Statistical Analysis Plan – Pregnant women

TRIAL FULL TITLE	**Reducing the Burden of Malaria in HIV-uninfected Pregnant Women and Infants – "Birth Cohort 1"**
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1 Abbreviations and Definitions

ACT	Artemisinin-based combination therapy
AE	Adverse Event
AL	Artemether-lumefantrine
ALT	Alanine transaminase (SGPT)
CAB	Community advisory board
CBC	CBC Complete blood cell
CRF	Case Report Form
DP	Dihydroartemisinin-piperaquine
DSMB	Data and Safety Monitoring Board
IDRC	Infectious Diseases Research Collaboration
IPT	Intermittent preventive therapy
IPTp	Intermittent preventive therapy in pregnancy
IRB	Institutional review board
ITN	Insecticide treated net
MOH	Ministry of Health
MU	Makerere University
NICHD	National Institute of Child Health and Human Development
NIH	National Institute of Health
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SP	Sulfadoxine-pyrimethamine
TDH	Tororo District Hospital
UCSF	University California San Francisco
WHO	WHO World Health Organization

2 Introduction

2.1 Preface

In sub-Saharan Africa approximately 25 million pregnant women are at risk of *P. falciparum* infection every year and 25% have evidence of placental infection.[1] Among pregnant women living in areas of stable transmission few infections lead to symptomatic malaria, however, infection is associated with maternal morbidity, such as anemia, and adverse birth outcomes including abortions, stillbirth, preterm delivery, low birth weight (LBW), and infant mortality.[2,3]

In Africa the only widely available tools for the prevention of malaria in pregnancy are insecticide treated bednets (ITNs) and intermittent preventive therapy (IPTp) with sulfadoxine-pyrimethamine (SP). Older studies demonstrated the efficacy of IPTp with SP in reducing the risk of placental malaria and LBW.[4–8] However, there are now concerns about the continued efficacy of IPTp given the spread of antifolate resistance. A 2007 review suggested that IPTp with SP remained beneficial in areas with antifolate resistance, however, this conclusion was based on communities where SP treatment failure rates in children remained at moderate levels.[9] More recently, reports from East Africa have documented SP failure rates over 65% in children when used for treatment[10] or prevention[11], and near saturation of common SP resistance alleles.[12,13] In a recent study from Tanzania IPTp with SP was not associated with a decreased risk of placental malaria, maternal anemia, or LBW, and unexpectedly associated with an increased risk of fetal anemia.[14] In a recent study from Uganda there was no significant difference in the risk of maternal infection, maternal anemia, and LBW for pregnant women receiving IPTp with SP plus ITNs vs. ITNs alone.[15] In addition, most IPTp studies have defined placental malaria on the basis of placental blood smears, which dramatically underestimate the true prevalence of placental malaria. In summary, there are several lines of evidence suggesting that IPTp with SP is no longer effective in areas of East Africa with widespread antifolate resistance. New interventions to reduce the burden of malaria in pregnancy in this region are desperately needed.

The artemisinin-based combination therapy (ACT) class of drugs offers an attractive alternative to SP for use in pregnancy. In a recent systematic review of parasitological efficacy for the treatment and prevention of falciparum malaria in pregnancy, placenta-positive rates were unacceptably high in a majority of SP trial arms and ACTs provided the lowest parasitological failure rates.[16] The authors

recommended that SP should no longer be used for treatment or prevention of malaria in pregnancy and that ACTs provide the most efficacious and safe alternative therapy. Two studies of the ACT artesunate (AS) + SP from Africa concluded that this drug was safe for the treatment of malaria in pregnant women.[17,18] More recent studies have focused on artemether-lumefantrine (AL), considering efficacy, pharmacokinetics, and safety. In a prospective study from Zambia, 495 pregnant women exposed to AL (including 156 in the 1st trimester) had similar risks of adverse maternal and infant outcomes compared to pregnant women exposed to SP.[19] In a recent study from Uganda, pregnant women in their 2nd or 3rd trimester with peripheral parasitemia treated with AL had a cure rate of 99%.[20] Dihydroartemisinin-piperaquine (DP) has also been safely used for the treatment of uncomplicated malaria in pregnancy in studies from Asia.[21,22] In two recent studies on the treatment of uncomplicated malaria with DP in pregnant and non-pregnant women, one concluded that there were no clinically important differences in piperaquine pharmacokinetics in pregnancy [23] and another concluded that pregnancy was associated with an unaltered total exposure to piperaquine but a shorter terminal elimination half-life.[24]

In summary, most African countries continue to recommend IPTp with SP, however, there are serious concerns about the efficacy of SP given widespread resistance, especially in East Africa. Available data have shown that ACTs are effective and safe for the treatment of malaria in pregnancy and are now recommended by the WHO as 1st line therapy for pregnant women in their 2nd or 3rd trimester.[25] However, there are no published studies evaluating the safety and efficacy of ACTs for use as preventive therapy in pregnant women.

2.2 Purpose of the analyses

This proposal will be the first clinical trial we are aware of to evaluate the efficacy and safety of DP for the prevention of malaria in pregnant women. We will perform a randomized, double-blinded, controlled trial to compare 2 dosing strategies of this novel intervention with the current standard of care of IPTp with SP in an area of high transmission intensity and widespread antifolate resistance. The primary outcome will be based on placental histopathology; in addition, pregnant women will undergo monthly blood sampling using a highly sensitive loop-mediated isothermal amplification (LAMP) assay to better define the timing and frequency of malaria infection during pregnancy.

3 Study Objectives and Endpoints

3.1 Study Objectives

(ICH E3; 8.)

We will test the hypothesis that IPT with DP will significantly reduce the burden of malaria in pregnancy. The specific study objectives are as follows:

3.1.1. Objective 1

To compare the risk of placental malaria among HIV uninfected pregnant women randomized to receive IPTp with 3 dose SP vs. 3 dose DP vs. monthly DP. We will test the hypothesis that pregnant women who receive IPTp with either 3 doses of DP or monthly DP will have a lower risk of placental malaria defined by histopathology compared to those who receive 3 doses of SP. Secondary outcomes will include maternal and fetal clinical outcomes. We will also compare the two different dosing strategies of DP.

3.2 Endpoints

(ICH E9; 2.2.2)

3.2.1. Primary Outcomes

The primary outcome for pregnant women will be the prevalence of placental malaria based on placental histopathology and dichotomized into any evidence of placental infection (parasites or pigment) vs. no evidence of placental infection. We will also evaluate placental malaria defined by histopathology as a categorical variable (active-acute, active-chronic, and past infection) based on the criteria developed by Rogerson et al.[26]

3.2.2. Secondary Outcomes

- **Prevalence of placental parasitemia**: Proportion of placental blood samples positive for parasites by microscopy or LAMP
- **Incidence of maternal malaria**: Episodes of fever and positive blood smear by microscopy

- **Prevalence of adverse birth outcomes**: Proportions of birth outcomes that include congenital malformations, preterm delivery, late spontaneous abortion, LBW (<2500g), stillbirth, or a composite of all of these
- **Incidence of adverse events**: Adverse events stratified by type, severity score and relationship to study drugs
- **Prevalence of anemia**: Proportion of routine hemoglobin measurements < 11 g/dL & < 8 g/dL
- **Prevalence of asymptomatic parasitemia**: Proportion of routine monthly samples positive for parasites by LAMP

3.3 Derived Variables

Composite adverse birth outcome will be a derived variable defined as the presence of any preterm delivery, congenital malformations, spontaneous abortion, low birth weight (<2500g), or stillbirth.

4 Study Methods

4.1 General Study Design and Plan

(ICH E3;9)

This will be a double-blinded randomized controlled phase III trial of 300 HIV uninfected pregnant women and the children born to them. The study interventions will be divided into two phases. In the first phase, HIV uninfected women enrolled at 12-20 weeks gestation will be randomized in equal proportions to one of three IPTp treatment arms: 1) 3 doses of SP, 2) 3 doses of DP, or 3) monthly DP. All three interventions arms will have either SP or DP placebo to ensure adequate blinding is achieved in the study. Follow-up for the pregnant women will end approximately 6 weeks after giving birth. In the second phase of the study, all children born to pregnant women enrolled in the study will be followed from birth until they reach 36 months of age. Children born to women randomized to receive 3 doses of SP during pregnancy will receive DP every 3 months between 2-24 months of age. Children born to women randomized to receive 3 doses of DP or monthly DP during pregnancy will receive either DP every 3 months or monthly DP between 2-24 months of age. To ensure adequate blinding, children who will receive DP every 3 months will be given DP placebo during the months they will not be taking DP. Children

will then be followed an additional year between 24-36 months of age following the interventions.

The details outlined in this statistical analyses plan will only focus on Phase I of the study involving pregnant women.

4.2 Equivalence or Non-Inferiority Studies

(ICH E3; 9.2, 9.7.1, 11.4.2.7. ICH E9; 3.3.2)

N/A – This trial is designed as a superiority trial.

4.3 Inclusion-Exclusion Criteria and General Study Population

(ICH E3;9.3. ICH E9;2.2.1)

4.3.1. Inclusion Criteria

1) Pregnancy confirmed by positive urine pregnancy test or intrauterine pregnancy by ultrasound

2) Estimated gestational age between 12-20 weeks

3) Confirmed to be HIV uninfected by rapid test

4) 16 years of age or older

5) Residency within 30km of the study clinic

6) Provision of informed consent by the pregnant woman for herself and her unborn child

7) Agreement to come to the study clinic for any febrile episode or other illness and avoid medications given outside the study protocol

8) Plan to deliver in the hospital

4.3.2. Exclusion Criteria

1) History of serious adverse event to SP or DP

2) Active medical problem requiring inpatient evaluation at the time of screening

3) Intention of moving more than 30km from the study clinic

4) Chronic medical condition requiring frequent medical attention

5) Prior SP preventive therapy or any other antimalarial therapy during this pregnancy

6) Early or active labor (documented by cervical change with uterine contractions)

4.4 Randomization and Blinding

(ICH E3; 9.4.3, 9.4.6. ICH E9; 2.3.1, 2.3.2)

Treatment Group Assignments

There will be 5 treatment arms which include both the intervention for the woman during pregnancy and her unborn child(ren) during infancy (Table 1). Non-singleton births from the same mother will be assigned to the same intervention. We will use a 2:1:1:1:1 randomization scheme targeting 100, 50, 50, 50, 50 pregnant women in treatment arms A-E respectively. This randomization scheme will result in a target of 100 women for each of the 3 different treatment arms during pregnancy. A randomization list will be computer generated by a member of the project who will not be directly involved in the conduct of the study. The randomization list will include consecutive treatment numbers with corresponding random treatment assignments. Randomized codes will correspond to the 5 treatment groups using permuted variable sized blocks of 6 and 12 (to account for treatment group A being represented twice as often as the other 4 treatment groups). Sealed copies of the original randomization list and documentation of the procedure used to generate the lists will be stored in the project administrative offices in San Francisco and Kampala. Prior to the onset of the study, a set of sequentially numbered, opaque, sealed envelopes will be prepared. Each envelope will be marked on the outside with the treatment allocation number. The inside of the envelope will contain a piece of paper with the treatment allocation number and treatment group assignment along with a piece of carbon paper.

Table 1. Treatment arms with assignment of study drugs during pregnancy and infancy

Phase of intervention	Treatment arm (target number)				
	A	B	C	D	E
During pregnancy	3 dose SP (100)	3 dose DP (100)		Monthly DP (100)	
During infancy	3 monthly DP (100)	3 monthly DP (50)	Monthly DP (50)	3 monthly DP (50)	Monthly DP (50)

4.5 Study Variables

(ICH E3; 9.5.1. ICH E9; 2.2.2)

See Data Dictionary Excel file for complete list of study variables.

Table 2. Schedule of routine assessments and procedures in pregnant women

Evaluations and Interventions	Enrollment	Weeks of gestation							Delivery	1 and 6 weeks postpartum
		16*	20	24	28	32	36	40		
Informed consent	X									
HIV testing[1]	X				X				X	
Pregnancy confirmation[2]	X									
Obstetrical ultrasound[3]	X									
Blood collected by phlebotomy for CBC, ALT, and immunology studies	X		X		X		X		X	
Blood collected by finger prick for dried blood spot and plasma	X	X	X	X	X	X	X	X	X	
Routine assessment in the study clinic[4]	X	X	X	X	X	X	X	X		X
Administration of study drugs		X	X	X	X	X	X	X		
Collection of cord blood and placental tissue									X	
Labor and delivery documentation[5]									X	

***** Only if study subject enrolled prior to 16 weeks gestation; If the woman is enrolled between 18-20 weeks, then the week 20 phlebotomy will not be performed.

Explanation of maternal schedule of events

1. HIV test will be done at enrollment and documented. A repeat rapid HIV test will be done at delivery. HIV testing shall be done using standard rapid HIV-testing algorithm.
2. Pregnancy confirmation by positive urine pregnancy test, or confirmed intrauterine pregnancy by ultrasound. A pregnancy test may be skipped if an intrauterine pregnancy has been noted on ultrasound at the screening visit.
3. Ultrasound will be done to confirm intrauterine pregnancy and estimate gestational age at enrollment. See Appendix B for dating criteria.
4. Targeted physical exam will include anthropometric measurements (e.g. weight) and vital signs (i.e. temperature, pulse, and blood pressure). Measurement of height at the enrollment visit only.
5. Labor & Delivery documentation will include: Peripartum history, mode of delivery, Apgar scores (when available), weight, length, and head circumference of the child at birth, approximate gestational age, duration of labor, signs of fetal distress (presence of meconium), summary of events in first days of life (including feeding, breathing patterns, jaundice, lethargy, or any additional abnormal findings), duration of admission if delivered in hospital.

Table 3. Study Case Report Forms

CRF#	Form/Questionnaire
1	BC-1 Screening
2	BC-1 Enrollment
3	BC-1 Clinic Visit Form
4	BC-1 Subject Hospital Admission
5	BC-1 Missed Visit Form
6	BC-1 Subject Death Record Form
7	BC-1 Mothers Delivery Form
8	BC-1 Postpartum Visit Form
9	BC-1 Child's Delivery Form
10	BC-1 Study Drug Dispensing Forms
11	BC-1 AE Forms
12	BC-1 Subject Withdrawal or Study Completion Form
13	BC-1 Household Survey Questionnaire
14	BC-1 GPS Coordinates
15	BC-1 Placental Histopathology

5 Sample Size

(ICH E3; 9.7.2. ICH E9; 3.5)

The number of pregnant women enrolled and the number of study participants reaching the various endpoints will determine the samples sizes for each of the primary outcomes of our study aims (Table 4). The 3 primary hypotheses for the complete study are as follows: 1) We will test the hypothesis that pregnant women who receive IPTp with either 3 dose DP or monthly DP will have a lower risk of placental malaria defined by histopathology compared to those who receive 3 doses of SP. We will also compare the two different dosing strategies of DP. 2) We will test the hypothesis that infants randomized to receive monthly DP between 2-24 months of age will have a lower incidence of malaria during the first 24 months of life compared to infants randomized to receive q 3 monthly DP. 3) We will test the hypotheses that a) infants born to mothers randomized to receive IPTp with 3 dose DP or monthly DP will have a lower incidence of malaria during the first 24 months of life compared to infants born to mothers who were randomized to receive IPTp with 3 doses of SP, and b) infants randomized to receive monthly DP between 2-24

months of age will have a lower incidence of malaria between 24-36 months of age after the intervention is stopped compared to infants randomized q 3 monthly DP between 2-24 months of age. The primary determinant of our target sample size was based on testing hypotheses 3 given that the magnitude of differences anticipated for this hypothesis would be smaller than those anticipated for hypothesis 1 and 2. We conservatively estimate that 90% of pregnant women enrolled will reach the primary study endpoint and we will lose 5% of follow-up time per year in the infants. The minimum relative differences detectable for the primary outcomes of the 3 hypotheses given our estimated sample sizes are summarized in Table 5 below. For hypotheses 1, we will be powered to detect a 33% relative difference in the prevalence of placental malaria in the 3 dose DP arm or the monthly DP arm compared to the 3 dose SP arm assuming a prevalence of placental malaria of 62% in the 3 dose SP arm based on prior data. We will also be powered to detect a 41-70% relative difference in the prevalence of placental malaria in the monthly DP arm compared to the 3 dose DP arm assuming a prevalence of placental malaria in the 3 dose DP arm ranging from 20-50%. For hypothesis 2, we will be powered to detect an 18-23% relative difference in the incidence of malaria between 0-24 months of age in the monthly DP arm compared to the q 3 monthly DP arm assuming an incidence of malaria in the q 3 monthly DP arm ranging from 3-5 episodes PPY. For hypothesis 3A, we will be powered to detect a 22-28% relative difference in the incidence of malaria between 0-24 months of age among children assigned to receive q 3 monthly DP when comparing those born to women who received 3 dose SP to those born to women who received 3 dose DP or monthly DP assuming an incidence of malaria in the control group ranging from 3-5 episodes PPY. For hypothesis 3B, we will also be powered to detect a 16-21% relative difference in the incidence of malaria between 24-36 months of age among children assigned to receive monthly DP during infancy compared to those who received q 3 monthly DP during infancy assuming an incidence of malaria in the control group ranging from 3-5 episodes PPY.

Table 4. Intervention arms during pregnancy and infancy

Study populations	**Intervention arms**				
Assigned treatment arms in pregnant women	3 doses of SP	3 doses of DP		monthly DP	
Estimated number of pregnant women enrolled	100	100		100	
Estimated number with placental outcomes	90	90		90	
Assigned treatment arms in infants	q 3 monthly DP	q 3 monthly DP	monthly DP	q 3 monthly DP	monthly DP
Estimated number of infants enrolled	90	45	45	45	45
Estimated number of infants reaching 24 months of age	81	41	41	41	41

Table 5. Minimum relative differences in outcomes detectable given estimated effective sample sizes

Hypothesis	Analysis population	Control group and estimated sample size	Comparison group and estimated sample size	Estimated outcome measure in control group	Minimum relative difference detectable*
1	Pregnant women with placental malaria measured by histopathology at birth	3 dose SP (n=90)	3 dose DP or monthly DP (n=90 each)	Prevalence of placental malaria = 62%	33%
		3 dose DP (n=90)	monthly DP (n=90)	Prevalence of placental malaria = 20-50%	41-70%
2	Infants 0-24 months of age born to women assigned to receive IPTp with 3 dose DP or monthly DP	Infants assigned q 3 monthly DP (n=90)	Infants assigned monthly DP (n=90)	Incidence of malaria = 3-5 episode PPY	18-23%
3	Infants 0-24 months of age randomized to q 3 monthly DP	Infants of women assigned 3 dose SP (n=90)	Infants of women assigned 3 dose DP or monthly DP (n=45 each)	Incidence of malaria = 3-5 episodes PPY	22-28%
	Infants 24-36 months of age after chemoprevention stopped	Infants assigned q 3 monthly DP (n=163)	Infants assigned monthly DP (n=82)	Incidence of malaria = 3-5 episodes PPY	16-21%

* Relative difference = (estimated outcome in control arm – estimated outcome in the comparison arm) / estimated outcome on the control arm (two-sided alpha = 0.05, power = 80%).

6 General Considerations

6.1 Timing of Analyses

The final trial analysis for Phase I of the study will be performed after the last enrolled woman has given birth and all outcome measures have been assessed. Prior to the final analysis we will perform an interim safety analysis when ~ ½ of the study subjects have given birth. The interim safety analyses will compare the incidence rate ratio of significant adverse events (grade 3/4 & SAEs).

6.2 Analysis Populations

(ICH E3; 9.7.1, 11.4.2.5. ICH E9; 5.2)

A modified intention-to-treat approach will be used for all analyses, including all women who were randomized and received at least one dose of study drugs, and who have evaluable data on specific outcomes regardless of whether the intervention was not given for any reason.

6.2.1 Full Analysis Population

- *All subjects who were randomized and received at least one dose of study drugs*
- *All subjects with evaluable data on specific outcomes*

6.2.2 Per Protocol Population

A per protocol analysis is not planned.

6.3 Covariates and Subgroups

(ICH E3; 9.7.1, 11.4.2.1. ICH E9; 5.7)

Covariates of interest will include baseline measures of maternal age, gestational age at enrollment, gravidity, bednet ownership, socio-economic status as estimated by a household wealth index, and prevalence of malaria parasites using LAMP.

Sub-group analyses will be performed based on categories of maternal age, gravidity, and gestational age at the time study drugs were first administered.

6.4 Missing Data

(ICH E3; 9.7.1, 11.4.2.2. ICH E9;5.3. EMA Guideline on Missing Data in Confirmatory Clinical Trials)

Women in the trial who are prematurely withdrawn from the study or are not able to provide data for specific outcomes will be considered un-evaluable and will not be included in the primary trial analysis. For some outcomes missing data will be imputed based on other information (see details in Table 10 below).

6.5 Interim Analyses and Data Monitoring

(ICH E3; 9.7.1, 11.4.2.3. ICH E9; 4.1, FDA Feb 2010 "Guidance for Industry Adaptive Design Clinical Trials for Drugs and Biologics")

6.5.1 Purpose of Interim Analyses

Over the course of the trial, we will perform an interim safety analysis in addition to a final safety analysis for a total of two sequential evaluations of study safety. The interim safety analyses will compare the incidence rate ratio of significant adverse events (grade 3/4 & SAEs).

6.5.2 Planned Schedule of Interim Analyses

The interim safety analysis for pregnant women will be performed when ~ ½ of the study subjects have given birth. A standardized test statistic will be calculated for the adverse event incidence rate ratio. If this statistic exceeds the nominal critical value calculated using the error spending function (Table 6), then a statistically significant result will have been achieved at the time of the analysis. In that event, the sponsor will be notified and a report submitted for review by the Data Safety Monitoring Board (DSMB). For the interim safety analysis, the study team will

present information on recruitment and the results of interim safety analyses to the DSMB, which will review the data and recommend a course of action.

Table 6. Schedule of interim safety analysis and boundaries to monitor study outcome

Number of Evaluable Subjects Accrued or % of Total Accrual Time	Test Statistic		Alpha	Cumulative Alpha
	Lower Bound	Upper Bound		
N=150 or 50% of accrual time	-2.51	2.51	0.00601	0.01210
N=300 or 100% of accrual time	-1.99	1.99	0.02313	0.05000

This analysis assumes α=0.05 (two-sided test), O'Brien-Fleming boundaries (DeMets error-spending function) and 300 trial participants. We will utilize Programs for Computing Group Sequential Boundaries Using the Lan-DeMets Method.

6.5.3 Scope of Adaptations

At the time of the interim analysis, the DSMB may decide to continue, stop, or modify the trial based on the interim safety analysis. This may include the discontinuation of a study arm and re-randomization or cessation of subject participation in the stopped arm.

6.5.4 Stopping Rules

The DSMB will determine whether to stop the study for early evidence of intervention safety problems after a thorough review of interim data. Interim reports will provide cumulative enrollment figures and cumulative adverse birth outcomes, serious adverse events (classified according to grade), sorted by study arm. Brief clinical descriptions of key events will also be provided. The PI will be responsible for immediately reporting to the funding agency any temporary or permanent suspension of the project and the reason for the suspension.

6.5.5 Adjustment of Confidence Intervals and p-values

As appropriate we will adjust p-values and confidence intervals taking into account the specified error spending functions and interim evaluation of the data.

6.5.6 Interim Analysis for Sample Size Adjustment

The sample size will not be adjusted based on the results of the interim analysis.

6.5.7 Practical Measures to Minimize Bias

The study will establish and control who will have access to what information at each stage of the trial. Uncontrolled reporting of interim analyses to study investigators responsible for recruiting subjects will not occur.

The following measure will be taken to minimize bias:

- Only the study statistician and assistant statistician will perform the interim analysis.
- Only the statisticians and the DSMB will see any data or analyses at the interim analysis
- No information will be publically available following an interim analysis
- Information will be provided to the sponsor and investigators as per recommendation of the study DSMB.
- Only the statisticians will be unblinded at for the interim analysis

6.5.8 Documentation of Interim Analyses

Snapshots of the data available at each interim analysis will be preserved, as will all documentation of analysis plans, programming code and reporting provided at the interim analysis.

6.6 Multi-center Studies

(ICH E3;9.7.1, 11.4.2.4. ICH E9; 3.2)

This is a single center study.

6.7 Multiple Testing

(ICH E3; 9.7.1, 11.4.2.5. ICH E9; 2.2.5)

This study has one primary endpoint. The preservation of the overall significance level is of importance and as there are more than two treatment groups, secondary analyses comparing multiple groups may require adjustment of p-values and confidence intervals.

7 Summary of Study Data

All continuous variables will be summarized using the following descriptive statistics: n (non-missing sample size), mean, standard deviation, median, maximum and minimum. The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures. All summary tables will be structured with a column for each treatment and will be annotated with the total population size relevant to that table/treatment, including any missing observations.

7.1 Trial Profile

The overall study profile will be presented as a figure following CONSORT guidelines. The total numbers of women screened, excluded during screening (including criteria for exclusion) and enrolled will be presented. The numbers of women enrolled in each treatment arm and followed through each stage of the trial profile are presented in skeleton Table 7 below.

Table 7. Trial profile

	Treatment arm		
	3 dose SP	3 dose DP	Monthly DP
Enrolled and randomized	###	###	###
Withdrawn before 1st dose of study drugs*	###	###	###
Received at least 1 dose of study drugs	###	###	###
Withdrawn before delivery*	###	###	###
Delivered	###	###	###
Withdrawn after delivery*	###	###	###
Completed 6 week post-partum visit	###	###	###

* Specific reasons for premature study withdrawal will be reported

7.2 Baseline Characteristics

Skeleton table of all baseline variables collected on the day of enrollment that will be presented are provided in Table 8 below.

Table 8. Baseline characteristics of study participants randomized to IPTp

Characteristic	Treatment arm		
	3 dose SP (n=XXX)	3 dose DP (n=XXX)	Monthly DP (n=XXX)
Age in years, mean (SD)			
Gestational age in weeks, mean (SD)			
Gestational age categories, n (%)			
12-16 weeks			
>16-20 weeks			
Previous pregnancies, n (%)			
0			
1			
≥ 2			
Bednet ownership, n (%)			
None			
Untreated net			
ITN			
Household wealth index, n (%)			
Lowest tertile			
Middle tertile			
Highest tertile			
Weight in kg, mean (SD)			
Height in cm, mean (SD)			
Laboratory values, mean (SD)			
WBC count per mm^3			
Neutrophil count per mm^3			
Platelet count per mm^3			
Hemoglobin g/dL			
ALT IU/L			
Detection of malaria parasites by LAMP, n (%)			

7.3 Treatment Adherence

During pregnancy, women will be given 1 of 3 treatment regimens: 1) SP given 3 times during pregnancy, 2) DP given 3 times during pregnancy, or 3) DP given every

4 weeks during pregnancy. Each treatment with SP will be given as a single dose consisting of 3 full strength tablets. Each treatment with DP will consist of 3 full strength tablets given once a day for 3 consecutive days. In addition, placebos will be used to mimic the identical dosing strategy such that every 4 weeks women will receive two drugs on day 1 (SP and placebo, DP and placebo, or two placebos) followed by one drug on days 2 and 3 (DP or placebo). Two placebos will be used, one that mimics the appearance of SP and one that mimics the appearance of DP. Administration of all study drugs will be double blinded. All doses of study drugs will be pre-packaged by a study pharmacist and administered by a study nurse blinded to the study participant's treatment regimen. All doses of SP (or SP placebo) administered will be directly observed in the clinic. For DP (or DP placebo), the first of the 3 daily doses will be directly observed in the clinic and the 2nd and 3rd daily doses will be administered at home using pre-packaged study drugs in opaque envelopes with dosing instructions written on the outside. For doses of study drugs administered in the clinic, if a study participant vomits the study drug within 30 minutes of administration, the drug will be re-administered. For doses of study drugs administered at home, if a study participant vomits the study drug within 30 minutes of administration or study drug is lost, the study participant will be instructed to come to the study clinic as soon as possible where the study drug will be re-administered/replaced. Measures of treatment adherence are summarized in skeleton Table 9 below.

Table 9. Measures of treatment adherence

	Treatment arm		
	3 dose SP	3 dose DP	Monthly DP
At the level of each individual woman receiving at least one dose of study drugs			
At least one dose of study drug held for adverse event	n/N (%)	n/N (%)	n/N (%)
Missed at least 1 course of study drugs (all 3 doses)	n/N (%)	n/N (%)	n/N (%)
Reported not taking at least 1 dose of study drug at home	n/N (%)	n/N (%)	n/N (%)
At the level of each scheduled dose of study drug			
Study drugs (all 3 doses) held for adverse event	n/N (%)	n/N (%)	n/N (%)
Study drugs (all 3 doses) missed	n/N (%)	n/N (%)	n/N (%)
Reported not taking day 2 dose of study drugs at home	n/N (%)	n/N (%)	n/N (%)
Reported not taking day 3 dose of study drugs at home	n/N (%)	n/N (%)	n/N (%)

8 Efficacy Analyses

8.1 Efficacy outcomes

Definitions and criteria used to generate estimates of all primary and secondary efficacy outcomes are presented in Table 10 below. For women with non-singleton birth (i.e. twins), dichotomous delivery outcomes at the level of the individual child/placenta will be based on whether the outcome was present in either child/placenta.

Table 10. Primary and secondary outcomes

Outcome	Category	Type of measurement	Timing of measurement	Numerator	Denominator	Missing data	Imputation
Placental malaria by histopathology	Primary outcome	Proportion	At delivery	Placental tissue samples with parasites or pigment detected	Number of placentas examined	No placental tissue sample collected	None
Placental parasitemia using microscopy	Secondary outcome	Proportion	At delivery	Placental blood samples with parasites detected by microscopy	Number of placental blood samples examined	No placental blood sample collected	None
Placental parasitemia using LAMP	Secondary outcome	Proportion	At delivery	Placental blood samples with parasites detected by LAMP	Number of placental blood samples examined	No placental blood sample collected	None
Maternal parasitemia using microscopy	Secondary outcome	Proportion	At delivery	Maternal blood samples with parasites detected by microscopy	Number of maternal blood samples examined	No maternal blood sample collected	None
Maternal parasitemia using LAMP	Secondary outcome	Proportion	At delivery	Maternal blood samples with parasites detected by LAMP	Number of maternal blood samples examined	No maternal blood sample collected	None
Cord blood parasitemia using microscopy	Secondary outcome	Proportion	At delivery	Cord blood samples with parasites detected by microscopy	Number of cord blood samples examined	No cord blood sample collected	None
Cord blood parasitemia using LAMP	Secondary outcome	Proportion	At delivery	Cord blood samples with parasites detected by LAMP	Number of cord blood samples examined	No cord blood sample collected	None
Spontaneous abortion	Secondary outcome	Proportion	At delivery	Delivery at < 28 weeks gestational age	All deliveries	Withdrawn prior to delivery	None
Stillbirth	Secondary outcome	Proportion	At delivery	Infant born deceased	Deliveries ≥ 28 weeks gestational age	Withdrawn prior to delivery or < 28 weeks gestational age	None

Low birth weight	Secondary outcome	Proportion	At delivery	Birth weight < 2500 gm	Deliveries ≥ 28 weeks gestational age	Withdrawn prior to delivery or < 28 weeks gestational age or no birth weight	Inferred if no birth weight but weight available with 7 days of birth
Preterm delivery	Secondary outcome	Proportion	At delivery	Gestational age < 37 weeks	Deliveries ≥ 28 weeks gestational age	Withdrawn prior to delivery or < 28 weeks gestational age	None
Congenital malformation	Secondary outcome	Proportion	At delivery	Any congenital malformation documented on exam	Deliveries ≥ 28 weeks gestational age	Withdrawn prior to delivery or < 28 weeks gestational age or infant unavailable for examination	None
Composite adverse birth outcome	Secondary outcome	Proportion	At delivery	Any of the following: spontaneous abortion, stillbirth, low birth weight, preterm delivery, or congenital malformation	All deliveries	Withdrawn prior to delivery	None
Symptomatic malaria during pregnancy	Secondary outcome	Incidence	Time at risk during pregnancy	Number of episodes of fever and positive blood smear by microscopy	Duration of observation from day following 1st dose of study drugs to delivery or premature study withdrawal	None	None
Parasitemia during pregnancy detected by LAMP	Secondary outcome	Proportion	At the time of each routine visit	Maternal blood samples with parasites detected by LAMP	Routine visits at 20, 24, 28, 32, 36, and 40 weeks of gestational age following 1st dose of study drugs to delivery or premature study withdrawal	Missed routine visits or samples not collected	None
Maternal anemia	Secondary outcome	Proportion	At the time of each routine visit and at delivery when phlebotomy done	Hemoglobin level < 11 g/dL	Routine visits at 20,28, 36 weeks of gestational age following 1st dose of study drugs to delivery or premature study withdrawal	Missed routine visits or sample not collected when scheduled for phlebotomy	None

8.2 Primary Efficacy Analysis

We will test the hypothesis that pregnant women who receive IPTp with either 3 dose DP or monthly DP will have a lower risk of placental malaria defined by histopathology compared to those who receive 3 doses of SP. We will also compare the two different dosing strategies of DP.

Primary Outcome: The primary outcome will be the prevalence of placental malaria based on placental histopathology and dichotomized into any evidence of placental infection (parasites or pigment) vs. no evidence of placental infection. We will also evaluate placental malaria defined by histopathology as a categorical variable (active-acute, active-chronic, and past infection) based on the criteria developed by Rogerson et al.[26]

Analysis Method: A modified intention-to-treat approach to all will be used, including all study participants randomized to therapy with the primary outcome measured, regardless of whether the intervention was not given for any reason.

Primary analysis. We will compare the prevalence of placental malaria defined by histopathology at birth between the study arms using the Chi-Square or Fisher's exact test for all pair-wise comparisons and presented as the risk ratio (RR) or reduction of the RR (1-RR x 100%) if the RR is lower than 1. We will explore for any differences of potential confounders between the treatment arms and if necessary adjust our analysis using multivariate logistic regression.

8.3 Secondary Efficacy Analyses

Secondary Outcomes: Secondary outcomes will include maternal outcomes during pregnancy and birth outcomes as listed in Table 10.

Analysis Method: A modified intention-to-treat approach will be used, including all study participants randomized to therapy with data on the secondary outcomes, regardless of whether the intervention was not given for any reason.

Secondary analyses. We will compare proportions between the study arms using the Chi-Square or Fisher's exact test for all pair-wise comparisons and presented as the risk ratio (RR) or reduction of the RR (1-RR x 100%) if the RR is lower than 1. For repeated measures in the same study participant (maternal parasitemia and anemia during pregnancy) we will use generalized estimating equations with a log-binomial family and robust standard errors. We will compare the incidence of symptomatic

malaria during pregnancy using Poisson or negative binomial regression models. The Poisson models will include the logarithm of the follow-up time as an offset. We will translate the fitted coefficients and their confidence bounds into percentage effects with the formula 100*[exp(coefficient)-1]. This approach is closely related to exponential survival models for analyzing events per follow-up time, but is better able to adjust for violated assumptions. Testing for overdispersion in the Poisson regression can detect violations of these assumptions, and variances can be adjusted accordingly to produce valid p-values and confidence intervals. If significant deviations from required distributions in study data are detected, we will employ negative-binomial or zero-inflated negative-binomial models to account for the observed pattern of data. If necessary, multivariate analyses will be performed to adjust for potential confounders and effect modifiers. Comparisons of incidence measures will be expressed at the incidence rate ratio (IRR) or the protective efficacy (PE = 1-IRR x 100%).

Skeleton tables for the presentation of primary and secondary efficacy outcomes are presented in Tables 11 and 12 below.

Table 11. Outcomes assessed at the time of delivery

Outcome	Treatment arm						
	3 dose SP[a]	3 dose DP			Monthly DP		
	Prevalence	Prevalence	RR (95% CI)	p-value	Prevalence	RR (95% CI)	p-value
Malaria positivity, by test							
Histopathology	n/N (%)	n/N (%)			n/N (%)		
Placental blood by microscopy	n/N (%)	n/N (%)			n/N (%)		
Placental blood by LAMP	n/N (%)	n/N (%)			n/N (%)		
Maternal blood by microscopy	n/N (%)	n/N (%)			n/N (%)		
Maternal blood by LAMP	n/N (%)	n/N (%)			n/N (%)		
Cord blood by microscopy	n/N (%)	n/N (%)			n/N (%)		
Cord blood by LAMP	n/N (%)	n/N (%)			n/N (%)		
Birth outcomes							
Spontaneous abortion	n/N (%)	n/N (%)			n/N (%)		
Stillbirth[b]	n/N (%)	n/N (%)			n/N (%)		
Low birth weight[b]	n/N (%)	n/N (%)			n/N (%)		
Preterm delivery[b]	n/N (%)	n/N (%)			n/N (%)		
Congenital anomaly[b]	n/N (%)	n/N (%)			n/N (%)		
Composite adverse birth outcome	n/N (%)	n/N (%)			n/N (%)		

[a] Reference group

[b] Only includes those at least 28 weeks gestational age

Table 12. Longitudinal outcomes assessed during pregnancy

Outcome	Treatment arm						
	3 dose SP[a]	3 dose DP			Monthly DP		
Incidence measures	**Events[b]**	**Events[b]**	**IRR (95% CI)**	**p-value**	**Events[b]**	**IRR (95% CI)**	**p-value**
Symptomatic malaria	xx (x.xx)	xx (x.xx)			xx (x.xx)		
Prevalence measures	**Prevalence**	**Prevalence**	**RR (95% CI)**	**p-value**	**Prevalence**	**RR (95% CI)**	**p-value**
Detection of malaria parasites by LAMP							
All routine visits	n/N (%)	n/N (%)			n/N (%)		
20 weeks gestational age	n/N (%)	n/N (%)			n/N (%)		
24 weeks gestational age	n/N (%)	n/N (%)			n/N (%)		
28 weeks gestational age	n/N (%)	n/N (%)			n/N (%)		
32 weeks gestational age	n/N (%)	n/N (%)			n/N (%)		
36 weeks gestational age	n/N (%)	n/N (%)			n/N (%)		
40 weeks gestational age	n/N (%)	n/N (%)			n/N (%)		
Anemia defined as hemoglobin level < 11 g/dL							
All routine visits and delivery	n/N (%)	n/N (%)			n/N (%)		
20 weeks gestational age	n/N (%)	n/N (%)			n/N (%)		
28 weeks gestational age	n/N (%)	n/N (%)			n/N (%)		
36 weeks gestational age	n/N (%)	n/N (%)			n/N (%)		
At the time of delivery	n/N (%)	n/N (%)			n/N (%)		

[a] Reference group

[b] Number of events (incidence per person year at risk)

9 Safety and Tolerability Analyses

Safety and tolerability will be evaluated during the period following the 1st dose of study drug administration through the end of the observation period (6 weeks post-partum) or premature study withdrawal.

9.1 Adverse Events

An adverse event will be defined as "any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment" (ICH Guidelines E2A). An adverse event can further be broadly defined as any untoward deviation from baseline health, which includes:

- Worsening of conditions present at the onset of the study
- Deterioration due to the primary disease
- Intercurrent illness
- Events related or possibly related to concomitant medications

(International Centers for Tropical Disease Research Network Investigator Manual, Monitoring and Reporting Adverse Events, 2003).

At each scheduled and unscheduled visit to the clinic, study clinicians will assess patients according to a standardized case record form. A severity grading scale, based on toxicity grading scales developed by the NIH Divisions of AIDS (DAIDS) and the Division of Microbiology and Infectious Diseases (DMID) Pediatric Toxicity Tables, will be used to grade severity of all symptoms, physical exam findings, and laboratory results. All participants, regardless of treatment arm, will be assessed using the same standardized case record form. Adverse event monitoring will occur during the period when study drugs are given and up to 6 weeks following delivery. Data will be captured on the incidence of all adverse events, regardless of severity. For each adverse event identified as severity grade 3-4 or a serious adverse event (SAE), an additional adverse event report form will be completed.

9.2 Serious Adverse Events

A Serious Adverse Event (SAE) will be define as any adverse event that results in any of the following outcomes:

- Death

- Life-threatening adverse experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital malformation/birth defect
- Any other experience that suggests a significant hazard, contraindication, side effect or precaution that may require medical or surgical intervention to prevent one of the outcomes listed above
- Event that changes the risk/benefit ratio of the study

9.3 Analytical Methods

We will test the hypothesis that pregnant women who receive IPTp with either 3 dose DP or monthly DP will have a lower incidence of adverse events and better tolerability compared to those who receive 3 doses of SP. A modified intention-to-treat approach to all will be used, including all study participants who received at least one dose of study drugs, regardless of whether subsequently the intervention was not given for any reason. We will compare the proportions of study participants with vomiting following each dose of study drugs using generalized estimating equations with a log-binomial family and robust standard errors to account for repeated measures in the same study participant. We will compare the incidence of various adverse events using Poisson or negative binomial regression models. The Poisson models will include the logarithm of the follow-up time as an offset. We will translate the fitted coefficients and their confidence bounds into percentage effects with the formula 100*[exp(coefficient)-1]. This approach is closely related to exponential survival models for analyzing events per follow-up time, but is better able to adjust for violated assumptions. Testing for overdispersion in the Poisson regression can detect violations of these assumptions, and variances can be adjusted accordingly to produce valid p-values and confidence interval. If significant deviations from required distributions in study data are detected, we will employ negative-binomial or zero-inflated negative-binomial models to account for the observed pattern of data. Comparisons of incidence measures will be expressed at the incidence rate ratio (IRR) or the protective efficacy (PE = 1-IRR x 100%). A skeleton table for the presentation of safety and tolerability outcomes are presented in Tables 13 below.

Table 13. Measures of safety and tolerability

Outcome	Treatment arm						
	3 dose SP[a]	3 dose DP			Monthly DP		
Prevalence measures	**Prevalence**	**Prevalence**	**RR (95% CI)**	**p-value**	**Prevalence**	**RR (95% CI)**	**p-value**
Vomiting following administration of study drugs							
Observed after administration of 1st dose in clinic	n/N (%)	n/N (%)			n/N (%)		
Reported after administration of 2nd dose at home	n/N (%)	n/N (%)			n/N (%)		
Reported after administration of 3rd dose at home	n/N (%)	n/N (%)			n/N (%)		
Incidence measures	**Events[b]**	**Events[b]**	**IRR (95% CI)**	**p-value**	**Events[b]**	**IRR (95% CI)**	**p-value**
Individual adverse events of any severity[c]							
XXXX	xx (x.xx)	xx (x.xx)			xx (x.xx)		
XXXX	xx (x.xx)	xx (x.xx)			xx (x.xx)		
XXXX	xx (x.xx)	xx (x.xx)			xx (x.xx)		
XXXX	xx (x.xx)	xx (x.xx)			xx (x.xx)		
XXXX	xx (x.xx)	xx (x.xx)			xx (x.xx)		
Individual grade 3-4 adverse events[c]							
XXXX	xx (x.xx)	xx (x.xx)			xx (x.xx)		
XXXX	xx (x.xx)	xx (x.xx)			xx (x.xx)		
XXXX	xx (x.xx)	xx (x.xx)			xx (x.xx)		
XXXX	xx (x.xx)	xx (x.xx)			xx (x.xx)		
XXXX	xx (x.xx)	xx (x.xx)			xx (x.xx)		
All grade 3-4 adverse events	xx (x.xx)	xx (x.xx)			xx (x.xx)		
Grade 3-4 adverse events possibly related to stud drugs	xx (x.xx)	xx (x.xx)			xx (x.xx)		
All serious adverse events	xx (x.xx)	xx (x.xx)			xx (x.xx)		

[a] Reference group

[b] Number of events (incidence per person year at risk)

[c] Includes only those categories with at least five total events

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