivery as per national guidelines  
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33 205 for prevention of opportunistic infections. Women will be asked to visit the ANC clinic monthly  
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35 206 to receive the amount of CTX tablets for the whole month. At each ANC clinic visit, adherence  
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37 207 to CTX prophylaxis will be assessed.

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41 208 ***c) ARV therapy and concomitant medications***

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45 209 Treatment for prevention of HIV MTCT (Option B+) will be given by the study health personnel,  
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47 210 according to national HIV/AIDS control guidelines[31]. Any other concomitant treatment  
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49 211 received by the study participants will be recorded in the study questionnaires.

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52 212 ***d) Long lasting insecticide treated nets***

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56 213 Regardless of gestational age at the time of recruitment, all women will receive a LLITN and  
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58 214 details about its use will be explained.

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3 215 **Study Outcomes**  
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6 216 The primary outcome of the trial will be the prevalence of maternal parasitaemia at delivery  
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8 217 defined by the presence of *Plasmodium falciparum* (*P. falciparum*) asexual parasites of any  
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10 218 density in peripheral blood (determined by microscopy). The secondary maternal and infant  
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13 219 endpoints can be found in Table 2.  
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16 220 **Sample size**  
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19 221 Based on previous estimations at the study sites and assuming a prevalence of peripheral  
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21 222 parasitaemia at delivery of 7.5% with CTXp, it is estimated that 298 women per arm will be  
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23 223 required to detect with 80% power a significant ( $p < 0.05$ ) decrease of 5% or more in the  
24  
25 224 prevalence of peripheral parasitaemia in the CTXp+IPTp-DHA-PPQ group [18]. In order to allow  
26  
27 225 for 10% losses to follow up, it is calculated that 332 women/study arm will need to be recruited  
28  
29 226 (total  $n=664$ ). Furthermore, assuming a 5% MTCT-HIV in the control group, this sample size will  
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31 227 have an 80% power to detect at the 5% level of significance, 2.2 times difference in the risk of  
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33 228 MTCT-HIV. Considering the prevalence of HIV infection among pregnant women in both sites  
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35 229 (please refer to Table 1), a total of 444 women are planned to be recruited in Mozambique and  
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37 230 220 women in Gabon to allow for a recruitment rate of 33 women/month on average. These  
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39 231 estimations are based on recruitment rates of previous IPTp clinical trials conducted among  
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41 232 pregnant women in the two study sites [18, 32, 33].  
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47 233 **Follow up and measurements of outcomes**  
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50 234 At baseline, the woman's demographic and obstetric information will be recorded in study  
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52 235 specific case report forms (CRF).  
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56 236 **a) Physical and clinical examination at enrolment**  
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3 237 The physical examination of the woman will include the following assessments: weight, height,  
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5 238 gestational age by bimanual palpation and measurement of middle-upper arm circumference  
6  
7 239 (MUAC). Ultrasound (US) will be performed to determine gestational age at enrolment and  
8  
9 240 confirm pregnancy viability.  
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13 241 ***b) Baseline biological samples***  
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16 242 At enrolment, a venous blood sample will be collected for analysis of haemoglobin level, CD4  
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18 243 cell counts, HIV viral load and malaria PCR.  
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21 244 ***c) Follow-up and household visits***  
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24 245 Women will be given an appointment to attend the subsequent ANC clinic visit one month after  
25  
26 246 the first one. The subsequent IPTp doses will be given at least four weeks apart from the previous  
27  
28 247 one. Study participants will be asked to visit the study facilities in case of any illness. Women  
29  
30 248 will be visited at home the day after recruitment to confirm residence status, assess drug  
31  
32 249 tolerability and the correct use of the net. Adherence to CTX prophylaxis, ARV therapy and  
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34 250 compliance with the LLITNs use will be assessed monthly at the ANC attendance.  
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39 251 ***d) Adverse events monitoring and reporting***  
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42 252 Active safety monitoring will consist in household visits to study participants two days after each  
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44 253 IPTp administration to assess drug tolerability and record all Adverse Events (AEs). In addition,  
45  
46 254 a health facility-based passive surveillance system will be established to capture unscheduled  
47  
48 255 visits of participants during follow-up. Information on unsolicited adverse events will be  
49  
50 256 collected at each scheduled and unscheduled visit. Any participant passively reporting being sick  
51  
52 257 during the study visits, will be referred to the clinical services as per routine system in place. A  
53  
54 258 blood smear will be collected in those presenting with malaria related signs/symptoms (fever  
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56 259 ( $\geq 37,5^{\circ}$  C) or having history of fever in the past 24 hours, arthromyalgias or headache). In case  
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3 260 of malaria parasitaemia or anaemia, they will be treated following national guidelines. Serious  
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5 261 Adverse Events (SAEs) will be reported by the site investigator within 24 hours of being made  
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7 262 aware to the trial's safety monitor and the Data Safety Monitoring Board (DSMB).  
8  
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11 263 ***e) End of pregnancy and infant assessments***  
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13  
14 264 At the end of pregnancy, maternal blood, placental and cord blood samples will be collected for  
15  
16 265 haematological and parasitological examination. The new-born will be examined, weighed and  
17  
18 266 measured and his/her gestational age assessed by the modified Ballard method [34].  
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22 267 ***f) Post-partum visit***  
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24  
25 268 Participants will be visited approximately after six weeks of end of pregnancy at the study health  
26  
27 269 facility where a blood smear for malaria screening will be collected. A summary of study  
28  
29 270 procedures is displayed in Table 3.  
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33 271 ***g) Infants follow up***  
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35  
36 272 Infants born to study participants will be followed up until one year of age. Mothers will be asked  
37  
38 273 to bring their child to the study facilities at weeks 4-6 of age and at 6, 9 and 12 months after  
39  
40 274 birth. At each scheduled visit, study infants will be physically examined and their psychomotor  
41  
42 275 and neurological development will be assessed following a standard protocol for African settings  
43  
44 276 [35-37]. Weight, height and axillary temperature will be measured and recorded. A capillary  
45  
46 277 blood sample will be taken from infants with fever (axillary temperature  $\geq 37.5^{\circ}\text{C}$ ) or history of  
47  
48 278 fever in the last 24 hours, or appearing pale, for malaria parasitaemia examination and  
49  
50 279 haematological determination. In case of malaria parasitaemia or anaemia, they will be treated  
51  
52 280 following national guidelines. At each scheduled visit, HIV DNA will be evaluated in the infant  
53  
54 281 from dried blood spot on filter paper (for PCR). As per routine healthcare national programs HIV-  
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56 282 exposed infants will receive CTX prophylaxis starting four to six weeks after birth. Infants will  
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3 283 also receive ARV, according to national guidelines. Infant's follow up visits and study procedures  
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5 284 are described in Table 4.  
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9 285 **Data management**

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11 286 Data are collected using case report forms (CRFs) developed for the study. Data is double  
12  
13 287 entered into the study database using the OpenClinica open source software (version 3.14) for  
14  
15 288 clinical data management (www.OpenClinica.com) at each research site. Automatic quality  
16  
17 289 checks are performed to ensure CRF completeness. Concomitant medications registered into  
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19 290 the database are coded using the WHO Drug Reference List, which employs the Anatomical  
20  
21 291 Therapeutic Chemical classification system. Medical history/current medical conditions and  
22  
23 292 adverse events are coded using the Medical dictionary for regulatory activities (MedDRA)  
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25 293 terminology.  
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29 294 **Analysis plan**

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32 295 The following populations of analysis have been defined: a) Intention to treat (ITT): it includes  
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34 296 all randomized pregnant women (target population for the efficacy analysis); b) According to  
35  
36 297 protocol (ATP): it includes participants who fulfil all the inclusion-exclusion criteria, took monthly  
37  
38 298 IPTp-DHA-PPQ/placebo study doses, received a LLITN, received CTXp and ARV drugs and from  
39  
40 299 whom data is available for the analysis; c) Safety: it includes participants who received at least  
41  
42 300 one dose of IPTp DHA-PPQ/placebo and had at least one post- baseline safety assessment. The  
43  
44 301 primary analysis of the trial will be the comparison of the proportion of pregnant women with  
45  
46 302 peripheral parasitaemia at delivery in the ITT and ATP cohorts, adjusted by gravidity, country,  
47  
48 303 seasonality and other variables associated with the prevalence of malaria. Proportions will be  
49  
50 304 compared between groups using the Fisher exact test and presented as relative risk ratio (RR)  
51  
52 305 or reduction of the RR ( $1 - RR * 100\%$ ) if RR lower than 1. Adjustment for co-variates and possible  
53  
54 306 confounders will be done using Poisson regression with a log link and robust estimate of the  
55  
56 307 covariance (Huber method), using the method proposed by Zou [38, 39]. Continuous variables  
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3 308 will be compared between groups using Wilcoxon rank sum test and the effect presented as  
4  
5 309 Mean Difference. Adjustment for co-variables and possible cofounders will be done using  
6  
7 310 ordinary least square regression. Incidence of clinical malaria, overall admissions and outpatient  
8  
9 311 attendances will be estimated as the number of episodes over the time at risk. Time at risk will  
10  
11 312 be estimated as the time from the start of follow up until the end of follow-up (visit one month  
12  
13 313 after end of pregnancy for mothers) or withdrawal due to censoring or death, whatever occurs  
14  
15 314 first. The total number of events will be compared between groups using Negative Binomial  
16  
17 315 regression models which takes into account a possible extra Poisson variation due to different  
18  
19 316 frailty of the subjects. The comparison will be expressed as relative rate ratio (RRate).

#### 23 24 317 ***Patient and public involvement***

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26  
27 318 Patients will not be directly involved in the design, conduct, reporting, or dissemination plans of  
28  
29 319 the study. However, a Community Advisory Board (CAB) has been set at each study site to  
30  
31 320 strengthen communication and interaction between the local study community and research  
32  
33 321 teams. The CAB also oversees and guides the study team on key issues such as potential risks  
34  
35 322 and burdens for participants or host communities that may be hidden from researchers, and  
36  
37 323 how to minimize them [40].

#### 38 39 40 41 324 ***Ethics and dissemination***

42  
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44  
45 325 The study is conducted in accordance with the EMA/ICH Guideline on Good Clinical Practice  
46  
47 326 and in total agreement with the applicable international, EU and national law of all the  
48  
49 327 participating countries[41]. The study protocol and the informed consent forms have been  
50  
51 328 reviewed and approved by the institutional and national ethics committees of Gabon  
52  
53 329 (042/2018/PR/SG/CNER) and Mozambique (504/CBNS/18) and the Hospital Clinic of  
54  
55 330 Barcelona (HCB/2018/0650,Spain). An independent DSMB monitors regularly the safety of  
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3 331 study participants. The trial is registered on clinicaltrials.gov  
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5 332 (<https://clinicaltrials.gov/ct2/show/NCT03671109>).

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8 333 A project communication has been developed in order to ensure timely, accurate, and effective  
9  
10 334 dissemination of project results. After concluding the trial's data analysis, findings will be made  
11  
12 335 available to all partners, key stakeholders and Ministries of Health. The project members will  
13  
14 336 actively disseminate information to the scientific community through reports, presentations at  
15  
16 337 scientific forums and publications in international open access journals. Trial results will also be  
17  
18 338 shared with the WHO Global Malaria Program and Malaria Policy Advisory Groups.  
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25 340 **Authors' contributions**

26  
27 341 RG and CM conceived the concept of the study. RG and CPD wrote the first draft of the  
28  
29 342 manuscript. RG and SS wrote sections on sample size, data management and analysis plan. All  
30  
31 343 authors participated in the design of the study, reviewed and approved the manuscript.  
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49  
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56 354 **Competing interests**

57  
58 355 None declared.  
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356 **References**

- 357 1. WHO. World Malaria report 2020. WHO. 2020;Available at  
358 <https://www.who.int/publications/i/item/9789240015791> [Accessed March 2021].
- 359 2. Mombo-Ngoma G, Mackanga JR, Gonzalez R, Ouedraogo S, Kakolwa MA, Manego RZ, et  
360 al. Young adolescent girls are at high risk for adverse pregnancy outcomes in sub-Saharan Africa:  
361 an observational multicountry study. *BMJ open*. 2016;6(6):e011783. Epub 2016/07/01. doi:  
362 10.1136/bmjopen-2016-011783. PubMed PMID: 27357200.
- 363 3. Group WAbCTAABS. Clinical determinants of early parasitological response to ACTs in  
364 African patients with uncomplicated falciparum malaria: a literature review and meta-analysis  
365 of individual patient data. *BMC Med*. 2015;13:212. Epub 2015/09/08. doi: 10.1186/s12916-015-  
366 0445-x. PubMed PMID: 26343145; PubMed Central PMCID: PMC4561425.
- 367 4. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoah K, Brabin B, et al. Epidemiology  
368 and burden of malaria in pregnancy. *Lancet Infect Dis*. 2007;7(2):93-104. Epub 2007/01/26. doi:  
369 S1473-3099(07)70021-X [pii]  
370 10.1016/S1473-3099(07)70021-X [doi]. PubMed PMID: 17251080.
- 371 5. Menendez C, D'Alessandro U, ter Kuile FO. Reducing the burden of malaria in pregnancy  
372 by preventive strategies. *Lancet Infect Dis*. 2007;7(2):126-35. Epub 2007/01/26. doi: S1473-  
373 3099(07)70024-5 [pii]  
374 10.1016/S1473-3099(07)70024-5 [doi]. PubMed PMID: 17251083.
- 375 6. Menendez C, Ordi J, Ismail MR, Ventura PJ, Aponte JJ, Kahigwa E, et al. The impact of  
376 placental malaria on gestational age and birth weight. *J Infect Dis*. 2000;181(5):1740-5. Epub  
377 2000/05/24. doi: 10.1086/315449. PubMed PMID: 10823776.
- 378 7. UNAIDS/WHO. Global report: UNAIDS report on the global AIDS Epidemic 2013. WHO  
379 Library Cataloguing-in-Publication Data 2013;UNAIDS(JC2502/1/E).
- 380 8. WHO. Malaria and HIV interactions and their implications for public health policy WHO.  
381 2005;ISBN 92 4 1593350.
- 382 9. ter Kuile FO, Parise ME, Verhoeff FH, Udhayakumar V, Newman RD, van Eijk AM, et al.  
383 The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant  
384 women in sub-saharan Africa. *Am J Trop Med Hyg*. 2004;71(2 Suppl):41-54. PubMed PMID:  
385 15331818.
- 386 10. Gonzalez R, Ataide R, Nanche D, Menendez C, Mayor A. HIV and malaria interactions:  
387 where do we stand? *Expert Rev Anti Infect Ther*. 2012;10(2):153-65. doi: 10.1586/eri.11.167.  
388 PubMed PMID: 22339190.
- 389 11. WHO. Intermittent Preventive Treatment of malaria in pregnancy using Sulfadoxine-  
390 Pyrimethamine (IPTp-SP). Updated WHO Policy Recommendation. WHO.  
391 2012;[http://www.who.int/malaria/iptp\\_sp\\_updated\\_policy\\_recommendation\\_en\\_102012.pdf](http://www.who.int/malaria/iptp_sp_updated_policy_recommendation_en_102012.pdf)  
392 .
- 393 12. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and  
394 preventing HIV infection Recommendations for a public health approach June 2013. 2013.
- 395 13. Sevene E, Gonzalez R, Menendez C. Current knowledge and challenges of antimalarial  
396 drugs for treatment and prevention in pregnancy. *Expert Opin Pharmacother*. 2010;11(8):1277-  
397 93. PubMed PMID: 20408744.
- 398 14. Ward SA, Sevene EJ, Hastings IM, Nosten F, McGready R. Antimalarial drugs and  
399 pregnancy: safety, pharmacokinetics, and pharmacovigilance. *Lancet Infect Dis*. 2007;7(2):136-  
400 44. Epub 2007/01/26. doi: S1473-3099(07)70025-7 [pii]  
401 10.1016/S1473-3099(07)70025-7 [doi]. PubMed PMID: 17251084.

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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. Peki 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: PMC4847718.
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: PMC5266794.
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: PMC5853208.
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.



- 1  
2  
3 453 28. Manego RZ, Mombo-Ngoma G, Witte M, Held J, Gmeiner M, Gebru T, et al. Demography,  
4 454 maternal health and the epidemiology of malaria and other major infectious diseases in the rural  
5 455 department Tsamba-Magotsi, Ngounie Province, in central African Gabon. *BMC Public Health*.  
6 456 2017;17(1):130. Epub 2017/01/29. doi: 10.1186/s12889-017-4045-x. PubMed PMID: 28129759;  
7 457 PubMed Central PMCID: PMC5273856.  
8 458 29. Caron M, Lekana-Douki SE, Makuwa M, Obiang-Ndong GP, Biba O, Nkoghe D, et al.  
9 459 Prevalence, genetic diversity and antiretroviral drugs resistance-associated mutations among  
10 460 untreated HIV-1-infected pregnant women in Gabon, central Africa. *BMC infectious diseases*.  
11 461 2012;12:64. Epub 2012/03/22. doi: 10.1186/1471-2334-12-64. PubMed PMID: 22433277;  
12 462 PubMed Central PMCID: PMC3359209.  
13 463 30. Lahuerta M, Aparicio E, Bardaji A, Marco S, Sacarlal J, Mandomando I, et al. Rapid spread  
14 464 and genetic diversification of HIV type 1 subtype C in a rural area of southern Mozambique. *AIDS*  
15 465 *Res Hum Retroviruses*. 2008;24(2):327-35. PubMed PMID: 18271719.  
16 466 31. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and  
17 467 preventing HIV infection 2016. Recommendations for a public health approach. Second edition.:  
18 468 World Health Organization; 2016.  
19 469 32. Gonzalez R, Mombo-Ngoma G, Ouedraogo S, Kakolwa MA, Abdulla S, Accrombessi M, et  
20 470 al. Intermittent Preventive Treatment of Malaria in Pregnancy with Mefloquine in HIV-Negative  
21 471 Women: A Multicentre Randomized Controlled Trial. *PLoS Med*. 2014;11(9):e1001733. Epub  
22 472 2014/09/24. doi: 10.1371/journal.pmed.1001733. PubMed PMID: 25247709.  
23 473 33. Menendez C, Bardaji A, Sigauque B, Romagosa C, Sanz S, Serra-Casas E, et al. A  
24 474 randomized placebo-controlled trial of intermittent preventive treatment in pregnant women  
25 475 in the context of insecticide treated nets delivered through the antenatal clinic. *PloS one*.  
26 476 2008;3(4):e1934. Epub 2008/04/10. doi: 10.1371/journal.pone.0001934 [doi]. PubMed PMID:  
27 477 18398460; PubMed Central PMCID: PMC2277457.  
28 478 34. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R. New Ballard Score,  
29 479 expanded to include extremely premature infants. *The Journal of pediatrics*. 1991;119(3):417-  
30 480 23. Epub 1991/09/01. PubMed PMID: 1880657.  
31 481 35. Gladstone M, Lancaster GA, Umar E, Nyirenda M, Kayira E, van den Broek NR, et al. The  
32 482 Malawi Developmental Assessment Tool (MDAT): the creation, validation, and reliability of a  
33 483 tool to assess child development in rural African settings. *PLoS Med*. 2010;7(5):e1000273. Epub  
34 484 2010/06/04. doi: 10.1371/journal.pmed.1000273. PubMed PMID: 20520849; PubMed Central  
35 485 PMCID: PMC2876049.  
36 486 36. Koura KG, Boivin MJ, Davidson LL, Ouedraogo S, Zoumenou R, Alao MJ, et al. Usefulness  
37 487 of child development assessments for low-resource settings in francophone Africa. *Journal of*  
38 488 *developmental and behavioral pediatrics : JDBP*. 2013;34(7):486-93. Epub 2013/08/01. doi:  
39 489 10.1097/DBP.0b013e31829d211c. PubMed PMID: 23899660; PubMed Central PMCID:  
40 490 PMC3821168.  
41 491 37. Prado EL, Abubakar AA, Abbeddou S, Jimenez EY, Some JW, Ouedraogo JB. Extending  
42 492 the Developmental Milestones Checklist for use in a different context in Sub-Saharan Africa.  
43 493 *Acta paediatrica (Oslo, Norway : 1992)*. 2014;103(4):447-54. Epub 2013/12/21. doi:  
44 494 10.1111/apa.12540. PubMed PMID: 24354938.  
45 495 38. Zou G. A modified poisson regression approach to prospective studies with binary data.  
46 496 *American journal of epidemiology*. 2004;159(7):702-6. Epub 2004/03/23. PubMed PMID:  
47 497 15033648.  
48 498 39. Zou GY, Donner A. Extension of the modified Poisson regression model to prospective  
49 499 studies with correlated binary data. *Statistical methods in medical research*. 2013;22(6):661-70.  
50 500 Epub 2011/11/11. doi: 10.1177/0962280211427759. PubMed PMID: 22072596.  
51 501 40. NHREC. Guidelines for Community Advisory Groups. NHREC. 2012;Available at :  
52 502 [http://research.ukzn.ac.za/Files/National%20Guidelines%20for%20Community%20Advisory%20](http://research.ukzn.ac.za/Files/National%20Guidelines%20for%20Community%20Advisory%20Groups%20for%20Research%20(2012).pdf)  
53 503 [Groups%20for%20Research%20\(2012\).pdf](http://research.ukzn.ac.za/Files/National%20Guidelines%20for%20Community%20Advisory%20Groups%20for%20Research%20(2012).pdf) [Accessed April 2021].



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3 504 41. EMA. Guideline for Good Clinical Practice E6 (R2). EMA. 2015;Available at  
4 505 [https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-good-clinical-  
6 507 practice-e6r2-4-step-2b\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-good-clinical-<br/>5 506 practice-e6r2-4-step-2b_en.pdf) [Accessed April 2021].  
7 508 42. UNAIDS. Mozambique country Fact sheet. 2015;Available at  
8 509 [http://www.unaids.org/sites/default/files/media/documents/UNAIDS\\_GlobalplanCountryfactsheet\\_mozambique\\_en.pdf](http://www.unaids.org/sites/default/files/media/documents/UNAIDS_GlobalplanCountryfactsheet_mozambique_en.pdf) [accessed February 2017].  
9 510 43. Ministère-de-la-Santé-et-de-la-Prévoyance-sociale-Gabon. Rapport National sur la  
10 511 Réponse au VIH/SIDA 2014. 2015;Available at  
11 512 [http://www.unaids.org/sites/default/files/country/documents/GAB\\_narrative\\_report\\_2015.p  
12 513 df](http://www.unaids.org/sites/default/files/country/documents/GAB_narrative_report_2015.pdf) [accessed February 7].  
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25 517 Figure Legends:  
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28 518 Figure 1. MAMAH trial design  
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519 **Tables**520 **Table 1. Malaria and HIV epidemiology in the study countries**

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

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

525 **Table 2. Study outcomes**

<b>Primary endpoint</b>	
	Prevalence of maternal parasitaemia at delivery (defined by the presence of <i>P. falciparum</i> asexual parasites of any density in peripheral blood determined by microscopy)
<b>Secondary endpoints</b>	
<b>Maternal</b>	
	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 <i>P. falciparum</i> 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 <i>P. falciparum</i> 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 <i>P. falciparum</i> peripheral parasitaemia at the post-partum visit
	Maternal mortality rate
<b>Infant</b>	
	Prevalence of <i>P. falciparum</i> 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

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528 **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			
<b>SCREENING AND ENROLMENT:</b>								
Eligibility screen	X							
Informed consent	X							
Randomization		X						
<b>INTERVENTIONS:</b>								
IPTp administration			X	X	X			
CTX administration			X	*	*	*	*	*
ARV administration			X	*	*	*	*	*
LLITN distribution			X					
<b>MATERNAL ASSESSMENTS:</b>								
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

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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

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

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

531 † Only in women passively reporting sick AND presenting with malaria related signs/symptoms (fever ( $\geq 37,5^{\circ}$  C) or having history of fever in the past 24  
532 hours, arthromyalgia or headache), as per national management guidelines

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

	TIMEPOINTS					
	Birth	1 month*	6 months	9 months	12 months	Unscheduled visits
<b>PROCEDURES:</b>						
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	#	#	#	#	#	#

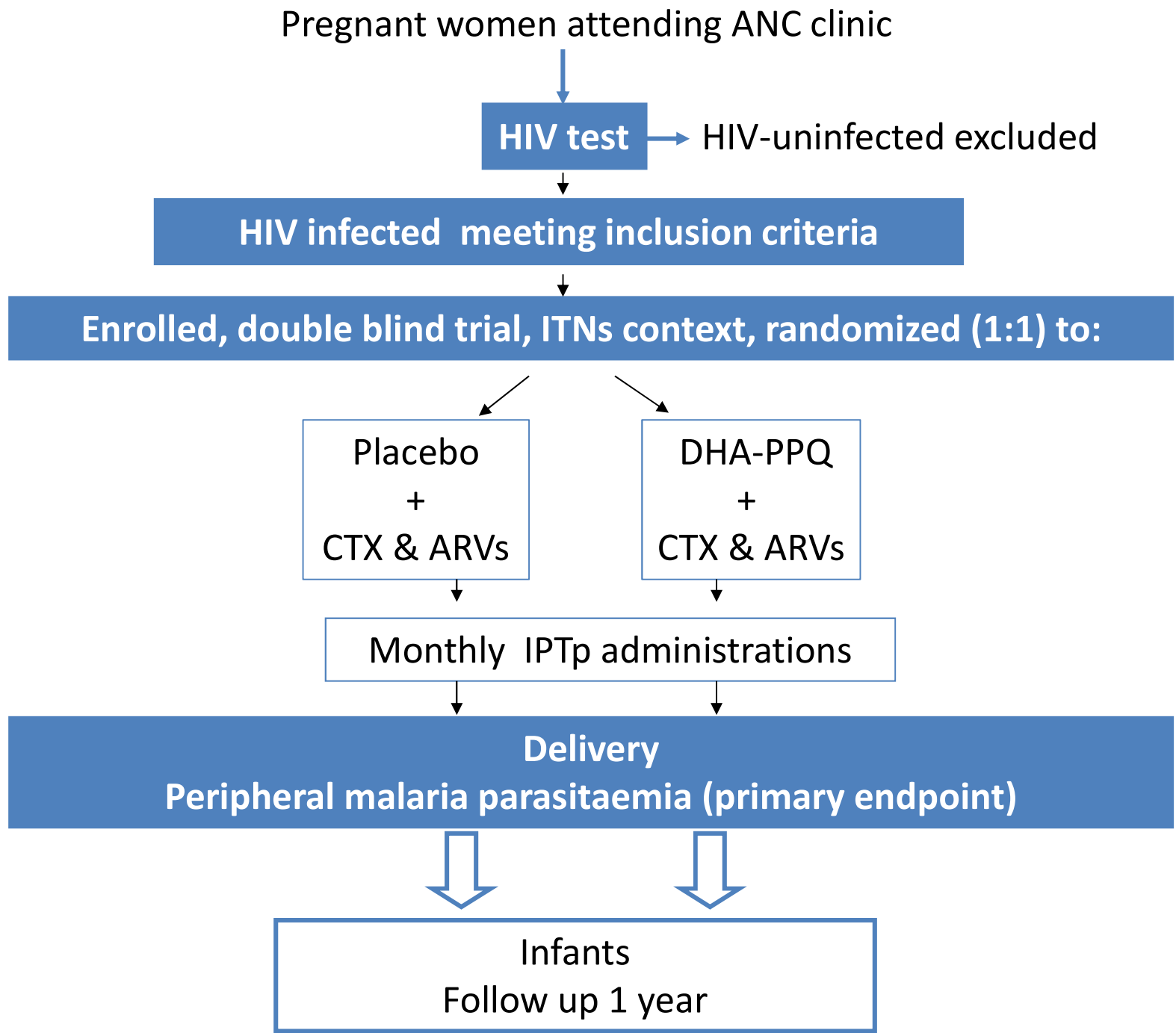
537 \* First visit will be scheduled 1 month after birth or coinciding with first EPI visit

538 † Only if fever ( $\geq 37,5^{\circ}$  C) or history of fever in the past 24 hours or signs suggestive of malaria.

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

540 # Adherence should be assessed at each visit.

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

Section/Topic	Item No	Checklist item	Reported on page No
<b>Title and abstract</b>			
	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
<b>Introduction</b>			
Background and objectives	2a	Scientific background and explanation of rationale	6
	2b	Specific objectives or hypotheses	8
<b>Methods</b>			
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	
<b>Randomisation:</b>			
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



1		assessing outcomes) and how	
2	11b	If relevant, description of the similarity of interventions	
3	Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes
4		12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses
5			15
6			16
7	<b>Results</b>		
8	Participant flow (a	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and
9	diagram is strongly		were analysed for the primary outcome
10	recommended)	13b	For each group, losses and exclusions after randomisation, together with reasons
11	Recruitment	14a	Dates defining the periods of recruitment and follow-up
12		14b	Why the trial ended or was stopped
13	Baseline data	15	A table showing baseline demographic and clinical characteristics for each group
14	Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was
15			by original assigned groups
16	Outcomes and	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its
17	estimation		precision (such as 95% confidence interval)
18		17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended
19	Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing
20			pre-specified from exploratory
21	Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)
22			
23	<b>Discussion</b>		
24	Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses
25	Generalisability	21	Generalisability (external validity, applicability) of the trial findings
26	Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence
27			5
28			5
29	<b>Other information</b>		
30	Registration	23	Registration number and name of trial registry
31	Protocol	24	Where the full trial protocol can be accessed, if available
32	Funding	25	Sources of funding and other support (such as supply of drugs), role of funders
33			4
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\*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](http://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:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-053197.R1
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<b>Primary Subject Heading</b>:	Global health
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Keywords:	HIV & AIDS < INFECTIOUS DISEASES, Maternal medicine < OBSTETRICS, Clinical trials < THERAPEUTICS, TROPICAL MEDICINE, Epidemiology < TROPICAL MEDICINE

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1 **Title:** Evaluation of the safety and efficacy of dihydroartemisinin-piperaquine for intermittent  
2 preventive treatment of malaria in HIV-infected pregnant women: protocol of a multi-centre,  
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31 **Word count:** 3837

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1  
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3 33 **Abstract**  
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6 34 **Introduction:** Malaria infection during pregnancy is an important driver of maternal and  
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8 35 neonatal health especially among HIV-infected women. Intermittent preventive treatment in  
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10 36 pregnancy (IPTp) with sulphadoxine-pyrimethamine is recommended for malaria prevention in  
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12 37 HIV-uninfected women but it is contraindicated in those HIV-infected on cotrimoxazole  
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14 38 prophylaxis (CTXp) due to potential adverse effects. Dihydroartemisinin-piperaquine (DHA-  
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16 39 PPQ), has been shown to improve antimalarial protection in HIV-uninfected pregnant women,  
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18 40 constituting a promising candidate for IPTp. The objective of this trial is to determine if monthly  
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20 41 three-day IPTp courses of DHA-PPQ added to daily CTXp are safe and superior to CTXp alone in  
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22 42 decreasing the proportion of peripheral malaria parasitaemia at the end of pregnancy.  
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27 43 **Methods and analysis:** This is a multi-centre, two-arm, placebo-controlled, individually  
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29 44 randomized trial in HIV-infected pregnant women receiving CTXp and antiretroviral treatment.  
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31 45 A total of 664 women will be enrolled at the first antenatal care clinic visit in sites from Gabon  
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33 46 and Mozambique. Participants will receive an insecticide-treated net and they will be  
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35 47 administered monthly IPTp with DHA-PPQ or placebo (1:1 ratio) as directly observed therapy  
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37 48 from the second trimester of pregnancy. Primary study outcome is the prevalence of maternal  
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39 49 parasitaemia at delivery. Secondary outcomes include prevalence of malaria-related maternal  
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41 50 and infant outcomes and proportion of adverse perinatal outcomes. Participants will be  
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43 51 followed until six weeks after the end of pregnancy and their infants until one year of age to also  
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45 52 evaluate the impact of DHA-PPQ on mother to child transmission of HIV. The analysis will be  
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47 53 done in the Intention to Treat and According to Protocol cohorts, adjusted by gravidity, country,  
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49 54 seasonality and other variables associated with malaria.  
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55 55 **Ethics and dissemination:** The protocol (version 1.0, 2<sup>nd</sup> May 2018) was reviewed and approved  
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57 56 by the institutional and national ethics committees of Gabon (042/2018/PR/SG/CNER) and  
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59 57 Mozambique (504/CBNS/18) and the Hospital Clinic of Barcelona (HCB/2018/0650,Spain).  
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3 59 **Strengths and limitations of this study**  
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- 5 60 • A major strength of this trial is its double blind placebo-controlled design which will allow to  
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7 61 yield conclusive results about the efficacy of the study intervention.  
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9 62 • The inclusion of pregnant women from different sub-Saharan countries will provide a wide  
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11 63 representation of different malaria endemicity areas and HIV subgroups.  
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13 64 • The study is also adequately powered to test the superiority hypothesis.  
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20 66 **Keywords**  
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23 67 Malaria, HIV, pregnancy, prevention, treatment.  
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29 69 **Trial registration**  
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33 70 ClinicalTrials.gov, NCT03671109. Registered on 14<sup>th</sup> September 2018. Recruitment is currently  
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35 71 ongoing.  
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## 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

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3 99 adverse effects. Thus, even though IPTp-SP is a life-saving and highly cost-effective intervention  
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5 100 it cannot be administered to the most vulnerable group, HIV-infected women [16-19].  
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7 101 Consequently and paradoxically the most susceptible women to malaria are currently the least  
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9 102 protected [20].  
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13 103 A placebo-controlled trial conducted in three sub-Saharan countries between 2010 and 2013  
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15 104 showed that an effective antimalarial added to CTXp and long-lasting ITNs (LLITNs) in HIV-  
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17 105 infected pregnant women has a significant impact in improving malaria prevention and maternal  
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19 106 health through reductions in hospital admissions [21]. However, the antimalarial used  
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21 107 (mefloquine) was not well tolerated, and most importantly, it was associated with a two-fold  
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23 108 increase in the frequency of mother to child transmission of HIV (MTCT-HIV), limiting its  
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25 109 potential for IPTp. These findings indicate the need to find drug alternatives with better  
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27 110 tolerability and safety profile to reduce malaria in this vulnerable group [21].  
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32 111 Dihydroartemisinin-piperaquine (DHA-PPQ) is an artemisinin-based combination therapy (ACT)  
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34 112 recommended by the WHO for treatment of uncomplicated malaria in adults and children aged  
35  
36 113  $\geq$  six months[22]. Among the available antimalarial drugs, DHA-PPQ constitutes one of the best  
37  
38 114 candidates for IPTp because it is not used as first-line malaria treatment, it is well tolerated, and  
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40 115 it is recommended for treatment of clinical malaria in the second and third trimesters of  
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42 116 pregnancy [22].  
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46 117 DHA-PPQ has been safely used for case management in HIV-uninfected pregnant women in  
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48 118 Thailand and in a multicentre trial in sub-Saharan Africa [23, 24]. Moreover, studies comparing  
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50 119 IPTp-SP with IPTp-DHA-PPQ in HIV-uninfected pregnant women from Kenya and Uganda  
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52 120 indicate that the drug could be an alternative to SP in terms of antimalarial efficacy and safety  
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54 121 in pregnancy [25, 26]. A recent meta-analysis of the safety and efficacy of repeated doses of  
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56 122 DHA-PPQ for prevention and treatment of malaria including 11 studies (among adults, children  
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58 123 and pregnant women) has concluded that monthly DHA-PPQ is well tolerated and effective for  
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3 124 IPT and that additional data are needed in pregnancy and to further explore the cardiac safety  
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5 125 with monthly dosing [27]. Scientific evidence shows that efficacy and safety findings from  
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7 126 malaria control strategies evaluated in HIV-uninfected pregnant women cannot be directly  
8  
9 127 extrapolated to HIV-infected women [21]. A trial comparing monthly IPTp with DHA-PPQ to CTXp  
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11 128 among 200 HIV-infected Ugandan women did not find differences in the risk of  
12  
13 129 histopathologically detected placental malarial infection and other outcomes between groups  
14  
15 130 [28]. However, authors acknowledge the limitations of their results due to the low prevalence  
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17 131 of malaria in the study area at the time of the trial [28]. Therefore, it is of highest public health  
18  
19 132 priority to provide conclusive evidence as to whether the most vulnerable population (HIV-  
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21 133 infected pregnant women) will benefit from the use of the currently most promising and  
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23 134 available alternative drug for IPTp, DHA-PPQ [20].  
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29 135 The objectives of the MAMAH (Improving **Maternal heAlth** by reducing **Malaria** in **African HiV**  
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31 136 women) trial are (1) to evaluate the safety, tolerability and efficacy of DHA-PPQ as IPTp for  
32  
33 137 malaria prevention in HIV-infected pregnant women receiving daily CTXp and ARV drugs, (2) to  
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35 138 assess the effect of DHA-PPQ as IPTp on mother to child transmission of HIV and (3) to evaluate  
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37 139 the effectiveness of CTXp in clearing malaria parasites in HIV-infected pregnant women.  
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## 43 141 **2. Methods**

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46 142 This is multi-centre, two-arm, placebo-controlled, individually randomized superiority clinical  
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48 143 trial with two study arms including HIV-infected pregnant women. The study will be carried out  
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50 144 and reported according to Consolidated Standards of Reporting Trials guidelines [29].  
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### 53 145 ***Study settings***

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57 146 The trial will be conducted in two countries from Central and South Eastern sub-Saharan Africa  
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59 147 (Gabon and Mozambique), where HIV prevalence among pregnant women ranges from 6 to 29%  
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3 148 [30, 31]. Malaria epidemiologic indicators and HIV prevalence in pregnancy in study sites are  
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5 149 shown in Table 1. The trial sites have been selected to provide representation of different  
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7 150 malaria endemicity and HIV subgroups (HIV-1 subtype C in Mozambique and HIV-1 CRF02\_AG in  
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9 151 Gabon, where HIV-2 also circulates [32, 33]).  
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### 13 152 ***Study population***

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15  
16 153 All pregnant women attending the study ANC services for the first time and/or who have not  
17  
18 154 received IPTp during their current pregnancy will be screened for participation in the trial.  
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20 155 Inclusion criteria are (1) permanent resident in the study area, (2) gestational age at the first  
21  
22 156 antenatal visit  $\leq$  28 weeks, (3) HIV seropositive status and (4) agreement to deliver in the study  
23  
24 157 site's maternity(ies) wards. Exclusion criteria are (1) planning to move out in the following 10  
25  
26 158 months from enrolment, (2) gestational age at the first antenatal visit  $>$  28 weeks of pregnancy,  
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28 159 (3) known history of allergy to CTX, (4) known history of allergy or contraindications to DHA-PPQ  
29  
30 160 and (5) participating in other intervention studies.  
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### 35 161 ***Informed consent***

36  
37  
38 162 All participants will receive information about study procedures, including knowledge about  
39  
40 163 malaria and HIV infection in pregnancy. A signed informed consent (or thumb-printed with a  
41  
42 164 witness whenever the woman is illiterate) will be obtained before any study tests or evaluations  
43  
44 165 are carried out. If the participant is under the legal age of maturity, she will sign the assent form  
45  
46 166 and her legal guardian will sign the informed consent according to national ethics local policies.  
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48 167 The informed consent will cover the woman and the new born infant.  
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### 53 168 ***Recruitment and randomization***

54  
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56 169 After the study details are explained and informed consent is signed, pregnant women will be  
57  
58 170 given a study number and automatically randomized to one of the study arms: 1) Daily CTX +  
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3 171 monthly IPTp-DHA-PPQ or 2) Daily CTX + Monthly IPTp-placebo. Each participant will be uniquely  
4  
5 172 identified in the study by a combination of her site code and participant number. Allocation of  
6  
7 173 participants to study arms will be done centrally by the trial's Sponsor (the Barcelona Institute  
8  
9 174 for Global Health, ISGlobal) by block randomization and stratified by country. This method will  
10  
11 175 ensure balanced allocation to both arms during different malaria seasons in the two study  
12  
13 176 countries. Each subject number will be related to a treatment number which assigns them to  
14  
15 177 one of the IPT arms. Study number allocation for each study participant will be concealed in  
16  
17 178 opaque sealed envelopes that will be opened only after recruitment. At each site the first  
18  
19 179 participant will be assigned a patient number, and consecutive numbers will be assigned to  
20  
21 180 subsequent women. A study identification card containing the individual study number and  
22  
23 181 basic demographic information will be given to the participant in order to facilitate identification  
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25 182 at all study contacts.  
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31 183 Figure 1 displays the study design.  
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#### 34 184 ***Blinding***

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37 185 Study tablets (IPTp-DHA-PPQ and IPTp-Placebo) will be identically packaged. Study personnel  
38  
39 186 will prepare and deliver the medication to the participant. All study personnel, investigators,  
40  
41 187 outcome assessors, data analysts and the participants will remain blinded throughout the trial.  
42  
43 188 Unblinding is only envisaged in case of a medical emergency (in such case, the investigator on  
44  
45 189 site will have justify to the Sponsor and the DSMB the need for unblinding).  
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#### 49 190 ***Interventions***

##### 51 52 53 191 ***a) IPTp administration***

54  
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56 192 Administration of the three-day IPTp course will always be done under fasting conditions  
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58 193 (following DHA-PPQ administration recommendations) and direct observation by study  
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3 194 personnel. The number of daily IPTp tablets to be administered will be based on bodyweight and  
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5 195 according to the treatment guidelines set by WHO [target dose (range) of 4 (2–10) mg/kg/ day  
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7 196 of DHA and 18 (16–27) mg/kg/day of PPQ given once a day for three days for adults]. Following  
8  
9  
10 197 physical examination, recruited women of gestational age $\geq$ 13 weeks will receive the assigned  
11  
12 198 IPTp drug. In case of gestational age $<$  13 weeks, first IPTp administration will be scheduled one  
13  
14 199 month later. If participants report malaria treatment in the preceding four weeks, first IPTp  
15  
16 200 administration will also be delayed one month. Administration of the second and third day  
17  
18 201 treatment course will be done by study personnel either at the study health facility or household  
19  
20 202 level. Women will be observed for 60 minutes after administration of the IPTp dose. Those  
21  
22 203 women vomiting within the first 30 minutes of IPTp administration will be given a second full  
23  
24 204 IPTp dose; women vomiting after 30-60 minutes of IPTp administration will be given an  
25  
26 205 additional half dose of the drug. Subsequent doses of IPTp will be given coinciding with the next  
27  
28 206 scheduled monthly ANC clinic visit, at least one month apart from the previous dose.  
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33 207 ***b) CTX administration***

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36 208 Cotrimoxazole (fixed combination drug containing 800 mg of trimethoprim and 160 mg of  
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38 209 sulfamethoxazole) will be taken daily from enrolment until delivery as per national guidelines  
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40 210 for prevention of opportunistic infections. Women will be asked to visit the ANC clinic monthly  
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42 211 to receive the amount of CTX tablets for the whole month. At each ANC clinic visit, adherence  
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44 212 to CTX prophylaxis will be assessed.  
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48 213 ***c) ARV therapy and concomitant medications***

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50  
51 214 Treatment for prevention of HIV MTCT (Option B+) will be given by the study health personnel,  
52  
53 215 according to national HIV/AIDS control guidelines[34]. Any other concomitant treatment  
54  
55 216 received by the study participants will be recorded in the study questionnaires.  
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59 217 ***d) Long lasting insecticide treated nets***

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3 218 Regardless of gestational age at the time of recruitment, all women will receive a LLITN and  
4  
5 219 details about its use will be explained.  
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### 8 220 ***Study Outcomes***

10  
11 221 The primary outcome of the trial will be the prevalence of maternal parasitaemia at delivery  
12  
13 222 defined by the presence of *Plasmodium falciparum* (*P. falciparum*) asexual parasites of any  
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15 223 density in peripheral blood (determined by microscopy). The secondary maternal and infant  
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17 224 endpoints can be found in Table 2.  
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### 20 21 225 ***Sample size***

22  
23  
24 226 Based on previous estimations at the study sites and assuming a prevalence of peripheral  
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26 227 parasitaemia at delivery of 7.5% with CTXp, it is estimated that 298 women per arm will be  
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28 228 required to detect with 80% power a significant ( $p < 0.05$ ) decrease of 5% or more in the  
29  
30 229 prevalence of peripheral parasitaemia in the CTXp+IPTp-DHA-PPQ group [21]. In order to allow  
31  
32 230 for 10% losses to follow up, it is calculated that 332 women/study arm will need to be recruited  
33  
34 231 (total  $n=664$ ). Considering the prevalence of HIV infection among pregnant women in both sites  
35  
36 232 (please refer to Table 1), a total of 444 women are planned to be recruited in Mozambique and  
37  
38 233 220 women in Gabon to allow for a recruitment rate of 33 women/month on average. These  
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40 234 estimations are based on recruitment rates of previous IPTp clinical trials conducted among  
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42 235 pregnant women in the two study sites [21, 35, 36].  
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### 48 236 ***Follow up and measurements of outcomes***

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51 237 At baseline, the woman's demographic and obstetric information will be recorded in study  
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53 238 specific case report forms (CRF).  
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#### 56 57 239 ***a) Physical and clinical examination at enrolment***



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3 240 The physical examination of the woman will include the following assessments: weight, height,  
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5 241 gestational age by bimanual palpation and measurement of middle-upper arm circumference  
6  
7 242 (MUAC). Ultrasound (US) will be performed to determine gestational age at enrolment and  
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9 243 confirm pregnancy viability.  
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13 244 ***b) Baseline biological samples***  
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16 245 At enrolment, a venous blood sample (5 mL) will be collected for analysis of haemoglobin level,  
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18 246 CD4 cell counts, HIV viral load and malaria PCR.  
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21 247 ***c) Follow-up and household visits***  
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24 248 Women will be given an appointment to attend the subsequent ANC clinic visit one month after  
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26 249 the first one. Participants will receive the standard ANC package of interventions, which includes  
27  
28 250 iron and folate supplementation, following national guidelines. The subsequent IPTp doses will  
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30 251 be given at least four weeks apart from the previous one. MAMAH participants will be asked to  
31  
32 252 visit the study facilities in case of any illness. A malaria blood smear will be collected in those  
33  
34 253 participants passively reporting sick and presenting with malaria related signs/symptoms (fever  
35  
36 254 ( $\geq 37.5^{\circ}$  C) or having history of fever in the past 24 hours, arthromyalgias or headache), as per  
37  
38 255 national management guidelines. Women will be visited at home the day after recruitment to  
39  
40 256 confirm residence status, assess drug tolerability and the correct use of the net. Adherence to  
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42 257 CTX prophylaxis, ARV therapy and compliance with the LLITNs use will be assessed monthly at  
43  
44 258 the ANC attendance.  
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50 259 ***d) Adverse events monitoring and reporting***  
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53 260 Active safety monitoring will consist in household visits to study participants two days after each  
54  
55 261 IPTp administration to assess drug tolerability and record all Adverse Events (AEs). In addition,  
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57 262 a health facility-based passive surveillance system will be established to capture unscheduled  
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3 263 visits of participants during follow-up. Information on unsolicited adverse events will be  
4  
5 264 collected at each scheduled and unscheduled visit. Any participant passively reporting being sick  
6  
7 265 during the study visits, will be referred to the clinical services as per routine system in place. A  
8  
9 266 blood smear will be collected in those presenting with malaria related signs/symptoms (fever  
10  
11 267 ( $\geq 37.5^{\circ}\text{C}$ ) or having history of fever in the past 24 hours, arthromyalgias or headache). In case  
12  
13 268 of malaria parasitaemia or anaemia, they will be treated following national guidelines. Serious  
14  
15 269 Adverse Events (SAEs) will be reported by the site investigator within 24 hours of being made  
16  
17 270 aware to the trial's safety monitor and the Data Safety Monitoring Board (DSMB).  
18  
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22 271 ***e) End of pregnancy and infant assessments***

23  
24  
25 272 At the end of pregnancy, maternal blood (5 mL), placental and cord blood samples (5 mL) will be  
26  
27 273 collected for haematological and parasitological examination. The new-born will be examined,  
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29 274 weighed and measured and his/her gestational age assessed by the modified Ballard method  
30  
31 275 [37].  
32  
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35 276 ***f) Post-partum visit***

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38 277 Participants will be visited approximately after six weeks of end of pregnancy at the study health  
39  
40 278 facility where a blood smear for malaria screening will be collected. A summary of study  
41  
42 279 procedures is displayed in Table 3.  
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45

46 280 ***g) Infants follow up***

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48  
49 281 Infants born to study participants will be followed up until one year of age. Mothers will be asked  
50  
51 282 to bring their child to the study facilities at weeks 4-6 of age and at 6, 9 and 12 months after  
52  
53 283 birth. At each scheduled visit, study infants will be physically examined and their psychomotor  
54  
55 284 and neurological development will be assessed following a standard protocol for African settings  
56  
57 285 [38-40]. Weight, height and axillary temperature will be measured and recorded. A capillary  
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3 286 blood sample will be taken from infants with fever (axillary temperature  $\geq 37.5^{\circ}\text{C}$ ) or history of  
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5 287 fever in the last 24 hours, or appearing pale, for malaria parasitaemia examination and  
6  
7 288 haematological determination. In case of malaria parasitaemia or anaemia, they will be treated  
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9  
10 289 following national guidelines. At each scheduled visit, HIV DNA will be evaluated in the infant  
11  
12 290 from dried blood spot on filter paper (for PCR). As per routine healthcare national programs HIV-  
13  
14 291 exposed infants will receive CTX prophylaxis starting four to six weeks after birth. Infants will  
15  
16 292 also receive ARV, according to national guidelines. Infant's follow up visits and study procedures  
17  
18 293 are described in Table 4.

## 22 294 **Laboratory tests**

### 25 295 *a) Parasitological and haematological determinations*

27 296 Thick and thin blood smears will be stained with Giemsa's stain and examined for *Plasmodium*  
28  
29 297 *spp.* following standard procedures. Also, blood haemoglobin will be determined following local  
30  
31  
32 298 SOPs.

### 35 299 *b) Detection of HIV and quantitative determination of viral load*

37 300 Quantitative PCR HIV viral load will be determined from the venous blood samples drawn at  
38  
39 301 enrolment and delivery. Additionally, vertical transmission of HIV will be determined by  
40  
41 302 qualitative DNA PCR performed on samples drawn from infants at one month and 12 months of  
42  
43  
44 303 age.

### 47 304 *c) Immunological determinations related to HIV status*

49 305 CD4+T cell count will be determined by flow cytometry after staining of whole blood with CD3,  
50  
51 306 CD8 and CD4 fluorochrometolabelled antibodies and acquisition using FACSCalibur (BD  
52  
53 307 Biosciences) and TruCOUNT tubes (Becton Dickinson, San Jose, CA; USA) or *MiniVidas device*.  
54  
55 308 HIV viral load will be determined from plasma cryopreserved at  $-80^{\circ}\text{C}$  using the devices in place  
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57  
58 309 *in the study sites (such as COBAS® AMPLICOR , AmpliPrep [Roche Diagnostics] or GeneXpert).*

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3 310 ***d) Placental samples analysis***  
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5 311 A tissue sample (approximately 2 cm<sup>3</sup>) will be collected from the maternal surface of the  
6  
7 312 placenta and will be immediately placed in 25 mL of 10% neutral buffered formalin and kept at  
8  
9 313 4°C until processed and embedded in paraffin wax by standard techniques. Paraffin sections  
10  
11 314 4µm thick from the placental tissue, will be stained with hematoxylin and eosin, Giemsa's stain  
12  
13 315 and the periodic acid-Schiff technique. Placentas will be classified histologically as: i) not  
14  
15 316 infected, ii) active infection and iii) past infection, depending on the presence or absence of  
16  
17 317 parasites, pigment or both [41]. Impression smears will be prepared from the placental blood  
18  
19 318 for parasitological examination. Blood from the placenta will also be collected onto filter paper  
20  
21 319 for PCR determination of malaria parasites.  
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27 321 ***Data management***  
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30 322 Data will be collected using paper case report forms (CRFs) developed for the trial by study  
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32 323 personnel at each scheduled and unscheduled visit. The quality of the data recorded in the study  
33  
34 324 source documents and CRFs will be monitored regularly following the principles of Good Clinical  
35  
36 325 Practices by the trials' clinical monitor [42]. Data will be double entered into the study database  
37  
38 326 using the OpenClinica open source software (version 3.14) for clinical data management  
39  
40 327 (www.OpenClinica.com) at each study site. Automatic quality checks will be performed to  
41  
42 328 ensure CRF completeness. Concomitant medications registered into the database are coded  
43  
44 329 using the WHO Drug Reference List, which employs the Anatomical Therapeutic Chemical  
45  
46 330 classification system. Medical history/current medical conditions and adverse events are coded  
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48 331 using the Medical dictionary for regulatory activities (MedDRA) terminology.  
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55 333 ***Analysis plan***  
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3 334 The following populations of analysis have been defined: a) Intention to treat (ITT): it includes  
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5 335 all randomized pregnant women who have data on outcome (target population for the efficacy  
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7 336 analysis); b) According to protocol (ATP): it includes participants who fulfil all the inclusion-  
8  
9 337 exclusion criteria, took monthly IPTp-DHA-PPQ/placebo study doses, received a LLITN, received  
10  
11 338 CTXp and ARV drugs and from whom data is available for the analysis; c) Safety: it includes  
12  
13 339 participants who received at least one dose of IPTp DHA-PPQ/placebo and had at least one post-  
14  
15 340 baseline safety assessment. No interim analyses of data is envisaged. The primary analysis of the  
16  
17 341 trial will be the comparison of the proportion of pregnant women with peripheral parasitaemia  
18  
19 342 at delivery in the ITT and ATP cohorts, adjusted by gravidity, country, seasonality and other  
20  
21 343 variables associated with the prevalence of malaria. Proportions will be compared between  
22  
23 344 groups using the Fisher exact test and presented as relative risk ratio (RR) or reduction of the RR  
24  
25 345  $(1 - RR * 100\%)$  if RR lower than 1. Adjustment for co-variates and possible confounders will be  
26  
27 346 done using Poisson regression with a log link and robust estimate of the covariance (Huber  
28  
29 347 method), using the method proposed by Zou [43, 44]. Continuous variables will be compared  
30  
31 348 between groups using Wilcoxon rank values. The differences in rank values will be assessed by  
32  
33 349 looking at the expected rank sums in each group under the null hypothesis. Adjustment for co-  
34  
35 350 variates and possible cofounders (such as country, gestational age, gravidity, anaemia, literacy,  
36  
37 351 Mid-Upper Arm Circumference (MUAC), viral load and CD4+T cell count) will be done using  
38  
39 352 ordinary least square regression. Subgroup analysis is not envisaged. Incidence of clinical  
40  
41 353 malaria, overall admissions and outpatient attendances will be estimated as the number of  
42  
43 354 episodes over the time at risk. Time at risk will be estimated as the time from the start of follow  
44  
45 355 up until the end of follow-up (visit one month after end of pregnancy for mothers) or withdrawal  
46  
47 356 due to censoring or death, whatever occurs first. The total number of events will be compared  
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49 357 between groups using Negative Binomial regression models which takes into account a possible  
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51 358 extra Poisson variation due to different frailty of the subjects. The comparison will be expressed  
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3 359 as relative rate ratio (RRate). Data analysis will be performed using Stata (Stata Corp., College  
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5 360 Station, TX, US).  
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9 361 ***Patient and public involvement***

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11 362 Patients will not be directly involved in the design, conduct, reporting, or dissemination plans of  
12  
13 363 the study. However, a Community Advisory Board (CAB) has been set at each study site to  
14  
15 364 strengthen communication and interaction between the local study community and research  
16  
17 365 teams. The CAB also oversees and guides the study team on key issues such as potential risks  
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19 366 and burdens for participants or host communities that may be hidden from researchers, and  
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21 367 how to minimize them [45].  
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26 368 ***Ethics and dissemination***

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29 369 The study is conducted in accordance with the EMA/ICH Guideline on Good Clinical Practice  
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31 370 and in total agreement with the applicable international, EU and national law of all the  
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33 371 participating countries[46]. The study protocol and the informed consent forms have been  
34  
35 372 reviewed and approved by the institutional and national ethics committees of Gabon  
36  
37 373 (042/2018/PR/SG/CNER) and Mozambique (504/CBNS/18) and the Hospital Clinic of  
38  
39 374 Barcelona (HCB/2018/0650,Spain). An independent DSMB monitors regularly the safety of  
40  
41 375 study participants. The trial is registered on [clinicaltrials.gov](https://clinicaltrials.gov)  
42  
43 376 (<https://clinicaltrials.gov/ct2/show/NCT03671109>).  
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48 377 The findings of the clinical trial will be submitted for publication in a peer-reviewed journal  
49  
50 378 within 12 months of study completion through an open access mechanism, or otherwise made  
51  
52 379 available publicly in compliance with H2020 open access requirements. Primary project raw data  
53  
54 380 will be published in the project website. At no stage will data containing personal information  
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56 381 of research participants be released.  
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3 382 A project communication has been developed in order to ensure timely, accurate, and effective  
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5 383 dissemination of project results. After concluding the trial's data analysis, findings will be made  
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7 384 available to all partners, key stakeholders and Ministries of Health. The project members will  
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9 385 actively disseminate information to the scientific community through reports, presentations at  
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11 386 scientific forums and publications in international open access journals. Trial results will also be  
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13 387 shared with the WHO Global Malaria Program and Malaria Policy Advisory Groups.  
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### 389 **Authors' contributions**

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

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404

### 405 **Competing interests**

406 None declared.

407 **References**

- 408 1. WHO. World Malaria report 2020. WHO. 2020;Available at  
409 <https://www.who.int/publications/i/item/9789240015791> [Accessed March 2021].
- 410 2. Mombo-Ngoma G, Mackanga JR, Gonzalez R, Ouedraogo S, Kakolwa MA, Manego RZ, et  
411 al. Young adolescent girls are at high risk for adverse pregnancy outcomes in sub-Saharan Africa:  
412 an observational multicountry study. *BMJ open*. 2016;6(6):e011783. Epub 2016/07/01. doi:  
413 10.1136/bmjopen-2016-011783. PubMed PMID: 27357200.
- 414 3. Group WAbCTAABS. Clinical determinants of early parasitological response to ACTs in  
415 African patients with uncomplicated falciparum malaria: a literature review and meta-analysis  
416 of individual patient data. *BMC Med*. 2015;13:212. Epub 2015/09/08. doi: 10.1186/s12916-015-  
417 0445-x. PubMed PMID: 26343145; PubMed Central PMCID: PMC4561425.
- 418 4. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoia K, Brabin B, et al. Epidemiology  
419 and burden of malaria in pregnancy. *Lancet Infect Dis*. 2007;7(2):93-104. Epub 2007/01/26. doi:  
420 S1473-3099(07)70021-X [pii]  
421 10.1016/S1473-3099(07)70021-X [doi]. PubMed PMID: 17251080.
- 422 5. Menendez C, D'Alessandro U, ter Kuile FO. Reducing the burden of malaria in pregnancy  
423 by preventive strategies. *Lancet Infect Dis*. 2007;7(2):126-35. Epub 2007/01/26. doi: S1473-  
424 3099(07)70024-5 [pii]  
425 10.1016/S1473-3099(07)70024-5 [doi]. PubMed PMID: 17251083.
- 426 6. Menendez C, Ordi J, Ismail MR, Ventura PJ, Aponte JJ, Kahigwa E, et al. The impact of  
427 placental malaria on gestational age and birth weight. *J Infect Dis*. 2000;181(5):1740-5. Epub  
428 2000/05/24. doi: 10.1086/315449. PubMed PMID: 10823776.
- 429 7. Bardají A, Sigauque B, Sanz S, Maixenchs M, Ordi J, Aponte JJ, et al. Impact of malaria at  
430 the end of pregnancy on infant mortality and morbidity. *J Infect Dis*. 2011;203(5):691-9. Epub  
431 2011/01/05. doi: 10.1093/infdis/jiq049. PubMed PMID: 21199881; PubMed Central PMCID:  
432 PMCPMC3071276.
- 433 8. Desai M, Hill J, Fernandes S, Walker P, Pell C, Gutman J, et al. Prevention of malaria in  
434 pregnancy. *Lancet Infect Dis*. 2018;18(4):e119-e32. Epub 2018/02/06. doi: 10.1016/s1473-  
435 3099(18)30064-1. PubMed PMID: 29395997.
- 436 9. UNAIDS/WHO. Global report: UNAIDS report on the global AIDS Epidemic 2013. WHO  
437 Library Cataloguing-in-Publication Data 2013;UNAIDS(JC2502/1/E).
- 438 10. WHO. Malaria and HIV interactions and their implications for public health policy WHO.  
439 2005;ISBN 92 4 1593350.
- 440 11. Kwenti TE. Malaria and HIV coinfection in sub-Saharan Africa: prevalence, impact, and  
441 treatment strategies. *Research and reports in tropical medicine*. 2018;9:123-36. Epub  
442 2018/08/14. doi: 10.2147/rrtm.S154501. PubMed PMID: 30100779; PubMed Central PMCID:  
443 PMCPMC6067790.
- 444 12. ter Kuile FO, Parise ME, Verhoeff FH, Udhayakumar V, Newman RD, van Eijk AM, et al.  
445 The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant  
446 women in sub-saharan Africa. *Am J Trop Med Hyg*. 2004;71(2 Suppl):41-54. PubMed PMID:  
447 15331818.
- 448 13. Gonzalez R, Ataide R, Nanche D, Menendez C, Mayor A. HIV and malaria interactions:  
449 where do we stand? *Expert Rev Anti Infect Ther*. 2012;10(2):153-65. doi: 10.1586/eri.11.167.  
450 PubMed PMID: 22339190.
- 451 14. WHO. Intermittent Preventive Treatment of malaria in pregnancy using Sulfadoxine-  
452 Pyrimethamine (IPTp-SP). Updated WHO Policy Recommendation. WHO.  
453 2012;[http://www.who.int/malaria/iptp\\_sp\\_updated\\_policy\\_recommendation\\_en\\_102012.pdf](http://www.who.int/malaria/iptp_sp_updated_policy_recommendation_en_102012.pdf)  
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57  
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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: PMC4847718.
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: PMC4847718.
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

- 1  
2  
3 506 Among HIV-Infected Pregnant Women. *J Infect Dis.* 2017;216(1):29-35. Epub 2017/03/23. doi:  
4 507 10.1093/infdis/jix110. PubMed PMID: 28329368; PubMed Central PMCID: PMC5853208.
- 5 508 29. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for  
6 509 reporting parallel group randomized trials. *Annals of internal medicine.* 2010;152(11):726-32.  
7 510 Epub 2010/03/26. doi: 10.7326/0003-4819-152-11-201006010-00232. PubMed PMID:  
8 511 20335313.
- 9 512 30. Gonzalez R, Munguambe K, Aponte J, Bavo C, Nhalungo D, Macete E, et al. High HIV  
11 513 prevalence in a southern semi-rural area of Mozambique: a community-based survey. *HIV Med.*  
12 514 2012. PubMed PMID: 22500780.
- 13 515 31. Manego RZ, Mombo-Ngoma G, Witte M, Held J, Gmeiner M, Gebru T, et al. Demography,  
14 516 maternal health and the epidemiology of malaria and other major infectious diseases in the rural  
15 517 department Tsamba-Magotsi, Ngounie Province, in central African Gabon. *BMC Public Health.*  
16 518 2017;17(1):130. Epub 2017/01/29. doi: 10.1186/s12889-017-4045-x. PubMed PMID: 28129759;  
17 519 PubMed Central PMCID: PMC5273856.
- 18 520 32. Caron M, Lekana-Douki SE, Makuwa M, Obiang-Ndong GP, Biba O, Nkoghe D, et al.  
20 521 Prevalence, genetic diversity and antiretroviral drugs resistance-associated mutations among  
21 522 untreated HIV-1-infected pregnant women in Gabon, central Africa. *BMC infectious diseases.*  
22 523 2012;12:64. Epub 2012/03/22. doi: 10.1186/1471-2334-12-64. PubMed PMID: 22433277;  
23 524 PubMed Central PMCID: PMC3359209.
- 24 525 33. Lahuerta M, Aparicio E, Bardaji A, Marco S, Sacarlal J, Mandomando I, et al. Rapid spread  
25 526 and genetic diversification of HIV type 1 subtype C in a rural area of southern Mozambique. *AIDS*  
26 527 *Res Hum Retroviruses.* 2008;24(2):327-35. PubMed PMID: 18271719.
- 27 528 34. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and  
28 529 preventing HIV infection 2016. Recommendations for a public health approach. Second edition.:  
30 530 World Health Organization; 2016.
- 31 531 35. Gonzalez R, Mombo-Ngoma G, Ouedraogo S, Kakolwa MA, Abdulla S, Accrombessi M, et  
32 532 al. Intermittent Preventive Treatment of Malaria in Pregnancy with Mefloquine in HIV-Negative  
33 533 Women: A Multicentre Randomized Controlled Trial. *PLoS Med.* 2014;11(9):e1001733. Epub  
34 534 2014/09/24. doi: 10.1371/journal.pmed.1001733. PubMed PMID: 25247709.
- 35 535 36. Menendez C, Bardaji A, Sigauque B, Romagosa C, Sanz S, Serra-Casas E, et al. A  
36 536 randomized placebo-controlled trial of intermittent preventive treatment in pregnant women  
37 537 in the context of insecticide treated nets delivered through the antenatal clinic. *PloS one.*  
38 538 2008;3(4):e1934. Epub 2008/04/10. doi: 10.1371/journal.pone.0001934 [doi]. PubMed PMID:  
39 539 18398460; PubMed Central PMCID: PMC2277457.
- 40 540 37. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R. New Ballard Score,  
41 541 expanded to include extremely premature infants. *The Journal of pediatrics.* 1991;119(3):417-  
42 542 23. Epub 1991/09/01. PubMed PMID: 1880657.
- 43 543 38. Gladstone M, Lancaster GA, Umar E, Nyirenda M, Kayira E, van den Broek NR, et al. The  
44 544 Malawi Developmental Assessment Tool (MDAT): the creation, validation, and reliability of a  
45 545 tool to assess child development in rural African settings. *PLoS Med.* 2010;7(5):e1000273. Epub  
46 546 2010/06/04. doi: 10.1371/journal.pmed.1000273. PubMed PMID: 20520849; PubMed Central  
47 547 PMCID: PMC2876049.
- 48 548 39. Koura KG, Boivin MJ, Davidson LL, Ouedraogo S, Zoumenou R, Alao MJ, et al. Usefulness  
49 549 of child development assessments for low-resource settings in francophone Africa. *Journal of*  
50 550 *developmental and behavioral pediatrics : JDBP.* 2013;34(7):486-93. Epub 2013/08/01. doi:  
51 551 10.1097/DBP.0b013e31829d211c. PubMed PMID: 23899660; PubMed Central PMCID:  
52 552 PMC3821168.
- 53 553 40. Prado EL, Abubakar AA, Abbeddou S, Jimenez EY, Some JW, Ouedraogo JB. Extending  
54 554 the Developmental Milestones Checklist for use in a different context in Sub-Saharan Africa.  
55 555 *Acta paediatrica (Oslo, Norway : 1992).* 2014;103(4):447-54. Epub 2013/12/21. doi:  
56 556 10.1111/apa.12540. PubMed PMID: 24354938.

- 1  
2  
3 557 41. Ismail MR, Ordi J, Menendez C, Ventura PJ, Aponte JJ, Kahigwa E, et al. Placental  
4 558 pathology in malaria: a histological, immunohistochemical, and quantitative study. Human  
5 559 pathology. 2000;31(1):85-93. Epub 2000/02/09. PubMed PMID: 10665918.  
6 560 42. Pharmalys. Pharmalys CRO. 2021; Available at <https://pharmalys.com/> [Accessed July  
7 561 2021].  
8 562 43. Zou G. A modified poisson regression approach to prospective studies with binary data.  
9 563 American journal of epidemiology. 2004;159(7):702-6. Epub 2004/03/23. PubMed PMID:  
10 564 15033648.  
11 565 44. Zou GY, Donner A. Extension of the modified Poisson regression model to prospective  
12 566 studies with correlated binary data. Statistical methods in medical research. 2013;22(6):661-70.  
13 567 Epub 2011/11/11. doi: 10.1177/0962280211427759. PubMed PMID: 22072596.  
14 568 45. NHREC. Guidelines for Community Advisory Groups. NHREC. 2012; Available at :  
15 569 [http://research.ukzn.ac.za/Files/National%20Guidelines%20for%20Community%20Advisory%20](http://research.ukzn.ac.za/Files/National%20Guidelines%20for%20Community%20Advisory%20Groups%20for%20Research%20(2012).pdf)  
16 570 [0Groups%20for%20Research%20\(2012\).pdf](http://research.ukzn.ac.za/Files/National%20Guidelines%20for%20Community%20Advisory%20Groups%20for%20Research%20(2012).pdf) [Accessed April 2021].  
17 571 46. EMA. Guideline for Good Clinical Practice E6 (R2). EMA. 2015; Available at  
18 572 [https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-good-clinical-](https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-good-clinical-practice-e6r2-4-step-2b_en.pdf)  
19 573 [practice-e6r2-4-step-2b\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-good-clinical-practice-e6r2-4-step-2b_en.pdf) [Accessed April 2021].  
20 574 47. Alonso PL, Sacarlal J, Aponte JJ, Leach A, Macete E, Milman J, et al. Efficacy of the  
21 575 RTS,S/AS02A vaccine against Plasmodium falciparum infection and disease in young African  
22 576 children: randomised controlled trial. Lancet. 2004;364(9443):1411-20. Epub 2004/10/19. doi:  
23 577 10.1016/s0140-6736(04)17223-1. PubMed PMID: 15488216.  
24 578 48. UNAIDS. Mozambique country Fact sheet. 2015; Available at  
25 579 [http://www.unaids.org/sites/default/files/media/documents/UNAIDS\\_GlobalplanCountryfacts](http://www.unaids.org/sites/default/files/media/documents/UNAIDS_GlobalplanCountryfactsheet_mozambique_en.pdf)  
26 580 [heet\\_mozambique\\_en.pdf](http://www.unaids.org/sites/default/files/media/documents/UNAIDS_GlobalplanCountryfactsheet_mozambique_en.pdf) [accessed February 2017].  
27 581 49. Sylla EH, Kun JF, Kreamsner PG. Mosquito distribution and entomological inoculation  
28 582 rates in three malaria-endemic areas in Gabon. Trans R Soc Trop Med Hyg. 2000;94(6):652-6.  
29 583 Epub 2001/02/24. doi: 10.1016/s0035-9203(00)90219-0. PubMed PMID: 11198649.  
30 584 50. Ministère-de-la-Santé-et-de-la-Prévoyance-sociale-Gabon. Rapport National sur la  
31 585 Réponse au VIH/SIDA 2014. 2015; Available at  
32 586 [http://www.unaids.org/sites/default/files/country/documents/GAB\\_narrative\\_report\\_2015.p](http://www.unaids.org/sites/default/files/country/documents/GAB_narrative_report_2015.pdf)  
33 587 [df](http://www.unaids.org/sites/default/files/country/documents/GAB_narrative_report_2015.pdf) [accessed February 7].  
34 588 51. Mourou JR, Coffinet T, Jarjaval F, Cotteaux C, Pradines E, Godefroy L, et al. Malaria  
35 589 transmission in Libreville: results of a one year survey. Malar J. 2012;11:40. Epub 2012/02/11.  
36 590 doi: 10.1186/1475-2875-11-40. PubMed PMID: 22321336; PubMed Central PMCID:  
37 591 PMCPMC3310827.

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56 596 Figure 1. MAMAH trial design  
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597 **Tables**598 **Table 1. Malaria and HIV epidemiology in the study sites**

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

599 \*EIR: Entomological Inoculation Rate

600 <sup>#</sup> Data from 2010-2012 in women receiving either two IPTp doses of mefloquine or SP (Tuikue-Ndam et al, unpublished). NI: No information.601 † The trial will be conducted at the ANC services of the Manhiça District Hospital where the *Centro de Investigaçãõ em Saúde de Manhiça* (CISM) is situated; the average monthly number of pregnant women attending first ANC clinic visit is 110.

602 ‡ 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.

603 \*\* 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.

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614 **Table 2. Study outcomes**

<b>Primary endpoint</b>	
	Prevalence of maternal parasitaemia at delivery (defined by the presence of <i>P. falciparum</i> asexual parasites of any density in peripheral blood determined by microscopy)
<b>Secondary endpoints</b>	
<b>Maternal</b>	
	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 <i>P. falciparum</i> 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 <i>P. falciparum</i> 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 <i>P. falciparum</i> peripheral parasitaemia at the post-partum visit
	Maternal mortality rate
<b>Infant</b>	
	Prevalence of <i>P. falciparum</i> 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

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617 **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			
<b>SCREENING AND ENROLMENT:</b>								
Eligibility screen	X							
Informed consent	X							
Randomization		X						
<b>INTERVENTIONS:</b>								
IPTp administration			X	X	X			
CTX administration			X	*	*	*	*	*
ARV administration			X	*	*	*	*	*
LLITN distribution			X					
<b>MATERNAL ASSESSMENTS:</b>								
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

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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

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

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

620 † Only in women passively reporting sick AND presenting with malaria related signs/symptoms (fever ( $\geq 37,5^{\circ}$  C) or having history of fever in the past 24  
621 hours, arthromyalgia or headache), as per national management guidelines

622



623 Table 4. Schedule of infant visits and procedures

	TIMEPOINTS					Unscheduled visits
	Birth	1 month*	6 months	9 months	12 months	
<b>PROCEDURES:</b>						
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

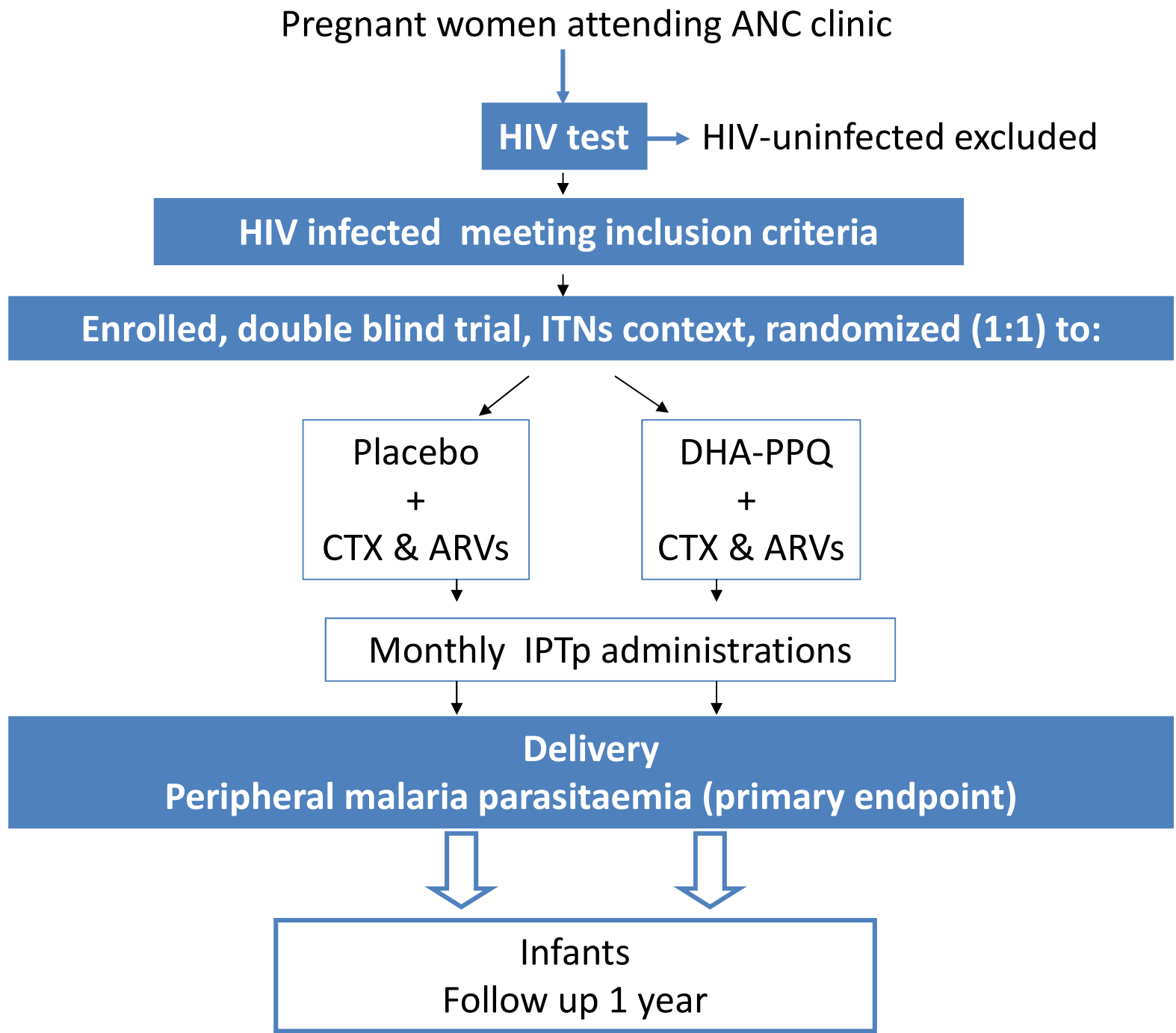
625 † Only if fever ( $\geq 37,5^{\circ}$  C) or history of fever in the past 24 hours or signs suggestive of malaria.

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

627 # Adherence should be assessed at each visit.

628







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

Section/item	Item No	Description	Addressed on page number
<b>Administrative information</b>			
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 ___

1	<b>Introduction</b>			
2				
3	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 ___
4				
5				
6		6b	Explanation for choice of comparators	___ 7,8 ___
7				
8	Objectives	7	Specific objectives or hypotheses	___ 9 ___
9				
10	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 ___
11				
12				
13				
14	<b>Methods: Participants, interventions, and outcomes</b>			
15				
16	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 ___
17				
18				
19	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 ___
20				
21				
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	___ 11,12 ___
23				
24		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 ___
25				
26		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	___ 14,15 ___
27				
28		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	___ 12-15 ___
29				
30	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 ___
31				
32				
33				
34	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 ___
35				
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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_____
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	_____13_____
5				
6				
7	<b>Methods: Assignment of interventions (for controlled trials)</b>			
8	Allocation:			
9				
10	Sequence	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_____
11	generation			
12				
13				
14				
15				
16	Allocation	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_____
17	concealment			
18	mechanism			
19				
20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____10-11_____
21				
22				
23				
24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	_____10-11_____
25				
26				
27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____11_____
28				
29				
30				
31	<b>Methods: Data collection, management, and analysis</b>			
32				
33	Data collection	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_____
34	methods			
35				
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39		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_____
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1	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___
2				
3				
4				
5	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___
6				
7				
8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	___18___
9				
10		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___
11				
12				
13				
14	<b>Methods: Monitoring</b>			
15				
16	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___
17				
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22		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___
23				
24				
25	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___
26				
27				
28	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___
29				
30				
31				
32	<b>Ethics and dissemination</b>			
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	___19___
35				
36				
37	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___
38				
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1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	_____10_____
2				
3				
4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____n/a_____
5				
6				
7	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_____
8				
9				
10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____20_____
11				
12				
13	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_____
14				
15				
16	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_____
17				
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19				
20	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_____
21				
22				
23				
24		31b	Authorship eligibility guidelines and any intended use of professional writers	_____20_____
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____19_____
27				
28				
29	<b>Appendices</b>			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	_____n/a_____
32				
33				
34	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_____
35				
36				

\*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”](https://creativecommons.org/licenses/by-nc-nd/3.0/) 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:	<i>BMJ Open</i>
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Keywords:	HIV & AIDS < INFECTIOUS DISEASES, Maternal medicine < OBSTETRICS, Clinical trials < THERAPEUTICS, TROPICAL MEDICINE, Epidemiology < TROPICAL MEDICINE

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

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30

31 **Word count:** 3907

32

For peer review only

1  
2  
3 **Abstract**  
4  
5

6 **Introduction:** Malaria infection during pregnancy is an important driver of maternal and  
7  
8 neonatal health especially among HIV-infected women. Intermittent preventive treatment in  
9  
10 pregnancy (IPTp) with sulphadoxine-pyrimethamine is recommended for malaria prevention in  
11  
12 HIV-uninfected women but it is contraindicated in those HIV-infected on cotrimoxazole  
13  
14 prophylaxis (CTXp) due to potential adverse effects. Dihydroartemisinin-piperaquine (DHA-  
15  
16 PPQ), has been shown to improve antimalarial protection, constituting a promising IPTp  
17  
18 candidate. This trial's objective is to determine if monthly three-day IPTp courses of DHA-PPQ  
19  
20 added to daily CTXp are safe and superior to CTXp alone in decreasing the proportion of  
21  
22 peripheral malaria parasitaemia at the end of pregnancy.  
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26  
27 **Methods and analysis:** This is a multi-centre, two-arm, placebo-controlled, individually  
28  
29 randomized trial in HIV-infected pregnant women receiving CTXp and antiretroviral treatment.  
30  
31 A total of 664 women will be enrolled at the first antenatal care clinic visit in sites from Gabon  
32  
33 and Mozambique. Participants will receive an insecticide-treated net and they will be  
34  
35 administered monthly IPTp with DHA-PPQ or placebo (1:1 ratio) as directly observed therapy  
36  
37 from the second trimester of pregnancy. Primary study outcome is the prevalence of maternal  
38  
39 parasitaemia at delivery. Secondary outcomes include prevalence of malaria-related maternal  
40  
41 and infant outcomes and proportion of adverse perinatal outcomes. Participants will be  
42  
43 followed until six weeks after the end of pregnancy and their infants until one year of age to also  
44  
45 evaluate the impact of DHA-PPQ on mother to child transmission of HIV. The analysis will be  
46  
47 done in the Intention to Treat and According to Protocol cohorts, adjusted by gravidity, country,  
48  
49 seasonality and other variables associated with malaria.  
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55 **Ethics and dissemination:** The protocol was reviewed and approved by the institutional and  
56  
57 national ethics committees of Gabon and Mozambique and the Hospital Clinic of Barcelona.  
58  
59 Project results will be presented to all stakeholders and published in open access journals.  
60

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3 59 **Strengths and limitations of this study**  
4

- 5 60 • A major strength of this trial is its double blind placebo-controlled design which will allow to  
6  
7 61 yield conclusive results about the efficacy of the study intervention.  
8  
9 62 • The inclusion of pregnant women from different sub-Saharan countries will provide a wide  
10  
11 63 representation of different malaria endemicity areas and HIV subgroups.  
12  
13 64 • The study is also adequately powered to test the superiority hypothesis.  
14  
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16  
17 65

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19  
20 66 **Keywords**  
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22  
23 67 Malaria, HIV, pregnancy, prevention, treatment.  
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26 68

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29 69 **Trial registration**  
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33 70 ClinicalTrials.gov, NCT03671109. Registered on 14<sup>th</sup> September 2018. Recruitment is currently  
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35 71 ongoing.  
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## 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

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3 99 adverse effects. Thus, even though IPTp-SP is a life-saving and highly cost-effective intervention  
4  
5 100 it cannot be administered to the most vulnerable group, HIV-infected women [16-19].  
6  
7 101 Consequently and paradoxically the most susceptible women to malaria are currently the least  
8  
9 102 protected [20].  
10  
11

12  
13 103 A placebo-controlled trial conducted in three sub-Saharan countries between 2010 and 2013  
14  
15 104 showed that an effective antimalarial added to CTXp and long-lasting ITNs (LLITNs) in HIV-  
16  
17 105 infected pregnant women has a significant impact in improving malaria prevention and maternal  
18  
19 106 health through reductions in hospital admissions [21]. However, the antimalarial used  
20  
21 107 (mefloquine) was not well tolerated, and most importantly, it was associated with a two-fold  
22  
23 108 increase in the frequency of mother to child transmission of HIV (MTCT-HIV), limiting its  
24  
25 109 potential for IPTp. These findings indicate the need to find drug alternatives with better  
26  
27 110 tolerability and safety profile to reduce malaria in this vulnerable group [21].  
28  
29  
30

31  
32 111 Dihydroartemisinin-piperaquine (DHA-PPQ) is an artemisinin-based combination therapy (ACT)  
33  
34 112 recommended by the WHO for treatment of uncomplicated malaria in adults and children aged  
35  
36 113  $\geq$  six months[22]. Among the available antimalarial drugs, DHA-PPQ constitutes one of the best  
37  
38 114 candidates for IPTp because it is not used as first-line malaria treatment, it is well tolerated, and  
39  
40 115 it is recommended for treatment of clinical malaria in the second and third trimesters of  
41  
42 116 pregnancy [22].  
43  
44  
45

46 117 DHA-PPQ has been safely used for case management in HIV-uninfected pregnant women in  
47  
48 118 Thailand and in a multicentre trial in sub-Saharan Africa [23, 24]. Moreover, studies comparing  
49  
50 119 IPTp-SP with IPTp-DHA-PPQ in HIV-uninfected pregnant women from Kenya and Uganda  
51  
52 120 indicate that the drug could be an alternative to SP in terms of antimalarial efficacy and safety  
53  
54 121 in pregnancy [25, 26]. A recent meta-analysis of the safety and efficacy of repeated doses of  
55  
56 122 DHA-PPQ for prevention and treatment of malaria including 11 studies (among adults, children  
57  
58 123 and pregnant women) has concluded that monthly DHA-PPQ is well tolerated and effective for  
59  
60



1  
2  
3 124 IPT and that additional data are needed in pregnancy and to further explore the cardiac safety  
4  
5 125 with monthly dosing [27]. Scientific evidence shows that efficacy and safety findings from  
6  
7 126 malaria control strategies evaluated in HIV-uninfected pregnant women cannot be directly  
8  
9 127 extrapolated to HIV-infected women [21]. A trial comparing monthly IPTp with DHA-PPQ to CTXp  
10  
11 128 among 200 HIV-infected Ugandan women did not find differences in the risk of  
12  
13 129 histopathologically detected placental malarial infection and other outcomes between groups  
14  
15 130 [28]. However, authors acknowledge the limitations of their results due to the low prevalence  
16  
17 131 of malaria in the study area at the time of the trial [28]. Therefore, it is of highest public health  
18  
19 132 priority to provide conclusive evidence as to whether the most vulnerable population (HIV-  
20  
21 133 infected pregnant women) will benefit from the use of the currently most promising and  
22  
23 134 available alternative drug for IPTp, DHA-PPQ [20].  
24  
25  
26  
27

28  
29 135 The objectives of the MAMAH (Improving **Maternal heAlth** by reducing **Malaria** in **African HiV**  
30  
31 136 women) trial are (1) to evaluate the safety, tolerability and efficacy of DHA-PPQ as IPTp for  
32  
33 137 malaria prevention in HIV-infected pregnant women receiving daily CTXp and ARV drugs, (2) to  
34  
35 138 assess the effect of DHA-PPQ as IPTp on mother to child transmission of HIV and (3) to evaluate  
36  
37 139 the effectiveness of CTXp in clearing malaria parasites in HIV-infected pregnant women.  
38  
39  
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41  
42

## 141 **2. Methods**

43  
44  
45  
46 142 This is multi-centre, two-arm, placebo-controlled, individually randomized superiority clinical  
47  
48 143 trial with two study arms including HIV-infected pregnant women. The study will be carried out  
49  
50 144 and reported according to Consolidated Standards of Reporting Trials guidelines [29].  
51  
52

### 145 ***Study settings***

53  
54  
55  
56  
57 146 The trial will be conducted in two countries from Central and South Eastern sub-Saharan Africa  
58  
59 147 (Gabon and Mozambique), where HIV prevalence among pregnant women ranges from 6 to 29%  
60

1  
2  
3 148 [30, 31]. Malaria epidemiologic indicators and HIV prevalence in pregnancy in study sites are  
4  
5 149 shown in Table 1. The trial sites have been selected to provide representation of different  
6  
7 150 malaria endemicity and HIV subgroups (HIV-1 subtype C in Mozambique and HIV-1 CRF02\_AG in  
8  
9 151 Gabon, where HIV-2 also circulates [32, 33]).  
10  
11  
12

### 13 152 ***Study population***

14  
15  
16 153 All pregnant women attending the study ANC services for the first time and/or who have not  
17  
18 154 received IPTp during their current pregnancy will be screened for participation in the trial.  
19  
20 155 Inclusion criteria are (1) permanent resident in the study area, (2) gestational age at the first  
21  
22 156 antenatal visit  $\leq$  28 weeks, (3) HIV seropositive status and (4) agreement to deliver in the study  
23  
24 157 site's maternity(ies) wards. Exclusion criteria are (1) planning to move out in the following 10  
25  
26 158 months from enrolment, (2) gestational age at the first antenatal visit  $>$  28 weeks of pregnancy,  
27  
28 159 (3) known history of allergy to CTX, (4) known history of allergy or contraindications to DHA-PPQ  
29  
30 160 and (5) participating in other intervention studies.  
31  
32  
33  
34

### 35 161 ***Informed consent***

36  
37  
38 162 All participants will receive information about study procedures, including knowledge about  
39  
40 163 malaria and HIV infection in pregnancy. A signed informed consent form (or thumb-printed with  
41  
42 164 a witness whenever the woman is illiterate) will be obtained before any study tests or  
43  
44 165 evaluations are carried out by study nurses in each site. The trial's informed consent is available  
45  
46 166 as Supplemental Material 1. If the participant is under the legal age of maturity, she will sign  
47  
48 167 the assent form and her legal guardian will sign the informed consent according to national  
49  
50 168 ethics local policies. The informed consent will cover the woman and the new born infant.  
51  
52  
53  
54

### 55 169 ***Recruitment and randomization***

1  
2  
3 170 After the study details are explained and informed consent is signed, pregnant women will be  
4  
5 171 given a study number and automatically randomized to one of the study arms: 1) Daily CTX +  
6  
7 172 monthly IPTp-DHA-PPQ or 2) Daily CTX + Monthly IPTp-placebo. Each participant will be uniquely  
8  
9 173 identified in the study by a combination of her site code and participant number. Allocation of  
10  
11 174 participants to study arms will be done centrally by the trial's Sponsor (the Barcelona Institute  
12  
13 175 for Global Health, ISGlobal) by block randomization and stratified by country. This method will  
14  
15 176 ensure balanced allocation to both arms during different malaria seasons in the two study  
16  
17 177 countries. Each subject number will be related to a treatment number which assigns them to  
18  
19 178 one of the IPT arms. Study number allocation for each study participant will be concealed in  
20  
21 179 opaque sealed envelopes that will be opened only after recruitment. At each site the first  
22  
23 180 participant will be assigned a patient number, and consecutive numbers will be assigned to  
24  
25 181 subsequent women. A study identification card containing the individual study number and  
26  
27 182 basic demographic information will be given to the participant in order to facilitate identification  
28  
29 183 at all study contacts.

30  
31  
32  
33  
34  
35 184 Figure 1 displays the study design.

### 36 37 38 185 **Blinding**

39  
40  
41 186 Study tablets (IPTp-DHA-PPQ and IPTp-Placebo) will be identically packaged. Study personnel  
42  
43 187 will prepare and deliver the medication to the participant. All study personnel, investigators,  
44  
45 188 outcome assessors, data analysts and the participants will remain blinded throughout the trial.  
46  
47 189 Unblinding is only envisaged in case of a medical emergency (in such case, the investigator on  
48  
49 190 site will have to justify to the Sponsor and the DSMB the need for unblinding).

### 50 51 52 53 191 **Interventions**

#### 54 55 56 57 192 **a) IPTp administration**

1  
2  
3 193 Administration of the three-day IPTp course will always be done under fasting conditions  
4  
5 194 (following DHA-PPQ administration recommendations) and direct observation by study  
6  
7 195 personnel. The number of daily IPTp tablets to be administered will be based on bodyweight and  
8  
9 196 according to the treatment guidelines set by WHO [target dose (range) of 4 (2–10) mg/kg/ day  
10  
11 197 of DHA and 18 (16–27) mg/kg/day of PPQ given once a day for three days for adults]. Following  
12  
13 198 physical examination, recruited women of gestational age $\geq$ 13 weeks will receive the assigned  
14  
15 199 IPTp drug. In case of gestational age $<$  13 weeks, first IPTp administration will be scheduled one  
16  
17 200 month later. If participants report malaria treatment in the preceding four weeks, first IPTp  
18  
19 201 administration will also be delayed one month. Administration of the second and third day  
20  
21 202 treatment course will be done by study personnel either at the study health facility or household  
22  
23 203 level. Women will be observed for 60 minutes after administration of the IPTp dose. Those  
24  
25 204 women vomiting within the first 30 minutes of IPTp administration will be given a second full  
26  
27 205 IPTp dose; women vomiting after 30-60 minutes of IPTp administration will be given an  
28  
29 206 additional half dose of the drug. Subsequent doses of IPTp will be given coinciding with the next  
30  
31 207 scheduled monthly ANC clinic visit, at least one month apart from the previous dose.  
32  
33  
34  
35

36  
37  
38 **b) CTX administration**  
39

40  
41 209 Cotrimoxazole (fixed combination drug containing 800 mg of trimethoprim and 160 mg of  
42  
43 210 sulfamethoxazole) will be taken daily from enrolment until delivery as per national guidelines  
44  
45 211 for prevention of opportunistic infections. Women will be asked to visit the ANC clinic monthly  
46  
47 212 to receive the amount of CTX tablets for the whole month. At each ANC clinic visit, adherence  
48  
49 213 to CTX prophylaxis will be assessed.  
50

51  
52  
53 **c) ARV therapy and concomitant medications**  
54  
55  
56  
57  
58  
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1  
2  
3 215 Treatment for prevention of HIV MTCT (Option B+) will be given by the study health personnel,  
4  
5 216 according to national HIV/AIDS control guidelines[34]. Any other concomitant treatment  
6  
7 217 received by the study participants will be recorded in the study questionnaires.  
8  
9

10  
11 218 **d) Long lasting insecticide treated nets**  
12

13  
14 219 Regardless of gestational age at the time of recruitment, all women will receive a LLITN and  
15  
16 220 details about its use will be explained.  
17

18  
19 221 **Study Outcomes**  
20

21  
22 222 The primary outcome of the trial will be the prevalence of maternal parasitaemia at delivery  
23  
24 223 defined by the presence of *Plasmodium falciparum* (*P. falciparum*) asexual parasites of any  
25  
26 224 density in peripheral blood (determined by microscopy). The secondary maternal and infant  
27  
28 225 endpoints can be found in Table 2.  
29  
30

31  
32 226 **Sample size**  
33

34  
35 227 Based on previous estimations at the study sites and assuming a prevalence of peripheral  
36  
37 228 parasitaemia at delivery of 7.5% with CTXp, it is estimated that 298 women per arm will be  
38  
39 229 required to detect with 80% power a significant ( $p < 0.05$ ) decrease of 5% or more in the  
40  
41 230 prevalence of peripheral parasitaemia in the CTXp+IPTp-DHA-PPQ group [21]. In order to allow  
42  
43 231 for 10% losses to follow up, it is calculated that 332 women/study arm will need to be recruited  
44  
45 232 (total  $n=664$ ). Considering the prevalence of HIV infection among pregnant women in both sites  
46  
47 233 (please refer to Table 1), a total of 444 women are planned to be recruited in Mozambique and  
48  
49 234 220 women in Gabon to allow for a recruitment rate of 33 women/month on average. These  
50  
51 235 estimations are based on recruitment rates of previous IPTp clinical trials conducted among  
52  
53 236 pregnant women in the two study sites [21, 35, 36].  
54  
55  
56  
57  
58

59 237 **Follow up and measurements of outcomes**  
60

1  
2  
3 238 At baseline, the woman's demographic and obstetric information will be recorded in study  
4  
5 239 specific case report forms (CRF).  
6  
7

8  
9 240 **a) Physical and clinical examination at enrolment**

10  
11 241 The physical examination of the woman will include the following assessments: weight, height,  
12  
13 242 gestational age by bimanual palpation and measurement of middle-upper arm circumference  
14  
15 243 (MUAC). Ultrasound (US) will be performed to determine gestational age at enrolment and  
16  
17 244 confirm pregnancy viability.  
18  
19

20  
21  
22 245 **b) Baseline biological samples**

23  
24  
25 246 At enrolment, a venous blood sample (5 mL) will be collected for analysis of haemoglobin level,  
26  
27 247 CD4 cell counts, HIV viral load and malaria PCR.  
28  
29

30  
31 248 **c) Follow-up and household visits**

32  
33 249 Women will be given an appointment to attend the subsequent ANC clinic visit one month after  
34  
35 250 the first one. Participants will receive the standard ANC package of interventions, which includes  
36  
37 251 iron and folate supplementation, following national guidelines. The subsequent IPTp doses will  
38  
39 252 be given at least four weeks apart from the previous one. MAMAH participants will be asked to  
40  
41 253 visit the study facilities in case of any illness. A malaria blood smear will be collected in those  
42  
43 254 participants passively reporting sick and presenting with malaria related signs/symptoms (fever  
44  
45 255 ( $\geq 37.5^{\circ}$  C) or having history of fever in the past 24 hours, arthromyalgias or headache), as per  
46  
47 256 national management guidelines. Women will be visited at home the day after recruitment to  
48  
49 257 confirm residence status, assess drug tolerability and the correct use of the net. Adherence to  
50  
51 258 CTX prophylaxis, ARV therapy and compliance with the LLITNs use will be assessed monthly at  
52  
53 259 the ANC attendance.  
54  
55  
56  
57  
58

59 260 **d) Adverse events monitoring and reporting**  
60

1  
2  
3 261 Active safety monitoring will consist in household visits to study participants two days after each  
4  
5 262 IPTp administration to assess drug tolerability and record all Adverse Events (AEs). In addition,  
6  
7 263 a health facility-based passive surveillance system will be established to capture unscheduled  
8  
9 264 visits of participants during follow-up. Information on unsolicited adverse events will be  
10  
11 265 collected at each scheduled and unscheduled visit. Any participant passively reporting being sick  
12  
13 266 during the study visits, will be referred to the clinical services as per routine system in place. A  
14  
15 267 blood smear will be collected in those presenting with malaria related signs/symptoms (fever  
16  
17 268 ( $\geq 37,5^{\circ}$  C) or having history of fever in the past 24 hours, arthromyalgias or headache). In case  
18  
19 269 of malaria parasitaemia or anaemia, they will be treated following national guidelines. Serious  
20  
21 270 Adverse Events (SAEs) will be reported by the site investigator within 24 hours of being made  
22  
23 271 aware to the trial's safety monitor and the Data Safety Monitoring Board (DSMB).  
24  
25  
26  
27

28  
29 272 ***e) End of pregnancy and infant assessments***  
30

31  
32 273 At the end of pregnancy, maternal blood (5 mL), placental and cord blood samples (5 mL) will be  
33  
34 274 collected for haematological and parasitological examination. The new-born will be examined,  
35  
36 275 weighed and measured and his/her gestational age assessed by the modified Ballard method  
37  
38 276 [37].  
39  
40

41  
42 277 ***f) Post-partum visit***  
43  
44

45 278 Participants will be visited approximately after six weeks of end of pregnancy at the study health  
46  
47 279 facility where a blood smear for malaria screening will be collected. A summary of study  
48  
49 280 procedures is displayed in Table 3.  
50  
51

52  
53 281 ***g) Infants follow up***  
54  
55

56 282 Infants born to study participants will be followed up until one year of age. Mothers will be asked  
57  
58 283 to bring their child to the study facilities at weeks 4-6 of age and at 6, 9 and 12 months after  
59  
60

1  
2  
3 284 birth. At each scheduled visit, study infants will be physically examined and their psychomotor  
4  
5 285 and neurological development will be assessed following a standard protocol for African settings  
6  
7 286 [38-40]. Weight, height and axillary temperature will be measured and recorded. A capillary  
8  
9 287 blood sample will be taken from infants with fever (axillary temperature  $\geq 37.5^{\circ}\text{C}$ ) or history of  
10  
11 288 fever in the last 24 hours, or appearing pale, for malaria parasitaemia examination and  
12  
13 289 haematological determination. In case of malaria parasitaemia or anaemia, they will be treated  
14  
15 290 following national guidelines. At each scheduled visit, HIV DNA will be evaluated in the infant  
16  
17 291 from dried blood spot on filter paper (for PCR). As per routine healthcare national programs HIV-  
18  
19 292 exposed infants will receive CTX prophylaxis starting four to six weeks after birth. Infants will  
20  
21 293 also receive ARV, according to national guidelines. Infant's follow up visits and study procedures  
22  
23 294 are described in Table 4.

### 295 **Laboratory tests**

#### 296 **a) Parasitological and haematological determinations**

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

#### 300 **b) Detection of HIV and quantitative determination of viral load**

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

#### 305 **c) Immunological determinations related to HIV status**

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



1  
2  
3 308 Biosciences) and TruCOUNT tubes (Becton Dickinson, San Jose, CA; USA) or *MiniVidas* device.  
4  
5 309 HIV viral load will be determined from plasma cryopreserved at -80°C using the devices in place  
6  
7 310 in the study sites (such as COBAS® AMPLICOR , AmpliPrep [Roche Diagnostics] or *GeneXpert*).  
8  
9

#### 10 311 *d) Placental samples analysis*

11  
12 312 A tissue sample (approximately 2 cm<sup>3</sup>) will be collected from the maternal surface of the  
13  
14 313 placenta and will be immediately placed in 25 mL of 10% neutral buffered formalin and kept at  
15  
16 314 4°C until processed and embedded in paraffin wax by standard techniques. Paraffin sections  
17  
18 315 4µm thick from the placental tissue, will be stained with hematoxylin and eosin, Giemsa's stain  
19  
20 316 and the periodic acid-Schiff technique. Placentas will be classified histologically as: i) not  
21  
22 317 infected, ii) active infection and iii) past infection, depending on the presence or absence of  
23  
24 318 parasites, pigment or both [41]. Impression smears will be prepared from the placental blood  
25  
26 319 for parasitological examination. Blood from the placenta will also be collected onto filter paper  
27  
28 320 for PCR determination of malaria parasites.  
29  
30  
31  
32

#### 33 321

#### 34 322 **Data management**

35  
36  
37 323 Data will be collected using paper case report forms (CRFs) developed for the trial by study  
38  
39 324 personnel at each scheduled and unscheduled visit. The quality of the data recorded in the study  
40  
41 325 source documents and CRFs will be monitored regularly following the principles of Good Clinical  
42  
43 326 Practices by the trials' clinical monitor [42]. Data will be double entered into the study database  
44  
45 327 using the OpenClinica open source software (version 3.14) for clinical data management  
46  
47 328 (www.OpenClinica.com) at each study site. Automatic quality checks will be performed to  
48  
49 329 ensure CRF completeness. The database system will be designed to protect the confidentiality  
50  
51 330 (sensitive data will be automatically encrypted) and integrity of the data and will include  
52  
53 331 authorization, authentication, auditing and availability features to safeguard the access and  
54  
55 332 usage of the data. Concomitant medications registered into the database will be coded using  
56  
57  
58  
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60

1  
2  
3 333 the WHO Drug Reference List, which employs the Anatomical Therapeutic Chemical  
4  
5 334 classification system. Medical history/current medical conditions and adverse events will be  
6  
7 335 coded using the Medical dictionary for regulatory activities (MedDRA) terminology.  
8  
9

10 336

11  
12 337 ***Analysis plan***  
13

14  
15 338 The following populations of analysis have been defined: a) Intention to treat (ITT): it includes  
16  
17 339 all randomized pregnant women who have data on outcome (target population for the efficacy  
18  
19 340 analysis); b) According to protocol (ATP): it includes participants who fulfil all the inclusion-  
20  
21 341 exclusion criteria, took monthly IPTp-DHA-PPQ/placebo study doses, received a LLITN, received  
22  
23 342 CTXp and ARV drugs and from whom data is available for the analysis; c) Safety: it includes  
24  
25 343 participants who received at least one dose of IPTp DHA-PPQ/placebo and had at least one post-  
26  
27 344 baseline safety assessment. No interim analyses of data is envisaged. The primary analysis of the  
28  
29 345 trial will be the comparison of the proportion of pregnant women with peripheral parasitaemia  
30  
31 346 at delivery in the ITT and ATP cohorts, adjusted by gravidity, country, seasonality and other  
32  
33 347 variables associated with the prevalence of malaria. Proportions will be compared between  
34  
35 348 groups using the Fisher exact test. Crude and adjusted analysis will be done using Poisson  
36  
37 349 regression with a log link and robust estimate of the covariance (Huber method), using the  
38  
39 350 method proposed by Zou [43, 44]. Relative risk ratio (RR) or reduction of the RR ( $1 - RR * 100\%$ )  
40  
41 351 if RR lower than 1 will be presented. Continuous variables will be compared between groups  
42  
43 352 using T-test and the Wilcoxon rank values according to variables' characteristics. The differences  
44  
45 353 in rank values will be assessed by looking at the expected rank sums in each group under the  
46  
47 354 null hypothesis. Adjustment for co-variates and possible cofounders (such as country,  
48  
49 355 gestational age, gravidity, anaemia, literacy, Mid-Upper Arm Circumference (MUAC), viral load  
50  
51 356 and CD4+T cell count) will be done using ordinary least square regression. Subgroup analysis is  
52  
53 357 not envisaged. Incidence of clinical malaria, overall admissions and outpatient attendances will  
54  
55 358 be estimated as the number of episodes over the time at risk. Time at risk will be estimated as  
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58  
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2  
3 359 the time from the start of follow up until the end of follow-up (visit one month after end of  
4  
5 360 pregnancy for mothers) or withdrawal due to censoring or death, whatever occurs first. The total  
6  
7 361 number of events will be compared between groups using Negative Binomial regression models  
8  
9 362 which takes into account a possible extra Poisson variation due to different frailty of the  
10  
11 363 subjects. The comparison will be expressed as relative rate ratio (RRate). Data analysis will be  
12  
13  
14 364 performed using Stata (Stata Corp., College Station, TX, US).

### 17 365 ***Patient and public involvement***

20 366 Patients will not be directly involved in the design, conduct, reporting, or dissemination plans of  
21  
22 367 the study. However, a Community Advisory Board (CAB) has been set at each study site to  
23  
24 368 strengthen communication and interaction between the local study community and research  
25  
26 369 teams. The CAB also oversees and guides the study team on key issues such as potential risks  
27  
28 370 and burdens for participants or host communities that may be hidden from researchers, and  
29  
30 371 how to minimize them [45].

### 35 372 ***Ethics and dissemination***

38 373 The study is conducted in accordance with the EMA/ICH Guideline on Good Clinical Practice  
39  
40 374 and in total agreement with the applicable international, EU and national law of all the  
41  
42 375 participating countries[46]. The study protocol (version 1.0, 2<sup>nd</sup> May 2018) and the informed  
43  
44 376 consent forms have been reviewed and approved by the institutional and national ethics  
45  
46 377 committees of Gabon (042/2018/PR/SG/CNER) and Mozambique (504/CBNS/18) and the  
47  
48 378 Hospital Clinic of Barcelona (HCB/2018/0650, Spain). An independent DSMB monitors  
49  
50 379 regularly the safety of study participants. The trial is registered on clinicaltrials.gov  
51  
52 380 (<https://clinicaltrials.gov/ct2/show/NCT03671109>).

57 381 The findings of the clinical trial will be submitted for publication in a peer-reviewed journal  
58  
59 382 within 12 months of study completion through an open access mechanism, or otherwise made

1  
2  
3 383 available publicly in compliance with H2020 open access requirements. Primary project raw data  
4  
5 384 will be published in the project website. At no stage will data containing personal information  
6  
7 385 of research participants be released.  
8  
9

10 386 A project communication has been developed in order to ensure timely, accurate, and effective  
11  
12 387 dissemination of project results. After concluding the trial's data analysis, findings will be made  
13  
14 388 available to all partners, key stakeholders and Ministries of Health. The project members will  
15  
16 389 actively disseminate information to the scientific community through reports, presentations at  
17  
18 390 scientific forums and publications in international open access journals. Trial results will also be  
19  
20 391 shared with the WHO Global Malaria Program and Malaria Policy Advisory Groups.  
21  
22  
23  
24  
25

392

#### 393 **Authors' contributions**

26  
27  
28 394 Conceived and designed the study: RG and CM. Gave inputs to protocol methodology: RG, TN,  
29  
30 395 GMN, JM, ME, AMT, CPD, LBD, BL, HL, LGO, RZM, MEG, SS, MP, ES, MR, FS, CM. Wrote the first  
31  
32 396 draft of the manuscript: RG and CPD. Wrote sections on sample size, data management and  
33  
34 397 analysis plan: SS and RG. Wrote, reviewed and approved the manuscript: RG, TN, GMN, JM, ME,  
35  
36 398 AMT, CPD, LBD, BL, HL, LGO, RZM, MEG, SS, MP, ES, MR, FS, CM  
37  
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39

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43  
44  
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5 409 **Competing interests**  
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411 **References**

- 412 1. WHO. World Malaria report 2020. WHO. 2020;Available at  
413 <https://www.who.int/publications/i/item/9789240015791> [Accessed March 2021].
- 414 2. Mombo-Ngoma G, Mackanga JR, Gonzalez R, Ouedraogo S, Kakolwa MA, Manego RZ, et  
415 al. Young adolescent girls are at high risk for adverse pregnancy outcomes in sub-Saharan Africa:  
416 an observational multicountry study. *BMJ open*. 2016;6(6):e011783. Epub 2016/07/01. doi:  
417 10.1136/bmjopen-2016-011783. PubMed PMID: 27357200.
- 418 3. Group WAbCTAABS. Clinical determinants of early parasitological response to ACTs in  
419 African patients with uncomplicated falciparum malaria: a literature review and meta-analysis  
420 of individual patient data. *BMC Med*. 2015;13:212. Epub 2015/09/08. doi: 10.1186/s12916-015-  
421 0445-x. PubMed PMID: 26343145; PubMed Central PMCID: PMC4561425.
- 422 4. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoia K, Brabin B, et al. Epidemiology  
423 and burden of malaria in pregnancy. *Lancet Infect Dis*. 2007;7(2):93-104. Epub 2007/01/26. doi:  
424 S1473-3099(07)70021-X [pii]  
425 10.1016/S1473-3099(07)70021-X [doi]. PubMed PMID: 17251080.
- 426 5. Menendez C, D'Alessandro U, ter Kuile FO. Reducing the burden of malaria in pregnancy  
427 by preventive strategies. *Lancet Infect Dis*. 2007;7(2):126-35. Epub 2007/01/26. doi: S1473-  
428 3099(07)70024-5 [pii]  
429 10.1016/S1473-3099(07)70024-5 [doi]. PubMed PMID: 17251083.
- 430 6. Menendez C, Ordi J, Ismail MR, Ventura PJ, Aponte JJ, Kahigwa E, et al. The impact of  
431 placental malaria on gestational age and birth weight. *J Infect Dis*. 2000;181(5):1740-5. Epub  
432 2000/05/24. doi: 10.1086/315449. PubMed PMID: 10823776.
- 433 7. Bardají A, Sigauque B, Sanz S, Maixenchs M, Ordi J, Aponte JJ, et al. Impact of malaria at  
434 the end of pregnancy on infant mortality and morbidity. *J Infect Dis*. 2011;203(5):691-9. Epub  
435 2011/01/05. doi: 10.1093/infdis/jiq049. PubMed PMID: 21199881; PubMed Central PMCID:  
436 PMC3071276.
- 437 8. Desai M, Hill J, Fernandes S, Walker P, Pell C, Gutman J, et al. Prevention of malaria in  
438 pregnancy. *Lancet Infect Dis*. 2018;18(4):e119-e32. Epub 2018/02/06. doi: 10.1016/s1473-  
439 3099(18)30064-1. PubMed PMID: 29395997.
- 440 9. UNAIDS/WHO. Global report: UNAIDS report on the global AIDS Epidemic 2013. WHO  
441 Library Cataloguing-in-Publication Data 2013;UNAIDS(JC2502/1/E).
- 442 10. WHO. Malaria and HIV interactions and their implications for public health policy WHO.  
443 2005;ISBN 92 4 1593350.
- 444 11. Kwenti TE. Malaria and HIV coinfection in sub-Saharan Africa: prevalence, impact, and  
445 treatment strategies. *Research and reports in tropical medicine*. 2018;9:123-36. Epub  
446 2018/08/14. doi: 10.2147/rrtm.S154501. PubMed PMID: 30100779; PubMed Central PMCID:  
447 PMC6067790.
- 448 12. ter Kuile FO, Parise ME, Verhoeff FH, Udhayakumar V, Newman RD, van Eijk AM, et al.  
449 The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant  
450 women in sub-saharan Africa. *Am J Trop Med Hyg*. 2004;71(2 Suppl):41-54. PubMed PMID:  
451 15331818.
- 452 13. Gonzalez R, Ataide R, Nanche D, Menendez C, Mayor A. HIV and malaria interactions:  
453 where do we stand? *Expert Rev Anti Infect Ther*. 2012;10(2):153-65. doi: 10.1586/eri.11.167.  
454 PubMed PMID: 22339190.
- 455 14. WHO. Intermittent Preventive Treatment of malaria in pregnancy using Sulfadoxine-  
456 Pyrimethamine (IPTp-SP). Updated WHO Policy Recommendation. WHO.  
457 2012;[http://www.who.int/malaria/iptp\\_sp\\_updated\\_policy\\_recommendation\\_en\\_102012.pdf](http://www.who.int/malaria/iptp_sp_updated_policy_recommendation_en_102012.pdf)  
458 .

- 1  
2  
3 459 15. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and  
4 460 preventing HIV infection Recommendations for a public health approach June 2013. 2013.  
5 461 16. Sevene E, Gonzalez R, Menendez C. Current knowledge and challenges of antimalarial  
6 462 drugs for treatment and prevention in pregnancy. *Expert Opin Pharmacother*. 2010;11(8):1277-  
7 463 93. PubMed PMID: 20408744.  
8 464 17. Ward SA, Sevene EJ, Hastings IM, Nosten F, McGready R. Antimalarial drugs and  
9 465 pregnancy: safety, pharmacokinetics, and pharmacovigilance. *Lancet Infect Dis*. 2007;7(2):136-  
10 466 44. Epub 2007/01/26. doi: S1473-3099(07)70025-7 [pii]  
11  
12 10.1016/S1473-3099(07)70025-7 [doi]. PubMed PMID: 17251084.  
13 467  
14 468 18. Menendez C, Bardaji A, Sigauque B, Sanz S, Aponte JJ, Mabunda S, et al. Malaria  
15 469 prevention with IPTp during pregnancy reduces neonatal mortality. *PloS one*. 2010;5(2):e9438.  
16 470 PubMed PMID: 20195472.  
17 471 19. Eisele TP, Larsen DA, Anglewicz PA, Keating J, Yukich J, Bennett A, et al. Malaria  
18 472 prevention in pregnancy, birthweight, and neonatal mortality: a meta-analysis of 32 national  
19 473 cross-sectional datasets in Africa. *Lancet Infect Dis*. 2012;12(12):942-9. Epub 2012/09/22. doi:  
20 474 10.1016/s1473-3099(12)70222-0. PubMed PMID: 22995852.  
21 475 20. Gonzalez R, Sevene E, Jagoe G, Slutsker L, Menendez C. A Public Health Paradox: The  
22 476 Women Most Vulnerable to Malaria Are the Least Protected. *PLoS Med*. 2016;13(5):e1002014.  
23 477 Epub 2016/05/04. doi: 10.1371/journal.pmed.1002014. PubMed PMID: 27139032.  
24 478 21. Gonzalez R, Desai M, Macete E, Ouma P, Kakolwa MA, Abdulla S, et al. Intermittent  
25 479 Preventive Treatment of Malaria in Pregnancy with Mefloquine in HIV-Infected Women  
26 480 Receiving Cotrimoxazole Prophylaxis: A Multicenter Randomized Placebo-Controlled Trial. *PLoS*  
27 481 *Med*. 2014;11(9):e1001735. Epub 2014/09/24. doi: 10.1371/journal.pmed.1001735. PubMed  
28 482 PMID: 25247995.  
29 483 22. WHO. Guidelines for the treatment of malaria. Second edition. WHO. 2010;ISBN  
30 484 9789241547925.  
31 485 23. Rijken MJ, McGready R, Boel ME, Barends M, Proux S, Pimanpanarak M, et al.  
32 486 Dihydroartemisinin-piperaquine rescue treatment of multidrug-resistant *Plasmodium*  
33 487 *falciparum* malaria in pregnancy: a preliminary report. *Am J Trop Med Hyg*. 2008;78(4):543-5.  
34 488 Epub 2008/04/04. PubMed PMID: 18385345.  
35 489 24. Pekyi D, Ampromfi AA, Tinto H, Traore-Coulibaly M, Tahita MC, Valea I, et al. Four  
36 490 Artemisinin-Based Treatments in African Pregnant Women with Malaria. *N Engl J Med*.  
37 491 2016;374(10):913-27. Epub 2016/03/11. doi: 10.1056/NEJMoa1508606. PubMed PMID:  
38 492 26962727.  
39 493 25. Desai M, Gutman J, L'Lanziva A, Otieno K, Juma E, Kariuki S, et al. Intermittent screening  
40 494 and treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine  
41 495 versus intermittent preventive treatment with sulfadoxine-pyrimethamine for the control of  
42 496 malaria during pregnancy in western Kenya: an open-label, three-group, randomised controlled  
43 497 superiority trial. *Lancet*. 2015. Epub 2015/10/03. doi: 10.1016/s0140-6736(15)00310-4. PubMed  
44 498 PMID: 26429700.  
45 499 26. Kakuru A, Jagannathan P, Muhindo MK, Natureeba P, Awori P, Nakalembe M, et al.  
46 500 Dihydroartemisinin-Piperaquine for the Prevention of Malaria in Pregnancy. *N Engl J Med*.  
47 501 2016;374(10):928-39. Epub 2016/03/11. doi: 10.1056/NEJMoa1509150. PubMed PMID:  
48 502 26962728; PubMed Central PMCID: PMC4847718.  
49 503 27. Gutman J, Kovacs S, Dorsey G, Stergachis A, Ter Kuile FO. Safety, tolerability, and efficacy  
50 504 of repeated doses of dihydroartemisinin-piperaquine for prevention and treatment of malaria:  
51 505 a systematic review and meta-analysis. *Lancet Infect Dis*. 2017;17(2):184-93. Epub 2016/11/21.  
52 506 doi: 10.1016/s1473-3099(16)30378-4. PubMed PMID: 27865890; PubMed Central PMCID:  
53 507 PMCPMC5266794.  
54 508 28. Natureeba P, Kakuru A, Muhindo M, Ochieng T, Ategeka J, Koss CA, et al. Intermittent  
55 509 Preventive Treatment With Dihydroartemisinin-Piperaquine for the Prevention of Malaria



- 1  
2  
3 510 Among HIV-Infected Pregnant Women. *J Infect Dis.* 2017;216(1):29-35. Epub 2017/03/23. doi:  
4 511 10.1093/infdis/jix110. PubMed PMID: 28329368; PubMed Central PMCID: PMC5853208.
- 5 512 29. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for  
6 513 reporting parallel group randomized trials. *Annals of internal medicine.* 2010;152(11):726-32.  
7 514 Epub 2010/03/26. doi: 10.7326/0003-4819-152-11-201006010-00232. PubMed PMID:  
8 515 20335313.
- 9 516 30. Gonzalez R, Munguambe K, Aponte J, Bavo C, Nhalungo D, Macete E, et al. High HIV  
10 517 prevalence in a southern semi-rural area of Mozambique: a community-based survey. *HIV Med.*  
11 518 2012. PubMed PMID: 22500780.
- 12 519 31. Manego RZ, Mombo-Ngoma G, Witte M, Held J, Gmeiner M, Gebru T, et al. Demography,  
13 520 maternal health and the epidemiology of malaria and other major infectious diseases in the rural  
14 521 department Tsamba-Magotsi, Ngounie Province, in central African Gabon. *BMC Public Health.*  
15 522 2017;17(1):130. Epub 2017/01/29. doi: 10.1186/s12889-017-4045-x. PubMed PMID: 28129759;  
16 523 PubMed Central PMCID: PMC5273856.
- 17 524 32. Caron M, Lekana-Douki SE, Makuwa M, Obiang-Ndong GP, Biba O, Nkoghe D, et al.  
18 525 Prevalence, genetic diversity and antiretroviral drugs resistance-associated mutations among  
19 526 untreated HIV-1-infected pregnant women in Gabon, central Africa. *BMC infectious diseases.*  
20 527 2012;12:64. Epub 2012/03/22. doi: 10.1186/1471-2334-12-64. PubMed PMID: 22433277;  
21 528 PubMed Central PMCID: PMC3359209.
- 22 529 33. Lahuerta M, Aparicio E, Bardaji A, Marco S, Sacarlal J, Mandomando I, et al. Rapid spread  
23 530 and genetic diversification of HIV type 1 subtype C in a rural area of southern Mozambique. *AIDS*  
24 531 *Res Hum Retroviruses.* 2008;24(2):327-35. PubMed PMID: 18271719.
- 25 532 34. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and  
26 533 preventing HIV infection 2016. Recommendations for a public health approach. Second edition.:  
27 534 World Health Organization; 2016.
- 28 535 35. Gonzalez R, Mombo-Ngoma G, Ouedraogo S, Kakolwa MA, Abdulla S, Accrombessi M, et  
29 536 al. Intermittent Preventive Treatment of Malaria in Pregnancy with Mefloquine in HIV-Negative  
30 537 Women: A Multicentre Randomized Controlled Trial. *PLoS Med.* 2014;11(9):e1001733. Epub  
31 538 2014/09/24. doi: 10.1371/journal.pmed.1001733. PubMed PMID: 25247709.
- 32 539 36. Menendez C, Bardaji A, Sigauque B, Romagosa C, Sanz S, Serra-Casas E, et al. A  
33 540 randomized placebo-controlled trial of intermittent preventive treatment in pregnant women  
34 541 in the context of insecticide treated nets delivered through the antenatal clinic. *PloS one.*  
35 542 2008;3(4):e1934. Epub 2008/04/10. doi: 10.1371/journal.pone.0001934 [doi]. PubMed PMID:  
36 543 18398460; PubMed Central PMCID: PMC2277457.
- 37 544 37. Ballard JL, Houry JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R. New Ballard Score,  
38 545 expanded to include extremely premature infants. *The Journal of pediatrics.* 1991;119(3):417-  
39 546 23. Epub 1991/09/01. PubMed PMID: 1880657.
- 40 547 38. Gladstone M, Lancaster GA, Umar E, Nyirenda M, Kayira E, van den Broek NR, et al. The  
41 548 Malawi Developmental Assessment Tool (MDAT): the creation, validation, and reliability of a  
42 549 tool to assess child development in rural African settings. *PLoS Med.* 2010;7(5):e1000273. Epub  
43 550 2010/06/04. doi: 10.1371/journal.pmed.1000273. PubMed PMID: 20520849; PubMed Central  
44 551 PMCID: PMC2876049.
- 45 552 39. Koura KG, Boivin MJ, Davidson LL, Ouedraogo S, Zoumenou R, Alao MJ, et al. Usefulness  
46 553 of child development assessments for low-resource settings in francophone Africa. *Journal of*  
47 554 *developmental and behavioral pediatrics : JDBP.* 2013;34(7):486-93. Epub 2013/08/01. doi:  
48 555 10.1097/DBP.0b013e31829d211c. PubMed PMID: 23899660; PubMed Central PMCID:  
49 556 PMC3821168.
- 50 557 40. Prado EL, Abubakar AA, Abbeddou S, Jimenez EY, Some JW, Ouedraogo JB. Extending  
51 558 the Developmental Milestones Checklist for use in a different context in Sub-Saharan Africa.  
52 559 *Acta paediatrica (Oslo, Norway : 1992).* 2014;103(4):447-54. Epub 2013/12/21. doi:  
53 560 10.1111/apa.12540. PubMed PMID: 24354938.



- 1  
2  
3 561 41. Ismail MR, Ordi J, Menendez C, Ventura PJ, Aponte JJ, Kahigwa E, et al. Placental  
4 562 pathology in malaria: a histological, immunohistochemical, and quantitative study. Human  
5 563 pathology. 2000;31(1):85-93. Epub 2000/02/09. PubMed PMID: 10665918.  
6 564 42. Pharmalys. Pharmalys CRO. 2021; Available at <https://pharmalys.com/> [Accessed July  
7 565 2021].  
8 566 43. Zou G. A modified poisson regression approach to prospective studies with binary data.  
9 567 American journal of epidemiology. 2004;159(7):702-6. Epub 2004/03/23. PubMed PMID:  
10 568 15033648.  
11 569 44. Zou GY, Donner A. Extension of the modified Poisson regression model to prospective  
12 570 studies with correlated binary data. Statistical methods in medical research. 2013;22(6):661-70.  
13 571 Epub 2011/11/11. doi: 10.1177/0962280211427759. PubMed PMID: 22072596.  
14 572 45. NHREC. Guidelines for Community Advisory Groups. NHREC. 2012; Available at :  
15 573 [http://research.ukzn.ac.za/Files/National%20Guidelines%20for%20Community%20Advisory%20](http://research.ukzn.ac.za/Files/National%20Guidelines%20for%20Community%20Advisory%20Groups%20for%20Research%20(2012).pdf)  
16 574 [OGroups%20for%20Research%20\(2012\).pdf](http://research.ukzn.ac.za/Files/National%20Guidelines%20for%20Community%20Advisory%20Groups%20for%20Research%20(2012).pdf) [Accessed April 2021].  
17 575 46. EMA. Guideline for Good Clinical Practice E6 (R2). EMA. 2015; Available at  
18 576 [https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-good-clinical-](https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-good-clinical-practice-e6r2-4-step-2b_en.pdf)  
19 577 [practice-e6r2-4-step-2b\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-good-clinical-practice-e6r2-4-step-2b_en.pdf) [Accessed April 2021].  
20 578 47. Alonso PL, Sacarlal J, Aponte JJ, Leach A, Macete E, Milman J, et al. Efficacy of the  
21 579 RTS,S/AS02A vaccine against Plasmodium falciparum infection and disease in young African  
22 580 children: randomised controlled trial. Lancet. 2004;364(9443):1411-20. Epub 2004/10/19. doi:  
23 581 10.1016/s0140-6736(04)17223-1. PubMed PMID: 15488216.  
24 582 48. UNAIDS. Mozambique country Fact sheet. 2015; Available at  
25 583 [http://www.unaids.org/sites/default/files/media/documents/UNAIDS\\_GlobalplanCountryfacts](http://www.unaids.org/sites/default/files/media/documents/UNAIDS_GlobalplanCountryfactsheet_mozambique_en.pdf)  
26 584 [heet\\_mozambique\\_en.pdf](http://www.unaids.org/sites/default/files/media/documents/UNAIDS_GlobalplanCountryfactsheet_mozambique_en.pdf) [accessed February 2017].  
27 585 49. Sylla EH, Kun JF, Kremsner PG. Mosquito distribution and entomological inoculation  
28 586 rates in three malaria-endemic areas in Gabon. Trans R Soc Trop Med Hyg. 2000;94(6):652-6.  
29 587 Epub 2001/02/24. doi: 10.1016/s0035-9203(00)90219-0. PubMed PMID: 11198649.  
30 588 50. Ministère-de-la-Santé-et-de-la-Prévoyance-sociale-Gabon. Rapport National sur la  
31 589 Réponse au VIH/SIDA 2014. 2015; Available at  
32 590 [http://www.unaids.org/sites/default/files/country/documents/GAB\\_narrative\\_report\\_2015.p](http://www.unaids.org/sites/default/files/country/documents/GAB_narrative_report_2015.pdf)  
33 591 [df](http://www.unaids.org/sites/default/files/country/documents/GAB_narrative_report_2015.pdf) [accessed February 7].  
34 592 51. Mourou JR, Coffinet T, Jarjaval F, Cotteaux C, Pradines E, Godefroy L, et al. Malaria  
35 593 transmission in Libreville: results of a one year survey. Malar J. 2012;11:40. Epub 2012/02/11.  
36 594 doi: 10.1186/1475-2875-11-40. PubMed PMID: 22321336; PubMed Central PMCID:  
37 595 PMCPMC3310827.

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598 Supplemental Material 1. Study Informed consent form

599 Figure Legends:

600 Figure 1. MAMAH trial design

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601 **Tables**602 **Table 1. Malaria and HIV epidemiology in the study sites**

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

603 \*EIR: Entomological Inoculation Rate

604 <sup>#</sup> Data from 2010-2012 in women receiving either two IPTp doses of mefloquine or SP (Tuikue-Ndam et al, unpublished). NI: No information.605  
606 <sup>†</sup> The trial will be conducted at the ANC services of the Manhiça District Hospital where the *Centro de Investigaçãõ em Saúde de Manhiça* (CISM) is situated; the average monthly number of pregnant women attending first ANC clinic visit is 110.607  
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609 <sup>‡</sup> 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 115.610  
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612 <sup>\*\*</sup> 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.

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618 **Table 2. Study outcomes**

<b>Primary endpoint</b>	
	Prevalence of maternal parasitaemia at delivery (defined by the presence of <i>P. falciparum</i> asexual parasites of any density in peripheral blood determined by microscopy)
<b>Secondary endpoints</b>	
<b>Maternal</b>	
	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 <i>P. falciparum</i> 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 <i>P. falciparum</i> 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 <i>P. falciparum</i> peripheral parasitaemia at the post-partum visit
	Maternal mortality rate
<b>Infant</b>	
	Prevalence of <i>P. falciparum</i> 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

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621 **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			
<b>SCREENING AND ENROLMENT:</b>								
Eligibility screen	X							
Informed consent	X							
Randomization		X						
<b>INTERVENTIONS:</b>								
IPTp administration			X	X	X			
CTX administration			X	*	*	*	*	*
ARV administration			X	*	*	*	*	*
LLITN distribution			X					
<b>MATERNAL ASSESSMENTS:</b>								
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

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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

622 # Only in the first household visit after the ANC visit of first IPTp administration.  
623 \* CTX and ARV adherence should be assessed at each scheduled visit.  
624 † 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  
625 hours, arthromyalgia or headache), as per national management guidelines  
626

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

	TIMEPOINTS					Unscheduled visits
	Birth	1 month*	6 months	9 months	12 months	
<b>PROCEDURES:</b>						
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	#	#	#	#	#	#

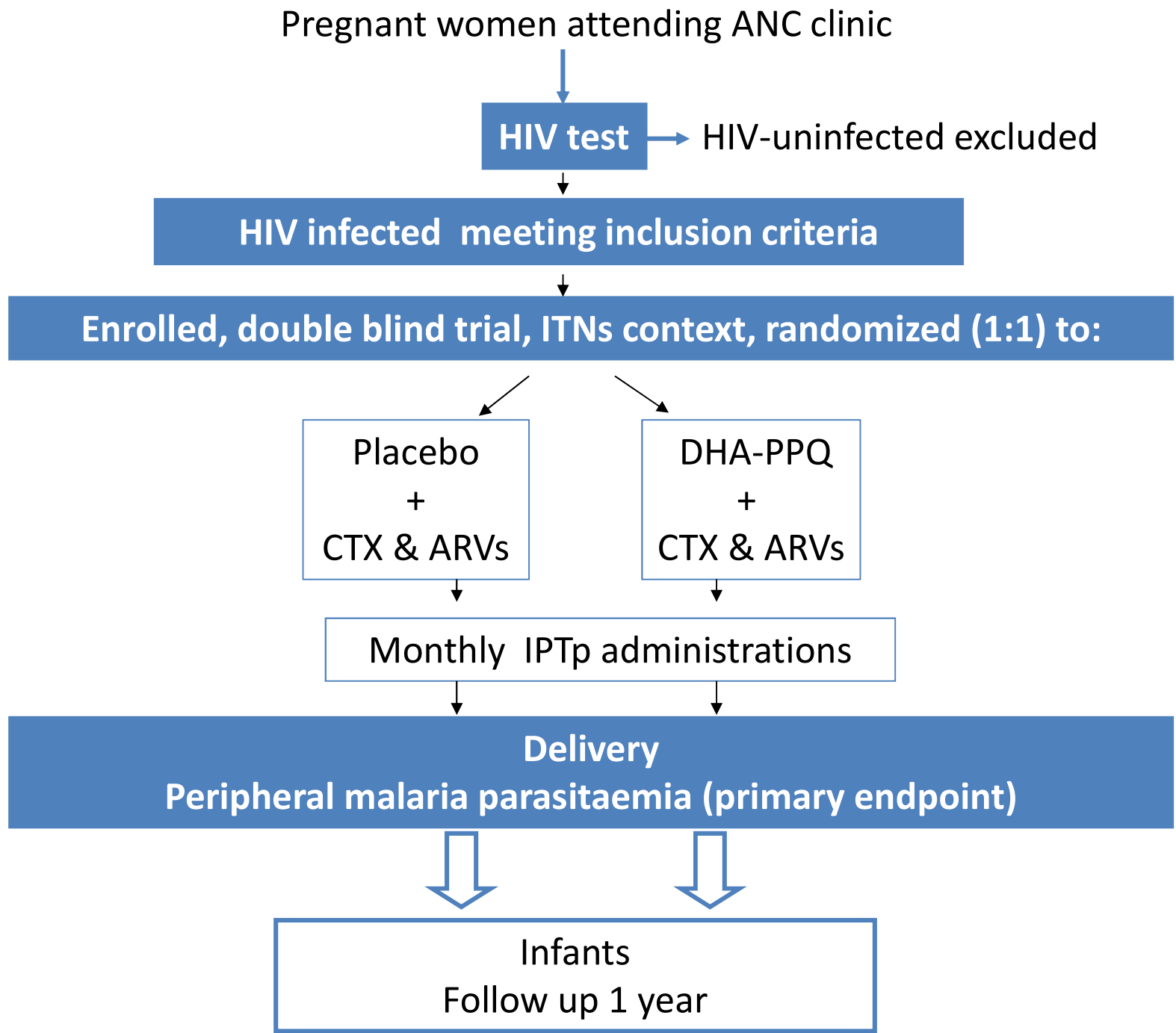
628 \* First visit will be scheduled 1 month after birth or coinciding with first EPI visit

629 † Only if fever ( $\geq 37,5^{\circ}$  C) or history of fever in the past 24 hours or signs suggestive of malaria.

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

631 # Adherence should be assessed at each visit.

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## 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).*

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### **Title of the study**

Evaluation of the safety and efficacy of dihydroartemisinin-piperazine (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 dihydroartemisinin-piperazine (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, dihydroartemisinin-piperazine 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 dihydroartemisinin-piperazine (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 dihydroartemisinin-piperazine 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.

1  
2  
3 Participants from both groups will have the same study visits. Before you learn about the study,  
4 it is important that you know that your participation in this study is voluntary and you may  
5 decide not to participate, not to have the tests, or to withdraw from the study at any time.  
6

7  
8 Let me explain to you what we mean by placebo. The placebo is a tablet that looks like  
9 dihydroartemisinin-piperaquine tablet but it does not have the ingredients that the  
10 dihydroartemisinin-piperaquine has and it will not prevent against malaria. You will receive  
11 either dihydroartemisinin-piperaquine or placebo by chance.  
12

### 13 ***What happens during the study***

14 If you agree to be in this study, your first visit will continue today, after you read, discuss, and  
15 sign or put thumbprint on this form.  
16

17  
18 You will be asked to come back to the clinic monthly before delivery. In addition, you must agree  
19 to deliver your baby at the study facility rather than at home. Because you have HIV virus, you  
20 will be offered drugs (antiretrovirals) for your treatment and for the prevention of mother to  
21 child transmission of HIV as per routine antenatal care and will be followed up as usual.  
22

23 If you agree to be in this study:

- 24 • We will first ask you some questions about yourself and your health
- 25 • We will ask you to give information on where you live and how to keep in contact with
- 26 you. The study staff will use this information to visit you at home to see how you are
- 27 feeling and to remind you about your study visits.
- 28 • A study clinician will examine you and will check your pregnancy status
- 29 • You will also be asked to give a venous blood sample at the first visit for tests of your
- 30 blood
- 31 • At the first study visit at the clinic, in the presence of the study nurse, you will take either
- 32 dihydroartemisinin-piperaquine or placebo (assigned by chance)
- 33 • The following day and the day after you will be visited by study personnel at home to
- 34 complete the three day course treatment of DHA-PPQ
- 35 • Subsequent doses of DHA-PPQ or placebo will be given to you at the next scheduled
- 36 monthly ANC clinic visit at least one month apart
- 37 • You will also receive cotrimoxazole and ARV drugs to take home and take it once a day
- 38 as per routine ANC care
- 39 • In case you will be unwell with malaria or other infection, you will have additional blood
- 40 tests done and if needed you will be given medicine and asked to come back here as
- 41 scheduled by study staff
- 42 • At enrolment you will receive a long lasting insecticide treated net and will be told how
- 43 to use it
- 44 • You and your baby will receive a unique identification number (ID) and identification
- 45 study card, which you will be requested to present to the study staff at every visit
- 46 • Even though you will receive drug for malaria prevention (if in dihydroartemisinin-
- 47 piperaquine group), it is possible that you may still get sick. Therefore you will be asked
- 48 to come to the clinic whenever you feel unwell, get fever or any other symptoms.
- 49 • At delivery you will be visited during in the labour ward and you and your new-born
- 50 baby will be examined by the study personnel.
- 51 • In addition to venous blood being collected from you, also a sample of cord blood will
- 52 be taken to be tested for malaria
- 53 • A piece of placenta will be examined at the study laboratory and also tested for malaria
- 54 • Blood sample will be taken from your baby for malaria tests
- 55
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- 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*

Dihydroartemisinin-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 dihydroartemisinin-piperazine 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?

For peer review only

1  
2  
3 **STATEMENT of CONSENT AND SIGNATURE**  
4

5 Participant and new-born approval:

6 The consent form has been explained to me and I agree to take part in this study. I also agree  
7 to let my new-born baby take part in this study. I understand that I am free to choose to be in  
8 the study and that saying "No" will not affect the treatment I get in this clinic, now and in  
9 future.

10 NOTE: You are not giving up any of your legal rights by signing this informed consent  
11 document.  
12

13  
14 If you agree circle YES  
15

16 \_\_\_\_\_  
17 Volunteer's Name  
18 (*print*)

16 \_\_\_\_\_  
17 Volunteer's Signature or  
18 Thumbprint (*if cannot write*)

16 \_\_\_\_\_  
17 Date

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26 Volunteer's Legal Guardian  
27 or Representative  
28 (as per country policy)  
29 (*print*)

25 \_\_\_\_\_  
26 Legal Guardian's Signature

25 \_\_\_\_\_  
26 Date

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36 \_\_\_\_\_  
37 Witness's Name  
38 (if participant illiterate)  
39 (*print*)

36 \_\_\_\_\_  
37 Witness's Signature

36 \_\_\_\_\_  
37 Date

40  
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43  
44 I have explained the purpose of this study to the volunteer. To the best of my knowledge, she  
45 understands the purpose, procedures, risks and benefits of this study.  
46  
47  
48

49 \_\_\_\_\_  
50 Investigator/Designee Name  
51 (*print*)

49 \_\_\_\_\_  
50 Investigator/Designee Signature

49 \_\_\_\_\_  
50 Date

52  
53  
54  
55 **NOTE:** This consent form with original signatures must be retained on file by the principal  
56 investigator. A copy must be given to the volunteer.  
57

58 If the woman refuses to take her copy of the consent with her, she states so below and signs  
59 and dates her decline statement.  
60



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

Section/item	Item No	Description	Addressed on page number
<b>Administrative information</b>			
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__

1	<b>Introduction</b>			
2				
3	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant	___ 7,8,9 ___
4	rationale		studies (published and unpublished) examining benefits and harms for each intervention	
5				
6		6b	Explanation for choice of comparators	___ 7,8 ___
7				
8	Objectives	7	Specific objectives or hypotheses	___ 9 ___
9				
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),	
11			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	___ 9 ___
12				
13				
14	<b>Methods: Participants, interventions, and outcomes</b>			
15				
16	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	___ 9,10 ___
17			be collected. Reference to where list of study sites can be obtained	
18				
19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	___ 10 ___
20			individuals who will perform the interventions (eg, surgeons, psychotherapists)	
21				
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be	___ 11,12 ___
23			administered	
24				
25		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose	___ n/a ___
26			change in response to harms, participant request, or improving/worsening disease)	
27				
28		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence	___ 14,15 ___
29			(eg, drug tablet return, laboratory tests)	
30				
31		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	___ 12-15 ___
32				
33	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood	___ 13, Table 2 ___
34			pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg,	
35			median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen	
36			efficacy and harm outcomes is strongly recommended	
37				
38	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for	___ 11,12, Figure 1
39			participants. A schematic diagram is highly recommended (see Figure)	
40				
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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_____
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	_____13_____
5				
6				
7	<b>Methods: Assignment of interventions (for controlled trials)</b>			
8	Allocation:			
9				
10	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_____
11				
12				
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15				
16	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_____
17				
18				
19				
20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____10-11_____
21				
22				
23				
24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	_____10-11_____
25				
26				
27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____11_____
28				
29				
30				
31	<b>Methods: Data collection, management, and analysis</b>			
32				
33	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_____
34				
35				
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39		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_____
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1	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___
2				
3				
4				
5	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___
6				
7				
8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	___18___
9				
10		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___
11				
12				
13				
14	<b>Methods: Monitoring</b>			
15				
16	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___
17				
18				
19				
20				
21				
22		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___
23				
24				
25	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___
26				
27				
28	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___
29				
30				
31				
32	<b>Ethics and dissemination</b>			
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	___19___
35				
36				
37	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___
38				
39				
40				
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42				

1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	_____10_____
2				
3				
4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____n/a_____
5				
6				
7	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_____
8				
9				
10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____20_____
11				
12				
13	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_____
14				
15				
16	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_____
17				
18				
19				
20	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_____
21				
22				
23				
24		31b	Authorship eligibility guidelines and any intended use of professional writers	_____20_____
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____19_____
27				
28				
29	<b>Appendices</b>			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	__Supplemental Material 1__
32				
33				
34	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_____
35				
36				

\*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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.