Trial title: Population differences in vaccine response: the role, reversibility, and mediators of immunomodulation by chronic infections in the tropics.
The effect of intermittent preventive treatment (IPT) for malaria with dihydroartemisinin-piperaquine on response to vaccines among rural adolescents (POPVAC B)

Statistical Analysis Plan
Version 1.0
12th October 2022

Table of Contents

This SAP has been written based on information contained in the POPVAC B protocol version 2 dated 19th November 2021.

1. Introduction

1.1. Background and rationale

Several important licensed and investigational vaccines have lower efficacy, and induce impaired immune responses, in low-income countries (LICs) compared to high-income countries and in rural, compared to urban, settings. Understanding these population differences is crucial for informing strategies towards optimisation of vaccine effectiveness in the tropics. We hypothesise that malaria infection suppresses immune responses to unrelated vaccines, and that this effect can be reversed, at least in part, by monthly preventive treatment of malaria in schools in high transmission settings.

To address this hypothesis, POPVAC B trial is being carried out among adolescents in rural sub-counties of Jinja district, Uganda to assess if monthly preventive treatment of malaria with dihyrodartemisinin-piperaquine (DP) will impact on vaccine responses in this population.

1.2. Research Objectives and hypothesis

Study objectives

The primary objective of this trial is to determine whether there are reversible effects of malaria infection on vaccine responses in adolescents, using an intervention study.

Secondary objectives are:

a) To determine effects on correlates of protective immunity (where recognised correlates exist).
b) To determine effects on vaccine response waning.
c) To determine whether there are differential effects on priming vs boosting immunisations.
d) To determine effects on current malaria infection prevalence and intensity.

The exploratory objective is to explore immunology among subsets of participants to further characterise effects of preventive treatment of malaria on vaccine responses.

Primary objective hypothesis

The null hypothesis is that there is no difference in the mean responses induced by BCG, YF-17D, Ty21a, HPV and Td vaccines between adolescents who received monthly DP and placebo treatment. The alternative hypothesis is that the mean vaccine responses will differ between adolescents receiving monthly DP and placebo treatment.

2. Study Methods

2.1. Trial design

This trial is a randomised, double-blind, placebo-controlled, parallel group trial comparing effect of monthly preventive treatment of malaria with dihyrodartemisinin-piperaquine (DP) versus placebo on vaccine response outcomes. Treatment allocation is a 1:1 ratio for the trial intervention.

2.2. Sample size considerations

The original target sample size was 640 healthy adolescents (320 in each arm) of whom 60% (384) were expected to have malaria infection at the outset will be enrolled into the trial. This target sample size was informed by the expected difference in vaccine responses between the two arms, with full details of sample size considerations presented in the protocol. However, due to the onset of the COVID-19 pandemic and the consequent restrictions in Uganda, it was not possible to achieve the original target sample size. Table 1 shows updated power estimation for the achieved sample size of 341.

Table 1. Updated power estimates for the primary analysis population of participants with malaria infection at baseline (5% significance level): n=341, assuming 60% malaria prevalence, and allowing for 20% loss to follow-up

	Log_{10} difference					
Standard deviation (log_{10})	**0.10**	**0.12**	**0.14**	**0.16**	**0.18**	**0.20**
102 DP vs 102 placebo (baseline malaria infected only), 20% loss to follow-up, i.e. analysis based on 82 vs 82						
0.3	56%	72%	84%	92%	97%	99%
0.4	36%	48%	61%	72%	82%	89%
0.5	25%	33%	43%	53%	63%	72%
0.6	19%	25%	32%	40%	48%	56%
Cells highlighted in grey correspond to >80% power.						

2.3. Randomisation

Eligible volunteers who consent will be enrolled and allocated sequentially to one of the two trial arms according to the randomisation code. The randomisation code was generated using a randomly permuted block size and will be used to allocate participants to either receive monthly DP (DP arm) or placebo (placebo arm).

2.4. Blinding

The trial is double blinded and will remain so until all data has been collected, cleaned, and analysed.

2.5. Statistical interim analyses and stopping guidance

Analyses will be conducted for safety reviews as outlined in the Data Safety Monitoring Board (DSMB) charter. For each safety analysis, the DSMB members will review data on trial progress and adverse events. Details of the safety holding rules are detailed in the protocol. Since recruitment will be conducted over a short time, there is no statistical interim analysis planned for comparison of vaccine responses between the two arms.

2.6. Timing for the primary outcome analysis

Analysis for comparison of each of the vaccine responses between the two trial arms will take place after enrolment of all participants in the two arms has been completed and samples for assessing the primary outcomes have been taken, laboratory assays for that particular vaccine primary outcome have been conducted and the data have been entered and cleaned.

2.7. Timing of outcome assessment

In the following section, week numbers in brackets are based on the schedule in the protocol, with BCG immunisation given at week 0, and YF-17D, Ty21a and first dose of HPV given at week 4 and second dose of HPV and Td given at week 28.

Timing of primary outcomes

BCG: *BCG-specific IFN-γ ELIspot response* eight weeks post BCG immunisation (week 8)

YF-17D: neutralising antibody titres (plaque-reduction neutralisation test) at four weeks post YF immunisation (week 8).

Ty21a: *Salmonella typhi* lipopolysaccharide (LPS)-specific immunoglobulin (Ig)G concentration at four weeks post Ty21a immunisation (week 8).

HPV: IgG specific for L1-proteins of HPV-16/18 at four weeks post HPV priming immunisation (week 8).

Td: tetanus and diphtheria toxoid-specific IgG concentration at 24 weeks post Td immunisation (week 52).

Timing of secondary outcomes

Protective immunity: Proportions with protective neutralising antibody (YF), i.e. neutralising antibody>1/5; protective IgG levels (TT), i.e. IgG>0.1 IU/ml; seroconversion rates (Ty21a) at four weeks post the corresponding immunisation (week 8).

Response waning: Primary outcome measures for all vaccines repeated at week 52 except for Td whose primary outcome responses are measured at week 52.

Priming versus boosting: HPV vaccine responses four weeks after the priming dose (week 8) and 24 weeks after the boosting dose (week 52).

Current malaria infection status and intensity at screening (week -8), weeks 0, 4, 8, 52.

3. Statistical Principles

3.1. Level of statistical significance

All applicable statistical tests will be 2-sided. All tests will be performed using a 5% significance level. 95% confidence intervals will be presented.

3.2. Compliance and Protocol Deviations

Compliance to the protocol schedule will be assessed based on the percent of participants who have attended the scheduled visits and received the appropriate assigned treatments and vaccines within the permitted time windows around the vaccinations and sample collection visits as specified in the schedule of visits and procedures in the protocol.

3.3. Analysis population

The primary analysis will be done on participants who are infected with malaria at baseline (based on the result of a malaria PCR test) to investigate the effect of parasite removal. The secondary analysis will include all randomised individuals. For all responses to vaccines, analysis will be done by intention to treat (ITT), that is, participants will be included in the arm to which they were randomised, regardless of whether they have received all allocated DP/Placebo treatments.

Analysis population for the primary outcomes

BCG-specific IFN-γ ELIspot: The analysis population for BCG-specific *IFN-γ ELIspot* response will include all enrolled participants who receive BCG at week 0 and have BCG-specific IFN-γ ELIspot response measurement at week 8.

YF-17D: The analysis population for YF-17D vaccine response will include all enrolled participants who receive yellow fever vaccine at week 4 and have YF-17D response measurement at week 8.

Ty21a: The analysis population for Typhoid vaccine (Ty21a) response will include all enrolled participants who receive Typhoid vaccine (Ty21a) at week 4 and have YF-17D response measurement at week 8.

HPV: The analysis population for HPV vaccine response will include all enrolled participants who receive HPV vaccine at week 4 and have HPV response measurement at week 8.

Td: The analysis population for Td vaccine response will include all enrolled participants who receive Tetanus and diphtheria vaccine at week 28 and have Td response measurements at week 52.

Analysis population for the secondary outcomes

Protective immunity: Analysis population for YF, Ty21a and Td protective immunity will include all enrolled participants who receive the respective vaccine (YF-17D, Ty21a at week 4, Td vaccine at week 28) and have responses to the respective vaccines at 4 weeks post vaccination for YF-17D, Ty21a and 24 weeks post Td vaccination.

Response waning: Analysis population will include all enrolled participants who receive any of BCG, YF-17D, Ty21a, HPV and Td vaccines; have corresponding responses to any of these vaccines at weeks 8, 28 (for Td vaccine) and 52 post vaccination.

Priming versus boosting: Analysis population will include all enrolled participants who receive a priming dose of HPV vaccine at week 4 and a boosting dose at week 28 and have corresponding responses to the vaccine (at weeks 8 and 52).

Current malaria infection status and intensity*:* Analysis population will include all enrolled participants who have serum/plasma samples and corresponding results at any of these time points; baseline (week -8), on immunisation days (weeks 0, 4, 8 and 28), and on primary (weeks 8 and 52) and secondary endpoint days (week 52).

4. Trial population

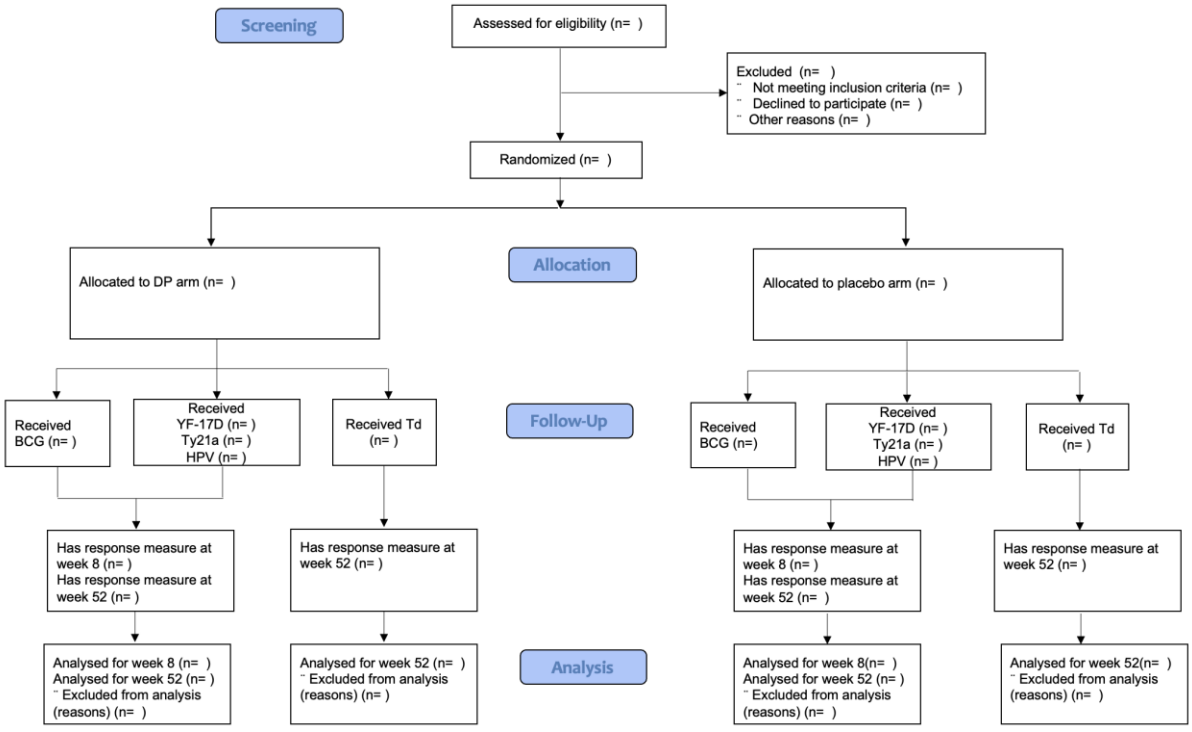
4.1. Screening, eligibility, recruitment and lost to follow up of participants.

A CONSORT flow diagram will be used to summarise the number of participants in each trial arm who were:

• assessed for eligibility at screening • ineligible at screening* • eligible and randomised • received vaccines • did not receive vaccines* • lost to follow-up* • included in the primary analysis • excluded from the primary analysis*

*reasons for these events will be provided.

CONSORT Flow Diagram



Note: Details of inclusion and exclusion criteria are listed in the protocol

4.2. Baseline characteristics

Participants will be described with respect to their baseline characteristics including age, sex, school, location of birth, prior vaccination status, malaria infection status both overall and separately for each arm.

Categorical variables will be summarised by numbers and percentages. Continuous variables will be summarised by mean, SD and range if data are normal or median, IQR and range if data are skewed. No statistical tests will be done to compare baseline characteristics between arms.

5. Analysis of outcomes

5.1. Outcome definitions

Timing for the outcome assessments is described in section 2.7

5.1.1. Outcomes for primary objective

- *BCG: BCG-specific IFN-γ ELIspot response*
- *YF-17D:* neutralising antibody titres
- *Ty21a: Salmonella typhi* lipopolysaccharide (LPS)-specific immunoglobulin (Ig)G concentration
- *HPV:* IgG specific for L1-proteins of HPV-16/18 at four weeks post HPV priming immunisation
- *Td:* tetanus and diphtheria toxoid-specific IgG concentration

5.1.2. Outcomes for secondary objective

- *Protective immunity:* Proportions with protective neutralising antibody (YF), i.e., >1/5; protective IgG levels (TT), i.e., >0.1 IU/mL; seroconversion rates (Ty21a).
- *Response waning:* Primary outcome measures for all vaccines repeated at week 52.
- *Priming versus boosting:* IgG specific for L1-proteins of HPV-16/18.
- Current malaria infection status and intensity: Infection status (infected and uninfected) and intensity (light, medium and heavy).

5.2. Analysis methods

5.2.1. Analysis of primary objective outcome measures

Unpaired t tests will be used to compare means of primary outcome responses between participants in the DP and placebo arms. The effect estimate will be presented as a mean difference in the responses reported with 95% confidence intervals and p values. There will be no adjustment for confounders. For each of the primary outcomes, we will investigate adjusting for the corresponding measure at baseline. Subgroup analyses by sex for all the primary outcome measures will be conducted and unpaired t tests will be used to compare

mean responses in each group, with interaction terms fitted to assess whether effects of the intervention on outcomes differ between subgroups.

5.2.2. Analysis of secondary objective outcome measures

Protective immunity: Proportions of participants in each category will be presented by trial arm and comparisons between trial arms will be made using chi square test.

Response waning: Area under the curve will used for analysis of response waning. Response measurements from weeks 8 and 52 will be used for this analysis.

Priming versus boosting: Mean HPV responses will be presented, and a paired t test will be used to compare HPV responses at weeks 8 and 52. In addition, a linear regression model including a time*trial arm interaction term will be fitted to assess whether the intervention has a differential effect on the response to priming and than to boosting.

Current malaria infection and intensity: Proportions of participants with malaria infection will be presented at each timepoint by trial arm and comparisons between trial arms will be made using chi square test. At each timepoint, the means (SD) of malaria infection intensity will be presented by trial arm, and comparisons between trial arms will be made using unpaired t-tests. For both malaria infection status and intensity, Generalised Estimating Equations (GEE) will be used to estimate and compare malaria status and intensity changes between the trial arms.

Checking assumptions

For statistical analyses that require data to be normally distributed, the normality assumption of the data will be checked using graphical assessment of normality (Q-Q plot) and a Kolmogorov-Smirnov test for normality. If the data are found to be non-normal, medians (IQR) or geometric means (SD) will be presented instead of ordinary means (SD). A log transformation will be applied to achieve normality and if not achieved, alternative tests such as the Wilcoxon rank sum test will be used to compare outcomes between trial arms. If normality is achieved through log transformation, then the parametric tests described in sections 5.2.1, 5.2.2 and 5.2.3 will be applied to the log transformed data and results back-transformed to be presented as geometric mean ratios (GMR) and 95% CIs. The assumption of equal variances will be checked by testing if standard deviations of the two sample outcome responses are approximately equal e.g., using the F test. If the variances are found to differ, a t test allowing for unequal variance will be used.

5.3. Missing data

Participants with missing data on BCG, YF-17D, Ty21a, HPV and Td vaccination and/or measurement for primary endpoints will be excluded from the respective analysis as detailed in the analysis population section. There will be no imputation of missing data.

5.4. Statistical software

The analysis will be carried out using Stata version 12 or above. Other packages such as R and SAS may be used if necessary.

5.5. Sample tables

5.5.1. Baseline characteristics of study participants

Table 2: Baseline characteristics of study participants by trial arm

	DP		Placebo	
Characteristic	**n**	**Col%**	**n**	**Col%**
Sex (Male)				
Age				
School				
Good hope day and boarding				
Etc…				
Malaria infection status				
Infected				
S. *mansoni* infection status				
Infected				
Etc...				

5.5.2. Comparison of primary outcome responses between trial arms

Table 3: Differences in responses between DP and Placebo arms at week 8 for BCG, YF-17D, Ty21a, HPV and week 52 for Td.

	DP	Placebo			
Vaccine	**Mean (SD)****	**Mean (SD)****	**Mean difference*****	**95% CI**	**P value***
BCG					
YF-17D					
Ty21a					
HPV					
Td					

*P values reported from unpaired t test

** If data are not normally distributed, median (IQR) or geometric mean (SD) will be presented

*** If data not normally distributed, Geometric Mean Ratios (GMR) will be presented

5.5.3. Comparison of secondary outcome responses between trial arms

Table 4: Proportions with protective immunity between DP and placebo arm participants at 4 weeks post the corresponding vaccination.

	DP		Placebo		Risk difference (95% CI)	P value*
	n/N	Col %	n/N	Col %		
Protective neutralising antibody (YF)						
Protective IgG levels (TT)						
Seroconversion rates (Ty21a)						

*P values reported from chi square test

Table 5: Priming versus boosting: Comparison of HPV responses after priming and boosting doses

	4 weeks after priming dose, mean (SD)**	**4 weeks after boosting dose, mean (SD)****	**P value***
Arm			
DP			
Placebo			

*P values reported from paired t test

** If data are non-normally distributed, median (IQR) or geometric mean (SD) will be presented

Table 6: Comparison of current malaria infection between DP and placebo arms at weeks -8, 0, 4, 8 and 52.

	Screening (week -8)#			**Week 0#**				
	DP	**Placebo**	**P value***	**DP**	**Placebo**	**P value***	**Estimate (95% CI)****	**P value****
	n(Col%)	n(Col%)		n (Col%)	n(Col%)			
Malaria infection status								
Positive								
Negative								
Malaria infection intensity								
Light								
Medium								
Heavy								

Shell table shows weeks -8 and 0 only for reasons of space in this document; additional columns in the same format as that shown for weeks -8 and 0 will be included for weeks 4, 8 and 52.

*P values reported from chi square test

** Estimate and P value from GEE