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# BMJ Open

## The effect of dose on the antimalarial efficacy of artesunate-mefloquine against *Plasmodium falciparum* malaria: a protocol for systematic review and individual patient data (IPD) meta-analysis

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Manuscripts

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4 1 The effect of dose on the antimalarial efficacy of artesunate-mefloquine  
5 2 against *Plasmodium falciparum* malaria: a protocol for systematic  
6 3 review and individual patient data (IPD) meta-analysis  
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## 24 Abstract

25 **Introduction:** Antimalarial posology based on weight-bands leave patients at the margins  
26 vulnerable to receiving either lower or higher weight-adjusted (mg/kg) dosages. This study  
27 aims to describe the distribution of artesunate and mefloquine dosage administered in patients  
28 with uncomplicated *P. falciparum* malaria treated with an artesunate-mefloquine (AS-MQ)  
29 regimen. Relationship between mg/kg dose and therapeutic outcomes will be assessed through  
30 a one-stage individual participant data (IPD) meta-analysis (MA).

31 **Methods and analysis:** Therapeutic efficacy studies with the AS-MQ regimen will be  
32 identified by searching the following databases: PUBMED, EMBASE, and Web of Science.  
33 The corresponding authors of the relevant studies will be requested to share the IPD for the  
34 purpose of this meta-analysis to a secured repository. All available studies will be standardised  
35 using a common data management protocol and pooled into a single database. The relationship  
36 between mg/kg dosage and treatment failures will be assessed using a Cox regression model  
37 with study sites considered as a shared frailty term. Safety parameters will be explored where  
38 available.

39 **Ethics and Dissemination:** This IPD meta-analysis met the criteria for waiver of ethical review  
40 as defined by the Oxford Tropical Research Ethics Committee (OxTREC) as the research  
41 consists of secondary analysis of existing anonymous data. The results of this analysis will be  
42 disseminated at conferences, WWARN website and any peer-reviewed publication arising will  
43 be made open-source.

44 **PROSPERO Registration:** CRD42018103776

45 **Keywords:** malaria, *Plasmodium falciparum*, efficacy, IPD meta-analysis, mefloquine,  
46 artemisinin, artemisinin combination therapy

## 47 **Strengths and limitations of this study**

- 48 • With the exception of recent studies in Southeast Asia, the regimen artesunate-mefloquine  
49 has consistently demonstrated an efficacy greater than 95% and treatment failures in any  
50 single antimalarial study are few. This IPD-MA will allow a robust exploration of host,  
51 parasite and drug factors associated with therapeutic outcomes, which otherwise would not  
52 be possible.
- 53 • The proposed IPD-MA will allow exploration of variations in the weight-adjusted dosage  
54 received by patients, which is not possible with aggregate data meta-analysis.
- 55 • The IPD-MA will be carried out as a study group under the auspices of the WorldWide  
56 Antimalarial Resistance Network (WWARN), which has championed responsible data  
57 sharing and advocates translational research.
- 58 • A limitation of this analysis will be heterogeneity between studies included in terms of  
59 design, patient population and the susceptibility of the parasites against the drug regimen.

## 60 Introduction

61 The combination artesunate-mefloquine (AS-MQ) was the first antimalarial regimen  
62 developed as an Artemisinin Combination Therapy (ACT) when mefloquine resistance became  
63 rampant along the Thai-Myanmar border in the early 1990s<sup>1</sup>. The efficacy of a combination  
64 regimen (artemisinin derivative + partner component) depends upon the ability of the partner  
65 component to mop up the residual parasites leftover after the initial and highly potent anti-  
66 parasitic activity of the artemisinin derivatives. This requires the dosage of the partner drug to  
67 be sufficient to ensure that blood concentrations exceed the Minimum Inhibitory Concentration  
68 (MIC) of the parasites until all the parasites have been killed. Manufacturers' recommendations  
69 regarding antimalarial posology are often pragmatic and the dose is administered based upon  
70 weight "banding". This approach inevitably results in some patients at the band margins  
71 receiving either lower or higher dosages when adjusted for body weight (Figure 1). Young  
72 children are particularly vulnerable to extreme total dosages especially when drug  
73 administration is based on tablets rather than paediatric formulations or suspensions. This may  
74 lead to sub-therapeutic drug concentrations in the blood plasma and such under-exposure has  
75 been related to poorer therapeutic response for some of the widely used ACTs<sup>2-4</sup>.

76 Until recently ACTs have consistently demonstrated an efficacy greater than 95% and  
77 treatment failures in any single antimalarial study are few thus limiting the ability to draw  
78 inferences regarding putative factors associated with therapeutic outcomes. Individual  
79 participant data (IPD) meta-analysis (MA) is being used increasingly to explore some of the  
80 putative factors which otherwise would not be possible through aggregate data meta-analysis<sup>5</sup>.  
81 Such a IPD-MA approach has been used to assess the dose-response relationships for the ACT  
82 regimens of artesunate-amodiaquine, artemether-lumefantrine and dihydroartemisinin-  
83 piperazine<sup>2,3,6</sup>. These studies have demonstrated that the drug formulation (fixed vs loose)  
84 and under-exposure in the paediatric population due to weight-banding are deterministic of

1  
2  
3 85 poorer therapeutic outcomes. A thorough evaluation of the dose-response relationship for the  
4  
5 86 regimen AS-MQ is lacking and this IPD-MA aims to address this research gap.  
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## 9 87 **Objectives**

10  
11 88 The overall aim of this study is to determine the mg/kg dosing range of artesunate (AS) and  
12  
13 89 mefloquine (MQ) adopted in clinical trials and to investigate the effects of mg/kg dosing on  
14  
15  
16 90 clinical outcome.  
17

18  
19 91 The specific objectives are:  
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- 21  
22  
23 92 • To investigate the effects of mefloquine and artesunate mg/kg dosing on early and late  
24  
25 93 clinical outcomes (treatment success or failure)  
26  
27 94 • To investigate the tolerability of AS-MQ across different study sites, population and age  
28  
29 95 groups  
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## 96 **Methods and analyses**

### 97 ***Criteria for study eligibility***

98 Studies identified will be deemed eligible for the purpose of this analysis if they meet the  
99 following criteria:

- 100 • Prospective clinical efficacy study of uncomplicated *P. falciparum* (either alone or  
101 mixed infections)
- 102 • Assessing the efficacy of a fixed-dose AS-MQ combination, either as single tablet  
103 type, or co-blister pack of more than one tablet type, or assessing the efficacy of a  
104 loose combination of AS-MQ
- 105 • Where AS was given over three days (with any number of doses per day) with a target  
106 total dose of 6-30mg/kg
- 107 • Where MQ was given over 1-3 days, on any of days 1-3 (with any number of doses  
108 per day) with a target total dose of 15-33mg/kg
- 109 • Where all AS and MQ were administered orally
- 110 • With a minimum of 28 days follow up
- 111 • With genotyping performed for late parasite recurrence
- 112 • With individual patient data on dosage of mefloquine received (actual or per protocol)  
113 by patients (dosage per tablets, number of tablets given per dose and duration of  
114 treatment)

### 115 ***Criteria for study exclusion***

- 116 • Where other antimalarial drugs were given in addition to the initial ASMQ treatment  
117 regimen, except for a single dose of primaquine of 0.25mg/kg in the first 3 days



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3 118 ***Types of study participants***  
4

5  
6 119 Patients with uncomplicated *P. falciparum* malaria will be included in this IPD-MA. The  
7  
8 120 following patients will be excluded from the analysis:  
9

- 10  
11 121 i. Severe *P. falciparum* malaria  
12  
13 122 ii. Pregnant women  
14  
15

16 123 ***Types of intervention/exposure and controls***  
17  
18  
19

- 20 124 • Fixed dose combination of AS-MQ, either as single tablet type, or co-blister pack of  
21  
22 125 more than one tablet type, or loose combination of AS-MQ. AS given over three days  
23  
24 126 (with any number of doses per day) with a target total dose of 6-30mg/kg. MQ given  
25  
26 127 over 1-3 days, on any of days 1-3 (with any number of doses per day) with a target  
27  
28 128 total dose of 15-33mg/kg  
29  
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31

32  
33 129 ***Types of outcomes***  
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- 35 130 i. Parasitological and clinical efficacy  
36  
37  
38 131 ii. Adverse events  
39  
40

41 132 ***Information sources and search strategy***  
42

43 133 We will carry out a systematic review and search PubMed, EMBASE, and Web of Science to  
44  
45 134 identify publications with artesunate-mefloquine between Jan 1990 to July 2018, the full search  
46  
47 135 terms are available from the PROSPERO registration (CRD42018103776). Any important  
48  
49 136 protocol amendments will be documented in the PROSPERO registration. Studies will be  
50  
51 137 included regardless of language and publication status. Study screenings will be carried by two  
52  
53 138 independent reviewers who will screen title, abstract, full text as necessary. The following  
54  
55 139 studies will be excluded: animal models (e.g. mouse malarias *P. berghei*, *P. chabaudi*),  
56  
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58  
59 140 publications only including severe malaria, studies with follow up <28 days, data previously  
60

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3 141 included in another published study, prophylaxis or mass drug administration studies, studies  
4  
5 142 in healthy volunteers/challenge studies, or studies in asymptomatic patients or pregnant women.  
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### 8 143 ***Data acquisition and data management***

#### 10 144 **Collating IPD**

11  
12  
13 145 Principal investigators of the published (or unpublished) studies identified from the literature  
14  
15 146 search will be invited to share IPD. At least three emails will be sent out in case of non-response.  
16  
17 147 Researchers agreeing to the terms and conditions of the submission will be requested to upload  
18  
19 148 anonymised IPD to the WWARN repository through a secure web portal. Figure 2 shows the  
20  
21 149 process map which depicts the different phases of data procurement; data acquisition from the  
22  
23 150 contributors, data standardisation, and their subsequent re-use in IPD meta-analysis.  
24  
25

26  
27 151 Data will be fully anonymised and handled in compliance with the UK Data Protection Act to  
28  
29 152 protect personal information and patient privacy. Original data will be stored on a secure server  
30  
31 153 hosted by the University of Oxford.  
32  
33

#### 34 154 **Data management**

35  
36  
37 155 Raw data from individual studies will be standardised using an open and transparent data  
38  
39 156 management and statistical analysis protocol<sup>7</sup>. Investigators will be further contacted for  
40  
41 157 validation or clarification, if required, and individual study protocols will be requested. On  
42  
43 158 standardisation, the data will be stored in a relational database of several tables containing  
44  
45 159 information on drug regimen, parasitological, clinical, and haematological assessments,  
46  
47 160 genotyping and therapeutic outcomes; all linked by a unique patient identifier.  
48  
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50

#### 51 161 **Statistical analysis plan**

#### 52 162 ***Study Population***

53  
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59 163 The following patients will be included in the analysis:  
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- 1  
2  
3 164 i. Information is available on drug dosage, either as exact number of tablets received,  
4  
5 165 exact mg/kg dose received or number of tablets planned per protocol  
6  
7  
8 166 ii. Date of the last day of follow-up or length of follow-up  
9  
10

11 167 The following patients will be excluded from the analysis:  
12  
13

- 14 168 i. Received other antimalarial drugs during follow-up before recorded *P. falciparum*  
15  
16 169 treatment failure  
17  
18 170 ii. Results of genotyping performed for late parasitological outcome are not available  
19  
20 171 iii. Missing confirmation of *P. falciparum* infection on enrolment  
21  
22 172 iv. Missing age or weight or gender  
23  
24 173 v. Other deviations, as defined in the data management plan<sup>7</sup> :  
25  
26 174 (a) Haemoglobin < 5 g/dL on day 0  
27  
28 175 (b) Haematocrit < 15% on day 0  
29  
30  
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33

34 176 **Outcomes**  
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- 37 177 Primary: PCR-corrected *P. falciparum* recrudescence  
38  
39  
40 178 Secondary: PCR-corrected *P. falciparum* reinfection  
41  
42  
43 179 PCR-uncorrected *P. falciparum* recurrence  
44  
45  
46 180 Early parasitological responses on days 1, 2 and 3  
47  
48  
49 181 Gametocyte carriage within 14 days of treatment initiation in patients without  
50  
51 182 gametocytaemia at enrolment  
52  
53  
54 183 Anaemia status on day 7  
55  
56  
57 184 Adverse symptoms developed after the drug administration  
58  
59  
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2  
3 185 The primary endpoint for this IPD-MA is Polymerase Chain Reaction (PCR) genotyping  
4  
5 186 corrected risk of *P. falciparum* recrudescence (treatment failure) on day 42. In the analysis of  
6  
7  
8 187 the primary endpoint, patients in whom new infections are observed during the study follow-  
9  
10 188 up, or those who are lost to follow-up will be censored; the former on the day new infection  
11  
12 189 was observed and the latter on their last recorded visit day. For the analysis of PCR corrected  
13  
14 190 new infections, patients with recrudescence and those who are lost to follow-up will be  
15  
16  
17 191 censored. Further definitions of status and other censorship are detailed in the Clinical Module  
18  
19 192 DMSAP <sup>7</sup>.

20  
21  
22  
23 193 Acute drug vomiting within an hour of treatment administration, general vomiting within 7  
24  
25 194 days of treatment initiation, diarrhoea within 7 days of treatment initiation, and,  
26  
27 195 neuropsychiatric adverse events (where available) are secondary endpoints.  
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## 196 **Variables and definition**

197 Artesunate and mefloquine doses received will be calculated from the number of tablets  
198 administered to each patient daily. If the actual number of tablets received is not recorded, the  
199 total dose in mg or mg/kg recorded as administered to each patient will be used. If none of  
200 these are available, administration as per protocol will be assumed. The current recommended  
201 daily mefloquine dose is 8.3 (range: 5 - 11) mg/kg<sup>8</sup>. A patient will be classified as under-dosed  
202 if the 3-day total mg/kg mefloquine dose is less than 15 mg/kg.

203 Nutritional status in children under 5 years of age will be assessed using standardised age,  
204 weight, height and gender specific growth reference standards according to the WHO 2006  
205 recommendations using igrowup Stata package<sup>9</sup>. Anthropometric indicators include weight-  
206 for-age (WAZ), height-for-age (HAZ), and weight-for-height (WHZ). The nutritional status of  
207 a child will be given as a Z-score and classified as stunted, underweight or wasted as defined  
208 in the WHO guidelines.

209 The falciparum malaria transmission intensity of the study sites will be assessed using the  
210 prevalence estimates generated by the Malaria Atlas Project (MAP) based on the latitude,  
211 longitude and the year the study was conducted<sup>10,11</sup>.

212 Anaemia will be defined as haemoglobin (Hb) < 10 g/dL or haematocrit (Ht) < 30%. Severe  
213 anaemia is defined as haemoglobin < 7 g/dL or haematocrit < 20%. Fever will be defined as  
214 body temperature > 37.5 degrees Celsius.

215 Parasite resistance status will be defined (data permitting) for each patient from South-East  
216 Asia region based on the reported prevalence of mutations of molecular markers (pfmdr1,  
217 kelch13) or the distribution of parasite clearance half-life for their study site and year of

1  
2  
3 218 admission<sup>12,13</sup>. For other locations, we will assume that parasites are sensitive to the ASMQ  
4  
5 219 regimen.  
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7

## 8 220 **Descriptive summary**

### 9 221 *Summary of the studies*

10  
11  
12  
13  
14 222 Summary of included studies will be presented with respect to: study location, years of study,  
15  
16 223 study population, duration of follow-up, AS-MQ drug formulation, methodology for parasite  
17  
18 224 quantification, methodology for PCR genotyping, supervision of drug administration. PCR-  
19  
20 225 corrected and uncorrected outcomes will be used to compute the Kaplan-Meier (K-M)  
21  
22 226 estimates using the censoring rules outlined above. The K-M estimates will be presented  
23  
24 227 graphically together with the number of patients in the risk set.  
25  
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27

### 28 228 *Summary of the patients*

29  
30  
31 229 Summary of baseline characteristics of the patients included in the analysis will be presented  
32  
33 230 for each study, by region and in overall. The following baseline characteristics of patients will  
34  
35 231 be presented: age; weight, parasitaemia on enrolment; presence of fever (body temperature >  
36  
37 232 37.5 degrees Celsius); haemoglobin (or haematocrit); anaemia (Hb < 10 g/dL) or severe  
38  
39 233 anaemia (Hb < 7 g/dL), gametocytes on presentation, description of infection (*P. falciparum*  
40  
41 234 or mixed infections), total mg/kg dose for each drug component, dosing strategies (age-based,  
42  
43 235 weight based etc.), dose formulation (fixed or loose etc.), and supervision of drug  
44  
45 236 administration. The number of available patients will be summarised for all variables,  
46  
47 237 proportion will be used for categorical or binary variables, and mean and standard deviation  
48  
49 238 (or median and range) will be used for continuous variables.  
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### 55 239 **Analysis of the primary endpoint**

56  
57 240 The efficacy estimates for each of the studies will be summarised using the Kaplan-Meier (K-  
58  
59 241 M) method.  
60

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2  
3 242 Cox regression analysis will be carried out to identify the predictors associated with parasitic  
4  
5 243 recrudescence using a one-stage IPD-MA. Random effects in the form of shared gamma frailty  
6  
7 244 parameters will be used to adjust for study-site effect and account for unobserved statistical  
8  
9 245 heterogeneity<sup>14</sup>. Schoenfeld residuals against transformed time will be used to determine if the  
10  
11 246 assumption of proportional hazard is met. Cox-Snell residuals will be examined to determine  
12  
13 247 the appropriateness of model fit. Martingale residuals will be used to assess the functional form  
14  
15 248 of the covariates. Potential non-linear relationships between continuous variable and the  
16  
17 249 treatment outcomes will be investigated using multivariable fractional polynomials (FP)<sup>15</sup>. If  
18  
19 250 the assumption of proportional hazards is not satisfied, alternative approaches such as  
20  
21 251 piecewise proportional hazards model, interaction with time, stratifying by the variable for  
22  
23 252 which the assumption isn't satisfied or flexible parametric models will be considered. Variable  
24  
25 253 selection process will follow a procedure described below.

#### 254 **Analyses of secondary endpoints**

255 ***P. falciparum* new infection:** The analysis of new infections will be similar to the analysis of  
256 the primary endpoint.

257 **Parasite clearance:** Early parasitological responses will be assessed by the parasite positivity  
258 rate (PPR), which is the proportion of patients remaining parasitaemic on days 1, 2 and 3 post  
259 treatment administration<sup>16</sup>. The relationship between mg/kg dosage of the artesunate and  
260 mefloquine on early parasitological responses will be explored using a logistic regression  
261 model with study sites fitted as random effect. Variable selection and additional sensitivity  
262 analyses will follow the plan as outlined for the primary endpoint.

263 **Gametocyte carriage:** Gametocyte carriage during the study follow-up will be stratified by  
264 the gametocytaemia status at baseline. For those with documented gametocytaemia at  
265 enrolment, proportion of patients in whom gametocyte has cleared will be reported. For those

1  
2  
3 266 without gametocytes on enrolment, the proportion of patients in whom gametocytes have  
4  
5 267 evolved will be presented. The relationship between mg/kg dosage of the artesunate and  
6  
7 268 mefloquine on gametocyte endpoints will be explored using a logistic regression model with  
8  
9 269 study sites fitted as random effect. Variable selection and additional sensitivity analyses will  
10  
11 270 follow the plan as outlined for the primary endpoint.  
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14

15 271 **Haematological insult:** Anaemia during the study follow-up will be stratified by the anaemia  
16  
17 272 status at baseline. For those who are anaemic at enrolment (, the proportion of patients who  
18  
19 273 have recovered will be reported. For those who are not anaemic at enrolment, the proportion  
20  
21 274 of patients whom are subsequently anaemic will be presented. The relationship between mg/kg  
22  
23 275 dosage of the artesunate and mefloquine on anaemia endpoints will be explored using a logistic  
24  
25 276 regression model with study sites fitted as random effect. Variable selection and additional  
26  
27 277 sensitivity analyses will follow the plan as outlined for the primary endpoint.  
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32 278 **Safety endpoints:** The proportion of patients with acute drug vomiting, vomiting and diarrhoea,  
33  
34 279 neuropsychiatric adverse events within a week of treatment initiation will be reported. The  
35  
36 280 relationship between the mg/kg mefloquine dose and safety endpoints will be evaluated using  
37  
38 281 a logistic regression model with study sites fitted as a random effect, if data permits. Variable  
39  
40 282 selection and additional sensitivity analyses will follow the plan as outlined for the primary  
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42 283 endpoint.  
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### 284 **Variable selection strategy**

285 The following covariates will be examined: age, sex, weight, baseline parasitaemia (except for  
286 new infection analysis), weight-for-age z-score (WAZ), underweight for age termed  
287 underweight (defined as  $WAZ < -2$ ), haemoglobin, gametocytes on presentation (except for  
288 new infection analysis), past history of malaria (if available); description of infection: mixed  
289 species infections (except for new infection analysis), presence of markers of drug resistance  
290 e.g. kelch13 mutations or *pfmdr1* amplification (if available), details of treatment received:  
291 total mg/kg dose of AS and MQ, regimen, drug supervision, and vomiting of medication. Year  
292 of enrolment will also be included to account for changes in parasite susceptibility over time.

293 A general strategy recommended by Collet (2015)<sup>17</sup> will be followed for the construction of  
294 multivariable regression model:

- 295 i) All possible risk factors will be examined in a univariable analysis. The log-likelihood  
296 estimates ( $-2 \times \text{Log}\hat{L}$ ) will be compared against the null model to assess if any of the  
297 variables reduce its value at 5% level of statistical significance.
- 298 ii) All the variables identified in step (i) will be fitted together in one model and variables  
299 which are not significant in the presence of other variables based on the results of the  
300 Wald test will be identified.
- 301 iii) A likelihood ratio test will be used to assess the impact of omitting variables identified  
302 in step (ii). If the omitted variable does not significantly impact the model log-  
303 likelihood, then they will be dropped. Only those variables which lead to significant  
304 change in log-likelihood are retained.
- 305 iv) All variables excluded from step (i) will be added to the model identified in step (iii)  
306 one by one to check if they provide any improvement to the model.
- 307 v) A final check of the model identified in step (iv) will be carried out to ensure that none  
308 of the variables in the model can be omitted without significantly increasing the model

1  
2  
3 309 log-likelihood, and none of the excluded variables significantly reduces the model log-  
4  
5 310 likelihood.

6  
7  
8 311 Comparison of likelihood between nested models will be conducted using Likelihood Ratio  
9  
10 312 Test (LRT). Akaike's Information Criterion (AIC) will be used to compare non-nested models.  
11  
12 313 Treatment dosage, drug formulation, and baseline parasitaemia will be included in the  
13  
14 314 multivariable model as *a priori* forced variables regardless of their statistical significance.  
15  
16 315 Variables with more than 50% observations missing will not be included in multivariable  
17  
18 316 analysis. Interactions will be assessed between dosing and the following variables: region, age  
19  
20 317 group, transmission intensity, hyperparasitaemia (parasitaemia > 100,000 parasites per  
21  
22 318 microlitre), date of enrollment.

### 23 24 25 26 27 319 **Assessment of statistical heterogeneity**

28  
29  
30 320 The multilevel logistic or Cox models will be used for explaining study-site heterogeneity.  
31  
32 321 Heterogeneity across study sites will be assessed by the variance of the shared frailty term  
33  
34 322 estimated in the random effect Cox model or variance of the random intercepts in logistic  
35  
36 323 regression. Additionally, intra-class correlation in logistic regression model will be reported.

### 37 38 39 40 324 **Subgroup analyses**

41  
42 325 Analyses will be conducted by geographical region, drug regimen and resistance status if data  
43  
44 326 permit.

### 45 46 47 48 327 **Sensitivity analyses**

49  
50 328 A model will be refitted with each study's data excluded, one at a time, and a coefficient of  
51  
52 329 variation around the parameter estimates will be calculated. This would identify any influential  
53  
54 330 studies, that is, studies with unusual results (due to variations in methodology, patient  
55  
56 331 population, and so on) that affect the overall pooled analysis findings. To assess the impact of  
57  
58 332 missing data (covariates, PCR genotyping results), sensitivity analysis will be performed to see

1  
2  
3 333 if our main conclusion is affected or not by the exclusion of patients with missing data. Multiple  
4  
5 334 Imputation (MI) will be used for handling missing data for missing covariates and missing  
6  
7  
8 335 outcomes. MI will be carried out for covariates with missing proportion less than 50%.<sup>18</sup>  
9

### 10 336 **Quality assessment/risk of bias assessment in studies included**

11  
12  
13 337 Two reviewers will independently assess risk of bias. The risk of bias within and across the  
14  
15 338 studies included in the analysis will be carried out using the GRADE guidelines<sup>19</sup>. Cochrane  
16  
17 339 risk of bias tool 2.0 will be used to assess risk of bias in individual randomised controlled  
18  
19  
20 340 trails. Publication bias will assessed by a funnel plot.  
21  
22

### 23 341 **Assessment of risk of potential bias in missing studies**

24  
25  
26 342 Despite best possible efforts, it is anticipated that raw data from all the identified studies will  
27  
28 343 not be available. Risk of potential bias in these studies will be assessed using a two-stage IPD-  
29  
30 344 MA for the reported efficacy outcomes.  
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3 345 **Further development of statistical analysis plan**  
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6 346 The main analysis is planned as described above. Modification or additional analyses may be  
7  
8 347 required as the data collection progresses. An updated statistical analysis plan will be available  
9  
10 348 on the WWARN study group website<sup>20</sup>.  
11  
12

13  
14 349 **Software**

15 350 All statistical analyses will be carried out using R (The R Foundation for Statistical Computing)  
16  
17  
18 351 or Stata (4905 Lakeway Drive, College Station, Texas 77845 USA). Using alternative  
19  
20 352 statistical software will not require amendment of this SAP.  
21  
22

23 353 **Ethics and dissemination**

24 354 This IPD meta-analysis met the criteria for waiver of ethical review as defined by the Oxford  
25  
26  
27 355 Tropical Research Ethics Committee (OxTREC) as the research consists of secondary analysis  
28  
29 356 of existing anonymous data<sup>21</sup>. All studies included in this analysis will have received local  
30  
31 357 ethical approvals and our pooled IPD meta-analysis will be addressing scientific questions that  
32  
33 358 are very similar to the original research questions.  
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37 359 Findings will be reported following the PRISMA-IPD guidelines<sup>22</sup> at open access peer-  
38  
39 360 reviewed journals. This systematic literature review and IPD meta-analysis is registered to  
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41 361 PROSPERO (CRD42018103776) and this protocol has been reported following the PRISMA-  
42  
43 362 P guidelines<sup>23</sup>. Any publications based on the findings of this IPD meta-analysis will be in  
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45 363 accordance with the guidelines of the International Committee of Medical Journal Editors.  
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## 365 Discussion

366 Large scale deployment of highly efficacious ACT regimens such as artesunate-mefloquine  
367 has been the cornerstone of global malaria control for over a decade and this has contributed  
368 to the global reduction of mortality and morbidity associated with malaria <sup>11</sup>. Maintaining these  
369 gains is highly dependent on efficient health systems, sustainable global funding and the  
370 current status of antimalarial drug resistance. The 2017 WHO report found that globally the  
371 number of malaria cases has stopped dropping and mortality has crept up compared to 2016,  
372 suggesting that the recent public health gains remain fragile <sup>24,25</sup>. To make things worse, a  
373 health calamity is looming large due to the emergence of resistance to artemisinins in South-  
374 East Asia which is threatening to reverse the remarkable progress achieved over the past decade  
375 <sup>26</sup>. In the absence of an alternative treatment class to replace the ACTs as first line therapy,  
376 preserving the currently available drugs remains the top-most priority and this requires the  
377 highest form of evidence regarding the susceptibility of the parasites against the antimalarial  
378 drugs. Artesunate-mefloquine retains extremely high efficacy in most locations except  
379 Thailand, and in any given trial, only few treatment failures have been observed which limits  
380 the utility of any single study in answering questions regarding the dose-response relationship.  
381 Individual participant data (IPD) meta-analysis provides an alternative strategy.

382 IPD meta-analysis is now considered the gold-standard for evidence synthesis and allows  
383 exploration of different risk factors which otherwise would not be possible through the  
384 aggregate data meta-analysis <sup>5</sup>. This IPD-MA is designed to explore the variability in drug  
385 dosage administered in patients with uncomplicated *P. falciparum* malaria, treated with  
386 artesunate-mefloquine (AS-MQ). The WHO-recommended AS-MQ regimen is administered  
387 as a three day course, with a total of 12 mg/kg artesunate and 25 mg/kg of mefloquine split  
388 over three days. Due to the poor tolerability of high dose mefloquine, the dose of mefloquine  
389 is usually divided into either two doses (15 and 10 mg/kg), or three as a fixed dose combination

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3 390 (8mg/kg/day). The fixed dose combination has been shown to provide better efficacy and  
4  
5 391 improve treatment adherence for artesunate-amodiaquine <sup>6</sup>. Such a comparison is yet to be  
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7  
8 392 made for the AS-MQ regimen, and in this IPD-MA we propose to compare the fixed and loose  
9  
10 393 formulations of the regimen with regards to the drug dosing, tolerability, efficacy and  
11  
12 394 practicality of the dose banding.

15 395 In conclusion, this pooled analysis will provide critical information regarding the relationship  
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17 396 between drug dosage and parasitological responses post-treatment with artesunate-mefloquine.  
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20 397 The assessment of the host, parasite and drug determinants that influence the treatment  
21  
22 398 response can provide evidence-based guidance for monitoring the early signs of artemisinin  
23  
24 399 resistance and effective case management that will be critical in optimising malaria control and  
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27 400 containment efforts.

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4 401 **Declarations**

5  
6  
7 402 **Author contributions**

8  
9  
10 403 Conceived the idea and wrote the first draft of the protocol: RM, PD, PG, EAA, and KS

11  
12 404 Systematic review of all published antimalarial studies: RB and GSH

13  
14 405 Data acquisition and standardisation: GSH, RB, EAA, KS

15  
16  
17 406 All authors read and approved the submission of the final draft of the study protocol.

18  
19  
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21  
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26  
27 410 did not participate in the study development, the writing of the paper, decision to publish, or  
28  
29 411 preparation of the manuscript.

30  
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32  
33 412 **Competing interests**

34  
35  
36 413 The authors declare that they have no competing interests.

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39 414 **Ethics approval**

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42 415 This individual patient data meta-analysis met the criteria for waiver of ethical review as  
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44 416 defined by the Oxford Tropical Research Ethics Committee (OxTREC) since the research  
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46 417 consists of secondary analysis of existing anonymous data. Each study included in the  
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48 418 analysis will have received local ethics approvals.

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52 419 **Acknowledgements**

53  
54  
55 420 We would like to thank Dr. Makoto Saito for his several helpful suggestions.  
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3 421 **List of Figures**  
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6 422 **Figure 1: Mg/kg dose variations for mefloquine**  
7

8  
9 423 **Legend:** The WHO recommendation on the 3-day fixed dose AS-MQ regimen is to administer  
10 424 once daily 50 mg of MQ for those weighing 5-8 kg, 100 mg for 9-17 kg, 200 mg for 18-29 kg  
11 425 and 400mg for those who weigh  $\geq 30$  kg. The horizontal dotted line represents the target MQ  
12 426 dose of 25 mg/kg. Since the dosing is based on weight-bands, this leads to the saw-tooth dosing  
13 427 curves.  
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20 429 **Figure 2: Data acquisition and standardisation process utilizing the WWARN database**  
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22 430 **Legend:** Individual participant data is shared by researchers to the WWARN secured portal  
23 431 (left panel). The studies are standardised using a common WWARN protocol and the tables of  
24 432 clinical, parasitological and drug measurements are stored in a secured repository with  
25 433 relational database (middle panel). On standardisation, the dataset is shared back with the study  
26 434 investigator, and subsequently used in meta-analyses to answer questions of public health  
27 435 importance (right panel).  
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For peer review only

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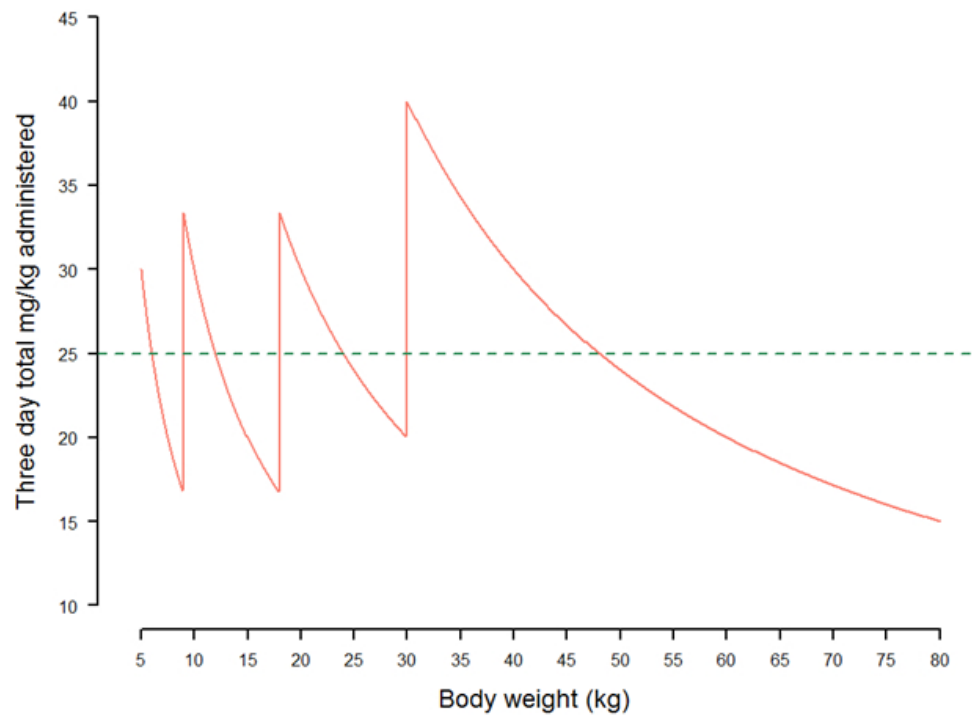


Figure 1: Mg/kg dose variations for mefloquine  
168x130mm (96 x 96 DPI)

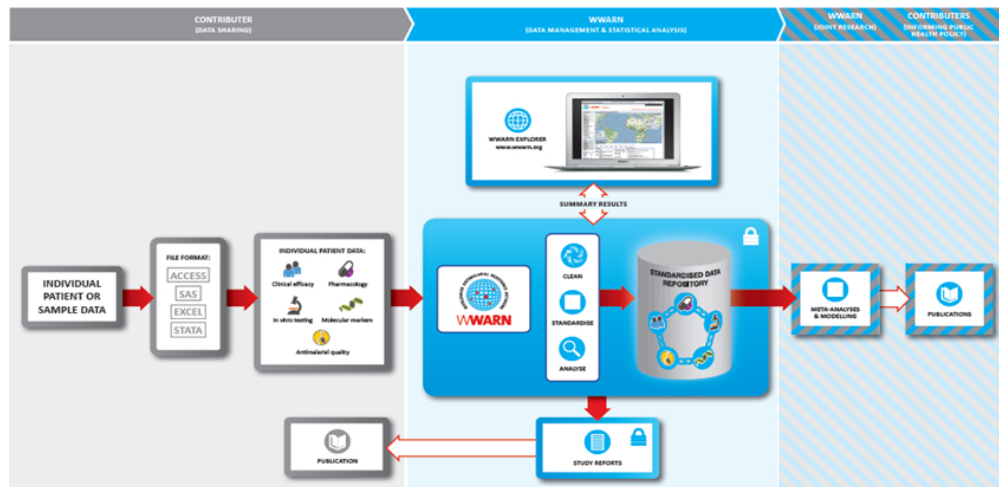


Figure 2: Data acquisition and standardisation process utilizing the WWARN database

223x108mm (96 x 96 DPI)

## PRISMA-P 2015 Checklist

This checklist has been adapted for use with protocol submissions to *Systematic Reviews* from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 4:1

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
<b>ADMINISTRATIVE INFORMATION</b>					
<b>Title</b>					
Identification	1a	Identify the report as a protocol of a systematic review	<input checked="" type="checkbox"/>	<input type="checkbox"/>	2-3
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	<input type="checkbox"/>	<input checked="" type="checkbox"/>	NA
<b>Registration</b>	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	<input checked="" type="checkbox"/>	<input type="checkbox"/>	44
<b>Authors</b>					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	<input checked="" type="checkbox"/>	<input type="checkbox"/>	5-22
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	<input checked="" type="checkbox"/>	<input type="checkbox"/>	402- 406
<b>Amendments</b>	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	<input type="checkbox"/>	<input checked="" type="checkbox"/>	NA
<b>Support</b>					
Sources	5a	Indicate sources of financial or other support for the review	<input checked="" type="checkbox"/>	<input type="checkbox"/>	407- 411
Sponsor	5b	Provide name for the review funder and/or sponsor	<input checked="" type="checkbox"/>	<input type="checkbox"/>	409
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	<input checked="" type="checkbox"/>	<input type="checkbox"/>	409-411
<b>INTRODUCTION</b>					
<b>Rationale</b>	6	Describe the rationale for the review in the context of what is already known	<input checked="" type="checkbox"/>	<input type="checkbox"/>	61- 86
<b>Objectives</b>	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	<input checked="" type="checkbox"/>	<input type="checkbox"/>	87- 95
<b>METHODS</b>					

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
<b>Eligibility criteria</b>	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	<input checked="" type="checkbox"/>	<input type="checkbox"/>	97-117
<b>Information sources</b>	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	<input checked="" type="checkbox"/>	<input type="checkbox"/>	132-142
<b>Search strategy</b>	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	<input checked="" type="checkbox"/>	<input type="checkbox"/>	132-142
<b>STUDY RECORDS</b>					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	<input checked="" type="checkbox"/>	<input type="checkbox"/>	154- 160
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	<input checked="" type="checkbox"/>	<input type="checkbox"/>	136- 138
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	<input checked="" type="checkbox"/>	<input type="checkbox"/>	144- 153
<b>Data items</b>	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	<input checked="" type="checkbox"/>	<input type="checkbox"/>	176- 219
<b>Outcomes and prioritization</b>	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	<input checked="" type="checkbox"/>	<input type="checkbox"/>	239-283
<b>Risk of bias in individual studies</b>	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	<input checked="" type="checkbox"/>	<input type="checkbox"/>	336- 340
<b>DATA</b>					
<b>Synthesis</b>	15a	Describe criteria under which study data will be quantitatively synthesized	<input checked="" type="checkbox"/>	<input type="checkbox"/>	176- 219
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., $I^2$ , Kendall's tau)	<input checked="" type="checkbox"/>	<input type="checkbox"/>	220-323
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	<input checked="" type="checkbox"/>	<input type="checkbox"/>	324-335
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	<input checked="" type="checkbox"/>	<input type="checkbox"/>	341-344
<b>Meta-bias(es)</b>	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	<input checked="" type="checkbox"/>	<input type="checkbox"/>	340
<b>Confidence in cumulative evidence</b>	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	<input checked="" type="checkbox"/>	<input type="checkbox"/>	337-340



# BMJ Open

## The effect of dose on the antimalarial efficacy of artesunate-mefloquine against *Plasmodium falciparum* malaria: a protocol for systematic review and individual patient data (IPD) meta-analysis

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-027738.R1
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Manuscripts

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4 1 The effect of dose on the antimalarial efficacy of artesunate-mefloquine  
5 2 against *Plasmodium falciparum* malaria: a protocol for systematic  
6 3 review and individual patient data (IPD) meta-analysis  
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56 27 Word count 3776  
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## 28 **Abstract**

29 **Introduction:** Antimalarial posology based on weight-bands leave patients at the margins  
30 vulnerable to receiving either lower or higher weight-adjusted (mg/kg) dosages. This article  
31 aims to describe the protocol for systematic review and individual patient meta-analysis for a  
32 study of the distribution of artesunate and mefloquine dosage administered in patients with  
33 uncomplicated *P. falciparum* malaria treated with an artesunate-mefloquine (AS-MQ) regimen.  
34 Relationship between mg/kg dose and therapeutic outcomes will be assessed through a one-  
35 stage individual participant data (IPD) meta-analysis (MA).

36 **Methods and analysis:** Therapeutic efficacy studies with the AS-MQ regimen will be  
37 identified by searching the following databases: PUBMED, EMBASE, and Web of Science.  
38 The corresponding authors of the relevant studies will be requested to share the IPD for the  
39 purpose of this meta-analysis to a secured repository. All available studies will be standardised  
40 using a common data management protocol and pooled into a single database. The relationship  
41 between mg/kg dosage and treatment failures will be assessed using a Cox regression model  
42 with study sites considered as a shared frailty term. Safety parameters will be explored where  
43 available.

44 **Ethics and Dissemination:** This IPD meta-analysis met the criteria for waiver of ethical review  
45 as defined by the Oxford Tropical Research Ethics Committee (OxTREC) as the research  
46 consists of secondary analysis of existing anonymous data. The results of this analysis will be  
47 disseminated at conferences, WWARN website and any peer-reviewed publication arising will  
48 be made open-source.

49 **PROSPERO Registration:** CRD42018103776

50 **Keywords:** malaria, *Plasmodium falciparum*, efficacy, IPD meta-analysis, mefloquine,  
51 artemisinin, artemisinin combination therapy

## 52 **Strengths and limitations of this study**

- 53 • With the exception of recent studies in Southeast Asia, the regimen artesunate-mefloquine  
54 has consistently demonstrated an efficacy greater than 95% and treatment failures in any  
55 single antimalarial study are few. This IPD-MA will allow a robust exploration of host,  
56 parasite and drug factors associated with therapeutic outcomes, which otherwise would not  
57 be possible.
- 58 • The proposed IPD-MA will allow exploration of variations in the weight-adjusted dosage  
59 received by patients, which is not possible with aggregate data meta-analysis.
- 60 • The IPD-MA will be carried out as a study group under the auspices of the WorldWide  
61 Antimalarial Resistance Network (WWARN), which has championed responsible data  
62 sharing and advocates translational research.
- 63 • A limitation of this analysis will be heterogeneity between studies included in terms of  
64 design, patient population and the susceptibility of the parasites against the drug regimen.

## 66 Introduction

67 The combination artesunate-mefloquine (AS-MQ) was the first antimalarial regimen  
68 developed as an Artemisinin Combination Therapy (ACT) when mefloquine resistance became  
69 rampant along the Thai-Myanmar border in the early 1990s<sup>1</sup>. The efficacy of a combination  
70 regimen (artemisinin derivative + partner component) depends upon the ability of the partner  
71 component to mop up the residual parasites leftover after the initial and highly potent anti-  
72 parasitic activity of the artemisinin derivatives. This requires the dosage of the partner drug to  
73 be sufficient to ensure that blood concentrations exceed the Minimum Inhibitory Concentration  
74 (MIC) of the parasites until all the parasites have been killed. Manufacturers' recommendations  
75 regarding antimalarial posology are often pragmatic and the dose is administered based upon  
76 weight "banding". This approach inevitably results in some patients at the band margins  
77 receiving either lower or higher dosages when adjusted for body weight (Figure 1). Young  
78 children are particularly vulnerable to extreme total dosages especially when drug  
79 administration is based on tablets rather than paediatric formulations or suspensions. This may  
80 lead to sub-therapeutic drug concentrations in the blood plasma and such under-exposure has  
81 been related to poorer therapeutic response for some of the widely used ACTs<sup>2-4</sup>.

82 Until recently ACTs have consistently demonstrated an efficacy greater than 95% and  
83 treatment failures in any single antimalarial study are few thus limiting the ability to draw  
84 inferences regarding putative factors associated with therapeutic outcomes. Individual  
85 participant data (IPD) meta-analysis (MA) is being used increasingly to explore some of the  
86 putative factors which otherwise would not be possible through aggregate data meta-analysis<sup>5</sup>.  
87 Such a IPD-MA approach has been used to assess the dose-response relationships for the ACT  
88 regimens of artesunate-amodiaquine, artemether-lumefantrine and dihydroartemisinin-  
89 piperazine<sup>2,3,6</sup>. These studies have demonstrated that the drug formulation (fixed vs loose)  
90 and under-exposure in the paediatric population due to weight-banding are deterministic of

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3 91 poorer therapeutic outcomes. A thorough evaluation of the dose-response relationship for the  
4  
5 92 regimen AS-MQ is lacking and this IPD-MA aims to address this research gap.  
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8

## 93 **Objectives**

94 The overall aim of this study is to determine the mg/kg dosing range of artesunate (AS) and  
95 mefloquine (MQ) adopted in clinical trials and to investigate the effects of mg/kg dosing on  
96 clinical outcome.  
97

97 The specific objectives are:

- 98 • To investigate the effects of mefloquine and artesunate mg/kg dosing on early and late  
99 clinical outcomes (treatment success or failure)
- 100 • To investigate the tolerability of AS-MQ across different study sites, population and age  
101 groups

102

## 103 **Methods and analyses**

### 104 ***Criteria for study eligibility***

105 Studies identified will be deemed eligible for the purpose of this analysis if they meet the  
106 following criteria:

- 107 • Prospective clinical efficacy study (defined as a trial which enrolled patients with  
108 confirmed diagnosis of malaria and who were follow-up for at least 28 days post-  
109 treatment) of uncomplicated *P. falciparum* (either alone or mixed infections) in  
110 patients of all ages
- 111 • Assessing the efficacy of a fixed-dose AS-MQ combination, either as single tablet  
112 type, or co-blister pack of more than one tablet type, or assessing the efficacy of a  
113 loose combination of AS-MQ
- 114 • Where AS was given over three days (with any number of doses per day) with a target  
115 total dose of 6-30mg/kg
- 116 • Where MQ was given over 1-3 days, on any of days 1-3 (with any number of doses  
117 per day) with a target total dose of 15-33mg/kg
- 118 • Where all AS and MQ were administered orally
- 119 • With a minimum of 28 days follow up
- 120 • With genotyping performed for late parasite recurrence
- 121 • With individual patient data on dosage of mefloquine received (actual or per protocol)  
122 by patients (dosage per tablets, number of tablets given per dose and duration of  
123 treatment)

### 124 ***Criteria for study exclusion***

- 125 • Where other antimalarial drugs were given in addition to the initial ASMQ treatment  
126 regimen, except for a single dose of primaquine of 0.25mg/kg in the first 3 days

1  
2  
3 127 ***Types of study participants***  
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5  
6 128 Patients with uncomplicated *P. falciparum* malaria will be included in this IPD-MA. The  
7  
8 129 following patients will be excluded from the analysis:  
9

- 10  
11 130 i. Severe *P. falciparum* malaria  
12  
13 131 ii. Pregnant women  
14  
15

16 132 ***Types of intervention/exposure and controls***  
17  
18  
19

- 20 133 • Fixed dose combination of AS-MQ, either as single tablet type, or co-blister pack of  
21  
22 134 more than one tablet type, or loose combination of AS-MQ. AS given over three days  
23  
24 135 (with any number of doses per day) with a target total dose of 6-30mg/kg. MQ given  
25  
26 136 over 1-3 days, on any of days 1-3 (with any number of doses per day) with a target  
27  
28 137 total dose of 15-33mg/kg  
29  
30  
31

32  
33 138 ***Types of outcomes***  
34

- 35 139 i. Parasitological and clinical efficacy  
36  
37  
38 140 ii. Adverse events  
39  
40

41 141 ***Information sources and search strategy***  
42

43 142 We will carry out a systematic review and search PubMed, EMBASE, and Web of Science to  
44  
45 143 identify publications with artesunate-mefloquine between Jan 1990 to July 2018, the full search  
46  
47 144 terms are available from the PROSPERO registration (CRD42018103776) and also presented  
48  
49 145 as supplementary file (Supplementary File 1). We do not plan to search grey literature for the  
50  
51 146 purpose of this review.  
52  
53

54  
55 147 Any important protocol amendments will be documented in the PROSPERO registration.  
56  
57 148 Studies will be included regardless of language and publication status. Study screenings will  
58  
59 149 be carried by two independent reviewers who will screen title, abstract, full text as necessary.  
60



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2  
3 150 The following studies will be excluded: animal models (e.g. mouse malaras *P. berghei*, *P.*  
4  
5 151 *chabaudi*), publications only including severe malaria, studies with follow up <28 days, data  
6  
7 152 previously included in another published study, prophylaxis or mass drug administration  
8  
9 153 studies, studies in healthy volunteers/challenge studies, or studies in asymptomatic patients or  
10  
11 154 pregnant women.  
12  
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14

## 15 ***Data acquisition and data management***

### 16 **Collating IPD**

17  
18 156 Principal investigators of the published (or unpublished) studies identified from the literature  
19  
20 157 search will be invited to share IPD. At least three emails will be sent out in case of non-response.  
21  
22 158 Researchers agreeing to the terms and conditions of the submission will be requested to upload  
23  
24 159 anonymised IPD to the WWARN repository through a secure web portal. Figure 2 shows the  
25  
26 160 process map which depicts the different phases of data procurement; data acquisition from the  
27  
28 161 contributors, data standardisation, and their subsequent re-use in IPD meta-analysis.  
29  
30  
31  
32

33  
34 163 Data will be fully anonymised and handled in compliance with the UK Data Protection Act to  
35  
36 164 protect personal information and patient privacy. Original data will be stored on a secure server  
37  
38 165 hosted by the University of Oxford.  
39  
40  
41

### 42 **Data management**

43  
44 167 Raw data from individual studies will be standardised using an open and transparent data  
45  
46 168 management and statistical analysis protocol<sup>7</sup>. Investigators will be further contacted for  
47  
48 169 validation or clarification, if required, and individual study protocols will be requested. On  
49  
50 170 standardisation, the data will be stored in a relational database of several tables containing  
51  
52 171 information on drug regimen, parasitological, clinical, and haematological assessments,  
53  
54 172 genotyping and therapeutic outcomes; all linked by a unique patient identifier.  
55  
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3 173 **Data contributors participation**  
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5 174 All the researchers who share individual patient data from eligible studies will become part of  
6  
7  
8 175 the study group; will have an opportunity to contribute to the analysis, interpretation of the  
9  
10 176 results, manuscript preparation; and will be listed as co-authors on the publication arising from  
11  
12 177 these analyses according to the WWARN publication policy.  
13  
14

15 178 **Statistical analysis plan**  
16  
17

18  
19 179 ***Study Population***  
20

21  
22 180 The following patients will be included in the analysis:  
23

- 24  
25 181 i. Information is available on drug dosage, either as exact number of tablets received,  
26  
27 exact mg/kg dose received or number of tablets planned per protocol  
28 182  
29  
30 183 ii. Date of the last day of follow-up or length of follow-up  
31  
32

33 184 The following patients will be excluded from the analysis:  
34

- 35  
36 185 i. Received other antimalarial drugs during follow-up before recorded *P. falciparum*  
37  
38 treatment failure  
39 186  
40  
41 187 ii. Results of genotyping performed for late parasitological outcome are not available  
42  
43 188 iii. Missing confirmation of *P. falciparum* infection on enrolment  
44  
45 189 iv. Missing age or weight or gender  
46  
47 190 v. Other deviations, as defined in the data management plan<sup>7</sup> :  
48  
49

50 191 (a) Haemoglobin < 5 g/dL on day 0  
51

52 192 (b) Haematocrit < 15% on day 0  
53  
54  
55

56 193 ***Outcomes***  
57  
58

59 194 Primary: PCR-corrected *P. falciparum* recrudescence  
60

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2  
3 195 Secondary: PCR-corrected *P. falciparum* reinfection  
4  
5  
6 196 PCR-uncorrected *P. falciparum* recurrence  
7  
8  
9 197 Early parasitological responses on days 1, 2 and 3  
10  
11  
12 198 Gametocyte carriage within 14 days of treatment initiation in patients without  
13  
14 199 gametocytaemia at enrolment  
15  
16  
17 200 Anaemia status on day 7  
18  
19  
20 201 Adverse symptoms developed after the drug administration  
21  
22

23  
24 202 The primary endpoint for this IPD-MA is Polymerase Chain Reaction (PCR) genotyping  
25  
26 203 corrected risk of *P. falciparum* recrudescence (treatment failure) on day 42. The day 42 was  
27  
28 204 selected as the primary endpoint based on the current recommendations from the WHO as  
29  
30 205 outlined in the 2009 protocol, which suggests that day 42 be the minimum follow-up period  
31  
32 206 for the mefloquine regimen<sup>8</sup>. In the analysis of the primary endpoint, patients in whom new  
33  
34 207 infections are observed during the study follow-up, or those who are lost to follow-up will be  
35  
36 208 censored; the former on the day new infection was observed and the latter on their last recorded  
37  
38 209 visit day. For the analysis of PCR corrected new infections, patients with recrudescence and  
39  
40 210 those who are lost to follow-up will be censored. Further definitions of status and other  
41  
42 211 censorship are detailed in the Clinical Module DMSAP<sup>7</sup>.

43  
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47  
48 212 Acute drug vomiting within an hour of treatment administration, general vomiting within 7  
49  
50 213 days of treatment initiation, diarrhoea within 7 days of treatment initiation, and,  
51  
52 214 neuropsychiatric adverse events (where available) are secondary endpoints.  
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## 215 **Variables and definition**

216 Artesunate and mefloquine doses received will be calculated from the number of tablets  
217 administered to each patient daily. If the actual number of tablets received is not recorded, the  
218 total dose in mg or mg/kg recorded as administered to each patient will be used. If none of  
219 these are available, administration as per protocol will be assumed. The current recommended  
220 daily mefloquine dose is 8.3 (range: 5 - 11) mg/kg<sup>9</sup>. A patient will be classified as under-dosed  
221 if the 3-day total mg/kg mefloquine dose is less than 15 mg/kg.

222 Nutritional status in children under 5 years of age will be assessed using standardised age,  
223 weight, height and gender specific growth reference standards according to the WHO 2006  
224 recommendations using igrowup Stata package<sup>10</sup>. Anthropometric indicators include weight-  
225 for-age (WAZ), height-for-age (HAZ), and weight-for-height (WHZ). The nutritional status of  
226 a child will be given as a Z-score and classified as stunted, underweight or wasted as defined  
227 in the WHO guidelines.

228 The falciparum malaria transmission intensity of the study sites will be assessed using the  
229 prevalence estimates generated by the Malaria Atlas Project (MAP) based on the latitude,  
230 longitude and the year the study was conducted<sup>11,12</sup>.

231 Anaemia will be defined as haemoglobin (Hb) < 10 g/dL or haematocrit (Ht) < 30%. Severe  
232 anaemia is defined as haemoglobin < 7 g/dL or haematocrit < 20%. Fever will be defined as  
233 body temperature > 37.5 degrees Celsius.

234 Parasite resistance status will be defined (data permitting) for each patient from South-East  
235 Asia region based on the reported prevalence of mutations of molecular markers (pfmdr1,  
236 kelch13) or the distribution of parasite clearance half-life for their study site and year of

237 admission<sup>13,14</sup>. For other locations, we will assume that parasites are sensitive to the ASMQ  
238 regimen.

## 239 **Descriptive summary**

### 240 *Summary of the studies*

241 Summary of included studies will be presented with respect to: study location, years of study,  
242 study population, duration of follow-up, AS-MQ drug formulation, methodology for parasite  
243 quantification, methodology for PCR genotyping, supervision of drug administration. PCR-  
244 corrected and uncorrected outcomes will be used to compute the Kaplan-Meier (K-M)  
245 estimates using the censoring rules outlined above. The K-M estimates will be presented  
246 graphically together with the number of patients in the risk set.

### 247 *Summary of the patients*

248 Summary of baseline characteristics of the patients included in the analysis will be presented  
249 for each study, by region and in overall. The following baseline characteristics of patients will  
250 be presented: age; weight, parasitaemia on enrolment; presence of fever (body temperature >  
251 37.5 degrees Celsius); haemoglobin (or haematocrit); anaemia (Hb < 10 g/dL) or severe  
252 anaemia (Hb < 7 g/dL), gametocytes on presentation, description of infection (*P. falciparum*  
253 or mixed infections), total mg/kg dose for each drug component, dosing strategies (age-based,  
254 weight based etc.), dose formulation (fixed or loose etc.), and supervision of drug  
255 administration. The number of available patients will be summarised for all variables,  
256 proportion will be used for categorical or binary variables, and mean and standard deviation  
257 (or median and range) will be used for continuous variables.

### 258 **Analysis of the primary endpoint**

259 The efficacy estimates for each of the studies will be summarised using the Kaplan-Meier (K-  
260 M) method.

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3 261 Cox regression analysis will be carried out to identify the predictors associated with parasitic  
4  
5 262 recrudescence using a one-stage IPD-MA. Random effects in the form of shared gamma frailty  
6  
7 263 parameters will be used to adjust for study-site effect and account for unobserved statistical  
8  
9 264 heterogeneity<sup>15</sup>. Schoenfeld residuals against transformed time will be used to determine if the  
10  
11 265 assumption of proportional hazard is met. Cox-Snell residuals will be examined to determine  
12  
13 266 the appropriateness of model fit. Martingale residuals will be used to assess the functional form  
14  
15 267 of the covariates. Potential non-linear relationships between continuous variable and the  
16  
17 268 treatment outcomes will be investigated using multivariable fractional polynomials (FP)<sup>16</sup>. If  
18  
19 269 the assumption of proportional hazards is not satisfied, alternative approaches such as  
20  
21 270 piecewise proportional hazards model, interaction with time, stratifying by the variable for  
22  
23 271 which the assumption isn't satisfied or flexible parametric models will be considered. Variable  
24  
25 272 selection process will follow a procedure described below.

### 26 273 **Analyses of secondary endpoints**

27 274 ***P. falciparum* new infection:** The analysis of new infections will be similar to the analysis of  
28  
29 275 the primary endpoint.

30  
31 276 **Parasite clearance:** Early parasitological responses will be assessed by the parasite positivity  
32  
33 277 rate (PPR), which is the proportion of patients remaining parasitaemic on days 1, 2 and 3 post  
34  
35 278 treatment administration<sup>17</sup>. The relationship between mg/kg dosage of the artesunate and  
36  
37 279 mefloquine on early parasitological responses will be explored using a logistic regression  
38  
39 280 model with study sites fitted as random effect. Variable selection and additional sensitivity  
40  
41 281 analyses will follow the plan as outlined for the primary endpoint.

42  
43 282 **Gametocyte carriage:** Gametocyte carriage during the study follow-up will be stratified by  
44  
45 283 the gametocytaemia status at baseline. For those with documented gametocytaemia at  
46  
47 284 enrolment, proportion of patients in whom gametocyte has cleared will be reported. For those  
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3 285 without gametocytes on enrolment, the proportion of patients in whom gametocytes have  
4  
5 286 evolved will be presented. The relationship between mg/kg dosage of the artesunate and  
6  
7 287 mefloquine on gametocyte endpoints will be explored using a logistic regression model with  
8  
9 288 study sites fitted as random effect. Variable selection and additional sensitivity analyses will  
10  
11 289 follow the plan as outlined for the primary endpoint.  
12  
13  
14

15 290 **Haematological insult:** Anaemia during the study follow-up will be stratified by the anaemia  
16  
17 291 status at baseline. For those who are anaemic at enrolment (, the proportion of patients who  
18  
19 292 have recovered will be reported. For those who are not anaemic at enrolment, the proportion  
20  
21 293 of patients whom are subsequently anaemic will be presented. The relationship between mg/kg  
22  
23 294 dosage of the artesunate and mefloquine on anaemia endpoints will be explored using a logistic  
24  
25 295 regression model with study sites fitted as random effect. Variable selection and additional  
26  
27 296 sensitivity analyses will follow the plan as outlined for the primary endpoint.  
28  
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32 297 **Safety endpoints:** The proportion of patients with acute drug vomiting, vomiting and diarrhoea,  
33  
34 298 neuropsychiatric adverse events within a week of treatment initiation will be reported. The  
35  
36 299 relationship between the mg/kg mefloquine dose and safety endpoints will be evaluated using  
37  
38 300 a logistic regression model with study sites fitted as a random effect, if data permits. Variable  
39  
40 301 selection and additional sensitivity analyses will follow the plan as outlined for the primary  
41  
42 302 endpoint.  
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### 303 **Variable selection strategy**

304 The following covariates will be examined: age, sex, weight, baseline parasitaemia (except for  
305 new infection analysis), weight-for-age z-score (WAZ), underweight for age termed  
306 underweight (defined as  $WAZ < -2$ ), haemoglobin, gametocytes on presentation (except for  
307 new infection analysis), past history of malaria (if available); description of infection: mixed  
308 species infections (except for new infection analysis), presence of markers of drug resistance  
309 e.g. kelch13 mutations or *pfmdr1* amplification (if available), details of treatment received:  
310 total mg/kg dose of AS and MQ, regimen, drug supervision, and vomiting of medication. Year  
311 of enrolment will also be included to account for changes in parasite susceptibility over time.

312 A general strategy recommended by Collet (2015)<sup>18</sup> will be followed for the construction of  
313 multivariable regression model:

- 314 i) All possible risk factors will be examined in a univariable analysis. The log-likelihood  
315 estimates ( $-2 \times \text{Log}\hat{L}$ ) will be compared against the null model to assess if any of the  
316 variables reduce its value at 5% level of statistical significance.
- 317 ii) All the variables identified in step (i) will be fitted together in one model and variables  
318 which are not significant in the presence of other variables based on the results of the  
319 Wald test will be identified.
- 320 iii) A likelihood ratio test will be used to assess the impact of omitting variables identified  
321 in step (ii). If the omitted variable does not significantly impact the model log-  
322 likelihood, then they will be dropped. Only those variables which lead to significant  
323 change in log-likelihood are retained.
- 324 iv) All variables excluded from step (i) will be added to the model identified in step (iii)  
325 one by one to check if they provide any improvement to the model.
- 326 v) A final check of the model identified in step (iv) will be carried out to ensure that none  
327 of the variables in the model can be omitted without significantly increasing the model



1  
2  
3 328 log-likelihood, and none of the excluded variables significantly reduces the model log-  
4  
5 329 likelihood.

6  
7  
8 330 Comparison of likelihood between nested models will be conducted using Likelihood Ratio  
9  
10 331 Test (LRT). Akaike's Information Criterion (AIC) will be used to compare non-nested models.  
11  
12 332 Treatment dosage, drug formulation, and baseline parasitaemia will be included in the  
13  
14 333 multivariable model as *a priori* forced variables regardless of their statistical significance.  
15  
16 334 Variables with more than 50% observations missing will not be included in multivariable  
17  
18 335 analysis. Interactions will be assessed between dosing and the following variables: region, age  
19  
20 336 group, transmission intensity, hyperparasitaemia (parasitaemia > 100,000 parasites per  
21  
22 337 microlitre), date of enrollment.

### 23 338 **Assessment of statistical heterogeneity**

24  
25 339 The multilevel logistic or Cox models will be used for explaining study-site heterogeneity.  
26  
27 340 Heterogeneity across study sites will be assessed by the variance of the shared frailty term  
28  
29 341 estimated in the random effect Cox model or variance of the random intercepts in logistic  
30  
31 342 regression. Additionally, intra-class correlation in logistic regression model will be reported.

### 32 343 **Subgroup analyses**

33 344 Analyses will be conducted by geographical region, drug regimen and resistance status if data  
34  
35 345 permit.

### 36 346 **Sensitivity analyses**

37 347 A model will be refitted with each study's data excluded, one at a time, and a coefficient of  
38  
39 348 variation around the parameter estimates will be calculated. This would identify any influential  
40  
41 349 studies, that is, studies with unusual results (due to variations in methodology, patient  
42  
43 350 population, and so on) that affect the overall pooled analysis findings. To assess the impact of  
44  
45 351 missing data (covariates, PCR genotyping results), sensitivity analysis will be performed to see

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2  
3 352 if our main conclusion is affected or not by the exclusion of patients with missing data. Multiple  
4  
5 353 Imputation (MI) will be used for handling missing data for missing covariates and missing  
6  
7  
8 354 outcomes. MI will be carried out for covariates with missing proportion less than 50%.<sup>19</sup>  
9

### 10 355 **Quality assessment/risk of bias assessment in studies included**

11  
12  
13 356 Two reviewers will independently assess risk of bias. The risk of bias within and across the  
14  
15 357 studies included in the analysis will be carried out using the GRADE guidelines<sup>20</sup>. Cochrane  
16  
17 358 risk of bias tool 2.0 will be used to assess risk of bias in individual randomised controlled  
18  
19  
20 359 trails. Publication bias will be assessed by a funnel plot<sup>21</sup>.  
21  
22

### 23 360 **Assessment of risk of potential bias in missing studies**

24  
25  
26 361 Despite best possible efforts, it is anticipated that raw data from all the identified studies will  
27  
28 362 not be available. Risk of potential bias in these studies will be assessed using a two-stage IPD-  
29  
30 363 MA for the reported efficacy outcomes.  
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### 364 **Further development of statistical analysis plan**

365 The main analysis is planned as described above. Modification or additional analyses may be  
366 required as the data collection progresses. An updated statistical analysis plan will be available  
367 on the WWARN study group website<sup>22</sup>.

### 368 **Software**

369 All statistical analyses will be carried out using R (The R Foundation for Statistical Computing)  
370 or Stata (4905 Lakeway Drive, College Station, Texas 77845 USA). Using alternative  
371 statistical software will not require amendment of this SAP.

### 372 **Ethics and dissemination**

373 This IPD meta-analysis met the criteria for waiver of ethical review as defined by the Oxford  
374 Tropical Research Ethics Committee (OxTREC) as the research consists of secondary analysis  
375 of existing anonymous data<sup>23</sup>. All studies included in this analysis will have received local  
376 ethical approvals and our pooled IPD meta-analysis will be addressing scientific questions that  
377 are very similar to the original research questions.

378 Findings will be reported following the PRISMA-IPD guidelines<sup>24</sup> at open access peer-  
379 reviewed journals. This systematic literature review and IPD meta-analysis is registered to  
380 PROSPERO (CRD42018103776) and this protocol has been reported following the PRISMA-  
381 P guidelines<sup>25</sup>. Any publications based on the findings of this IPD meta-analysis will be in  
382 accordance with the guidelines of the International Committee of Medical Journal Editors.

### 383 **Patient and Public Involvement**

384 Patients were not involved in the development of the research question, outcome measure or  
385 study design.

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387

## 388 Discussion

389 Large scale deployment of highly efficacious ACT regimens such as artesunate-mefloquine  
390 has been the cornerstone of global malaria control for over a decade and this has contributed  
391 to the global reduction of mortality and morbidity associated with malaria <sup>12</sup>. Maintaining these  
392 gains is highly dependent on efficient health systems, sustainable global funding and the  
393 current status of antimalarial drug resistance. The 2017 WHO report found that globally the  
394 number of malaria cases has stopped dropping and mortality has crept up compared to 2016,  
395 suggesting that the recent public health gains remain fragile <sup>26,27</sup>. To make things worse, a  
396 health calamity is looming large due to the emergence of resistance to artemisinins in South-  
397 East Asia which is threatening to reverse the remarkable progress achieved over the past decade  
398 <sup>28</sup>. In the absence of an alternative treatment class to replace the ACTs as first line therapy,  
399 preserving the currently available drugs remains the top-most priority and this requires the  
400 highest form of evidence regarding the susceptibility of the parasites against the antimalarial  
401 drugs. Artesunate-mefloquine retains extremely high efficacy in most locations except  
402 Thailand, and in any given trial, only few treatment failures have been observed which limits  
403 the utility of any single study in answering questions regarding the dose-response relationship.  
404 Individual participant data (IPD) meta-analysis provides an alternative strategy.  
405 IPD meta-analysis is now considered the gold-standard for evidence synthesis and allows  
406 exploration of different risk factors which otherwise would not be possible through the  
407 aggregate data meta-analysis <sup>5</sup>. This IPD-MA is designed to explore the variability in drug  
408 dosage administered in patients with uncomplicated *P. falciparum* malaria, treated with  
409 artesunate-mefloquine (AS-MQ). The WHO-recommended AS-MQ regimen is administered  
410 as a three day course, with a total of 12 mg/kg artesunate and 25 mg/kg of mefloquine split  
411 over three days. Due to the poor tolerability of high dose mefloquine, the dose of mefloquine  
412 is usually divided into either two doses (15 and 10 mg/kg), or three as a fixed dose combination

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3 413 (8mg/kg/day). The fixed dose combination has been shown to provide better efficacy and  
4  
5 414 improve treatment adherence for artesunate-amodiaquine <sup>6</sup>. Such a comparison is yet to be  
6  
7 415 made for the AS-MQ regimen, and in this IPD-MA we propose to compare the fixed and loose  
8  
9 416 formulations of the regimen with regards to the drug dosing, tolerability, efficacy and  
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11 417 practicality of the dose banding.  
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15 418 In conclusion, this pooled analysis will provide critical information regarding the relationship  
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17 419 between drug dosage and parasitological responses post-treatment with artesunate-mefloquine.  
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19 420 The assessment of the host, parasite and drug determinants that influence the treatment  
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21 421 response can provide evidence-based guidance for monitoring the early signs of artemisinin  
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23 422 resistance and effective case management that will be critical in optimising malaria control and  
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25 423 containment efforts.  
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4 424 **Declarations**  
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7 425 **Author contributions**  
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10 426 Conceived the idea and wrote the first draft of the protocol: RM, PD, PG, EAA, and KS

11  
12 427 Systematic review of all published antimalarial studies: GSH  
13

14 428 Data acquisition and standardisation: GSH, EAA, KS  
15

16  
17 429 All authors read and approved the submission of the final draft of the study protocol.  
18  
19

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22

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24

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26

27 433 did not participate in the study development, the writing of the paper, decision to publish, or  
28

29 434 preparation of the manuscript.  
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33 435 **Competing interests**  
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36 436 The authors declare that they have no competing interests.  
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39 437 **Ethics approval**  
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42 438 This individual patient data meta-analysis met the criteria for waiver of ethical review as  
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44 439 defined by the Oxford Tropical Research Ethics Committee (OxTREC) since the research  
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46 440 consists of secondary analysis of existing anonymous data. Each study included in the  
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48 441 analysis will have received local ethics approvals.  
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52 442 **Acknowledgements**  
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56

57 444 thank Brittany Maguire for her help with the manuscript.  
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3 446 **List of Figures**  
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6 447 **Figure 1: Mg/kg dose variations for mefloquine**  
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9 448 **Legend:** The WHO recommendation on the 3-day fixed dose AS-MQ regimen is to administer  
10 449 once daily 50 mg of MQ for those weighing 5-8 kg, 100 mg for 9-17 kg, 200 mg for 18-29 kg  
11 450 and 400mg for those who weigh  $\geq 30$  kg. The horizontal dotted line represents the target MQ  
12 451 dose of 25 mg/kg. Since the dosing is based on weight-bands, this leads to the saw-tooth dosing  
13 452 curves.  
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20 454 **Figure 2: Data acquisition and standardisation process utilizing the WWARN database**  
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22 455 **Legend:** Individual participant data is shared by researchers to the WWARN secured portal  
23 456 (left panel). The studies are standardised using a common WWARN protocol and the tables of  
24 457 clinical, parasitological and drug measurements are stored in a secured repository with  
25 458 relational database (middle panel). On standardisation, the dataset is shared back with the study  
26 459 investigator, and subsequently used in meta-analyses to answer questions of public health  
27 460 importance (right panel).  
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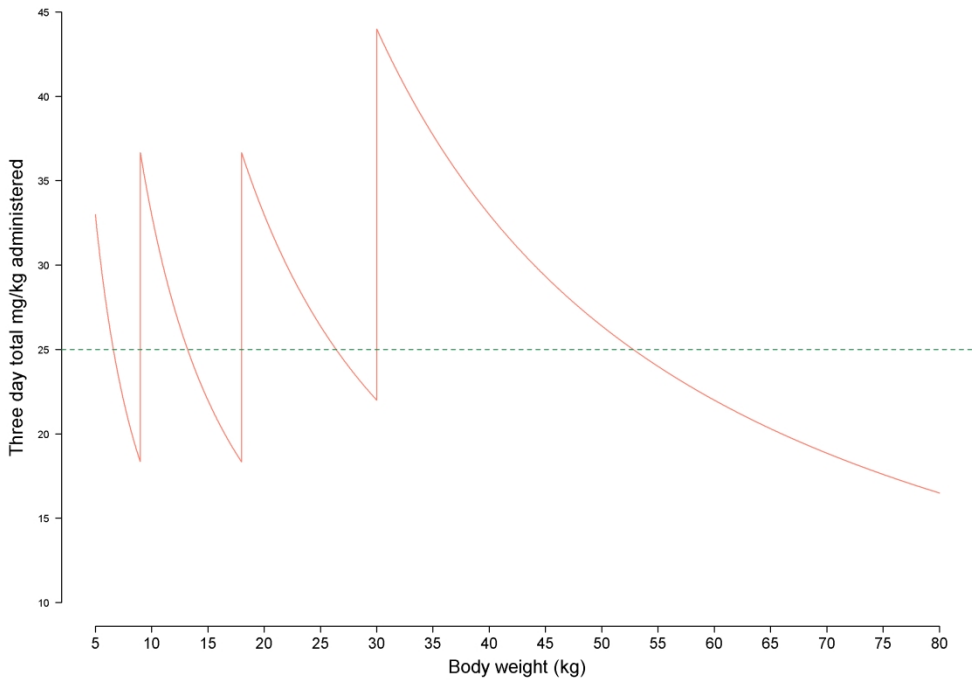
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Caption : Figure 1:Mg/kg dose variations for mefloquine  
Legend: The WHO recommendation on the 3-day fixed dose AS-MQ regimen is to administer once daily 50 mg of MQ for those weighing 5-8 kg, 100 mg for 9-17 kg, 200 mg for 18-29 kg and 400mg for those who weigh  $\geq 30$  kg. The horizontal dotted line represents the target MQ dose of 25 mg/kg. Since the dosing is based on weight-bands, this leads to the saw-tooth dosing curves.

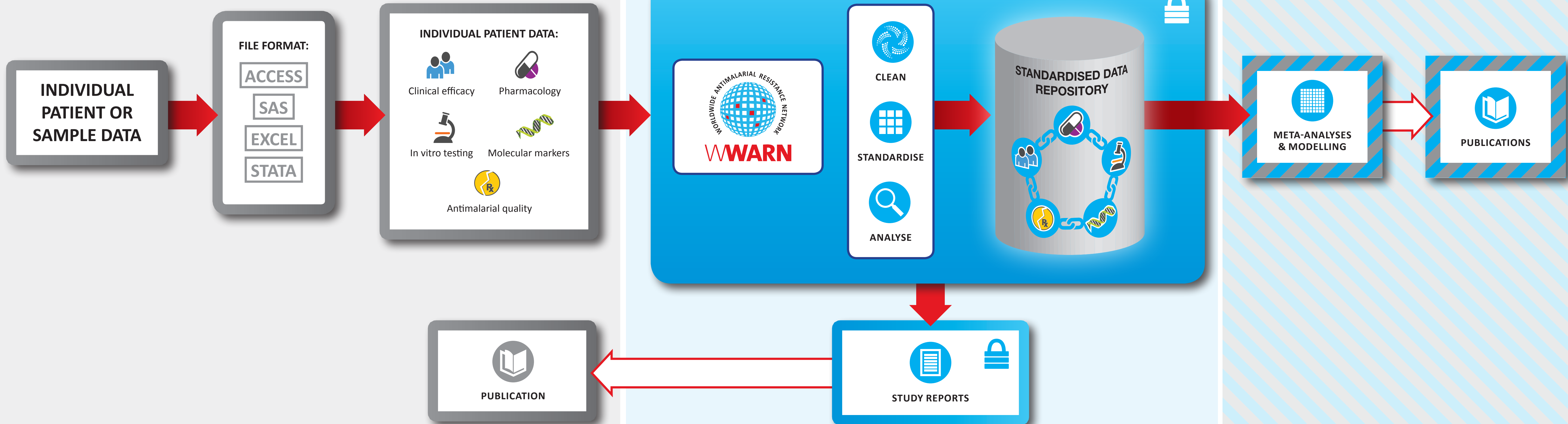
239x179mm (300 x 300 DPI)

CONTRIBUTER  
(DATA SHARING)

WWARN  
(DATA MANAGEMENT & STATISTICAL ANALYSIS)

WWARN  
(JOINT RESEARCH)

CONTRIBUTERS  
(INFORMING PUBLIC  
HEALTH POLICY)



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## Supplementary file: The effect of dose on the antimalarial efficacy of artesunate-mefloquine against *Plasmodium falciparum* malaria: a protocol for systematic review and individual patient data (IPD) meta-analysis

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## Literature Search Strategy

The following search terminologies were used for searching relevant publications in the given libraries

### PubMed via PubMed

((("plasmodium falciparum"[MeSH Terms] OR ("plasmodium"[All Fields] AND "falciparum"[All Fields]) OR "plasmodium falciparum"[All Fields]) OR falciparum[All Fields]) OR ("malaria"[MeSH Terms] OR "malaria"[All Fields])) AND (((artesunate-mefloquine[All Fields] OR artesunate/mefloquine[All Fields]) OR ASMQ[All Fields]) OR AS-MQ[All Fields]) OR AS/MQ[All Fields]) OR (("artesunate"[Supplementary Concept] OR "artesunate"[All Fields]) AND (("mefloquine"[MeSH Terms] OR "mefloquine"[All Fields]) OR MQ[All Fields]))))

### EMBASE via OVID

(plasmodium falciparum.mp. OR Plasmodium falciparum/ OR falciparum.mp. OR (falciparum.mp. AND (Plasmodium/ OR plasmodium.mp.)) OR malaria.mp. OR malaria/ AND (artesunatemefloquine.mp. OR ASMQ.mp. OR AS-MQ.mp. OR ((artesunate/ OR artesunate.mp.) AND (mefloquine/ OR mefloquine.mp. OR MQ.mp.))

### BIOSIS via Web of Science

(TS=(plasmodium falciparum) OR (TS=(falciparum AND plasmodium)) OR TS=(falciparum) OR TS=(malaria)) AND TS=(artesunate-mefloquine) OR TS=(ASMQ) OR TS=(AS-MQ) OR (TS=(artesunate) AND TS=(mefloquine OR MQ))

### Web of Science Core Collection via Web of Science

(TS=(plasmodium falciparum) OR (TS=(falciparum AND plasmodium)) OR TS=(falciparum) OR TS=(malaria)) AND TS=(artesunate-mefloquine) OR TS=(ASMQ) OR TS=(AS-MQ) OR (TS=(artesunate) AND TS=(mefloquine OR MQ))



## PRISMA-P 2015 Checklist

This checklist has been adapted for use with protocol submissions to *Systematic Reviews* from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 4:1

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
<b>ADMINISTRATIVE INFORMATION</b>					
<b>Title</b>					
Identification	1a	Identify the report as a protocol of a systematic review	<input checked="" type="checkbox"/>	<input type="checkbox"/>	2-3
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	<input type="checkbox"/>	<input checked="" type="checkbox"/>	NA
<b>Registration</b>	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	<input checked="" type="checkbox"/>	<input type="checkbox"/>	44
<b>Authors</b>					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	<input checked="" type="checkbox"/>	<input type="checkbox"/>	5-22
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	<input checked="" type="checkbox"/>	<input type="checkbox"/>	402- 406
<b>Amendments</b>	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	<input type="checkbox"/>	<input checked="" type="checkbox"/>	NA
<b>Support</b>					
Sources	5a	Indicate sources of financial or other support for the review	<input checked="" type="checkbox"/>	<input type="checkbox"/>	407- 411
Sponsor	5b	Provide name for the review funder and/or sponsor	<input checked="" type="checkbox"/>	<input type="checkbox"/>	409
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	<input checked="" type="checkbox"/>	<input type="checkbox"/>	409-411
<b>INTRODUCTION</b>					
<b>Rationale</b>	6	Describe the rationale for the review in the context of what is already known	<input checked="" type="checkbox"/>	<input type="checkbox"/>	61- 86
<b>Objectives</b>	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	<input checked="" type="checkbox"/>	<input type="checkbox"/>	87- 95
<b>METHODS</b>					



Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
<b>Eligibility criteria</b>	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	<input checked="" type="checkbox"/>	<input type="checkbox"/>	97-117
<b>Information sources</b>	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	<input checked="" type="checkbox"/>	<input type="checkbox"/>	132-142
<b>Search strategy</b>	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	<input checked="" type="checkbox"/>	<input type="checkbox"/>	132-142
<b>STUDY RECORDS</b>					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	<input checked="" type="checkbox"/>	<input type="checkbox"/>	154- 160
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	<input checked="" type="checkbox"/>	<input type="checkbox"/>	136- 138
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	<input checked="" type="checkbox"/>	<input type="checkbox"/>	144- 153
<b>Data items</b>	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	<input checked="" type="checkbox"/>	<input type="checkbox"/>	176- 219
<b>Outcomes and prioritization</b>	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	<input checked="" type="checkbox"/>	<input type="checkbox"/>	239-283
<b>Risk of bias in individual studies</b>	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	<input checked="" type="checkbox"/>	<input type="checkbox"/>	336- 340
<b>DATA</b>					
<b>Synthesis</b>	15a	Describe criteria under which study data will be quantitatively synthesized	<input checked="" type="checkbox"/>	<input type="checkbox"/>	176- 219
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., $I^2$ , Kendall's tau)	<input checked="" type="checkbox"/>	<input type="checkbox"/>	220-323
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	<input checked="" type="checkbox"/>	<input type="checkbox"/>	324-335
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	<input checked="" type="checkbox"/>	<input type="checkbox"/>	341-344
<b>Meta-bias(es)</b>	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	<input checked="" type="checkbox"/>	<input type="checkbox"/>	340
<b>Confidence in cumulative evidence</b>	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	<input checked="" type="checkbox"/>	<input type="checkbox"/>	337-340