"Novel Strategies to Prevent Malaria and Improve Maternal-Child Health in Africa" (PROMOTE II)

A UCSF/ MAKERERE UNIVERSITY COLLABORATION

Title: Prevention of Malaria in HIV-uninfected Pregnant Women and Infants

Short Title: PROMOTE Birth Cohort 3

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GLOSSARY

AE	Adverse event
ACT	Artemisinin-based combination therapy
AL	Artemether-lumefantrine
ALT	Alanine transaminase (SGPT)
CAB	Community advisory board
CBC	Complete blood cell count
CRF	Case report form
DP	Dihydroartemisinin-piperaquine
DSMB	Data and Safety Monitoring Board
IDRC	Infectious Diseases Research Collaboration
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
SP	Sulfadoxine-pyrimethamine
UCSF	University California San Francisco
WHO	World Health Organization

SCHEMA

Title	Prevention of Malaria in HIV-uninfected Pregnant Women and Infants
Description	Double blinded randomized controlled trial
Study Objectives	1. To compare the risk of adverse birth outcomes among HIV-uninfected pregnant women randomized to receive IPTp with monthly SP vs. monthly DP. 2. To compare the incidence of malaria among infants born to mothers randomized to receive IPTp with monthly SP vs. monthly DP. 3. To validate and adapt a gestational dating by metabolic profile at birth algorithm and to evaluate the added benefits of using metabolic profile to identify newborns at increased risk for neonatal death or serious complications.
Participants and Sample Size	782 HIV-uninfected pregnant women and the children born to them
Clinical Site	The study will be conducted in Busia District, Uganda. A designated study clinic will be located within Masafu General Hospital. The study clinic will be open daily from 8:00 am to 5:00 pm and after-hours care will be available with the hospital complex.
Selection Criteria	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 Busia District of Uganda 6. Provision of informed consent 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 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 outside of Busia District Uganda 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)
Treatment assignment	HIV-uninfected pregnant women will be randomized at the time of enrollment
Treatment arms	1. Monthly SP 2. Monthly DP
Follow-up and Diagnosis of Malaria	Study participants will be followed for all of their outpatient medical care in our study clinic. Women will be followed until 6 weeks postpartum and children will be followed until 12 months of age. Routine assessments will be performed in the study clinic for all study participants every 4 weeks. Patients presenting with a new episode of fever will undergo standard evaluation for the diagnosis of malaria.
Primary study outcome	The risk of having an adverse birth outcome defined as the occurrence of any of the following: 1) Low birth weight (< 2500 gm) 2) Preterm delivery (< 37 weeks gestational age) 3) Small for gestational age (< 10^{th} percentile relative to an external growth reference)

1. INTRODUCTION

1.1. Background

Malaria in pregnancy: burden of disease and prevention. In sub-Saharan Africa over 30 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 with SP 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 needed.

The 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] The ACT dihydroartemisinin-piperaquine (DP) has also been safely used for the treatment of uncomplicated malaria in pregnancy in studies from Asia.[21,22] In two 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]

Two recent published studies have compared IPTp with DP versus SP. In a study from Kenya, HIV negative pregnant women were randomly assigned to receive intermittent screening and treatment with DP vs. standard dosing of IPTp with either SP or DP.[25] Intermittent screening and treatment with DP was not found to be a suitable alternative to IPTp with SP. However, compared to IPTp with SP, IPTp with DP was associated with a lower incidence of malaria infection and clinical malaria during pregnancy and a lower prevalence of malaria infection at delivery (3% vs. 10%, $p<0.001$). Serious adverse events were also lower in the IPTp with DP arm compared to the IPTp with SP arm. In a recent study from our group in Uganda, study participants were randomized to receive IPTp with 3 dose SP, 3 dose DP, or monthly DP.[26] Compared to the 3 dose SP arm, monthly DP was associated with a significant reduction in the risk of symptomatic malaria, infection with malaria parasites, and maternal anemia during pregnancy, and the risk of placental malaria measured at delivery. In addition, compared to the 3 dose DP arm, monthly DP was associated with a significant reduction in the risk of symptomatic malaria and infection with malaria parasites during pregnancy, with a trend towards a lower risk of maternal anemia during pregnancy and placental malaria measured at delivery. All IPTp regimens were equally well tolerated with no differences in the risk of adverse events.

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.[27] More recently, two published studies have documented that IPTp with DP is superior to IPTp with SP for the prevention of malaria during pregnancy and the risk of placental malaria. In July 2015 the WHO sponsored an "Expert Review Group" meeting to review IPTp policy recommendations. Data from both recently published studies evaluating IPTp with DP were presented. The WHO panel felt that based on these 2 new studies, DP is the most promising alternative to SP. However, several limitations of these

studies were noted during the discussion, and knowledge gaps regarding evidence of clinical benefit in birth outcomes and safety need to be filled prior to a policy change. These include:

Lack of statistical power to detect a difference in birth outcomes known to be associated with placental malaria: Although a majority of RCTs evaluating IPTp have been powered to detect a difference in measures of malaria during pregnancy, the primary goal of IPTp is to reduce the risk of adverse birth outcomes, namely small for gestational age, low birth weight and premature delivery. Because these outcomes are relatively uncommon and can be caused by factors other than malaria in pregnancy, the sample size needed to detect differences in birth outcomes is larger than needed to detect differences in measures of malaria during pregnancy. To better justify a change in policy from IPTp with SP to IPTp with DP, one would ideally be able to show that this alternative regimen is associated with a significantly lower risk of adverse birth outcomes associated with malaria during pregnancy.

*Control arm did not reflect current Uganda/Global Guideline*s: In the study from Kenya, both SP and DP were given a median of 3 times during pregnancy at intervals of 4-6 weeks. In our study from Uganda, SP was given 3 times during pregnancy (at 20, 28, and 36 weeks of gestation) in line with Ugandan guidelines in place at the time the study was designed. In contrast, in our monthly DP arm active study drugs were given every 4 weeks starting as early as 16 weeks of gestational age. Updated Ugandan and global guidelines now recommend that IPTp with SP be started as early as possible during the 2nd trimester and continued at each scheduled antenatal care visit at least 1 month apart up to the time of delivery in line with updated recommendations from the WHO.[28]

Safety assessments: More data are needed on the safety of IPTp with DP, especially with regards to the risk of QTc prolongation. Our study is the only study of IPTp with DP conducted to date that included ECG evaluations, but we only included a subset of 42 women; 12 assigned to 3 dose SP, 17 assigned to 3 dose DP, and 13 assigned to monthly DP. QTc intervals were also only measured before and after study drug treatment at 28 weeks of gestational age.

Impact of exposure to malaria during pregnancy on the development of immunity in the fetus. Increasing evidence suggests that maternal infection during pregnancy affects the developing immune system of the fetus independent of potential vertical transmission.[29] Several studies indicate that placental malaria is associated with altered parasite-specific immune responses in neonates that could affect response to malaria after birth.[30-33] In addition, several clinical studies have reported that infants born to mothers with placental malaria have a higher risk of death [34] and malaria during infancy.[35-38] However, all of these were observational studies in which it is very difficult to control for exposure, which is tightly linked between

pregnant women and their infants. It remains unclear whether, as we hypothesize in this proposal, prevention of malaria during pregnancy improves the development of antimalarial immunity and reduces the risk of malaria in the first year of life.

Using metabolic profiles in the newborns for gestational age dating and evaluation of outcomes. A number of metabolites measured in newborn serum and bloodspots (i.e. acylcarnitines, amino acids, thyroid stimulating hormone) have been shown to be associated with intra-uterine growth restriction, mortality, and complications of prematurity including, for example, respiratory distress syndrome, patent ductus arteriosus, and necrotizing enterocolitis.[39-44] Recently our group found that well-performing gestational dating algorithms could be built using newborn characteristics and metabolites measured within the first week of life (acylcarnitines, amino acids, thyroid stimulating hormone, 17-hydroxyprogesterone, and galactose-1-phosphate-uridyl-transferase).[45] Associations between these target markers, gestational age, and outcomes appears to be related to poor metabolic regulation – particularly with respect to glucose homeostasis[46-51] and are also related to hepatic immaturity.[52] Evaluating these patterns in the context of the other aims of this study provides an opportunity to understand these patterns more fully in the population of study and also provides data that may prove critical for understanding the immune status of neonates over the course of the first year of life – particularly in those born preterm, with intra-uterine growth restriction, and in those with complications with close links to prematurity.

Improved point-of-care testing for the detection of infection with malaria parasites during pregnancy. The detection of infection with malaria parasite during pregnancy could have important implications for screening and treating women with the goal of reducing the risk of complications due to malaria in pregnancy. Currently available rapid diagnostic tests (RDTs) lack the sensitivity to detect low level parasitemia common during pregnancy among women living in highly endemic areas.[25] Malaria RDTs are lateral flow assays (LFA) that detect circulating malaria antigens. LFAs are uniquely applicable in a point-of-care setting because of their ease-of-use and short time required for diagnosis. To date, most LFAs are limited by the requirement to accumulate a certain level of analyte-antibody-nanoparticle (dye) conjugate, commonly including a gold nanoparticle (GNP), sufficient to enable visual detection of a test line. This limits their potential in the clinical management, screening and surveillance of many infectious diseases. One result of this limitation is a failure to detect low-density (often asymptomatic) *P. falciparum* infections, which can lead to placental malaria. We plan to evaluate two new technologies aimed to improve the sensitivity of RDTs used to detect malaria parasites during pregnancy. The first is an LFA-enhancement technology based on photo-thermal spectroscopy (PTS) which can reduce the limit of detection (and thereby increase the sensitivity) of RDTs currently on the market by 4-5 fold. Photo-thermal spectroscopy involves the selective heating

of gold nanoparticles on the wick of a LFA, and detection of gold accumulation through an increase in temperature relative to its surrounding substrate. Heating is attained through excitation of gold nanoparticles with a laser of certain wavelength, and detection through use of a thermal camera or other device to detect temperature change. This device achieves lower thresholds of detection and thus enables the user of the LFA to detect target antigen or antibody at lower concentration, and/or at an earlier stage. The LFA-reader is approximately laptop sized and comprises the reader unit and disposable assay cartridges. The reader can be operated under battery power for 1 day and can be recharged using standard power sources. Both the cartridges and the readers can be operated under typical room conditions, not requiring air conditioning or refrigeration. Approximately 5 µL of whole blood is placed on the cartridge, which is inserted into the reader, and the reader will take approximately 1-3 minutes to complete the testing. The graphical user interface (GUI) guides the user through the assay process. In addition to the Photo-Thermal Reader, we plan to evaluate an experimental RDT which has been designed to increase clinical sensitivity compared to commercially available RDTs.

1.2. Preliminary studies

Intermittent preventive therapy in pregnancy with sulfadoxine-pyrimethamine. We conducted a cross-sectional study of 565 HIV-uninfected women giving birth between February – July 2011 at Tororo District Hospital.[53] The primary objective of the study was to measure associations between use of SP during pregnancy from antenatal records and the risk of adverse outcomes including placental malaria, low birth weight, maternal parasitemia and maternal anemia. The proportion of women who reported taking 0, 1, 2, and 3 doses of SP during pregnancy was 5.7%, 35.8%, 56.6% and 2.0% respectively. Overall, the prevalence of placental malaria was 17.5%, 28.1%, and 66.2% by placental smear, PCR, and histopathology, respectively. In multivariate analyses controlling for potential confounders, $\geq$ 2 doses of SP was associated with non-significant trends towards lower odds of placental malaria by placental smear (OR=0.75, p=0.25), placental malaria by PCR (OR=0.93, p=0.71), placental malaria by histopathology (OR=0.75, p=0.16), low birth weight (OR=0.63, p=0.11), maternal parasitemia (OR=0.88, p=0.60) and maternal anemia (OR=0.88, p=0.48). Using a composite outcome, $\geq$ 2 doses of SP was associated with a significantly lower odds of placental malaria, low birth weight, maternal parasitemia, or maternal anemia (OR=0.52, p=0.01). In this area with intense malaria transmission, the prevalence of placental malaria by histopathology was high even among women who reported taking at least 2 doses of SP during pregnancy. The reported use of $\geq$ 2 doses of SP was not associated with protection against individual birth and maternal outcome.

Antifolate Resistance in Uganda. Surveillance of key mutations in *P. falciparum dhfr* and *dhps* genes, which encode the target enzymes of SP, has been proposed as a means of monitoring antifolate drug resistance in Africa. We have studied the association between the five key mutations commonly reported in Africa and clinical treatment failure in children treated with SP for uncomplicated malaria in Kampala [54,55]. The prevalences of the *dhfr* 108N (98%) and 51I (95%) mutations were very high, and therefore these mutations were not useful independent predictors of treatment outcome. Considering combinations of mutations, there was generally a "dose response" relationship, with an increasing number of mutations resulting in stronger associations with treatment failure. Infections with parasites containing the quintuple mutant (*dhfr* 108N + 51I +59R; *dhps* 437G + 540E) was associated with over 10 times the odds of treatment failure compared to infections with parasites containing only the 108N and 51I mutations (OR = 10.7, 95%CI 1.8-64.4, p = 0.009).

We have measured the prevalence of key antifolate resistance-conferring mutations from subjects living in Tororo (nearby the site of this proposal) over a 4 year period. From 2002-2006 the prevalence of the *dhfr* triple mutant increased from 40% to almost 80% and the prevalence of the *dhps* triple mutant increased from 60% to almost 100%. This temporal increase in the prevalence of molecular markers of antifolate resistance corresponds to the 2001 implementation of a national policy change from CQ to CQ+SP as the recommended first-line treatment for malaria in Uganda [56]. We have also measured the prevalence of key antifolate resistance-conferring mutations from the 9 children in a cohort of HIV-infected children who developed symptomatic malaria while taking TS prophylaxis [57]. All of these samples contained the *dhfr/dhps* quintuple mutant and one sample contained an additional mutation (*dhfr* 164L) associated with high-level antifolate resistance (probably leading to complete loss of antimalarial activity of antifolates) that is rare in Africa [58] and was only very rarely detected in hundreds of prior samples from Uganda evaluated by our group. These data provided further evidence that SP, currently the regimen used for IPTp in Uganda, may be faced with diminishing protective efficacy. Thus, alternative regimens and strategies, such as DP as proposed in our protocol, should be considered.

IPTp with DP vs. SP. As mentioned in the background section, we recently conducted a double-blinded randomized controlled trial comparing 3 dose SP versus 3 dose DP versus monthly DP in 300 HIV-uninfected pregnant women in Tororo, Uganda, where SP resistance is widespread and transmission intensity has historically been high. The primary outcome was the prevalence of placental malaria by histopathology. A total of 106, 94, and 100 women were randomized to 3 dose SP, 3 dose DP and monthly DP, respectively. The prevalence of placental malaria by histopathology was significantly higher in the 3 dose SP arm (50.0%) compared with the 3 dose DP (34.1%, P=0.03) or monthly DP (27.1%, P=0.001) arms. The prevalence of a composite adverse birth outcome was lower in the monthly DP arm (9.2%) compared to the 3 dose SP

(18.6%, P=0.05) and 3 dose DP (21.3%, P=0.02) arms. During pregnancy, the incidence of symptomatic malaria was significantly higher in the 3 dose SP arm (41 episodes over 43.0 person years at risk) compared to the 3 dose DP (12 episodes over 38.2 person years at risk, P=0.001) or monthly DP (0 episodes over 42.3 person years at risk, P<0.001) arms, and parasite prevalence significantly higher in the 3 dose SP arm (40.5%) compared to the 3 dose DP (16.6%, P<0.001) or monthly DP (5.2%, P<0.001) arms. The risk of vomiting following the administration of study drugs was < 0.4% in all 3 treatment arms and there were no significant differences in the risk of adverse events.

Currently, the children born to the pregnant women enrolled in this study are being randomized to receive monthly vs. 3 monthly DP from 2-24 months of age and then followed for an additional one year (to 36 months of age) after chemoprevention is stopped. One of the objectives of this study is to compare the incidence of malaria in children born to mothers who received different IPTp regimens. However, shortly after women started giving birth in this study, the district of Tororo implemented indoor residual spraying of insecticide (IRS) for the first time with the plan to conduct IRS every 6 months for 3 years (December 2014 through December 2017). Due to the combination of IRS and chemoprevention among the children, the incidence of malaria has been extremely low (< 0.1 episodes PPY) precluding our ability to test the hypothesis that the incidence of malaria will be different in children born to mothers randomized to IPTp with SP vs. DP. The study outlined in this proposal will address this development but recruiting study participants from an area of Eastern Uganda where IRS is not being conducted and chemoprevention will not be given to children, which remains the current standard of care in Uganda.

Using metabolic profiles in the newborns for gestational age dating and evaluation of outcomes. As is noted in the background section, our group is experienced in examining the relationship between metabolic profiles at birth, gestational age, and newborn outcomes. Specifically, we recently evaluated whether metabolic markers in newborns could be used to develop a population-level metabolic gestational dating algorithm that is robust despite intrauterine-growth restriction and could be used when fetal ultrasound dating is not available. We focused specifically on the ability of these markers to differentiate preterm births (<37 weeks) from term births and to assign a specific gestational age in the preterm birth group. In a cohort of 729,503 singleton newborns we used multivariate backward stepwise regression to test for associations and linear discriminate analyses to create a linear function for preterm birth and to assign a specific week of gestation. These efforts resulted in a finding that when considered with birth weight and timing of test by hours after birth at blood draw, 35 of the 51 metabolic markers evaluated were able to sort preterm and term births accurately with sensitivities and specificities of 95% or more in both the training and testing subsets. Assignment of a specific week of gestation in those identified as preterm births resulted in the

correct assignment of week +/-2 weeks in 89.8% of all newborns in the training and 91.7% of those in the testing subsets.[45]

Our group is also uniquely prepared to investigate the relationships between metabolic markers and newborn outcomes given extensive work with similar data and specimens. Of particular note is work from our group that has shown that preterm neonates are metabolically different than their term peers.[42-44] Also important are our findings demonstrating differences in glucose-related markers in preterm birth neonates within the first few days of life that go on to develop complications of prematurity including respiratory distress syndrome and necrotizing enterocolitis. Recently we found that that acylcarnitine patterns within the first few days of life can help identify more than 80% of preterm neonates that develop necrotizing enterocolitis.

1.3. Rationale

Malaria remains one of the most important infectious diseases worldwide with an estimated 3.3 billion people at risk leading to hundreds of millions of cases and 660,000 deaths each year.[59,60] In 2010, 81% of cases and 91% of deaths were estimated to have occurred in Africa, with children under five years of age and pregnant women most severely affected.[60] The primary tools currently available for malaria prevention in Africa include ITNs and IPTp. However, there are limitations with these interventions and the burden of malaria remains high in many parts of Africa despite recent increases in coverage levels. ITNs do not fully protect against malaria and there is concern for waning efficacy given the emergence of resistance to pyrethroid insecticides (the only class used in ITNs).[61,62] The only drug widely used for IPTp is SP, and there are recent reports suggesting that IPTp with SP is no longer effective, especially in East Africa, where resistance to this drug is now widespread.[14,63] Recent increases in funding for malaria control offer an unprecedented opportunity to expand preventative interventions; however, new strategies are urgently needed to reduce the burden of malaria for those at greatest risk.

One of the most important malaria control interventions is the use of ACTs for the treatment of malaria. ACTs are highly efficacious, and now the recommended first-line treatment for uncomplicated falciparum malaria in all countries of sub-Saharan Africa , including the treatment of malaria in pregnant women during the 2nd and 3rd trimesters.[27] ACTs also offer the opportunity to greatly reduce the burden of malaria in pregnant women and young children if their role is expanded to chemoprevention. IPT with the ACTs artesunate-amodiaquine, artesunate-SP, and DP have been shown to be effective and safe for the prevention of malaria in African children.[64-66] IPT with DP is especially attractive given its prolonged post-treatment prophylactic effect, due to the unusually long half-life of piperaquine.[67]

Two recent studies from East Africa have shown that IPTp with DP is more effective than SP at reducing the risk of malaria during pregnancy and the risk of placental malaria at delivery.[25,26] However, there were limitations to these studies and WHO policy recommendations continue to recommend the use of IPTp with SP. This proposal will address some of these limitations by using a dosing strategy in-line with updated WHO recommendations, power the study to detect a difference in the risk of adverse birth outcomes, undertake a more thorough assessment of adverse events including repeated assessments of QTc prolongation in all study participant, and assess the impact of different IPTp regimens on the incidence of malaria during infancy. We will perform a randomized, double-blinded, controlled trial to compare IPTp with SP vs. DP delivered every 4 weeks starting as early in the 2nd trimester as possible in an area of high transmission intensity and widespread antifolate resistance. Pregnant women will undergo frequent sampling using a highly sensitive molecular assay to better define the timing and frequency of malaria infection during pregnancy and the primary outcome will be based on a composite adverse birth outcome of low birth weight (LBW), preterm delivery, or small for gestational age (SGA). The overall theme of this proposal will be that in areas of high malaria transmission intensity where the burden of malaria remains high despite the use of currently available control interventions, aggressive and strategic use of highly effective drugs for IPTp may improve birth outcomes and reduce the risk of malaria during infancy.

With respect to examining metabolites in newborns, given the established links between metabolic markers, gestational age, survival and morbidity, we suspect that this information may prove useful not only for determining gestational age when there is no ultrasound (a common occurrence in Uganda and in other developing countries), but we suspect that eventually, monitoring of these metabolites may provide useful information for tailoring care to an individual neonates' metabolic profile. For example, carnitine levels might inform nutritional supplementation strategies given the link between total parenteral nutrition and carnitine deficiency which can impair fatty acid oxidation.[68] In the context of the present study, we also suspect that these markers may prove useful for understanding immune patterns in some infants given that they may point to systems that are more or less mature. Such data may prove especially useful when evaluating drug efficacy.

This metabolite assay is not validated for clinical use; therefore the results will not be routinely reported to clinicians or to participants and their families. However, abnormal results from some chemicals in the assay can be associated with certain diseases. Patterns of adult hemoglobins [HbA(A) and variants (S,C,D,E, B-thal)] as well as hemoglobin peak percentages (HGB-FAST, HGB-F1, HGB-F, HGB-F+F1, HGBFAST+ F1, HGB-Other, HGB-A] can suggest of Sickle Cell Disease (SCD) or other hemoglobinopathies like thalassemia. The prevalence of sickle cell disease is ~ 1.5% in eastern Uganda, so it is quite likely that children with the disease will be in our study cohort of likely > 780 children[69]. High levels of thyroid stimulating hormone (TSH) in

the assay would be suggestive of hypothyroidism. The incidence of congenital hypothyroidism is generally considered to be ~ 1 in 2,000 to 4,000.[70] There are no published precise estimates of the prevalence of congenital hypothyroidism in Uganda, but one small study of neonatal TSH levels suggests iodine deficiency is a persistent problem among neonates in Uganda.[71] If this investigational assay yields values concerning for either SCD or hypothyroidism we will report the results to the parents and guardians and offer to perform additional testing using clinically validated assays to confirm or exclude the disease, free of charge. If the disease is confirmed, the study will offer care for these conditions during the study follow up free of charge, and referral to local providers at the end of the study. The decision to act on the results of an investigational assay is based on the clinical and ethical rationale that early identification and treatment for these SCD and hypothyroidism has the potential to improve clinical outcomes for infants within the resources health care setting of Uganda.[70] We believe that those potential benefits outweigh the risks, that include anxiety for families and phlebotomy for the cases shown to not actually have disease, risk from the additional phlebotomy for confirmatory testing, and risks of standard clinical treatment for the conditions if found to be present. Additional details about the management of test results and participant follow up are provided in Section 6.10.4.

2. STUDY OBJECTIVES

We will test the hypothesis that IPTp with DP will significantly reduce the burden of malaria in pregnancy and infancy compared to IPTp with SP. The specific study objectives are as follows:

2.1. Objective 1

To compare the risk of adverse birth outcomes among HIV-uninfected pregnant women randomized to receive IPTp with monthly SP vs. monthly DP. We will test the hypothesis that pregnant women who receive IPTp monthly DP will have a lower risk of a composite adverse birth outcome defined as LBW, preterm delivery or SGA compared to those who receive monthly SP. Secondary outcomes will include incidence of malaria and adverse events during pregnancy, risk of asymptomatic parasitemia and anemia during pregnancy, and measures of placental malaria at the time of delivery.

2.2. Objective 2

To compare the incidence of malaria among infants born to mothers randomized to receive IPTp with monthly SP vs. monthly DP. We will test the hypothesis that infants born to mothers randomized to IPTp with monthly DP will have a lower incidence of malaria in the 1st year of life compared to infants born to mothers randomized to IPTp with monthly SP. Secondary outcomes will include the incidence of complicated malaria, hospitalizations, infant mortality rate, and the prevalence of asymptomatic parasitemia and anemia.

2.3. Objective 3

To validate and adapt a gestational dating by metabolic profile at birth algorithm and to evaluate the added benefits of using metabolic profile to identify newborns at increased risk for neonatal death or serious complications. We will test the hypotheses that a metabolic dating algorithm developed in a western setting can be applied to gestational dating in Uganda. We will also test the hypothesis that metabolic markers (especially those related to glucose metabolism) can be used to identify newborns at more or less risk for death and serious morbidities.

2.4 Objective 4

To evaluate experimental malaria diagnostic tests during pregnancy and infancy. 1) We will evaluate a Photo-Thermal Reader with the Ugandan public sector's primarily used rapid diagnostic test (RDT), CareStart Pf HRP-2, and an experimental, highly-sensitive HRP2-based RDT to detect malaria parasites during pregnancy. We will estimate the sensitive and specificity of the Photo-Thermal Reader using a standard RDT and an experimental, highly-sensitive RDT using quantitative PCR (qPCR) as the gold standard. RDT results will be used for research purposes only and not for clinical care. **2) We will evaluate the relative performance of a hemozoin-based rapid diagnostic test versus Ugandan public sector's primarily used Rapid Diagnostic Test (RDT), CareStart Pf HRP-2, and an experimental, highly-sensitive HRP2-based RDT to detect malaria parasites in infants.** We will estimate the sensitivity and specificity of the Magneto-Optical Detection (MOD) test using standard RDT and microscopy test , using quantitative PCR (qPCR) as the gold standard. All results will be used for research purposes only and not for clinical care.

3. STUDY DESIGN

This will be a double-blinded randomized controlled phase III trial of 782 HIV uninfected pregnant women and the children born to them. HIV uninfected women at 12-20 weeks gestation will be randomized in equal proportions to one of two IPTp treatment arms: 1) monthly SP, or 2) monthly DP. Both interventions arms will have either SP or DP placebo to ensure adequate blinding is achieved in the study as outlined in appendix D. Follow-up for the pregnant women will end approximately 6 weeks after giving birth. All children born to mothers enrolled in the study will be followed from birth until they reach 12 months of age.

4. SELECTION AND ENROLLMENT OF SUBJECTS

4.1. 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) Resident of Busia District, Uganda
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

4.2. 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 outside of Busia District, Uganda
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)

4.3. Initial Screening

We will recruit pregnant women presenting for routine care at the Masafu General Hospital antenatal clinic, at local health centers within Busia District, or referred by the Uganda government voluntary health teams. Pregnant women will be approached about participating in the study and will be provided an information sheet about the requirements of the study (Appendix A). If women are initially agreeable to screening for participation in the study and are $\leq$ 20 weeks gestation by LMP, 16 years of age or older, and not known to be HIV infected, they will either be escorted to the study clinic or made an appointment to return at a later date. At our study clinic study physicians will assess for initial eligibility criteria through conversation with the woman (confirming the age of the woman, location of residence, agreement to come to the study clinic for any febrile episode or other illness and avoid medications given outside the study protocol, plan to deliver in the hospital, no history of serious adverse event to SP or DP, no active medical problems requiring inpatient evaluation at the time of screening, no intention of moving outside the study district, no chronic medical condition requiring frequent medical attention, and no prior SP preventive therapy or any other antimalarial therapy during

this pregnancy). Women who pass initial screening based on conversation with study physicians will undergo the following additional screening procedures: 1) rapid HIV testing to confirm HIV negative status, 2) ultrasound dating to confirm intrauterine pregnancy and gestational age of 12-20 weeks in conjunction with LMP (Appendix B), and 3) a pelvic examination to exclude early or active labor if clinically indicated. Women who are not eligible for the study will be referred back to their local antenatal clinic.

4.4. Study Enrollment Procedures and Baseline Evaluation

Informed consent will be obtained from women who pass initial screening. Study physicians will conduct the informed consent discussion in the study clinic. Informed consent will include both participation of the pregnant woman and her unborn child (or children in the case of non-singleton pregnancies). Informed consent will be conducted in the appropriate language and a translator will be used if necessary. The study will be described and consent obtained in one of 4 languages (Samia, Swahili, Luganda, or English). The consent forms will be translated into each language and back-translated into English to check for any loss or change of meaning. Following the informed consent discussion, pregnant women will be asked by the study physicians to sign a written consent form approved by the UCSF Committee for Human Research (UCSF CHR), Makerere University School of Biomedical Sciences - Research and Ethics Committee (SBS-REC), and the Uganda National Council for Science and Technology (UNCST) for their own participation in the research study and a second approved consent form for the future use of biological specimens obtained during the course of the study. If the pregnant woman is unable to read or write, her fingerprint will substitute for a signature, and a signature from an impartial witness to the informed consent procedures will be obtained. The father will also be asked to provide written consent or a fingerprint to allow participation of the unborn child. If a father is unknown or unavailable, the woman will document in accordance with UNCST guidance and participation will continue for both the woman and infant. If the biological father refuses consent for the child's participation, the child will not be enrolled but the mother may still be enrolled.

Women will be enrolled in study on the same day that they provide informed consent for participation in the research study. On the day of enrollment, women will undergo a standardized history and physical examination, and have blood collected by venipuncture (15 cc's) for filter paper sample (for future molecular studies), routine baseline laboratory testing and storage. Routine baseline laboratory testing will consist of a thick blood smear for malaria parasites (will be read later by laboratory technicians not involved in study participants care and will not used for clinical care), RDT testing including a standard RDT using a PTS reader and an experimental RDT (results will not be used for clinical care), qPCR for malaria parasites, CBC and ALT measurement. Women who have history of fever in the previous 24 hours or a temperature $\geq$ 38.0ºC (tympanic) will have a thick blood smear read urgently in the study clinic. Women with history of fever in the previous 24 hours or a temperature $\geq$ 38.0ºC (tympanic) and a positive blood smear will be diagnosed with malaria and treated as described in section 6.2. At the end of the enrolment visit all study participants will be given a long lasting insecticide treated bed net (ITN) and a household survey appointment will be scheduled within

2 weeks to collect household-level information on the use of bednets, house members, household characteristics, and GPS coordinates (Appendix C).

5. STUDY TREATMENT

5.1. Treatment Group Assignments

There will be 2 treatment arms for the woman during pregnancy; monthly SP or monthly DP. We will use a 1:1 randomization scheme targeting 391 pregnant women in each treatment arm. 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 2 treatment arms using permuted variable sized blocks of 4 and 8. 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.

5.2. Treatment Allocation

On the day of enrollment, pregnant women will be referred to a study pharmacist responsible for treatment allocation. The study pharmacist will assign treatment arms as follows:

1. Select next available envelope
2. Note treatment number on the outside of the envelope
3. Write date, time, and study number on the outside of the envelope
4. Open envelope
5. Remove form containing code for treatment arm and date, time, and study number (transferred to form via carbon paper inside of envelope)
6. Store form in lockable file box in study pharmacy
7. Record onto the treatment allocation master list the study number, enrollment date, treatment assignment code, treatment arm, and study medications to be given during pregnancy
8. Store treatment allocation Master list in a lockable cabinet in study pharmacy
9. Record treatment number in the study participant's file

5.3. Study Drug Dosing and Formulations

During pregnancy, women will be given 1 of 2 treatment regimens: 1) SP given every 4 weeks during pregnancy, or 2) 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 or DP and placebo) 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. Dosing schedules for each treatment regimen according to gestational age are presented in Appendix D. The study drugs, DP (Duo-Cotecxin) and SP (Kamsidar) will be supplied by Holley-Cotec, Beijing and Kampala Pharmaceutical Industries (KPI), Uganda, respectively. Holley-Cotec will manufacture the DP placebo of exact specifications as that of the active DP. The DP placebo will be exactly the same in color, size, shape and packaging as the active DP. KPI will manufacture the SP placebo of exact specifications as that of the active SP. The SP placebo will be exactly the same in color, size, shape and packaging as the active SP. Details of the study drug formulations are included in Table 1.

Table 1. Drug formulation and labeling

Drug	Formulations	Trade name (Manufacturer)
Sulfadoxine-Pyrimethamine (SP)	500mg/25mg tabs	Kamsidar (KPI)
Dihydroartemisinin-Piperaquine (DP)	40mg/320mg tabs	Duo-Cotexin (Holley-Cotec)

5.4. Blinding, Study Drug Administration, and Duration

Administration of all study drugs will be double blinded such that study participants and study staff will be blinded to study treatments with the exception of the study pharmacist and pharmacy technician, who will not be involved with patient care or assessment of study outcomes. All doses of study drugs will be prepackaged 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. For pregnant women all doses of study drugs will be given between 16 and 40 weeks gestation as outlined in Appendix D.

5.5. Study Drug Accountability

The study pharmacist will maintain complete records of all study drugs received in the study pharmacy. Lot number and number of pills given to each study participant will be recorded. A registry of all study medication, current product labels, and Certificates of Analysis, provided by suppliers will be maintained within the regulatory binder for the study. The date received, lot number, expiration date, and date used will be recorded for each of the study medications. Monthly inventory of all study medications will be conducted and a record log of investigational medications will be kept at the study clinic.

6. SUBJECT MANAGEMENT

6.1. Subject Follow-up

Pregnant women will be scheduled to be seen in the clinic every 4 weeks during their pregnancy and then 1 and 6 weeks following delivery. In addition, pregnant women will be instructed to come to the study clinic for all their medical care and avoid the use of any outside medications. Children will be scheduled to be seen in the clinic at 1, 4, 6, and 8 weeks of age and then every 4 weeks until they reach 52 weeks of age. Parents /guardians will be instructed to bring their children to the study clinic for all medical care and avoid the use of any outside medications. The study clinic will remain open 7 days a week from 8 a.m. to 5 p.m. Pregnant women and children who are not seen on the day of their regularly scheduled visits will be visited at home and instructed to come to the clinic as soon as possible.

Each time a study participant is seen in the clinic a standardized history and physical exam will be performed including temperature, pulse, and blood pressure measurement. Patients who are febrile (tympanic temperature $\geq$ 3 8.0˚C) or report history of fever in the past 24 hours will have blood obtained by finger prick for a thick blood smear (in very young children, heel sticks may be substituted for finger pricks). If the thick blood smear is positive, the patient will be diagnosed with malaria. If the thick blood smear is negative, the patient will be managed by study physicians for a non-malarial febrile illness (Section 6.3). If the patient is afebrile and does not report a recent fever, a thick blood smear will not be obtained, except when following routine testing schedules (Section 6.5).

6.2. Diagnosis and Management of Malaria

Patients found to have malaria based on laboratory confirmation will have a second finger prick for a thin smear (to determine parasite species), hemoglobin measurement using a portable spectrophotometer (HemoCue), and collection of plasma, with residual blood used for testing of an experimental hemozoin-based rapid diagnostic test(MOD) or experimental magnetic levitation-based parasite detection (in children only). All episodes of malaria will be classified as uncomplicated or complicated based on the following criteria:

Uncomplicated malaria (all of the following)

1) Fever (≥ 38.0ºC tympanic) or history of fever in the previous 24 hours
2) Positive thick blood smear
3) Absence of complicated malaria

Complicated malaria (any of the following)

1) Evidence of severe malaria (Appendix E) and parasitemia
2) Danger signs present in children (Appendix E) and parasitemia

Episodes of malaria will also be classified into the following categories according to the timing of previous malaria episodes for treatment purposes:

1. New episodes of malaria will be defined as any first episode or any episode occurring > 14 days after the diagnosis of a previous episode
2. Treatment failures will be defined as any of the following:
    a. Complicated malaria occurring 1-14 days after the diagnosis of a previous episode
    b. Fever (≥ 38.0°C tympanic) or history of fever in the previous 24 hours with a parasite density ≥ the parasite density of an episode of malaria diagnosed 2 days prior
    c. Fever (≥ 38.0°C tympanic) or history of fever in the previous 24 hours with a parasite density ≥ 25% of the parasite density of an episode of malaria diagnosed 3 days prior
    d. Fever (≥ 38.0°C tympanic) or history of fever in the previous 24 hours with a positive thick blood smear of any parasite density occurring 4-14 days after the diagnosis of a previous episode

All patients diagnosed with new episodes of uncomplicated malaria will be prescribed AL, the recommended first-line treatment in Uganda for children and pregnant women in their 2nd or 3rd trimester. Patients with complicated malaria will be prescribed quinine or artesunate according to national malaria treatment guidelines. Children who are less than 4 months of age or weigh <5kg, will be treated with quinine for uncomplicated malaria in accordance with the

Uganda Ministry of Health Guidelines. Patients with treatment failure within 14 days following treatment with AL will be prescribed quinine according to national malaria treatment guidelines. Patients with treatment failure within 14 days following treatment with quinine or artesunate will be treated with quinine plus clindamycin or artesunate.

6.3. Management of Non-Malaria Illnesses

Patients who are found to have illnesses other than malaria will receive standard-of-care treatment in the study clinic, according to standardized algorithms, or will be referred to the local hospital. We will avoid the routine use of non-study medications with antimalarial activity, including antifolates (with the exception of assigned chemopreventive regimens), and macrolide antibiotics, when acceptable alternatives are available. In addition, drugs with known risk of torsades de pointes or CYP3A inhibitors (Appendix J and K) will be avoided when prescribing treatment to pregnant women during the time they are taking study drugs. If the study clinician deems treatment with one of the drugs listed on Appendix J or K is required, the clinical management team will be consulted. During follow-up for non-malarial illnesses, blood smears will be done at the discretion of the study physician if the subjects are febrile (tympanic temperature $\geq$ 38.0˚C) or report history of fever in the past 24 hours. If the blood smear is positive, the patient will be diagnosed with a new episode of malaria and managed per study protocol.

6.4. After Hours Visits

Pregnant women will be encouraged to come to Masafu General Hospital maternity ward (open 24 hours a day) and parents/guardians will be encouraged to bring their child to Masafu General Hospital pediatric inpatient ward (open 24 hours a day) when urgent care is needed outside of study clinic hours. Pregnant women or parents/guardians of children will be instructed to inform hospital personnel of their involvement in the study at the time of registration and to visit the study clinic on the following day. If a patient is diagnosed with uncomplicated malaria they will receive treatment from a hospital supply of AL and the doctors will be instructed to refer patients to our study clinic when it opens at 8 am the following day. If a patient is diagnosed with severe malaria, he/she will receive quinine or artesunate following standard treatment guidelines. Patients with non-malarial illnesses will be managed at the discretion of the hospital staff. Upon discharge, patients will receive follow-up at the study clinic as outlined above.

6.5. Routine Assessments

Routine assessments will be done in the clinic every 4 weeks for both pregnant women and children. Study participants not seen in the clinic for their every 4 week routine visits will be visited at home and requested to come to the study clinic as soon as possible. Pregnant women and children will receive standards of care as designated in the Uganda MOH guidelines (Appendix F). Routine antenatal care will include screening and treatment for sexually transmitted Infections, blood pressure assessment, urine dipstick for proteinuria, prescription of iron, folate, multivitamins and mebendazole. Routine care in children will include immunizations, vitamin A supplementation, and management of anemia using Integrated Management of Childhood Illness (IMCI) guidelines. During routine assessments subjects will be asked about visits to outside health facilities and the use of any medications outside the study protocol. The study protocol will be reinforced with discussion regarding the need to come to the study clinic promptly upon the onset of any illness and to avoid use of outside medications. Standardized assessment of adherence will also be done for study drugs administered at home and ITN use. A routine history and physical exam will be performed using a standardized clinical assessment form. Blood will be collected by finger prick (in very young children, heel sticks may be substituted for finger pricks) for thick smear; capillary plasma, RDT testing, qPCR (for routine visits where phlebotomy is not done); and filter paper samples. In a subset of visits, less than 10 µL of blood from finger prick or phlebotomy, if performed, will be used for magnetic levitation-based detection of malaria parasites. If a pregnant woman or parent/guardian of a child reports a fever in the last 24 hours or the patient has a documented temperature $\geq$ 38.0˚C tympanic, the patient's thick blood smear will be read immediately and if positive the patient will be diagnosed and treated for malaria (see section 6.2). In pregnant mothers, thick blood smears (other than those done when a mother has fever) and RDT testing will not be used for clinical care of study participants. Phlebotomy for routine laboratory tests (CBC and ALT) to monitor for potential adverse events from study medications, storage of plasma, RDT testing using PTS reader and experimental RDT, qPCR, and for immunology studies will be performed every 8 weeks in pregnant women. Phlebotomy for routine laboratory tests (hemoglobin measurement using a portable spectrophotometer (HemoCue Angholm, Sweden)), hemozoin based experimental rapid diagnostic test (experimentalMOD), standard RDT, microscopy, qPCR and immunology studies will be performed at 12, 28, and 52 weeks of age in children. For pregnant women, study drugs will be administered at the time of each routine visit as described in sections 5.3 and 5.4. ECGs will be performed to measure the QTc interval in all pregnant women just prior to the 1st dose of study drugs and 2-3 hours after their 3rd dose of study drugs at 20, 28 and 36 weeks of gestation. In addition a finger prick capillary plasma sample will be collected just prior to performing the ECGs after the 3rd dose of study drugs at 20, 28, and 36 weeks of gestation in pregnant women.

6.6. Delivery visit

Addressing one of the study outcomes, placenta malaria, requires collection and processing of specimens at delivery. Systems will be in place to facilitate a birth plan which will encourage women to come to the hospital for delivery, including access to transportation 24 hours a day. However for women who are unable to travel to the hospital for delivery or choose to deliver at home, a study staff member will be driven to the home to follow study procedures. Study staff will document details of the delivery, including date and time, type of delivery, estimated blood loss and any maternal, obstetrical or neonatal complications. Study staff will document the infant's Apgar score and birth weight with calibrated scales. Biological samples collected at the time of delivery will include maternal venous blood (for thick blood smear, filter paper samples, CBC, ALT, and immunology studies), cord blood (for thick blood smear, filter paper samples, hemoglobin measurement, and immunology studies) and placental tissue. Following delivery, neonatal care, as per national guidelines, will include polio and BCG immunization, ophthalmic tetracycline, and vitamin K. On the day of birth newborns will have a heel stick or finger prick done for collection of blood for metabolic testing.

At the time of delivery, women will undergo repeat rapid HIV testing based on national guidelines. If women are found to have become HIV-infected during pregnancy, both the mother and their newborn will be withdrawn from the study and immediately referred for care following local prevention of mother-to-child transmission (PMTCT) guidelines.

6.7. Postpartum visits.

Women will be evaluated within 1 week following delivery and 6 weeks postpartum as part of routine care. Both visits will include an abdominal exam, syndromic management of STIs, and follow-up on any obstetrical complications that occurred including evaluation of the neonate for any congenital abnormalities. Pelvic and breast exam will be done if clinically indicated. Contraceptive counseling, nutritional assessment and infant feeding and support will be provided. Following the 6 weeks visit, women will no longer be considered study participants, although most will remain primary care givers for their children enrolled in the study.

Summaries of all procedures done during routine visits in pregnant women and children are presented in Appendix G and H, respectively.

6.8. Medical Care Outside the Study Clinic

We will provide routine medical care in our clinic free of charge, including medications, to the extent possible given resources available. Study participants and their guardians will be reimbursed for costs of any transportation to and from our clinic. In addition, we will

reimburse the cost of tests and drugs for referrals of study participants made by study physicians to other clinics and services as well as after-hours hospital visits. We anticipate reimbursing the cost of most diagnostic tests (including laboratory tests, X-rays, and ultrasounds) and medications resulting from these referrals, using available funds. However, reimbursement of all diagnostic tests and treatment recommended outside the study clinic cannot be guaranteed in all circumstances. Decisions on reimbursement will be made by the study coordinator and the investigators, in conjunction with the funding agency if necessary.

6.9. Duration of Follow-up and Criteria for Premature Study Withdrawal

Pregnant women will be followed until their 6 week postpartum visit. Children will be followed until they reach 12 months of age. Study participants will be prematurely withdrawn from the study for: 1) movement out of study area, 2) inability to be located for > 60 consecutive days, 3) withdrawal of informed consent, 4) inability to comply with the study schedule and procedures, 5) at the discretion of the site investigator if the study is not in the best interest of the participant, 6) subject or parent/guardian judged by the site investigator to be at significant risk of failing to comply with the study protocol as to cause them harm or seriously interfere with the validity of study results, 7) women found to have become HIV infected at the time of delivery (child withdrawal only). If a subject is withdrawn for reasons # 1-3, we will be unable to perform any additional study procedures. If a subject is withdrawn for reasons # 4-6, plans to obtain appropriate follow-up tests outside of the study will be individualized for each subject depending on the health status of the subject at the time of withdrawal and the willingness of the participant and his or her parent/guardian to proceed with additional testing. If a subject is withdrawn for #7, they will be referred for appropriate care following local PMTCT guidelines.

6.10. Diagnostic and Laboratory Testing

Diagnostic and laboratory testing shall take place using accredited local laboratories, as described below. In the event that local capacity is not available for specialized testing, reasonable efforts will be made to build local capacity. In the event that local capacity is not available, samples will be exported with all required permissions and permits to external testing laboratories. Table 2 lists examples of specialized testing, laboratories and rationale required to meet study aims.

Table 2: Laboratory Evaluations Requiring Sample Export

Evaluation	Testing Laboratory	Rationale
Pharmacokinetic assessment of antimalarial drug levels	UCSF Drug Research Unit Francesca Aweeka, PharmD University of California, San Francisco Department of Pharmacology	Measure piperaquine concentrations in venous specimens using liquid chromatography/ tandem mass spectrometry (LC/MS-MS). Testing equipment and expertise not available in-country.
Assessment of cord blood metabolites	Iowa State Hygienic Laboratory Ankeny, Iowa	Need for standardized mass spectrometry guidelines and referents
Immunologic studies of infants	Department of Pediatrics Margaret Feeney, MD University of California, San Francisco Division of Experimental Medicine	Testing equipment and expertise not available in-country for high-throughput immunologic assays, including RNA-sequencing

6.10.1. Microscopy

Thick and thin blood smears will be stained with 2% Giemsa and read by experienced laboratory technologists. Parasite densities will be calculated by counting the number of asexual parasites per 200 leukocytes (or per 500 leukocytes, if the count is <10 asexual parasites/200 leukocytes), assuming a leukocyte count of 8,000/μl. A blood smear will be considered negative when the examination of 100 high power fields does not reveal asexual parasites. Gametocytemia will also be determined from thick smears. Thin smears will be used for parasite species identification. For quality control, all slides will be read by a second microscopist and a third reviewer will settle any discrepant readings.

6.10.2. Clinical Laboratory Studies

At enrollment and every 8 weeks during follow-up for pregnant women venipuncture blood samples will be collected for routine clinical laboratory studies, including CBC and ALT measurements. In children venipuncture blood samples will be collected for a routine hemoglobin measurement using a portable spectrophotometer (HemoCue Angholm, Sweden) at 12, 28, and 52 weeks of age. Additional venipunctures will be performed, as appropriate, for laboratory testing to evaluate non-malarial medical illnesses at the discretion of study physicians. Results will be made available to study physicians in a timely manner for patient management decision-making. Additional hemoglobin measurements will be performed each time a patient is diagnosed with malaria using a portable spectrophotometer (HemoCue, Angholm, Sweden) and results will be immediately available.

6.10.3. Placental Studies

Cord blood will be collected for immunology studies described below. Placental blood collected for Giemsa-stained blood smears and future molecular studies will be obtained by making a small incision on the maternal surface of the placenta within 1 hour of delivery, collecting blood from the intervillous space via syringe, and then placing the blood in an EDTA tube. Placental tissue will be collected for histological assessment. Two 1 cm-wide full thickness biopsies from each placenta, obtained about 5 cm from the cord, will be obtained within 1 hour of delivery and placed in 10% neutral buffered formalin. Biopsy specimens will be embedded in paraffin wax, sectioned into 3 µM slices using a rotary microtome, fixed to glass slides, and dehydrated in sequential ethanol baths. Separate slides will then be stained in 0.1% hematoxylin and 1% eosin for 5 and 1 min, respectively, or in 2% Giemsa for 30 minutes. All necessary expertise and infrastructure for these studies is in place in Tororo. For assessment of histologic evidence of placental malaria, placentas will be graded into 5 categories using a standardized approach.[72] The presence of intervillous parasite-infected erythrocytes and of pigment in monocyte/macrophages or fibrin will be noted. Quantitative assessments of placental malaria will be as follows. First, 1000 intervillous blood cells will be counted under high power. Percentages of intervillous erythrocytes infected with parasites and of monocyte/macrophages containing malarial pigment will be counted. Placental specimens will be examined by two experts, and any discrepant readings will be resolved by a third reader.

6.10.4. Molecular and Parasitology Studies

Each time a thick blood smear is obtained; blood will also be collected onto filter paper. Samples will be collected by venipuncture or by finger prick sampling. Blood will be placed onto filter paper in approximately 25 µl aliquots per blood spot (4 blood spots per sample). The samples will be labeled with study numbers and dates, air-dried, and stored in small, sealed sample bags at ambient temperature or 4°C with desiccant. Molecular studies will include the extraction of DNA from filter paper and followed by characterization of parasite genetics using standard molecular procedures including loop-mediated isothermal amplification (LAMP), DNA hybridization, and/or restriction enzyme digestion. Additional molecular studies will include analyses of polymorphisms in parasite genes and measurement of piperaquine drug levels from plasma samples. Molecular studies will be performed only for research purposes and will have no impact on the clinical management of study patients. Molecular studies will be formed in Uganda whenever possible and at UCSF when facilities/equipment are not available in Uganda. One exception will be for metabolic analyses of newborn bloodspots and cord blood for the purposes of gestational dating and examination of metabolite-outcomes relationships. Given

the need to evaluate relationships using standardized mass spectrometry guidelines and referents, these tests will be done at the Iowa State Hygienic Laboratory in Ankeny, Iowa. Specimens will be collected using Gutherie card stock provided by the laboratory. Specifically, a 5 millimeter spot of 1) blood collected by heel stick or finger prick and 2) cord blood at birth (< 10 drops) will be deposited on card stock from the same lot that is provided by the Iowa lab. All specimens will be delivered to Iowa from Uganda by mail via freezer packs within one week of draw.

Blood collected by venipuncture or finger prick on the day malaria in child is diagnosed will be used in selected subjects for parasite culture, experimental MOD, standard RDT, microscopy, qPCR, parasite detection by magnetic levitation, and/or immunology studies (below). For these studies the skin will be prepped with 3 washes with a betadine or equivalent sterilizing solution, and then approximately 3-5 mls of blood will be collected in an anticoagulated sterile tubes and transferred promptly (generally within 30 minutes) to our molecular laboratory. Parasites will be cultured following standard protocols. In brief, erythrocytes will be separated from plasma by centrifugation and removal of the supernatant and buffy coat, and the infected erythrocytes will then be cultured in RPMI medium supplemented with human serum or Albumax serum substitute. Cultured parasites will be evaluated for in vitro drug sensitivity, molecular characteristics, and other features to characterize antimalarial drug resistance and other aspects of malaria. Information from the parasitology studies will have no impact on patient care.

CareStart Pf HRP-2 (Access Bio Inc USA) RDT testing using the PTS reader will be performed at enrollment and every 4 weeks at the time of routine visits during pregnancy. Approximately 5 µL of whole blood will be placed on the cartridge, which is inserted into the reader, and the reader will take approximately 1-3 minutes to complete the testing. The graphical user interface (GUI) guides the user through the assay process. Quantitative PCR will be performed at enrollment and at the time of routine visits using standardized procedures on ~ 100ul of whole blood. A HRP2-based experimental RDT will be tested at enrollment and every 8 weeks at the time of routine phlebotomy during pregnancy using ~ 100ul of whole blood. An RDT result will be considered positive if both the control line and the test line are visible after the development time. A result will be considered negative if the control line is visible, but no test line appears. The result will be considered invalid if no control line is visible, regardless of whether or not a test line appears. A hemozoin-based experimental rapid diagnostic test (MOD) will be performed at routine visits in a subset of children using ~ 50ul of whole blood collected by finger prick or phlebotomy. Approximately 50ul of whole blood will be placed in a special cuvette with a custom dilute, which is inserted into the sonicator and then the reader, and it will take approximately 2 minutes to complete the testing. For samples undergoing parasite detection by magnetic levitation, less than 10 µL of blood will be mixed with a paramagnetic

agent such as gadolinium, placed in close proximity to the earth magnets, and visualized by microscopy and/or a magnified imaging system.

Procedures for reporting and follow up of incidental concerning metabolite testing results
Participants will be considered at elevated risk for Sickle Cell Disease (SCD) or Congenital Hypothyroidism (CH) according to the following criteria:

- SCD: Patterns of adult hemoglobins [HbA(A) and variants (S,C,D,E, B-thal)] as well as hemoglobin peak percentages (HGB-FAST, HGB-F1, HGB-F, HGB-F+F1, HGBFAST+ F1, HGB-Other, HGB-A] based on the interpretation of the Iowa testing laboratory.
- CH: thyroid stimulating hormone> levels >50 mIU/L from cord blood sample

The metabolite laboratory will send results and IDs for all participants with concerning results. The Ugandan team will ask the participant and parents to come to clinic when the will explain that the metabolite testing results suggest the possibility of a disease, but do not constitute a true diagnosis. The team will explain offer to perform confirmatory testing using standard clinical tests; if the participant is found to have the disease of concern, the study team will offer treatment free of charge. A blood specimen would then be obtained for confirmatory testing by a local commercial lab, including Hemoglobin Electrophoresis for concern of SCD and TSH and T4 testing for concern for hypothyroidism. Patients will receive care for these conditions by the study team during study follow up and be referred to local providers for care at the conclusion of the study

6.10.5. Immunology Studies

Venipuncture blood samples collected at enrollment, during routine visits done every 8 weeks in pregnant women and at 12, 28, and 52 weeks of age in children, and in select subjects on the day malaria is diagnosed (above) will be made available for immunology studies. Approximately 15mls of blood in pregnant women and 5mls of blood in children will be collected and separated into plasma and peripheral blood mononuclear cells (PBMC) using a Ficoll gradient, following standard protocols. Plasma will be stored at -80°C for future immunology studies, which may include measurement of levels of cytokines, antibodies, and other features related to the host immune response. PBMCs will be stored in liquid nitrogen to maintain viability, and will be evaluated using flow cytometry, ELISPOT, RT-PCR, gene expression microarrays and other assays to assess the host immune response. Stool will be collected in in select children at 4 and 52 weeks of age and stored for future studies analyzing the influence of parasitic and bacterial infections on immunity. Information from immunology studies will have no impact on patient care. Immunology studies will be done in Uganda whenever possible. However, due to technological limitations some immunology studies may be done at UCSF.

6.10.6. Sample Storage for Future Use

Certain biologic specimens (placenta, filter paper, plasma, stool, and PBMC) will be stored in our laboratories for future molecular and immunology studies, pending participant consent for future use of biological specimens obtained during the course of the study. Samples will generally be stored in our laboratory in Tororo, Uganda. Certain specimens requiring long term freezer storage (plasma, PBMC) may be transferred for long-term storage to our laboratory in Kampala, Uganda (IDRC Molecular laboratory) or a specialized specimen bank with our collaborators at UCSF (Department of Medicine, 1001 Potrero Avenue, Building 3, Room 511). With consent for future use, sample retention will generally be open ended. Samples that do not have consent for future use, as detailed above, will be destroyed upon completion of clinical studies. Samples will be destroyed following SOPs which comply with local regulations and guidance.

6.11. Co-enrollment Guidelines

The pregnant women and children from this study may be co-enrolled in observational studies. They may not be co-enrolled in protocols that utilize concomitant study medications. Co-enrollment in other studies will be determined on a case-by-case basis by the protocol team.

6.12. Management of Adverse Events Potentially Related to Study Drugs

The following section outlines management of adverse events potentially related to study drugs (SP or DP) among pregnant women. Given the double blinded nature of the intervention, study clinicians and study participants will not be aware of what study drugs are being administered. Therefore all women will be considered potentially exposed to either study drug during the period study drugs are first given up to the completion of follow-up (6 weeks post-partum).

6.13. Grade 1 or 2 Adverse Events

Participants who develop grade 1 or 2 adverse events may continue study drugs. The study clinicians will manage the grade 1 or 2 events according to standard practice.

6.14. Grade 3 or 4 Adverse Events

<u>Management will be as follows:</u>

- Repeat observation or lab test within 72 hours of observation or of receiving lab results report.

- Work-up to exclude other causes.
- For grade 3 or non-life threatening grade 4 adverse events, subjects may continue taking study drugs pending clinic visit or repeat laboratory tests. Clinician has the option of immediately stopping the study drugs if subject cannot be examined in clinic, if a repeat laboratory test cannot be performed within 72 hours, or if the clinician determines that the continuation of study drugs is unsafe while awaiting clinic exam or test results.
- For grade 4 life-threatening adverse events, study clinicians should hold study drugs pending laboratory confirmation.
- For all grade 3 or 4 adverse events supported by repeat clinical exam or laboratory test results, study drugs will be held, and laboratory tests will be repeated every 1-2 weeks, until the adverse event resolves to $\leq$ grade 2 unless there is strong evidence that the adverse event is not related to either study drug.
- If the adverse event persists at grade 3 or 4 for more than 28 days or recurs on re-challenge, and the adverse event is thought to be possibly related to one of the study drugs, the study drugs will be permanently discontinued.

In the event that study drugs are permanently discontinued, study participants will remain in the study, following our intention-to-treat analysis approach. In the event that study drugs are permanently discontinued, study clinicians may become un-blinded to the study participant assigned treatment regimen if this knowledge may assist in the management of the grade 3 or 4 adverse event(s) that lead to the permanent discontinuation of the study drugs.

7. MONITORING OF ADVERSE EVENTS AND MANAGEMENT

7.1. Monitoring and Reporting of Adverse Events

7.1.1. Definitions

An adverse event is 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
- Concurrent illness
- Events related or possibly related to concomitant medications

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

7.1.2. Identification of Adverse Events

At each scheduled and unscheduled visit to the clinic, study clinicians will assess in all pregnant women who have started study drugs, 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) Toxicity Tables, will be used to grade severity of all symptoms, physical exam findings, and laboratory results (Appendix I). All pregnant women, 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 post-partum.

Data will be captured on the incidence of all adverse events, regardless of severity. For each adverse event identified and graded as severe or life threatening and felt to be possibly, probably or definitely related to study drugs, an adverse event report form will be completed. In addition, an adverse event form will be completed for all serious adverse events and unexpected events, regardless of severity. An adverse event report form will not be completed for events classified as mild or moderate (unless they are serious or unexpected), as mild and moderate symptoms are common and difficult to distinguish from signs and symptoms due to malaria and other common illnesses. The following information will be recorded for all adverse experiences that are reported:

1) Description of event
2) Date of event onset
3) Date event reported
4) Maximum severity of the event
5) Maximum suspected relationship of the event to study drugs (either SP or DP)
6) Whether the event is a serious adverse event
7) Initials of the person reporting the event
8) Outcome
9) Date event resolved

7.1.3. Reporting of Adverse Events

Guidelines for reporting of adverse events provided by NICHD, UCSF Committee for Human Research (CHR), and the Food and Drug Administration (FDA) in the U.S. and the Makerere

University IRB (SBSREC), and National Drug Authority (NDA) in Uganda will be followed as summarized in Table 2 below.

Table 2. Guidelines for reporting adverse events

Institution	Type of Adverse Events	When to Report
UCSF-CHR	External (off-site) Adverse Events are: • AEs that occur in study participants who are not enrolled at a UCSF or affiliated study site. These AEs occur at sites that are under the oversight of another IRB.	• Not applicable
MU-SBSREC	• All Serious and Unexpected events irrespective of relationship	• Fatal or life-threatening events within 3 working days of awareness • All other SAEs within 7 calendar days
NDA	• All Serious and Unexpected events irrespective of relationship	• Within 7 calendar days of awareness
FDA	• Definitely, Probably or Possibly related **AND BOTH** Serious* AND Unexpected±	• For fatal or life-threatening events, by telephone or fax within 7 calendar days of first awareness • All other reportable events within 15 calendar days of first awareness

Serious Adverse Event (SAE) is any AE 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 anomaly/birth defect, or cancer, or
- 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.

Unexpected Adverse Event An adverse event is defined as being unexpected if the event exceeds the nature, severity, or frequency described in the protocol, consent form and investigator brochure (when applicable). An unexpected AE also includes any AE that meets any of the following criteria:

- Results in subject withdrawal from study participation,
- Due to an overdose of study medication, or
- Due to a deviation from the study protocol

8. STATISTICAL CONSIDERATIONS

8.1. Hypothesis 1

We will test the hypothesis that pregnant women who receive IPTp with monthly DP will have a lower risk of a composite adverse birth outcome defined as LBW, preterm delivery or SGA compared to those who receive monthly SP. Secondary outcomes will include incidence of malaria and adverse events during pregnancy, risk of asymptomatic parasitemia and anemia during pregnancy, and measures of placental malaria at the time of delivery. 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.

8.1.1. Primary Outcome

The primary outcome will be the risk of having an adverse birth outcome defined as the occurrence of any of the following:

4) Low birth weight (< 2500 gm)
5) Preterm delivery (< 37 weeks gestational age)
6) Small for gestational age (< 10^{th} percentile relative to an external growth reference)[73]

8.1.2. Secondary Outcomes

Secondary outcomes are summarized in Table 3 below.

Table 3. Secondary outcomes

Secondary outcome	Definition
Placental malaria by histopathology	Any evidence of placental infection (parasites or pigment)
Placental parasitemia	Proportion of placental blood samples positive for parasites by microscopy or LAMP
Maternal malaria	Any treatment for malaria during pregnancy
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 microscopy and LAMP

8.1.3. Analyses

A modified intention-to-treat approach to all analyses will be used, including all study participants randomized to therapy and have the outcome of interest measured including all

follow-up time until the study participants complete the study or early study termination regardless of whether the intervention was stopped due to an adverse event.

Primary analysis. We will compare the risk of having any adverse birth outcome between the study arms using the Chi-Square or Fisher's exact test. We will explore for any differences of potential confounders between the treatment arms and if necessary adjust our analysis using multivariate logistic regression. We will also perform sub-group analyses comparing the risks of having individual adverse birth outcome (LBW, preterm delivery, SGA) between the study arms using the Chi-Square or Fisher's exact test.

Secondary analyses. We will compare the prevalence of different definitions of placental malaria (placental parasitemia by microscopy, placental malaria by LAMP, placental malaria by histopathology) using the Chi-Square or Fisher's exact test. We will compare the incidence of maternal malaria and 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. We will compare the prevalence of maternal anemia and asymptomatic parasitemia using generalized estimating equations with adjustments for repeated measures in the same study participant. If necessary, multivariate analyses will be performed to adjust for potential confounders and effect modifiers.

8.2. Hypothesis 2

We will test the hypothesis that infants born to mothers randomized to IPTp with monthly DP will have a lower incidence of malaria in the 1st year of life compared to infants born to mothers randomized to IPTp with monthly SP. Secondary outcomes will include the incidence of complicated malaria, hospitalizations, infant mortality rate, and the prevalence of asymptomatic parasitemia and anemia.

8.2.1. Primary Outcome

The primary outcome will be the incidence of malaria, defined as the number of incident episodes per time at risk. Incident cases will include all treatments for malaria not proceeded by another treatment in the previous 14 days. Time at risk will begin at birth and will end when study participants reaches 12 months of age or early study termination (if prior to 12 months of age).

8.2.2. Secondary Outcomes

Secondary outcomes are summarized in Table 4 below.

Table 4. Secondary outcomes

Secondary outcome	Definition
Incidence of complicated malaria	Any treatment for malaria meeting criteria for severe malaria or danger sings
Incidence of hospital admissions	Admission to the pediatric ward for any cause
Infant mortality rate	Any deaths occurring after birth
Prevalence of anemia	Proportion of routine hemoglobin measurements < 10 g/dL & < 8 g/dL
Prevalence of asymptomatic parasitemia	Proportion of routine samples (by microscopy or LAMP) positive for asexual parasites.

8.2.3. Analyses

A modified intention-to-treat approach to all analyses will be used, including all live infants born and including all follow-up time until the study participants reach 12 months of age or early study termination regardless of whether the infant's mother received her assigned IPTp regimen.

Primary analysis. We will compare the incidence of malaria using Poisson or negative binomial regression models as described in section 8.1.3. If necessary, multivariate analyses will be performed to adjust for potential confounders and effect modifiers.

Secondary analyses. We will compare the incidence of complicated malaria and hospitalizations using Poisson or negative binomial regression models as described in section 8.1.3. We will compare the prevalence of anemia and asymptomatic parasitemia using generalized estimating equations with adjustments for repeated measures in the same study participant. If necessary, multivariate analyses will be performed to adjust for potential confounders and effect modifiers.

8.3. Hypothesis 3

We will test the hypotheses that a metabolic dating algorithm developed in a western setting can also be used for gestational dating in Uganda. We will also test the hypothesis that metabolic markers – especially those related to glucose metabolism can be used to identify newborns at more or less risk for death and serious morbidities.

8.3.1. Primary Outcomes

The primary outcomes will be preterm birth (< 37 completed weeks gestation) as determined by ultrasound dating and an existing gestational dating by metabolic profile algorithm. With respect to newborn outcomes, primary outcomes will be survival to 24 hours, 48 hours, 7 days, 30 days, and to the first year of life as well as diagnoses of any major morbidity including necrotizing enterocolitis, respiratory distress syndrome, and retinopathy of prematurity or blindness.

8.3.2. Secondary Outcomes

Secondary outcomes of interest include SGA birth outcome and the capacity of metabolic markers to correctly date a pregnancy in the context of intra-uterine growth restriction. Also, we will evaluate whether metabolic associations with survival and morbidity differ by SGA status.

8.3.3. Analyses

Analyses will focus on model validation for both the gestational dating and neonatal survival and morbidity using predictive models that have been or will be built using a population of more than 720,000 newborns in California using linear discriminate analyses.

Primary analysis. We will compare categorization of a newborn as preterm or not and specific week of gestation using the metabolic algorithm versus ultrasound dating. We will also compare predicted survival and diagnoses based on metabolic prediction versus true events and diagnoses. Performance will be measured using sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio.

Secondary analyses. We will compare performance statistics in newborns with and without SGA (sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio).

8.4. Sample size and power

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. The primary determinant of our target sample size was based on testing hypotheses 1. Based on our previous studies, we estimate that 95% 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 study will be powered to detect a significant difference in the risk of our composite adverse birth outcome between the 2 study arms. Considering pregnancies resulting in either low birth weight (< 2500 gm), small for gestational age, or premature delivery (< 37 weeks) in our previous study, the risk was 21.4% in the monthly DP arm compared to 30.4% in the 3 dose SP arm. Assuming the same risks, we would need to enroll 782 women (assuming 5% loss to follow-up during pregnancy) to have 80% power (2-side alpha = 0.05) to detect a 30% or greater reduction in the relative risk of our composite birth outcome. For hypothesis 2, we will have 80% power (2-side alpha = 0.05) to detect an 18-23% relative difference in the incidence of malaria between 0-12 months of age among children born to mothers randomized to the 2 IPTp arms assuming an incidence of malaria ranging from 3-5 episodes PPY among children born to mothers randomized to receive IPTp with monthly SP. Hypothesis 3 relies on the collected study data and samples to validate existing gestational dating, survival, and morbidity algorithms and as such, no limitations in statistical power are anticipated.

8.5. Data and Safety Monitoring Plan

The proposed study will conform to rigorous standard monitoring procedures, standardized reporting of adverse events (Adverse Event Report Forms are completed by study coordinators and sent immediately to the investigators), and regular review of the study by a Data and Safety Monitoring Board (DSMB). The PI has primary responsibility for the overall conduct of the study, including the safety of human subjects. The PI will ensure appropriate (1) conduct of the informed consent process (e.g. that informed consent is obtained before proceeding with study procedures); (2) enrollment of study subjects; (3) collection and analysis of data; (4) implementation of study procedures to ensure consistent monitoring of subjects for possible adverse events; (5) review of adverse events and reporting to the DSMB and the IRBs; and (6) maintenance of the privacy and confidentiality of study subjects. The PI maintains ultimate

responsibility for the project and for the safety of study participants. The PI will be in contact with the research team on a regular basis to review the progress of the study and address any human subject issues that occur. These discussions may involve adverse event prevention measures, recruiting of appropriate study subjects, research staff training on protection of human subjects, as well as occurrence of adverse events, unexpected incidents, or protocol problems.

8.5.1. Data and Safety Monitoring Board

A DSMB will be established by the study team in cooperation with the sponsor to assess at intervals the progress of a clinical trial, safety data, and critical efficacy variables and recommend to the sponsor whether to continue, modify or terminate a trial. The DSMB will have written operating procedures and maintain records of all its meetings, including interim results; these will be available for review when the trial is complete. The DSMB will be a separate entity from the US and host-country Institutional Review Boards (IRBs). The independence of the DSMB is intended to control the sharing of important comparative information and to protect the integrity of the clinical trial from adverse impact resulting from access to trial information. DSMB members will not participate in the study as investigators and will not have conflicts of interest regarding the study or the investigational product. The composition of the DSMB will include at minimum:

DSMB Chair, having experience and expertise in clinical trials
Scientist with expertise in malaria and malaria inpregnancy
Biostatistician with expertise in clinical trials.

A member of the sponsor, NICHD, will be invited to attend and thus have access to unblinded information, and control of dissemination of interim trial results within the sponsor organization. The DSMB will meet at least annually to review progress of the clinical trial and safety data.

The DSMB will review the study for progress and safety. The PI will provide information that will allow the DSMB to review and assess the following:

- The research protocol, informed consent documents and plans for data safety and monitoring;
- Periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, and other factors that can affect study outcome;
- Factors external to the study when relevant information, such as scientific or

therapeutic developments, may have an impact on the safety of the participants or the ethics of the trial;
- Study performance to make recommendations and assist in the resolution of problems;
- The safety of the study participants;
- The safety and scientific progress of the trial;
- The continuation, termination or other modifications of the trial based on the observed beneficial or adverse effects of the treatment under study;
- The confidentiality of the data and the results of monitoring; and
- Any problems with study conduct, enrollment, sample size and/or data collection.

The first meeting of the DSMB will take place prior to the initiation of the study to discuss the protocol and the Data Safety Monitoring Plan. Meetings of the DSMB shall be held according to the plan outlined above. Meetings shall be closed to the public because discussions may address confidential patient data. Meetings may be convened as conference calls as well as in person. An emergency meeting of the Board may be called at any time should questions of patient safety arise. The DSMB may request the presence of study investigators at such meetings.

The study PI will distribute study information to the DSMB at least 10 days prior to a scheduled meeting. The DSMB may request additions and other modifications to this information on a one-time or continuing basis. This information will consist of two parts: (1) information on study progress such as accrual, baseline characteristics, and other general information on study status and (2) any confidential data on study outcomes, including safety data. A formal report from the DSMB should be supplied to the PI within 6 weeks of each meeting. Each report should conclude with a recommendation to continue or to terminate the study. This recommendation should be made by formal majority vote. A recommendation to terminate the study should be transmitted to the PI, IRBs and NIH as rapidly as possible, by immediate telephone and fax if sufficiently urgent. In the event of a split vote in favor of continuation, a minority report should be contained within the regular DSMB report.

8.5.2. Interim safety analysis

Over the course of the trial, we will perform one interim safety analysis in addition to a final safety analysis for a total of two sequential evaluations of study safety for the pregnant women. The interim safety analysis will be performed when ½ of the study subjects have given birth. A standardized test statistic will be calculated for the incident rate ratio of significant adverse

events (grade 3/4 & SAEs). If this statistic exceeds the nominal critical value calculated using the error spending function (Table 5), then a statistically significant result will have been achieved at the time of that analysis. In that event, the sponsor will be notified and a report submitted for review by the Data Safety Monitoring Board (DSMB). The study team will present the results of the interim safety analyses to the DSMB, which will review the data and recommend a course of action.

Table 5. Schedule of interim safety analysis and boundaries to monitor study outcome

Number of Evaluable Subjects Accrued or % of Total Accrual	Test Statistic		Alpha	Cumulative Alpha
	Lower Bound	Upper Bound		
N=391 or 50% of accrual	-2.51	2.51	0.00601	0.01210
N=782 or 100% of accrual	-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 782 trial participants. We will utilize Programs for Computing Group Sequential Boundaries Using the Lan-DeMets Method.

8.5.3. 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.

9. DATA COLLECTION AND MONITORING

9.5. Record Keeping

All clinical data will be recorded onto standardized case record forms (CRFs) by study clinicians. Blood smear results will be recorded in a laboratory record book by the study laboratory technologists and then transferred to the case record forms by study coordinators, who will review the case record forms frequently for completeness and accuracy. Other laboratory data (CBC, ALT measurements) will be entered into the CRFs and hard copies of the original results will be stored in a file. Data will be entered directly from CRFs into a computerized database or transferred from the CRFs onto standardized data extraction forms and then into a computerized database. All computerized data will be double entered to verify accuracy of entry. Electronic data including all study databases and supporting electronic documentation

will be archived to large-scale digital tape on a daily basis. On a monthly basis, a complete backup tape will be transported off-site to the Kampala Data Management Center for rotating secure storage.

9.6. Data Quality Assurance and Monitoring

In order to insure data quality, the study Data Manager will perform a quarterly data quality audit. For this audit a 1% random sample of study forms entered into the data management system from the previous 2 weeks will be selected and compared for accuracy with the original case-report forms and source documents. In addition the study Data Manager will perform monthly reviews of the 100% double data entry data verification logs and the data management system audit trail log to identify potential data quality issues**.** The data will be owned by the Makerere University-University of California, San Francisco Research Collaboration.

10. HUMAN SUBJECTS

10.5. Subject Selection Criteria

Study subjects will be HIV-uninfected pregnant women and the children born to them who meet our selection criteria and provide informed consent. We plan to recruit only Ugandan residents and will recruit both pregnant women age 16 and above and male or female children.

10.6. Risks and Discomforts

10.6.1. Privacy

Care will be taken to protect the privacy of subjects, as described in this protocol. However, there is a risk that others may inadvertently see patients' medical information, and thus their privacy may be compromised.

10.6.2. Finger Pricks, Heel Sticks, and Venipuncture

Risks of these procedures include pain, transient bleeding and soft-tissue infection.

Risks of Study Medications
Risk of Sulfadoxine-Pyrimethamine

Although technically a combination regimen, SP is generally considered a single antimalarial agent, as its success depends on the synergistic action of its two component inhibitors of folate synthesis. SP is currently the standard of care of IPTp throughout sub-Saharan Africa. Adverse reactions listed on the SP package insert are blood dyscrasias (agranulocytosis, aplastic anemia, thrombocytopenia), allergic reactions (erythema multiforme and other dermatological conditions), gastrointestinal reactions (glossitis, stomatitis, nausea, emesis, abdominal pain, hepatitis, diarrhea), central nervous system reactions (headache, peripheral neuritis, convulsions, ataxia, hallucinations), respiratory reactions (pulmonary infiltrates), and miscellaneous reactions (fever, chills, nephrosis); based on widespread experience with the drug, all of these reactions appear to be uncommon or rare with short-term therapeutic use. The best-documented severe adverse effects with SP are cutaneous reactions, primarily noted when SP was used for long-term chemoprophylaxis in non-African populations. Reported rates of serious reactions to SP in the UK, with long-term use for chemoprophylaxis, were 1:2100, with 1:4900 serious dermatological reactions and 1:11,100 deaths.[74] Estimated rates of toxicity in the US were 1:5000-8000 severe cutaneous reactions and 1:11,000-25,000 deaths.[75] Clinical experience suggests that risks of severe toxicity are much lower with malaria treatment regimens in Africa.

The WHO currently recommends IPTp with SP in areas with moderate-to-high malaria transmission.[28] In a recent systematic review and meta-analysis of 7 trials from sub-Saharan Africa, IPTp with 3 or more doses of SP was associated with a higher birth weight and lower risk of low birth weight compared to 2 doses of SP. In addition there were no differences in the rates of serious adverse events between the two groups.[76]

Risk of Dihydroartemisinin-piperaquine

Risks associated with DP among adults and children. Minyt and colleagues conducted a systematic review of DP efficacy and safety for treatment of malaria using data from 14 clinical trials involving adults and children.[77] There were 2636 study participants treated with DP in 13 trials in which safety data were reported. Overall, DP was associated with fewer adverse events compared to comparator medications. The most common adverse events were dizziness, nausea and vomiting, though generally the medication was well-tolerated by both adults and children (Table 6). Of note, the only serious adverse events in these 14 studies included 5 deaths (2 adults, 3 children) that were thought unrelated to DP.

Table 6. Summary of adverse events following treatment with dihydroartemisinin-piperaquine

Table 2 Summary of adverse events following treatment with dihydroartemisinin-piperaquine

Study/site	Nausea	Vomiting	Anorexia	Dizziness	Headache	Diarrhoea	Abdominal pain	Sleep disturbance	Neuropsychiatric adverse events	Cardiovascular dysfunction	Haematological dysfunction	Hepatological dysfunction	Dermatological adverse events	Total no. of evaluated patients
Denis et al. (2002) Cambodia	5 (4.7)	NR	4 (3.8)	5 (4.7)	0 (0)	5 (4.7)	5 (4.7)	NR	NR	NR	NR	NR	1 (0.9)	106
Wilairatana et al. (2002) Thailand	8 (3.4)	0 (0)	NR	11 (4.7)	9 (3.8)	NR	NR	0 (0)	0 (0)	NR	NR	NR	NR	234
Ying et al. (2003) China	3 (5)	1 (1.6)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	60
Hien et al. (2004) Vietnam	8 (2)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	399
Karunajeewa et al. (2004) Cambodia	NR	NR	NR	NR	NR	NR	NR	NR	NR	0 (0)	0 (0)	0 (0)	NR	62
Hung et al. (2004) Cambodia	3 (4)	NR	1 (1)	12 (14)	30 (36)	NR	9 (11)	1 (1)	NR	NR	NR	NR	NR	80
Giao et al. (2004) Vietnam	NR	NR	NR	NR	1 (1.2)	NR	NR	NR	NR	NR	NR	NR	1 (1.2)	82
Ashley et al. (2004) Bangkok, Thailand	11 (9.3)	NR	NR	9 (7.6)	12 (10.2)	NR	NR	NR	NR	NR	NR	NR	NR	118
Ashley et al. (2004) Mae Sot, Thailand	30 (8.5)	18 (1.7)	NR	51 (14.5)	NR	20 (5.7)	35 (9.9)	20 (7.4)	NR	0 (0)	0 (0)	0 (0)	1 (0.3)	353
Ashley et al. (2005) Thailand	37 (11.1)	23 (6.9)	NR	37 (11.1)	NR	33 (9.9)	28 (8.4)	42 (12.6)	NR	NR	NR	NR	3 (0.9)	333
Tangpukdee et al. (2005) Thailand	5 (4.2)	0 (0)	0 (0)	4 (3.3)	4 (3.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	120
Smithuis et al. (2006) Myanmar	39 (11.9)	6 (1.8)	10 (8.3)	104 (31.8)	0 (0)	11 (9.2)	3 (0.9)	0 (0)	NR	NR	0 (0)	NR	0 (0)	327
Mayxay et al. (2006) Lao PDR	6 (5.5)	3 (3)	10 (9)	12 (11)	11 (10)	8 (7)	12 (11)	17 (15.5)	0 (0)	NR	NR	NR	0 (0)	110
Karema et al. (2006) Rwanda	2 (0.8)	5 (2)	3 (1.2)	1 (0.4)	2 (0.8)	8 (3.2)	6 (2.4)	0 (0)	0 (0)	NR	NR	NR	0 (0)	252

NR: not reported.
Data are given as *n* (%).

More recently Lwin et al conducted a randomized controlled trial of monthly vs. bimonthly DP IPT among 961 adults at high risk of malaria at the Northwest border of Thailand.[78] Overall, 69% of the participants included in the final analysis reported at least one adverse event. There was no difference in the proportion of those reporting at least one adverse event among participants in the monthly vs. bimonthly vs. placebo arms. There was an increased risk of joint pain among participants randomized to the placebo arm, but otherwise there were no differences noted in adverse events by study arm. There was only one serious adverse event not related to the use of DP (Table 7).

Table 7. Frequency, incidence, and risk of the 20 most frequently reported adverse events

Adverse event	No. of participants in indicated treatment group who reported adverse event (%)[a]			Incidence rate for indicated group[b]			IRR (95% CI) for DPm and DPalt	IRR (95% CI) for DPm and placebo
	DPm	DPalt	Placebo	DPm	DPalt	Placebo		
Dizziness	127 (32.8)	119 (31.2)	49 (25.4)	0.66	0.62	0.69	0.93 (0.72–1.21)	1.05 (0.74–1.47)
Headache	115 (29.7)	108 (28.4)	49 (25.4)	0.60	0.56	0.69	0.93 (0.71–1.23)	1.16 (0.81–1.63)
Soft stool	99 (25.6)	82 (21.5)	29 (15.0)	0.52	0.42	0.41	0.82 (0.61–1.12)	0.80 (0.51–1.22)
Abdominal pain	67 (17.3)	57 (15.0)	28 (14.5)	0.35	0.30	0.40	0.85 (0.58–1.22)	1.14 (0.70–1.79)
Muscle pain	65 (16.8)	63 (16.5)	35 (18.1)	0.34	0.33	0.50	0.96 (0.67–1.38)	1.46 (0.94–2.24)
Fever	47 (12.1)	50 (13.1)	27 (14.0)	0.24	0.26	0.38	1.06 (0.70–1.61)	1.56 (0.94–2.56)
Cough	44 (11.4)	42 (11.9)	17 (8.81)	0.23	0.22	0.24	0.95 (0.61–1.48)	1.05 (0.56–1.88)
Joint pain	40 (10.3)	50 (13.1)	30 (15.5)	0.21	0.26	0.42	1.24 (0.80–1.93)	2.04 (1.23–3.36)
Dry mouth	33 (8.53)	33 (8.66)	11 (5.70)	0.17	0.17	0.16	0.99 (0.60–1.66)	0.91 (0.41–1.84)
Insomnia	34 (8.79)	36 (9.45)	17 (8.81)	0.18	0.19	0.24	1.05 (0.64–1.73)	1.36 (0.71–2.50)
Sleep disturbance.	32 (8.27)	33 (8.66)	14 (7.25)	0.17	0.17	0.20	1.03 (0.61–1.72)	1.19 (0.59–2.29)
Anorexia	24 (6.20)	26 (6.82)	10 (5.18)	0.13	0.13	0.14	1.08 (0.59–1.96)	1.13 (0.48–2.46)
Nausea	22 (5.68)	24 (6.30)	11 (5.70)	0.11	0.12	0.16	1.09 (0.58–2.03)	1.36 (0.60–2.92)
Diarrhea	20 (5.17)	24 (6.30)	5 (2.59)	0.10	0.12	0.07	1.19 (0.63–2.28)	0.68 (0.20–1.87)
Itching	15 (3.88)	8 (2.10)	7 (3.63)	0.08	0.04	0.10	0.53 (0.19–1.33)	1.27 (0.44–3.31)
Vomiting	15 (3.88)	10 (2.62)	3 (1.55)	0.08	0.05	0.04	0.66 (0.27–1.58)	0.54 (0.10–1.92)
Fatigue	15 (3.88)	17 (4.46)	11 (5.70)	0.08	0.09	0.16	1.13 (0.53–2.42)	1.99 (0.83–4.65)
Skin rash	4 (1.03)	4 (1.05)	2 (1.04)	0.02	0.02	0.03	0.99 (0.19–5.34)	1.36 (0.12–9.48)
Palpitation	3 (0.78)	5 (1.31)	3 (1.55)	0.02	0.03	0.04	1.66 (0.32–10.7)	2.72 (0.36–20.3)
Back pain	2 (0.52)	4 (1.05)	2 (1.04)	0.01	0.02	0.03	1.99 (0.29–22.0)	2.72 (0.20–37.5)

[a] Number of participants who reported an adverse event at least once.
[b] Per person-year at risk.

Risks associated the use of DP for IPT in infants. Relevant to this protocol is the PROMOTE Chemoprevention study, an open label randomized clinical trial conducted by our group evaluating the protective efficacy of 3 different chemoprevention regimens against malaria compared to the current standard of care of no chemoprevention. This was the first study to evaluate the safety of DP when used for IPT in infants. DP was shown to be very well-tolerated and associated with a significantly lower rate of all grade 3-4 adverse events, elevated temperature, anemia, and thrombocytopenia compared to the control arm (Table 8).[79] In addition, 145 ECGs performed 19 study participants randomized to DP documented all QTc intervals to be within normal limits.

Table 8. Comparison of adverse events among children between 6-24 months of age

Characteristic	Number of events (incidence per PYAR) by treatment arm			
	Control	Monthly SP	Daily TS	Monthly DP
All grade 3-4 adverse events	169 (1·159)	202 (1·415)	135 (0·914)	87 (0·611) ‡
All serious adverse events	26 (0·178)	52 (0·364)	29 (0·196)	13 (0·091)
Grade 3-4 adverse events possibly related to study drugs	N/A	8 (0·056)	8 (0·054)	3 (0·021)
Individual grade 3-4 adverse events*				
Elevated temperature	79 (0·542)	78 (0·546)	58 (0·393)	46 (0·323) †
Anaemia	56 (0·384)	86 (0·602)	47 (0·318)	24 (0·168) †
Thrombocytopenia	18 (0·123)	17 (0·119)	9 (0·061)	5 (0·035)α
Elevated aspartate aminotransferase	7 (0·048)	8 (0·056)	6 (0·041)	3 (0·021)
Elevated alanine aminotransferase	4 (0·027)	4 (0·028)	4 (0·027)	3 (0·021)
Neutropenia	3 (0·021)	6 (0·042)	2 (0·014)	1 (0·007)

PYAR = person-years at risk.

SP = sulfadoxine-pyrimethamine. TS = trimethoprim-sulfamethoxazole. DP = dihydroartemisinin-piperaquine.

* Only includes those with at least 5 total events.

α p-value < 0.05 compared to control group; † p-value < 0.01 compared to control group; ‡ p-value < 0.001 compared to control group.

Risks associated with DP during pregnancy. While data are limited, preclinical animal studies [80-82] and clinical studies involving pregnant women [22,23] have not demonstrated significant safety concerns with the use of DP. In a recent study from Kenya, serious adverse events were less frequent in women randomized to IPTp with DP compared to women randomized to IPTp with SP.[25] In a recent study from our group comparing IPTp with 3 dose SP vs. 3 dose DP vs. monthly DP in Tororo Uganda, study drugs were well tolerated and there were no significant differences in the risk of adverse events.[26] Vomiting occurred < 0.2% of the time after administration of study drugs with no differences between study arms (Table 9). There were no significant differences in the incidence of any adverse events apart from dysphagia, which was higher in the monthly DP arm compared to the 3 dose DP arm. All episodes of dysphagia were mild in severity and we are not aware of any previous reports of DP being associated with dysphagia. Only one grade 3-4 adverse event was possibly related to study drugs; an episode of anemia which occurred both after the 1st and 2nd doses of monthly DP (study drug was subsequently withheld after the 2nd dose)(Table 9). Among 42 women who underwent ECG measurements at 28 weeks gestational age, all pre- and post-dosing QTc intervals were within normal limits ($\leq$ 450 msec) and no clinical adverse events consistent with cardiotoxicity occurred during the course of the study. Median change in QTc intervals was greater in the 3 dose DP (20 msec) and monthly DP (30 msec) arms compared to the 3 dose SP arm (5 msec), but these differences were not statistically significant.

Table 9. Measures of safety and tolerability from recent IPTp study conducted in Tororo, Uganda

Outcome	Treatment arm		
	3 dose SP	3 dose DP	Monthly DP
Prevalence measures	**no./total no. (%)**	**no./total no. (%)**	**no./total no. (%)**
Vomiting following administration of study drugs			
Observed after administration of 1st dose in clinic	2/617 (0.32)	0/542 (0)	1/594 (0.17)
Reported after administration of 2nd or 3rd dose at home	2/1222 (0.16)	0/1067 (0)	5/1180 (0.42)
Incidence measures	**Events [a]**	**Events [a]**	**Events [a]**
Individual adverse events of any severity [b]			
Abdominal pain	172 (3.14)	122 (2.52)	132 (2.47)
Cough	94 (1.72)	71 (1.47)	77 (1.44)
Headache	90 (1.64)	70 (1.45)	78 (1.46)
Chills	21 (0.38)	14 (0.29)	12 (0.22)
Diarrhea	12 (0.22)	10 (0.21)	13 (0.24)
Malaise	16 (0.29)	9 (0.19)	8 (0.15)
Dysphagia	9 (0.16)	2 (0.04)	14 (0.26) [c]
Vomiting	8 (0.15)	8 (0.17)	8 (0.15)
Nausea	2 (0.04)	4 (0.08)	2 (0.04)
Urinary tract infection	3 (0.05)	2 (0.04)	2 (0.04)
Anorexia	2 (0.04)	0 (0)	4 (0.07)
Individual grade 3-4 adverse events			
Anemia	12 (0.22)	4 (0.08)	6 (0.11)
Congenital anomaly	2 (0.04)	4 (0.08)	0 (0)
Stillbirth	1 (0.02)	1 (0.02)	1 (0.02)
Thrombocytopenia	2 (0.04)	0 (0)	0 (0)
Threatened abortion	1 (0.02)	0 (0)	0 (0)
Retained products of conception	0 (0)	1 (0.02)	0 (0)
Preeclampsia	0 (0)	0 (0)	1 (0.02)
Hypotension	0 (0)	0 (0)	1 (0.02)
Pyelonephritis	0 (0)	1 (0.02)	0 (0)
Respiratory distress	0 (0)	1 (0.02)	0 (0)
All grade 3-4 adverse events	18 (0.33)	12 (0.25)	9 (0.17)
Grade 3-4 adverse events possibly related to study drugs	0 (0)	0 (0)	1 (0.02)
All serious adverse events	6 (0.11)	9 (0.19)	4 (0.07)

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

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

[c] P=0.02 comparing monthly DP with 3 dose DP

10.6.4 Risks of reporting and treatment of incidental findings

Reporting the results of the experimental metabolite assay: Risks undue anxiety in families about diagnoses and child may not ultimately have.

Treatment of hypothyroidism incidentally identified by study procedures: Risks of standard of care treatment include the side effects of treatment with thyroxine which include tachycardia, arrhythmias, and aspiration from avid suckling.

Treatment of sickle cell disease incidentally identified by study procedures: Risks of standard of care treatment of sickle cell disease include the side effects of folate which include flushing and allergic reaction, and antibiotic prophylaxis, which include allergic reaction, serum sickness, and hemolytic anemia.

10.7 Treatment and Compensation for Injury

If the participant is injured as a result of being in this study, treatment will be available through Masafu General Hospital. The costs of the treatment may be covered by the study sponsor, NICHD, depending on a number of factors. A clinical trial liability insurance policy has been provided to cover all trial participants for "Bodily injury" caused to the health of the person provided that causal-connection between harm, bodily injury or death resulting from participation in the trial is established.

10.8 Costs to the Subjects

There will be no cost to the participant or their parents/guardians for participation in this study.

10.9 Reimbursement of Subjects

Participants will not be paid for their participation in the study. The study will provide all routine medical care, including evaluations, medications available in our clinic, and cost of any transportation free of charge. In addition, we will reimburse the cost of consultation for referrals made by study physicians to other clinics and services and visits the cost of most diagnostic tests (including laboratory test, X-rays, and ultrasounds) and medications resulting from referrals by the study team, using available funds. However, reimbursement of all diagnostic tests and treatment recommended outside the study clinic cannot be guaranteed in all circumstances.

10.10 Institutional Review Board (IRB) Review and Informed Consent

This protocol, all procedures and consent forms, and any subsequent modifications must be reviewed and approved by the IRBs of all the participating institutions in both the U.S. and in Uganda. This includes the UCSF Committee on Human Research (CHR), the MU School of Medicine - Research and Ethics Committee (SOM-REC) or MU School of Biomedical Sciences – Research and Ethics Committee (SBS-REC), and the Uganda National Council of Science and Technology (UNCST).

All consent forms will be translated into the local languages (Samia, Swahili, Luganda, and English) and back-translated into English to ensure correct use of language. Consent forms will be read aloud to parents by trained study interviewers. The informed consent will describe the purpose of the study, all the procedures involved, and the risks and benefits of participation. Study physicians will ask parents/guardians of study participants to summarize the study and explain the reasons why they want to participate. Either a signature or a thumbprint (for parents/guardians who cannot read) will be acceptable to confirm informed consent for participation in the study.

Participant's parents / guardians will be approached to have the "consent amendment" explained, and then either sign or decline participation at a routine visit. If an urgent value in the metabolite assay has prompted follow up prior to the next scheduled visit, they will be contacted by phone and asked to come to the study clinic to review test results; the consent amendment will be reviewed and consent obtained/declined at that time. "

10.11 Definition of Parent/Guardianship.

For this study, we will define a parent/primary guardian of the child enrolled in the study as the women giving birth to the child. However to the extent possible, consent of the father will be also obtained.

12. PUBLICATION OF RESEARCH FINDINGS

The findings from this study may be published in a medical journal. No individual identities will be used in any reports or publications resulting from the study. The researchers will publish results of the trial in accordance with NICHD, UNCST, UCSF, and Makerere University guidelines.

13. BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel involved in the drawing of blood, exposure to blood and secretions, and shipping and handling of all specimens for this study. We will follow the current guidelines set forth by the Centers for Disease Control and Prevention and the NIH. All infectious specimens will be transported using packaging mandated in the Federal Code of Regulations, CDC 42 CFR Part 72.

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15. APPENDICES

Appendix A. Information Sheet

UCSF

A STUDY ON PREVENTING MALARIA IN PREGNANT WOMEN AND YOUNG CHILDREN

Makerere University in Uganda and the University of California, San Francisco in the United States are combining efforts in Busia district, Uganda to study new ways of using malaria drugs to prevent malaria in pregnant women and their babies.

Malaria during pregnancy can have a harmful effect on you or your child. We want to study 2 different malaria drugs to see if they can be used to prevent malaria if taken during pregnancy.

Our study clinic is located at Masafu General Hospital next to the antenatal clinic and is open every day from 8:00 am to 5:00 pm

We want to enroll pregnant women who are at least 16 years old and follow them during pregnancy.

We will then follow the child born up to 12 months of age.

Women and children in this study will receive free medical care

We shall also give reimbursement for transport to and from our study clinic

For more information, please come to our study clinic where our doctors will be happy to talk to you and see if you and your baby can be in the study.

Appendix B. Determination of Gestational Age

Gestational age will be based on the first day of the last menstrual period (LMP) and the earliest available ultrasound performed at ≥ 6 weeks gestation. The estimated due date (EDD) is calculated as 280 days following the LMP.

If the first available ultrasound is consistent with a gestational age of 6 to 12 weeks, and the ultrasound gestational age is within 7 days of that given LMP, then the LMP will be used to determine gestational age. However, if the ultrasound gestational age differs from the LMP gestational age by more than 7 days, then the ultrasound will be used to determine gestational age.

If the first available ultrasound is consistent with a gestational age of 13-24 weeks, and the ultrasound gestational age is within 14 days of that given by the LMP, then the LMP will be used to determine gestational age. However, if the ultrasound gestational age differs from the LMP by more than 14 days, then the ultrasound will be used to determine gestational age.

If the first available ultrasound is consistent with a gestational age of 25 weeks or more, and the ultrasound gestational age is within 21 days of that given by the LMP, then the LMP will be used to determine gestational age. However, if the ultrasound gestational age differs from the LMP by more than 21 days, then the ultrasound will be used to determine gestational age. Care here should be taken to rule out intrauterine growth restriction (IUGR).

If the LMP is not known, then the earliest ultrasound performed at ≥ 6 weeks will be used to determine gestational age.

Appendix C. Household survey

The household survey will be administered through a completely paperless QDS software system using hand-held tablet computers. A list of questions that will be used in the survey is provided below.

Ques. No.	Variable Name	Question
Section 1: Identification		
1	VISDATE	Date of final visit
2	STARTIME	Start time of interview
4	BC	Birth Cohort Number
3	PPTIDA	Study Participant ID
5	PPTID	To ensure data integrity, please re-enter the Participant ID.
7	INTNUM	Interviewer number
8	AGREE	Are you going to conduct the interview with this household?
Section 2: Household Characteristics		
9	SWATER	What is the main source of drinking water for members of your household?
10	OTHERSCS	Specify other source of water
11	TFACLTY	What kind of toilet facility do members of your household usually use?
12	OTHERFCY	Specify other kind of toilet facilities
13	ELECTRIC	Does your household have... ...Electricity?
14	RADIO	...Radio?
15	CASSETTE	…Cassette player?
16	TV	…Television?
17	MOBILE	…Mobile phone?
18	PHONE	…Fixed phone?
19	FRIDGE	…Refrigerator?
20	TABLE	…Table?
21	CHAIR	…Chairs?
22	SOFA	…Sofa set?
23	BED	…Bed?
24	CUPBOARD	…Cupboard?
25	CLOCK	…Clock?
26	FUELTYPE	What type of fuel does your household mainly use for cooking?
27	OTHERFUE	Specify other type of fuel used
28	SENERGY	What is the main source of energy for lighting in the household?
29	OTHERENG	Specify other source of energy for lighting
30	MMFLOOR	MAIN MATERIAL OF THE FLOOR RECORD OBSERVATION. MARK ONLY ONE.
31	OTHERMMF	Specify other material of the floor
32	MMROOF	MAIN MATERIAL OF THE ROOF. RECORD OBSERVATION. MARK ONLY ONE.

33	OTHERMMR	Specify other material of the roof
34	MMEWALLS	MAIN MATERIAL OF THE EXTERIOR WALLS. RECORD OBSERVATION. MARK ONLY ONE.
35	OTHERMME	Specify other material of the exterior walls
36	HHROOMS	How many rooms in your household are used for sleeping? (INCLUDING ROOMS OUTSIDE THE MAIN DWELLING) If there are 15 or more rooms, enter 15
37	HHSPACES	How many sleeping spaces like mats, mattresses, or beds are available in your household? If there are 25 or more sleeping places, enter 25
38	WATCH	Does any member of your household own or have… …A watch?
39	BICYCLE	….A bicycle?
40	SCOOTER	…A motorcycle or motor scooter?
41	CART	…An animal-drawn cart?
42	CAR	…A car or truck?
43	MBOAT	…A boat with a motor?
44	NOMBOAT	…A boat without a motor?
45	BANKACCO	…A bank account?
46	NUMALAND	How many acres of agricultural land do members of this household own?
47	DMARKT	How far is it to the nearest market place?
48	HHMEALS	Now I would like to ask you about the food your household eats. How many meals does your household usually have per day?
49	HHNUMT	In the past week, on how many days did the household eat meat?
50	HHPSF	How often in the last year did you have problems in satisfying the food needs of the household?
51	DHFCTY	How far is it to the nearest health facility?
52	MTHFCTY	If you were to go this facility,how would you <u>most likely</u> go there?
53	OTHERMTH	Specify other means of transport to the health facility
54	PSPRAY	At any time in the past 12 months, has anyone asked permission to come into your dwelling to spray the interior walls against mosquitoes?
55	GPSPRAY	Did you grant them permission to spray the interior walls of your dwelling?
56	RGSPRAY	What was the primary reason that you did not grant permission to spray the interior walls of your dwelling against mosquitoes?
57	OTHERRGS	Specify other reasons for not granting permission to spray the interior walls of your dwelling against mosquitoes
58	TSPRAY	How many months ago was the dwelling last sprayed?
59	WSPRAY	Who sprayed the dwelling?
60	OTHERWSP	Specify other people who sprayed the dwelling
61	DSPRAY	Did you pay for the dwelling to be sprayed?
62	PPWALLS	Since the spraying, have the walls in your dwelling been plastered or painted?
63	TPPWALLS	How many months ago were the walls plastered or painted?
64	MSPRAY	In the past 12 months, have you seen or heard any messages about spraying the interior walls of your dwelling against mosquitoes?
65	MSGA	Where did you hear or see message(s)? ...Radio?

66	MSGB	...TV?
67	MSGC	...Newspaper/Leaflet?
68	MSGD	…Health worker/CMD?
69	MSGE	…Neighbour/Relative/Friend
70	MSGF	...Community Leader?
71	MSGG	…Village public adress system
72	MSGH	...Don't know
73	MSGI	…Other
74	OTHERMSG	Specify other
75	AHWKER	Is there a community health worker (community medicine distributor/CMD, village health team/VHT, community own resource person/CORP) who distributes malaria medicines in your village or community?
76	AMCHWKER	Does the community health worker currently have malaria medicines available?
Section 3: Study Participants Sleeping Area Characteristics All questions in this section will be repeated with variable names entopen1, entcov1 , etc. for each entryway and window in the study participants room		
77	SRENTRY	OBSERVATION: How many entryways into the room are there?
78	ENTOPN1	OBSERVATION: Does it open to the outside?
79	ENTCOV1	OBSERVATION: Is the entry way covered?
80	ETMM1	OBSERVATION: Main material is the covering made of.
81	OTHCOV1	Specify Other covering type
82	SRWINDOW	OBSERVATION: How many windows are in the room?
83	WNDCOV1	OBSERVATION: Is the window covered?
84	WNDOPN1	OBSERVATION: Does the window open to the outside
85	SREAVES	OBSERVATION: Does the room have eaves?
86	EAVESCOV	OBSERVATION: If room has eaves, are the eaves covered?
87	EAVESOPN	OBSERVATION: Do the eaves open to the outside?
88	AIRBRICK	OBSERVATION: Does the room have airbricks?
89	AIRBRCOV	OBSERVATION: If the room has airbricks, are the airbricks covered?
90	AIRBROPN	OBSERVATION: Do the airbricks open to the outside?
91	AIRBRNUM	OBSERVATION: How many airbricks are in the room?
92	SLEEP	Where does the study participant usually sleep?
93	OTHERSL	Specify other sleeping area
94	SRSLNUM	Usually, how many people sleep in the same room as the study participant (excluding the study participant)?
95	SRSLNUM5	How many of those people are under 5 years old (excluding the study participant)?
96	SLAREAS	How many sleeping areas are in the room where the study participant sleeps?
97	SASLNUM	How many people sleep in the same bed/sleeping area as the study participant under the mosquito net (excluding the study participant)?
98	SASLNUM5	How many of those people are under 5 years old (excluding the study participant)?
Section 4: Bednets All questions in this section will be repeated with variable names obs2,mnths2,where2, etc. for each mosquito net in the household (hhnumnet)		
99	HHAMNETS	IMMEDIATELY BEFORE Enrollment, did your household have any mosquito nets that can be used while sleeping?
100	HNUMNETS	IMMEDIATELY BEFORE study enrollment, How many mosquito nets did your household have?
101	OBS1	May I have a look at (all) the net(s) to establish the brand?
102	MNTHS1	How many months ago did your household obtain the mosquito net?
103	WHERE1	Where did you get the mosquito net from?
104	SPCFRO1	Specify other sources of the mosquito net
105	BRAND1	OBSERVE OR ASK THE BRAND OR TYPE OF MOSQUITO NET.
106	OTHERB1	Specify other brands or types of mosquito net

107	SMNET1	Since you got the mosquito net, was it ever soaked or dipped in a liquid to repel mosquitoes or bugs?
108	TSMNET1	How many months ago was the net last soaked or dipped?
109	SLPNET1	Did anyone sleep under this mosquito net last night?
110	NUSED1A	What are some of the reasons why this net was not used? ...Too hot
111	NUSED1B	...Don't like smell
112	NUSED1C	...No mosquitoes
113	NUSED1D	...Net too old/too many holes
114	NUSED1E	...Net not hung
115	NUSED1F	...Net too dirty
116	NUSED1G	...Net no longer kill insects
117	NUSED1H	...Don't know
118	NUSED1I	...Other
119	NTHUNG1	If not hung, why not?
120	OTHRNT1	Specify other reason why the net was not hung.
Section 5: Interviewer Details		
122	STOPTIME	End time of interview
123	VSTATUS	Result of Visit
124	OVSTATUS	Specify other result
125	TOTVISIT	Total number of visits
126	COMMENTS	Interviewer's Comments

Appendix D. Administration of study drugs and placebos

Timing of administration of study drugs during pregnancy

Weeks of gestation	Monthly SP (treatment arm A)	Monthly DP (treatment arm B)
16	SP + DP placebo	DP + SP placebo
20	SP + DP placebo	DP + SP placebo
24	SP + DP placebo	DP + SP placebo
28	SP + DP placebo	DP + SP placebo
32	SP + DP placebo	DP + SP placebo
36	SP + DP placebo	DP + SP placebo
40	SP + DP placebo	DP + SP placebo

Appendix E. WHO Criteria for Severe Malaria and Danger Signs

Criteria for severe malaria

Cerebral malaria - defined as unarousable coma not attributable to any other cause in a patient with falciparum malaria
Generalized convulsions (≥ 3 convulsions over 24 hours period)
Severe normocytic anemia (Hb < 5 gm/dL)
Hypoglycemia
Metabolic acidosis with respiratory distress
Fluid and electrolyte disturbances
Acute renal failure
Acute pulmonary edema and adult respiratory distress syndrome (ARDS)
Circulatory collapse, shock, septicemia ("algid malaria")
Abnormal bleeding
Jaundice

Danger signs (in children only)

Less than 3 convulsions over 24 hour period
Inability to sit up or stand
Vomiting everything
Unable to breastfeed or drink
Lethargy

Appendix F. Uganda Ministry of Health Guidelines for Routine Care of Pregnant and Postpartum Women, and Newborns

In addition to receiving medical care as described above in the protocol, women and infants enrolled in the study will receive standard routine prenatal and postpartum care according to Uganda Ministry of Health guidelines. These standard procedures are subject to availability at the local health care facilities.

Routine antenatal care. Women enrolled in the study will receive routine care as designated in the Uganda Ministry of Health Guidelines. Routine antenatal care includes screening and treating for syphilis and syndromic management of sexually transmitted infections (STIs). Pregnant women will receive iron and folic acid supplementation. In addition, women will be given multivitamins that will be given once daily. In addition, women receive mebendazole 500mg as a single dose as early as possible after the 1st trimester. Each antenatal visit also includes blood pressure assessment and urine dip stick for proteinuria.

Routine intrapartum/delivery care. Routine delivery care for in-hospital births will include labor management by the midwifery staff and management of obstetrical complications as per Ministry of Health guidelines. Immediate postpartum infant care will include polio and BCG immunization, ophthalmic tetracycline, and vitamin K.

Routine postpartum care. All postpartum women will receive vitamin A supplementation (200,000 IU) immediately following delivery. Depending on clinical circumstances and based on local standard of care, women may receive 2 weeks of multivitamins twice a day. Common indications for postpartum multivitamins include anemia, postpartum hemorrhage and prolonged labor. Women will be seen at 1 week after delivery as per Ugandan standards of care. Women also undergo a 6 weeks postpartum visit as part of routine care. These visits include an abdominal exam, syndromic management of STIs, and follow-up on any obstetrical complications that occurred. In addition, women receive vitamin A at this visit, if not given immediately postpartum, and are continued on iron and folic acid supplementation. Pelvic and breast exam will be done if clinically indicated. Contraceptive counseling is performed at this visit as is a nutritional assessment and infant feeding and support. Screening for cervical cancer will be performed postpartum by clinical staff if available at Masafu General Hospital.

Routine infant care. Infants will be referred to Masafu General Hospital antenatal clinic for routine immunizations at 6 weeks, 10 weeks, 14 weeks, 6 and 9 months of life.

Appendix G. 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	
Obstetrical ultrasound[2]	X									
Blood collected by phlebotomy for CBC, ALT, and immunology studies	X		X		X		X		X	
Blood collected by finger prick for blood smear and dried blood spot	X	X	X	X	X	X	X	X	X	
Blood collected by finger prick for standard RDT and qPCR	X	X	X	X	X	X	X	X		
Blood collected by phlebotomy for experimental RDT	X		X		X		X			
Routine assessment in the study clinic[3]	X	X	X	X	X	X	X	X		X
ECG (pre and post study drugs) [4]			X		X		X			
Administration of study drugs		X	X	X	X	X	X	X		
Collection of cord blood and placental blood/tissue									X	
Collection of heel stick blood in newborn									X	
Labor and delivery documentation[5]									X	
Standard Care										
Obstetrical exam[6]	X	X	X	X	X	X	X	X	X	X
Syphilis screening	X									
Iron and Folic Acid	X	X	X	X	X	X	X	X		
Prenatal vitamins	X									
Mebendazole[7]		X								
Screening for non-malarial parasitic infections[8]		X								
Vitamin A[9]									X	
Insecticide treated bednet	X								X	

***** Only if study subject enrolled prior to 16 weeks gestation; If the woman is enrolled between 18 or 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. Ultrasound will be done to confirm intrauterine pregnancy and estimate gestational age at enrollment. See Appendix B for dating criteria.
3. 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.
4. Also includes collection of finger prick capillary plasma sample just prior to performing the post study drug ECG
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.

6. Obstetrical exam includes estimation of gestational age at study entry, fundal height measurement, fetal heart tones and urine dipstick for protein. A cervical exam will also be performed at screening and during antepartum study visits as clinically indicated.
7. Mebendazole is typically given as 500mg as a single dose as early as possible after the 1st trimester (16 or 20 week visit).
8. Screening for non-malarial parasitic infections will be done prior to administering Mebendazole and will include stool ova and parasite examination, circulating filarial antigens, and blood smear for microfilaremia.
9. Vitamin A supplementation is dosed as 200,000 IU.

Appendix H. Schedule of routine assessments and procedures in infants

Weeks of age	Blood collected by finger prick		Blood collected by phlebotomy for immunology studies and hemoglobin measurement	Stool collected	Routine assessment in the study clinic
	Blood smear	Dried blood spots			
1					X
4	X	X		X	X
6					X
8	X	X			X
12	X	X	X		X
16	X	X			X
20	X	X			X
24	X	X			X
28	X	X	X		X
32	X	X			X
36	X	X			X
40	X	X			X
44	X	X			X
48	X	X			X
52	X	X	X	X	X

Appendix I. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events Version 2.0 November 2014

Citation:
U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0. [November 2014]. Available from: http://rsc.tech-res.com/Document/safetyandpharmacovigilance/DAIDS_AE_GRADING_TABLE_v2_NOV2014.pdf

Selected sections of the above document are listed here:

Introduction

The Division of AIDS (DAIDS) oversees clinical trials throughout the world which it sponsors and supports. The clinical trials evaluate the safety and efficacy of therapeutic products, vaccines, and other preventive modalities. Adverse event (AE) data collected during these clinical trials form the basis for subsequent safety and efficacy analyses of pharmaceutical products and medical devices. Incorrect and inconsistent AE severity grading can lead to inaccurate data analyses and interpretation, which in turn can impact the safety and well-being of clinical trial participants and future patients using pharmaceutical products.

The DAIDS AE grading table is a shared tool for assessing the severity of AEs (including clinical and laboratory abnormalities) in participants enrolled in clinical trials. Over the years as scientific knowledge and experience have expanded, revisions to the DAIDS AE grading table have become necessary.

The Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0 replaces the grading table published in 2004 and updated in 2009. In version 2.0, AEs not previously included, but which now are deemed medically important events, are included while other AEs have been removed. Some AE severity grading descriptions have been revised to more appropriately reflect the presentation of these events in clinical settings and their impact on clinical trials. For example, DAIDS performed an extensive literature search and reviews of select DAIDS clinical trial data in revising certain hematology parameters (i.e., hemoglobin, white cell counts, and absolute neutrophil counts). DAIDS also took into consideration the U.S. Food and Drug Administration's guidance regarding the use of local laboratory reference values and ethnic differences among certain healthy adolescent and adult populations in defining parameter limits. Finally, the revised DAIDS AE grading table also contains an updated glossary and acronyms section, an expanded instructions for use section, and an appendix that provides more age-specific information for an AE of concern to DAIDS.

Instructions for Use

General Considerations

The Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0 consists of parameters, or AEs, with severity grading guidance that are to be used in DAIDS clinical trials for safety data reporting to maintain accuracy and consistency in the evaluation of AEs. The term "severe" is not the same as the term "serious" in classifying AEs. The severity of a specific event describes its intensity, and it is the intensity which is graded. Seriousness, which is not graded, relates to an outcome of an AE and is a regulatory definition.

Clinical sites are encouraged to report parameters in the DAIDS AE grading table as they are written to maintain data consistency across clinical trials. However, since some parameters can be reported with more specificity, clinical sites are encouraged to report parameters that convey additional clinical information. For example, diarrhea could be reported as neonatal diarrhea; seizures, as febrile seizures; and pain, as jaw pain.

The DAIDS AE grading table provides an AE severity grading scale ranging from grades 1 to 5 with descriptions for each AE based on the following general guidelines:

- Grade 1 indicates a mild event
- Grade 2 indicates a moderate event
- Grade 3 indicates a severe event
- Grade 4 indicates a potentially life-threatening event
- Grade 5 indicates death (Note: This grade is not specifically listed on each page of the grading table).

Other points to consider include:

- Use parameters defined by age and sex values as applicable.
- Male and female sex are defined as sex at birth.
- Unless noted, laboratory values are for term neonates. Preterm neonates should be assessed using local laboratory normal ranges.
- Where applicable, Standard International (SI) units are included in italics.

Selecting and Reporting a Primary AE Term

When selecting a primary AE term to report, sites should select the term that best describes what occurred to the participant. For example, a participant may present with itching, urticaria, flushing, angioedema of the face, and dyspnea. If the underlying diagnosis is determined to be an acute allergic reaction, sites should report “Acute Allergic Reaction” as the primary AE term.

Primary AE terms should be reported using the DAIDS Adverse Experience Reporting System (DAERS) only if they meet expedited reporting criteria. However, all primary AE terms should be reported using protocol-specific case report forms (CRFs). Because the reported information is stored in different databases (i.e., safety and clinical), sites should report primary AE terms using the same terminology for data consistency.

When reporting using DAERS, other clinically significant events associated with a primary AE term that more fully describe the nature, severity, or complications of the primary AE term should be entered in the “Other Events” section. However, the severity grade for these events must be lower than or equal to the severity grade of the primary AE term. In the example above, dyspnea and angioedema of the face may be entered in the “Other Events” section, because they are more descriptive and provide additional information on the severity of the acute allergic reaction. However, their severity grades must be lower than or equal to the severity grade of the primary AE term of “Acute Allergic Reaction”.

Differences exist in the reporting and recording of information (e.g., signs and symptoms, clinically significant events) in DAERS and CRFs. Therefore, sites should refer to their protocols and CRF requirements for further instructions.

Grading Adult and Pediatric AEs

When a single parameter is not appropriate for grading an AE in both adult and pediatric populations, separate parameters with specified age ranges are provided. If no distinction between adult and pediatric populations has been made, the listed parameter should be used for grading an AE in both populations.

Reporting Pregnancy Outcomes

In the Pregnancy, Puerperium, and Perinatal section, all parameters are pregnancy outcomes and should be reported using the mother's participant ID. If an infant is not enrolled in the same study as the mother, any identified birth defects should be reported using the mother's participant ID. However, if an infant is enrolled in the same study as the mother or in another study, any identified birth defects should be reported using the infant's participant ID. Sites should refer to the applicable network standards for reporting abnormal pregnancy outcomes on the CRFs.

Determining Severity Grade for Parameters between Grades

If the severity of an AE could fall in either one of two grades (i.e., the severity of an AE could be either grade 2 or grade 3), sites should select the higher of the two grades.

Laboratory Values

General. An asymptomatic, abnormal laboratory finding without an accompanying AE should not be reported to DAIDS in an expedited timeframe unless it meets protocol-specific reporting requirements. Sites should refer to the applicable network standards for reporting abnormal laboratory findings on the CRFs.

Values below Grade 1. Any laboratory value that is between the ULN and grade 1 (for high values) or the LLN and grade 1 (for low values) should not be graded or reported as an AE. Sites should consult the Manual for Expedited Reporting of Adverse Events to DAIDS, Version 2.0 and their protocol when making an assessment of the need to report an AE.

Overlap of Local Laboratory Normal Values with Grading Table Ranges. When local laboratory normal values fall within grading table laboratory ranges, the severity grading is based on the ranges in the grading table unless there is a protocol-specific grading criterion for the laboratory value. For example, “Magnesium, Low" has a grade 1 range of 1.2 to < 1.4 mEq/L, while a particular laboratory’s normal range for magnesium may be 1.3 to 2.8 mEq/L. If a study participant’s magnesium laboratory value is 1.3 mEq/L, the laboratory value should be graded as grade 1.

Estimating Severity Grade for Parameters Not Identified in the Grading Table. The functional table below should be used to grade the severity of an AE that is not specifically identified in the grading table. In addition, all deaths related to an AE are to be classified as grade 5.

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Clinical adverse event **NOT** identified elsewhere in the grading table	Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated	Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death

Major Clinical Conditions
Cardiovascular

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Arrhythmia (by ECG or physical examination) *Specify type, if applicable*	No symptoms AND No intervention indicated	No symptoms AND Non-urgent intervention indicated	Non-life-threatening symptoms AND Non-urgent intervention indicated	Life-threatening arrhythmia OR Urgent intervention indicated
Blood Pressure Abnormalities[1]				
Hypertension *(with the lowest reading taken after repeat testing during a visit)* ≥ *18 years of age*	140 to < 160 mmHg systolic OR 90 to < 100 mmHg diastolic	≥ 160 to < 180 mmHg systolic OR ≥ 100 to < 110 mmHg diastolic	≥ 180 mmHg systolic OR ≥ 110 mmHg diastolic	Life-threatening consequences in a participant not previously diagnosed with hypertension (e.g., malignant hypertension) OR Hospitalization indicated
< *18 years of age*	> 120/80 mmHg	≥ 95th to < 99th percentile + 5 mmHg adjusted for age, height, and gender (systolic and/or diastolic)	≥ 99th percentile + 5 mmHg adjusted for age, height, and gender (systolic and/or diastolic)	Life-threatening consequences in a participant not previously diagnosed with hypertension (e.g., malignant hypertension) OR Hospitalization indicated
Hypotension	No symptoms	Symptoms corrected with oral fluid replacement	Symptoms AND IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure
Cardiac Ischemia or Infarction *Report only one*	NA	NA	New symptoms with ischemia (stable angina) OR New testing consistent with ischemia	Unstable angina OR Acute myocardial infarction
Heart Failure	No symptoms AND Laboratory or cardiac imaging abnormalities	Symptoms with mild to moderate activity or exertion	Symptoms at rest or with minimal activity or exertion (e.g., hypoxemia) OR Intervention indicated (e.g., oxygen)	Life-threatening consequences OR Urgent intervention indicated (e.g., vasoactive medications, ventricular assist device, heart transplant)
Hemorrhage (with significant acute blood loss)	NA	Symptoms AND No transfusion indicated	Symptoms AND Transfusion of ≤ 2 units packed RBCs indicated	Life-threatening hypotension OR Transfusion of > 2 units packed RBCs (for children, packed RBCs > 10 cc/kg) indicated

Cardiovascular

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Prolonged PR Interval or AV Block *Report only one* *> 16 years of age*	PR interval 0.21 to < 0.25 seconds	PR interval ≥ 0.25 seconds OR Type I 2nd degree AV block	Type II 2nd degree AV block OR Ventricular pause ≥ 3.0 seconds	Complete AV block
≤ 16 years of age	1st degree AV block (PR interval > normal for age and rate)	Type I 2nd degree AV block	Type II 2nd degree AV block OR Ventricular pause ≥ 3.0 seconds	Complete AV block
Prolonged QTc Interval[2]	0.45 to 0.47 seconds	> 0.47 to 0.50 seconds	> 0.50 seconds OR ≥ 0.06 seconds above baseline	Life-threatening consequences (e.g., Torsade de pointes, other associated serious ventricular dysrhythmia)
Thrombosis or Embolism *Report only one*	NA	Symptoms AND No intervention indicated	Symptoms AND Intervention indicated	Life-threatening embolic event (e.g., pulmonary embolism, thrombus)

Dermatologic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Alopecia (scalp only)	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	NA	NA
Bruising	Localized to one area	Localized to more than one area	Generalized	NA
Cellulitis	NA	Non-parenteral treatment indicated (e.g., oral antibiotics, antifungals, antivirals)	IV treatment indicated (e.g., IV antibiotics, antifungals, antivirals)	Life-threatening consequences (e.g., sepsis, tissue necrosis)
Hyperpigmentation	Slight or localized causing no or minimal interference with usual social & functional activities	Marked or generalized causing greater than minimal interference with usual social & functional activities	NA	NA
Hypopigmentation	Slight or localized causing no or minimal interference with usual social & functional activities	Marked or generalized causing greater than minimal interference with usual social & functional activities	NA	NA
Petechiae	Localized to one area	Localized to more than one area	Generalized	NA
Pruritus[3] (without skin lesions)	Itching causing no or minimal interference with usual social & functional activities	Itching causing greater than minimal interference with usual social & functional activities	Itching causing inability to perform usual social & functional activities	NA
Rash *Specify type, if applicable*	Localized rash	Diffuse rash OR Target lesions	Diffuse rash AND Vesicles or limited number of bullae or superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Stevens-Johnson syndrome OR Toxic epidermal necrolysis

Endocrine and Metabolic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Diabetes Mellitus	Controlled without medication	Controlled with medication OR Modification of current medication regimen	Uncontrolled despite treatment modification OR Hospitalization for immediate glucose control indicated	Life-threatening consequences (e.g., ketoacidosis, hyperosmolar non-ketotic coma, end organ failure)
Gynecomastia	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing pain with greater than minimal interference with usual social & functional activities	Disfiguring changes AND Symptoms requiring intervention or causing inability to perform usual social & functional activities	NA
Hyperthyroidism	No symptoms AND Abnormal laboratory value	Symptoms causing greater than minimal interference with usual social & functional activities OR Thyroid suppression therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., thyroid storm)
Hypothyroidism	No symptoms AND Abnormal laboratory value	Symptoms causing greater than minimal interference with usual social & functional activities OR Thyroid replacement therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., myxedema coma)
Lipoatrophy[4]	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	Disfiguring changes	NA
Lipohypertrophy[5]	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	Disfiguring changes	NA

[4] Definition: A disorder characterized by fat loss in the face, extremities, and buttocks.
[5] Definition: A disorder characterized by abnormal fat accumulation on the back of the neck, breasts, and abdomen.

Gastrointestinal

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Anorexia	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss	Life-threatening consequences OR Aggressive intervention indicated (e.g., tube feeding, total parenteral nutrition)
Ascites	No symptoms	Symptoms AND Intervention indicated (e.g., diuretics, therapeutic paracentesis)	Symptoms recur or persist despite intervention	Life-threatening consequences
Bloating or Distension *Report only one*	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Cholecystitis	NA	Symptoms AND Medical intervention indicated	Radiologic, endoscopic, or operative intervention indicated	Life-threatening consequences (e.g., sepsis, perforation)
Constipation	NA	Persistent constipation requiring regular use of dietary modifications, laxatives, or enemas	Obstipation with manual evacuation indicated	Life-threatening consequences (e.g., obstruction)
Diarrhea *≥ 1 year of age*	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 to 6 stools over baseline per 24-hour period	Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g., hypotensive shock)
< 1 year of age	Liquid stools (more unformed than usual) but usual number of stools	Liquid stools with increased number of stools OR Mild dehydration	Liquid stools with moderate dehydration	Life-threatening consequences (e.g., liquid stools resulting in severe dehydration, hypotensive shock)
Dysphagia or Odynophagia *Report only one and specify location*	Symptoms but able to eat usual diet	Symptoms causing altered dietary intake with no intervention indicated	Symptoms causing severely altered dietary intake with intervention indicated	Life-threatening reduction in oral intake
Gastrointestinal Bleeding	Not requiring intervention other than iron supplement	Endoscopic intervention indicated	Transfusion indicated	Life-threatening consequences (e.g., hypotensive shock)

Gastrointestinal

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Mucositis or Stomatitis *Report only one and specify location*	Mucosal erythema	Patchy pseudomembranes or ulcerations	Confluent pseudomembranes or ulcerations OR Mucosal bleeding with minor trauma	Life-threatening consequences (e.g., aspiration, choking) OR Tissue necrosis OR Diffuse spontaneous mucosal bleeding
Nausea	Transient (< 24 hours) or intermittent AND No or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 to 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Pancreatitis	NA	Symptoms with hospitalization not indicated	Symptoms with hospitalization indicated	Life-threatening consequences (e.g., circulatory failure, hemorrhage, sepsis)
Perforation (colon or rectum)	NA	NA	Intervention indicated	Life-threatening consequences
Proctitis	Rectal discomfort with no intervention indicated	Symptoms causing greater than minimal interference with usual social & functional activities OR Medical intervention indicated	Symptoms causing inability to perform usual social & functional activities OR Operative intervention indicated	Life-threatening consequences (e.g., perforation)
Rectal Discharge	Visible discharge	Discharge requiring the use of pads	NA	NA
Vomiting	Transient or intermittent AND No or minimal interference with oral intake	Frequent episodes with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)

Musculoskeletal

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Arthralgia	Joint pain causing no or minimal interference with usual social & functional activities	Joint pain causing greater than minimal interference with usual social & functional activities	Joint pain causing inability to perform usual social & functional activities	Disabling joint pain causing inability to perform basic self-care functions
Arthritis	Stiffness or joint swelling causing no or minimal interference with usual social & functional activities	Stiffness or joint swelling causing greater than minimal interference with usual social & functional activities	Stiffness or joint swelling causing inability to perform usual social & functional activities	Disabling joint stiffness or swelling causing inability to perform basic self-care functions
Myalgia (generalized)	Muscle pain causing no or minimal interference with usual social & functional activities	Muscle pain causing greater than minimal interference with usual social & functional activities	Muscle pain causing inability to perform usual social & functional activities	Disabling muscle pain causing inability to perform basic self-care functions
Osteonecrosis	NA	No symptoms but with radiographic findings AND No operative intervention indicated	Bone pain with radiographic findings OR Operative intervention indicated	Disabling bone pain with radiographic findings causing inability to perform basic self-care functions
Osteopenia[6] *≥ 30 years of age*	BMD t-score -2.5 to -1	NA	NA	NA
< 30 years of age	BMD z-score -2 to -1	NA	NA	NA
Osteoporosis[6] *≥ 30 years of age*	NA	BMD t-score < -2.5	Pathologic fracture (e.g., compression fracture causing loss of vertebral height)	Pathologic fracture causing life-threatening consequences
< 30 years of age	NA	BMD z-score < -2	Pathologic fracture (e.g., compression fracture causing loss of vertebral height)	Pathologic fracture causing life-threatening consequences

Neurologic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Acute CNS Ischemia	NA	NA	Transient ischemic attack	Cerebral vascular accident (e.g., stroke with neurological deficit)
Altered Mental Status (for Dementia, see *Cognitive, Behavioral, or Attentional Disturbance* below)	Changes causing no or minimal interference with usual social & functional activities	Mild lethargy or somnolence causing greater than minimal interference with usual social & functional activities	Confusion, memory impairment, lethargy, or somnolence causing inability to perform usual social & functional activities	Delirium OR Obtundation OR Coma
Ataxia	Symptoms causing no or minimal interference with usual social & functional activities OR No symptoms with ataxia detected on examination	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Disabling symptoms causing inability to perform basic self-care functions
Cognitive, Behavioral, or Attentional Disturbance (includes dementia and attention deficit disorder) *Specify type, if applicable*	Disability causing no or minimal interference with usual social & functional activities OR Specialized resources not indicated	Disability causing greater than minimal interference with usual social & functional activities OR Specialized resources on part-time basis indicated	Disability causing inability to perform usual social & functional activities OR Specialized resources on a full-time basis indicated	Disability causing inability to perform basic self-care functions OR Institutionalization indicated
Developmental Delay *< 18 years of age* *Specify type, if applicable*	Mild developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Moderate developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Severe developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Developmental regression, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting
Headache	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Hospitalization indicated OR Headache with significant impairment of alertness or other neurologic function

Neurologic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Neuromuscular Weakness (includes myopathy and neuropathy) *Specify type, if applicable*	Minimal muscle weakness causing no or minimal interference with usual social & functional activities OR No symptoms with decreased strength on examination	Muscle weakness causing greater than minimal interference with usual social & functional activities	Muscle weakness causing inability to perform usual social & functional activities	Disabling muscle weakness causing inability to perform basic self-care functions OR Respiratory muscle weakness impairing ventilation
Neurosensory Alteration (includes paresthesia and painful neuropathy) *Specify type, if applicable*	Minimal paresthesia causing no or minimal interference with usual social & functional activities OR No symptoms with sensory alteration on examination	Sensory alteration or paresthesia causing greater than minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing inability to perform usual social & functional activities	Disabling sensory alteration or paresthesia causing inability to perform basic self-care functions
Seizures *New Onset Seizure* *≥ 18 years of age*	NA	NA	1 to 3 seizures	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
< 18 years of age (includes new or pre-existing febrile seizures)	Seizure lasting < 5 minutes with < 24 hours postictal state	Seizure lasting 5 to < 20 minutes with < 24 hours postictal state	Seizure lasting ≥ 20 minutes OR > 24 hours postictal state	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
Pre-existing Seizure	NA	Increased frequency from previous level of control without change in seizure character	Change in seizure character either in duration or quality (e.g., severity or focality)	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
Syncope	Near syncope without loss of consciousness (e.g., pre-syncope)	Loss of consciousness with no intervention indicated	Loss of consciousness AND Hospitalization or intervention required	NA

Pregnancy, Puerperium, and Perinatal

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Fetal Death or Stillbirth (report using mother's participant ID) *Report only one*	NA	NA	Fetal loss occurring at ≥ 20 weeks gestation	NA
Preterm Delivery[7] (report using mother's participant ID)	Delivery at 34 to < 37 weeks gestational age	Delivery at 28 to < 34 weeks gestational age	Delivery at 24 to < 28 weeks gestational age	Delivery at < 24 weeks gestational age
Spontaneous Abortion or Miscarriage[8] (report using mother's participant ID) *Report only one*	Chemical pregnancy	Uncomplicated spontaneous abortion or miscarriage	Complicated spontaneous abortion or miscarriage	NA

Psychiatric

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Insomnia	Mild difficulty falling asleep, staying asleep, or waking up early	Moderate difficulty falling asleep, staying asleep, or waking up early	Severe difficulty falling asleep, staying asleep, or waking up early	NA
Psychiatric Disorders (includes anxiety, depression, mania, and psychosis) *Specify disorder*	Symptoms with intervention not indicated OR Behavior causing no or minimal interference with usual social & functional activities	Symptoms with intervention indicated OR Behavior causing greater than minimal interference with usual social & functional activities	Symptoms with hospitalization indicated OR Behavior causing inability to perform usual social & functional activities	Threatens harm to self or others OR Acute psychosis OR Behavior causing inability to perform basic self-care functions
Suicidal Ideation or Attempt *Report only one*	Preoccupied with thoughts of death AND No wish to kill oneself	Preoccupied with thoughts of death AND Wish to kill oneself with no specific plan or intent	Thoughts of killing oneself with partial or complete plans but no attempt to do so OR Hospitalization indicated	Suicide attempted

Respiratory

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Acute Bronchospasm	Forced expiratory volume in 1 second or peak flow reduced to ≥ 70 to < 80% OR Mild symptoms with intervention not indicated	Forced expiratory volume in 1 second or peak flow 50 to < 70% OR Symptoms with intervention indicated OR Symptoms causing greater than minimal interference with usual social & functional activities	Forced expiratory volume in 1 second or peak flow 25 to < 50% OR Symptoms causing inability to perform usual social & functional activities	Forced expiratory volume in 1 second or peak flow < 25% OR Life-threatening respiratory or hemodynamic compromise OR Intubation
Dyspnea or Respiratory Distress *Report only one*	Dyspnea on exertion with no or minimal interference with usual social & functional activities OR Wheezing OR Minimal increase in respiratory rate for age	Dyspnea on exertion causing greater than minimal interference with usual social & functional activities OR Nasal flaring OR Intercostal retractions OR Pulse oximetry 90 to < 95%	Dyspnea at rest causing inability to perform usual social & functional activities OR Pulse oximetry < 90%	Respiratory failure with ventilator support indicated (e.g., CPAP, BPAP, intubation)

Sensory

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Hearing Loss *≥ 12 years of age*	NA	Hearing aid or intervention not indicated	Hearing aid or intervention indicated	Profound bilateral hearing loss (> 80 dB at 2 kHz and above) OR Non-serviceable hearing (i.e., >50 dB audiogram and <50% speech discrimination)
< 12 years of age (based on a 1, 2, 3, 4, 6 and 8 kHz audiogram)	> 20 dB hearing loss at ≤ 4 kHz	> 20 dB hearing loss at > 4 kHz	> 20 dB hearing loss at ≥ 3 kHz in one ear with additional speech language related services indicated (where available) OR Hearing loss sufficient to indicate therapeutic intervention, including hearing aids	Audiologic indication for cochlear implant and additional speech-language related services indicated (where available)
Tinnitus	Symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Symptoms causing inability to perform usual social & functional activities	NA
Uveitis	No symptoms AND Detectable on examination	Anterior uveitis with symptoms OR Medicamylasal intervention indicated	Posterior or pan-uveitis OR Operative intervention indicated	Disabling visual loss in affected eye(s)
Vertigo	Vertigo causing no or minimal interference with usual social & functional activities	Vertigo causing greater than minimal interference with usual social & functional activities	Vertigo causing inability to perform usual social & functional activities	Disabling vertigo causing inability to perform basic self-care functions
Visual Changes (assessed from baseline)	Visual changes causing no or minimal interference with usual social & functional activities	Visual changes causing greater than minimal interference with usual social & functional activities	Visual changes causing inability to perform usual social & functional activities	Disabling visual loss in affected eye(s)

Systemic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Acute Allergic Reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with intervention indicated OR Mild angioedema with no intervention indicated	Generalized urticaria OR Angioedema with intervention indicated OR Symptoms of mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR Laryngeal edema
Chills	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Cytokine Release Syndrome[9]	Mild signs and symptoms AND Therapy (i.e., antibody infusion) interruption not indicated	Therapy (i.e., antibody infusion) interruption indicated AND Responds promptly to symptomatic treatment OR Prophylactic medications indicated for ≤ 24 hours	Prolonged severe signs and symptoms OR Recurrence of symptoms following initial improvement	Life-threatening consequences (e.g., requiring pressor or ventilator support)
Fatigue or Malaise *Report only one*	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Incapacitating symptoms of fatigue or malaise causing inability to perform basic self-care functions
Fever (non-axillary temperatures only)	38.0 to < 38.6°C or 100.4 to < 101.5°F	≥ 38.6 to < 39.3°C or ≥ 101.5 to < 102.7°F	≥ 39.3 to < 40.0°C or ≥ 102.7 to < 104.0°F	≥ 40.0°C or ≥ 104.0°F
Pain[10] (not associated with study agent injections and not specified elsewhere) *Specify location*	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities	Pain causing inability to perform usual social & functional activities	Disabling pain causing inability to perform basic self-care functions OR Hospitalization indicated
Serum Sickness[11]	Mild signs and symptoms	Moderate signs and symptoms AND Intervention indicated (e.g., antihistamines)	Severe signs and symptoms AND Higher level intervention indicated (e.g., steroids or IV fluids)	Life-threatening consequences (e.g., requiring pressor or ventilator support)

[9] Definition: A disorder characterized by nausea, headache, tachycardia, hypotension, rash, and/or shortness of breath.
[10] For pain associated with injections or infusions, see the *Site Reactions to Injections and Infusions* section (page 23).
[11] Definition: A disorder characterized by fever, arthralgia, myalgia, skin eruptions, lymphadenopathy, marked discomfort, and/or dyspnea.

Systemic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Underweight[12] *> 5 to 19 years of age*	NA	WHO BMI z-score < -2 to ≤ -3	WHO BMI z-score < -3	WHO BMI z-score < -3 with life-threatening consequences
2 to 5 years of age	NA	WHO Weight-for-height z-score < -2 to ≤ -3	WHO Weight-for-height z-score < -3	WHO Weight-for-height z-score < -3 with life-threatening consequences
< 2 years of age	NA	WHO Weight-for-length z-score < -2 to ≤ -3	WHO Weight-for-length z-score < -3	WHO Weight-for-length z-score < -3 with life-threatening consequences
Weight Loss (excludes postpartum weight loss)	NA	5 to < 9% loss in body weight from baseline	≥ 9 to < 20% loss in body weight from baseline	≥ 20% loss in body weight from baseline OR Aggressive intervention indicated (e.g., tube feeding, total parenteral nutrition)

Urinary

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Urinary Tract Obstruction	NA	Signs or symptoms of urinary tract obstruction without hydronephrosis or renal dysfunction	Signs or symptoms of urinary tract obstruction with hydronephrosis or renal dysfunction	Obstruction causing life-threatening consequences

Site Reactions to Injections and Infusions

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Injection Site Pain or Tenderness *Report only one*	Pain or tenderness causing no or minimal limitation of use of limb	Pain or tenderness causing greater than minimal limitation of use of limb	Pain or tenderness causing inability to perform usual social & functional activities	Pain or tenderness causing inability to perform basic self-care function OR Hospitalization indicated
Injection Site Erythema or Redness[13] *Report only one* *> 15 years of age*	2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area AND Symptoms causing no or minimal interference with usual social & functional activities	≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm^2 surface area OR Symptoms causing greater than minimal interference with usual social & functional activities	≥ 10 cm in diameter OR ≥ 100 cm^2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage OR Symptoms causing inability to perform usual social & functional activities	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
≤ 15 years of age	≤ 2.5 cm in diameter	> 2.5 cm in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	≥ 50% surface area of the extremity segment involved (e.g., upper arm or thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Injection Site Induration or Swelling *Report only one* *> 15 years of age*	Same as for **Injection Site Erythema or Redness**, *> 15 years of age*	Same as for **Injection Site Erythema or Redness**, *> 15 years of age*	Same as for **Injection Site Erythema or Redness**, *> 15 years of age*	Same as for **Injection Site Erythema or Redness**, *> 15 years of age*
≤ 15 years of age	Same as for **Injection Site Erythema or Redness**, *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness**, *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness**, *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness**, *≤ 15 years of age*
Injection Site Pruritus	Itching localized to the injection site that is relieved spontaneously or in < 48 hours of treatment	Itching beyond the injection site that is not generalized OR Itching localized to the injection site requiring ≥ 48 hours treatment	Generalized itching causing inability to perform usual social & functional activities	NA

[13] Injection Site Erythema or Redness should be evaluated and graded using the greatest single diameter or measured surface area.

Laboratory Values
Chemistries

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Acidosis	NA	pH ≥ 7.3 to < LLN	pH < 7.3 without life-threatening consequences	pH < 7.3 with life-threatening consequences
Albumin, Low (g/dL; *g/L*)	3.0 to < LLN *30 to < LLN*	≥ 2.0 to < 3.0 *≥ 20 to < 30*	< 2.0 *< 20*	NA
Alkaline Phosphatase, High	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Alkalosis	NA	pH > ULN to ≤ 7.5	pH > 7.5 without life-threatening consequences	pH > 7.5 with life-threatening consequences
ALT or SGPT, High *Report only one*	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Amylase (Pancreatic) or Amylase (Total), High *Report only one*	1.1 to < 1.5 x ULN	1.5 to < 3.0 x ULN	3.0 to < 5.0 x ULN	≥ 5.0 x ULN
AST or SGOT, High *Report only one*	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Bicarbonate, Low (mEq/L; *mmol/L*)	16.0 to < LLN *16.0 to < LLN*	11.0 to < 16.0 *11.0 to < 16.0*	8.0 to < 11.0 *8.0 to < 11.0*	< 8.0 *< 8.0*
Bilirubin ***Direct Bilirubin[14], High*** *> 28 days of age*	NA	NA	> ULN	> ULN with life-threatening consequences (e.g., signs and symptoms of liver failure)
≤ 28 days of age	ULN to ≤ 1 mg/dL	> 1 to ≤ 1.5 mg/dL	> 1.5 to ≤ 2 mg/dL	> 2 mg/dL
Total Bilirubin, High *> 28 days of age*	1.1 to < 1.6 x ULN	1.6 to < 2.6 x ULN	2.6 to < 5.0 x ULN	≥ 5.0 x ULN
≤ 28 days of age	See Appendix A. Total Bilirubin for Term and Preterm Neonates	See Appendix A. Total Bilirubin for Term and Preterm Neonates	See Appendix A. Total Bilirubin for Term and Preterm Neonates	See Appendix A. Total Bilirubin for Term and Preterm Neonates
Calcium, High (mg/dL; *mmol/L*) *≥ 7 days of age*	10.6 to < 11.5 *2.65 to < 2.88*	11.5 to < 12.5 *2.88 to < 3.13*	12.5 to < 13.5 *3.13 to < 3.38*	≥ 13.5 *≥ 3.38*
< 7 days of age	11.5 to < 12.4 *2.88 to < 3.10*	12.4 to < 12.9 *3.10 to < 3.23*	12.9 to < 13.5 *3.23 to < 3.38*	≥ 13.5 *≥ 3.38*

[14] Direct bilirubin > 1.5 mg/dL in a participant < 28 days of age should be graded as grade 2, if < 10% of the total bilirubin.

Chemistries

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Calcium (Ionized), High (mg/dL; *mmol/L*)	> ULN to < 6.0 *> ULN to < 1.5*	6.0 to < 6.4 *1.5 to < 1.6*	6.4 to < 7.2 *1.6 to < 1.8*	≥ 7.2 *≥ 1.8*
Calcium, Low (mg/dL; *mmol/L*) *≥ 7 days of age*	7.8 to < 8.4 *1.95 to < 2.10*	7.0 to < 7.8 *1.75 to < 1.95*	6.1 to < 7.0 *1.53 to < 1.75*	< 6.1 *< 1.53*
< 7 days of age	6.5 to < 7.5 *1.63 to < 1.88*	6.0 to < 6.5 *1.50 to < 1.63*	5.50 to < 6.0 *1.38 to < 1.50*	< 5.50 *< 1.38*
Calcium (Ionized), Low (mg/dL; *mmol/L*)	< LLN to 4.0 *< LLN to 1.0*	3.6 to < 4.0 *0.9 to < 1.0*	3.2 to < 3.6 *0.8 to < 0.9*	< 3.2 *< 0.8*
Cardiac Troponin I, High	NA	NA	NA	Levels consistent with myocardial infarction or unstable angina as defined by the local laboratory
Creatine Kinase, High	3 to < 6 x ULN	6 to < 10 x ULN	10 to < 20 x ULN	≥ 20 x ULN
Creatinine, High	1.1 to 1.3 x ULN	> 1.3 to 1.8 x ULN OR Increase of > 0.3 mg/dL above baseline	> 1.8 to < 3.5 x ULN OR Increase of 1.5 to < 2.0 x above baseline	≥ 3.5 x ULN OR Increase of ≥ 2.0 x above baseline
Creatinine Clearance[15] **or eGFR, Low** *Report only one*	NA	< 90 to 60 ml/min or ml/min/1.73 m^2 OR 10 to < 30% decrease from baseline	< 60 to 30 ml/min or ml/min/1.73 m^2 OR ≥ 30 to < 50% decrease from baseline	< 30 ml/min or ml/min/1.73 m^2 OR ≥ 50% decrease from baseline or dialysis needed
Glucose (mg/dL; *mmol/L*) ***Fasting, High***	110 to 125 *6.11 to < 6.95*	> 125 to 250 *6.95 to < 13.89*	> 250 to 500 *13.89 to < 27.75*	> 500 *≥ 27.75*
Nonfasting, High	116 to 160 *6.44 to < 8.89*	> 160 to 250 *8.89 to < 13.89*	> 250 to 500 *13.89 to < 27.75*	> 500 *≥ 27.75*
Glucose, Low (mg/dL; *mmol/L*) *≥ 1 month of age*	55 to 64 *3.05 to 3.55*	40 to < 55 *2.22 to < 3.05*	30 to < 40 *1.67 to < 2.22*	< 30 *< 1.67*
< 1 month of age	50 to 54 *2.78 to 3.00*	40 to < 50 *2.22 to < 2.78*	30 to < 40 *1.67 to < 2.22*	< 30 *< 1.67*
Lactate, High	ULN to < 2.0 x ULN without acidosis	≥ 2.0 x ULN without acidosis	Increased lactate with pH < 7.3 without life-threatening consequences	Increased lactate with pH < 7.3 with life-threatening consequences

[15] Use the applicable formula (i.e., Cockroft-Gault in mL/min or Schwatrz in mL/min/1.73m^2).

Chemistries

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Lipase, High	1.1 to < 1.5 x ULN	1.5 to < 3.0 x ULN	3.0 to < 5.0 x ULN	≥ 5.0 x ULN
Lipid Disorders (mg/dL; *mmol/L*)				
Cholesterol, Fasting, High				
≥ 18 years of age	200 to < 240 *5.18 to < 6.19*	240 to < 300 *6.19 to < 7.77*	≥ 300 *≥ 7.77*	NA
< 18 years of age	170 to < 200 *4.40 to < 5.15*	200 to < 300 *5.15 to < 7.77*	≥ 300 *≥ 7.77*	NA
LDL, Fasting, High				
≥ 18 years of age	130 to < 160 *3.37 to < 4.12*	160 to < 190 *4.12 to < 4.90*	≥ 190 *≥4.90*	NA
> 2 to < 18 years of age	110 to < 130 *2.85 to < 3.34*	130 to < 190 *3.34 to < 4.90*	≥ 190 *≥4.90*	NA
Triglycerides, Fasting, High	150 to 300 *1.71 to 3.42*	>300 to 500 *>3.42 to 5.7*	>500 to < 1,000 *>5.7 to 11.4*	> 1,000 *> 11.4*
Magnesium[16], Low (mEq/L; *mmol/L*)	1.2 to < 1.4 *0.60 to < 0.70*	0.9 to < 1.2 *0.45 to < 0.60*	0.6 to < 0.9 *0.30 to < 0.45*	< 0.6 *< 0.30*
Phosphate, Low (mg/dL; *mmol/L*)				
> 14 years of age	2.0 to < LLN *0.81 to < LLN*	1.4 to < 2.0 *0.65 to < 0.81*	1.0 to < 1.4 *0.32 to < 0.65*	< 1.0 *< 0.32*
1 to 14 years of age	3.0 to < 3.5 *0.97 to < 1.13*	2.5 to < 3.0 *0.81 to < 0.97*	1.5 to < 2.5 *0.48 to < 0.81*	< 1.5 *< 0.48*
< 1 year of age	3.5 to < 4.5 *1.13 to < 1.45*	2.5 to < 3.5 *0.81 to < 1.13*	1.5 to < 2.5 *0.48 to < 0.81*	< 1.5 *< 0.48*
Potassium, High (mEq/L; *mmol/L*)	5.6 to < 6.0 *5.6 to < 6.0*	6.0 to < 6.5 *6.0 to < 6.5*	6.5 to < 7.0 *6.5 to < 7.0*	≥ 7.0 *≥ 7.0*
Potassium, Low (mEq/L; *mmol/L*)	3.0 to < 3.4 *3.0 to < 3.4*	2.5 to < 3.0 *2.5 to < 3.0*	2.0 to < 2.5 *2.0 to < 2.5*	< 2.0 *< 2.0*
Sodium, High (mEq/L; *mmol/L*)	146 to < 150 *146 to < 150*	150 to < 154 *150 to < 154*	154 to < 160 *154 to < 160*	≥ 160 *≥ 160*
Sodium, Low (mEq/L; *mmol/L*)	130 to < 135 *130 to < 135*	125 to < 130 *125 to < 135*	121 to < 125 *121 to < 125*	≤ 120 *≤ 120*
Uric Acid, High (mg/dL; *mmol/L*)	7.5 to < 10.0 *0.45 to < 0.59*	10.0 to < 12.0 *0.59 to < 0.71*	12.0 to < 15.0 *0.71 to < 0.89*	≥ 15.0 *≥ 0.89*

[16] To convert a magnesium value from mg/dL to mmol/L, laboratories should multiply by 0.4114.

Hematology

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Absolute CD4+ Count, Low (cell/mm^3; *cells/L*)				
> 5 years of age (not HIV infected)	300 to < 400 *300 to < 400*	200 to < 300 *200 to < 300*	100 to < 200 *100 to < 200*	< 100 *< 100*
Absolute Lymphocyte Count, Low (cell/mm^3; *cells/L*)				
> 5 years of age (not HIV infected)	600 to < 650 *0.600 x 10^9 to < 0.650 x 10^9*	500 to < 600 *0.500 x 10^9 to < 0.600 x 10^9*	350 to < 500 *0.350 x 10^9 to < 0.500 x 10^9*	< 350 *< 0.350 x 10^9*
Absolute Neutrophil Count (ANC), Low (cells/mm^3; *cells/L*)				
> 7 days of age	800 to 1,000 *0.800 x 10^9 to 1.000 x 10^9*	600 to 799 *0.600 x 10^9 to 0.799 x 10^9*	400 to 599 *0.400 x 10^9 to 0.599 x 10^9*	< 400 *< 0.400 x 10^9*
2 to 7 days of age	1,250 to 1,500 *1.250 x 10^9 to 1.500 x 10^9*	1,000 to 1,249 *1.000 x 10^9 to 1.249 x 10^9*	750 to 999 *0.750 x 10^9 to 0.999 x 10^9*	< 750 *< 0.750 x 10^9*
≤ 1 day of age	4,000 to 5,000 *4.000 x 10^9 to 5.000 x 10^9*	3,000 to 3,999 *3.000 x 10^9 to 3.999 x 10^9*	1,500 to 2,999 *1.500 x 10^9 to 2.999 x 10^9*	< 1,500 *< 1.500 x 10^9*
Fibrinogen, Decreased (mg/dL; *g/L*)	100 to < 200 *1.00 to < 2.00* OR 0.75 to < 1.00 x LLN	75 to < 100 *0.75 to < 1.00* OR ≥ 0.50 to < 0.75 x LLN	50 to < 75 *0.50 to < 0.75* OR 0.25 to < 0.50 x LLN	< 50 *< 0.50* OR < 0.25 x LLN OR Associated with gross bleeding
Hemoglobin[17]**, Low** (g/dL; *mmol/L*)[18]				
≥ 13 years of age (male only)	10.0 to 10.9 *6.19 to 6.76*	9.0 to < 10.0 *5.57 to < 6.19*	7.0 to < 9.0 *4.34 to < 5.57*	< 7.0 *< 4.34*
≥ 13 years of age (female only)	9.5 to 10.4 *5.88 to 6.48*	8.5 to < 9.5 *5.25 to < 5.88*	6.5 to < 8.5 *4.03 to < 5.25*	< 6.5 *< 4.03*

[17] Male and female sex are defined as sex at birth.
[18] The conversion factor used to convert g/dL to mmol/L is 0.6206 and is the most commonly used conversion factor. For grading hemoglobin results obtained by an analytic method with a conversion factor other than 0.6206, the result must be converted to g/dL using the appropriate conversion factor for the particular laboratory.

Hematology

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
57 days of age to < 13 years of age (male and female)	9.5 to 10.4 *5.88 to 6.48*	8.5 to < 9.5 *5.25 to < 5.88*	6.5 to < 8.5 *4.03 to < 5.25*	< 6.5 *< 4.03*
36 to 56 days of age (male and female)	8.5 to 9.6 *5.26 to 5.99*	7.0 to < 8.5 *4.32 to < 5.26*	6.0 to < 7.0 *3.72 to < 4.32*	< 6.0 *< 3.72*
22 to 35 days of age (male and female)	9.5 to 11.0 *5.88 to 6.86*	8.0 to < 9.5 *4.94 to < 5.88*	6.7 to < 8.0 *4.15 to < 4.94*	< 6.7 *< 4.15*
8 to ≤ 21 days of age (male and female)	11.0 to 13.0 *6.81 to 8.10*	9.0 to < 11.0 *5.57 to < 6.81*	8.0 to < 9.0 *4.96 to < 5.57*	< 8.0 *< 4.96*
≤ 7 days of age (male and female)	13.0 to 14.0 *8.05 to 8.72*	10.0 to < 13.0 *6.19 to < 8.05*	9.0 to < 10.0 *5.59 to < 6.19*	< 9.0 *< 5.59*
INR, High (not on anticoagulation therapy)	1.1 to < 1.5 x ULN	1.5 to < 2.0 x ULN	2.0 to < 3.0 x ULN	≥ 3.0 x ULN
Methemoglobin (% hemoglobin)	5.0 to < 10.0%	10.0 to < 15.0%	15.0 to < 20.0%	≥ 20.0%
PTT, High (not on anticoagulation therapy)	1.1 to < 1.66 x ULN	1.66 to < 2.33 x ULN	2.33 to < 3.00 x ULN	≥ 3.00 x ULN
Platelets, Decreased (cells/mm^3; *cells/L*)	100,000 to < 124,999 *100.000 x 10^9 to < 124.999 x 10^9*	50,000 to < 100,000 *50.000 x 10^9 to < 100.000 x 10^9*	25,000 to < 50,000 *25.000 x 10^9 to < 50.000 x 10^9*	< 25,000 *< 25.000 x 10^9*
PT, High (not on anticoagulation therapy	1.1 to < 1.25 x ULN	1.25 to < 1.50 x ULN	1.50 to < 3.00 x ULN	≥ 3.00 x ULN
WBC, Decreased (cells/mm^3; *cells/L*)				
> 7 days of age	2,000 to 2,499 *2.000 x 10^9 to 2.499 x 10^9*	1,500 to 1,999 *1.500 x 10^9 to 1.999 x 10^9*	1,000 to 1,499 *1.000 x 10^9 to 1.499 x 10^9*	< 1,000 *< 1.000 x 10^9*
≤ 7 days of age	5,500 to 6,999 *5.500 x 10^9 to 6.999 x 10^9*	4,000 to 5,499 *4.000 x 10^9 to 5.499 x 10^9*	2,500 to 3,999 *2.500 x 10^9 to 3.999 x 10^9*	< 2,500 *< 2.500 x 10^9*

Urinalysis

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Glycosuria (random collection tested by dipstick)	Trace to 1+ or ≤ 250 mg	2+ or > 250 to ≤ 500 mg	> 2+ or > 500 mg	NA
Hematuria (not to be reported based on dipstick findings or on blood believed to be of menstrual origin)	6 to < 10 RBCs per high power field	≥ 10 RBCs per high power field	Gross, with or without clots OR With RBC casts OR Intervention indicated	Life-threatening consequences
Proteinuria (random collection tested by dipstick)	1+	2+	3+ or higher	NA

Appendix J. List of drugs associated with known risk of torsades de pointes

Substantial evidence supports the conclusion that these drugs prolong the QT interval AND are clearly associated with a risk of TdP, even when taken as directed in official labeling.
Reference: https://www.crediblemeds.org/

CredibleMeds Filtered QTDrug List

The last revision date: August 01, 2014

Filters: ; TdP Risk Category --> "Drugs with known TdP risk"

Generic Name	Brand Names	Drug Class	Therapeutic Use	Risk Category	Route
Amiodarone	Cordarone®, Pacerone®, Nexterone®	Anti-arrhythmic	Abnormal heart rhythm	Risk of TdP	oral, injection
Anagrelide	Agrylin®, Xagrid®	Phosphodiesterase 3 inhibitor	Thrombocythemia	Risk of TdP	oral
Arsenic trioxide	Trisenox®	Anti-cancer	Leukemia	Risk of TdP	injection
Astemizole (Off US mkt)	Hismanal®	Antihistamine	Allergic rhinitis	Risk of TdP	oral
Azithromycin	Zithromax®, Zmax®	Antibiotic	Bacterial infection	Risk of TdP	oral, injection
Bepridil (Off US mkt)	Vascor®	Anti-anginal	Angina Pectoris (heart pain)	Risk of TdP	oral
Chloroquine	Aralen®	Anti-malarial	Malaria infection	Risk of TdP	oral
Chlorpromazine	Thorazine®, Largactil®, Megaphen®	Anti-psychotic / Anti-emetic	Schizophrenia/ nausea	Risk of TdP	oral, injection, suppository
Cisapride (Off US mkt)	Propulsid®	GI stimulant	Heartburn	Risk of TdP	oral
Citalopram	Celexa®, Cipramil®	Anti-depressant, SSRI	Depression	Risk of TdP	oral
Clarithromycin	Biaxin®, Prevpac®	Antibiotic	Bacterial infection	Risk of TdP	oral
Cocaine	Cocaine	Local anesthetic	Topical anesthetic	Risk of TdP	topical
Disopyramide	Norpace®	Anti-arrhythmic	Abnormal heart rhythm	Risk of TdP	oral
Dofetilide	Tikosyn®	Anti-arrhythmic	Abnormal heart rhythm	Risk of TdP	oral
Domperidone (Not on US mkt)	Motilium®, Motillium®, Motinorm Costi®, Nomit®	Anti-nausea	Nausea	Risk of TdP	oral, injection, suppository
Dronedarone	Multaq®	Anti-arrhythmic	Atrial Fibrillation	Risk of TdP	oral
Droperidol	Inapsine®, Droleptan®, Dridol®, Xomolix®	Anti-psychotic / Anti-emetic	Anesthesia adjunct, nausea	Risk of TdP	injection
Erythromycin	E.E.S.®, Robimycin®, EMycin®, Erymax®, Ery-Tab®, Eryc Ranbaxy®, Erypar®, Eryped®, Erythrocin Stearate Filmtab®, Erythrocot®, E-Base®, Erythroped®, Ilosone®, MY-E®, Pediamycin®, Zineryt®, Abboticin®, Abboticin-ES®, Erycin®, PCE Dispertab®, Stiemycine®, Acnasol®, Tiloryth®	Antibiotic	Bacterial infection; increase GI motility	Risk of TdP	oral, injection

Generic Name	Brand Names	Drug Class	Therapeutic Use	Risk Category	Route
Escitalopram	Cipralex®, Lexapro®, Nexito®, Anxiset-E® (India), Exodus® (Brazil), Esto® (Israel), Seroplex®, Elicea®, Lexamil®, Lexam®, Entact® (Greece), Losita® (Bangladesh), Reposil® (Chile), Animaxen® (Colombia), Esitalo® (Australia), Lexamil® (South Africa)	Anti-depressant, SSRI	Major depression/ Anxiety disorders	Risk of TdP	oral
Flecainide	Tambocor®, Almarytm®, Apocard®, Ecrinal®, Flécaine®	Anti-arrhythmic	Abnormal heart rhythm	Risk of TdP	oral
Grepafloxacin (Off market worldwide)	Raxar®	Antibiotic	Bacterial infection	Risk of TdP	oral
Halofantrine	Halfan®	Anti-malarial	Malaria infection	Risk of TdP	oral
Haloperidol	Haldol® (US & UK), Aloperidin®, Bioperidolo®, Brotopon®, Dozic®, Duraperidol® (Germany), Einalon S®, Eukystol®, Halosten®, Keselan®, Linton®, Peluces®, Serenace®, Serenase®, Sigaperidol®	Anti-psychotic	Schizophrenia, agitation	Risk of TdP	oral, injection
Ibutilide	Corvert®	Anti-arrhythmic	Abnormal heart rhythm	Risk of TdP	injection
Levofloxacin	Levaquin®, Tavanic®	Antibiotic	Bacterial infection	Risk of TdP	oral, injection
Levomethadyl (Off US mkt)	Orlaam®	Opiate	Pain control, narcotic dependence	Risk of TdP	oral
Mesoridazine (Off US mkt)	Serentil®	Anti-psychotic	Schizophrenia	Risk of TdP	oral
Methadone	Dolophine®, Symoron®, Amidone®, Methadose®, Physeptone®, Heptadon®	Opiate	Pain control, narcotic dependence	Risk of TdP	oral, injection
Moxifloxacin	Avelox®, Avalox®, Avelon®	Antibiotic	Bacterial infection	Risk of TdP	oral, injection
Ondansetron	Zofran®, Anset®, Ondemet®, Zuplenz®, Emetron®, Ondavell®, Emeset®, Ondisolv®, Setronax®	Anti-emetic	Nausea, vomiting	Risk of TdP	oral, injection
Pentamidine	Pentam®	Antibiotic	Pneumocystis pneumonia	Risk of TdP	injection
Pimozide	Orap®	Anti-psychotic	Tourette's tics	Risk of TdP	oral
Probucol (Off US mkt)	Lorelco®	Antilipemic	Hypercholesterolemia	Risk of TdP	oral
Procainamide (Oral off US mkt)	Pronestyl®, Procan®	Anti-arrhythmic	Abnormal heart rhythm	Risk of TdP	injection

Generic Name	Brand Names	Drug Class	Therapeutic Use	Risk Category	Route
Quinidine	Quinaglute®, Duraquin®, Quinact®, Quinidex®, Cin-Quin®, Quinora®	Anti-arrhythmic	Abnormal heart rhythm	Risk of TdP	oral, injection
Sevoflurane	Ulane®, Sojourn®	Anesthetic, general	Anesthesia	Risk of TdP	inhaled
Sotalol	Betapace®, Sotalex®, Sotacor®	Anti-arrhythmic	Abnormal heart rhythm	Risk of TdP	oral
Sparfloxacin (Off US mkt)	Zagam®	Antibiotic	Bacterial infection	Risk of TdP	oral
Sulpiride (Not on US Mkt.)	Dogmatil®, Dolmatil®, Eglonyl®, Espiride®, Modal®, Sulpor®	Anti-psychotic, atypical	Schizophrenia	Risk of TdP	oral
Terfenadine (Off US mkt)	Seldane®	Antihistamine	Allergic rhinitis	Risk of TdP	oral
Thioridazine	Mellaril®, Novoridazine®, Thioril®	Anti-psychotic	Schizophrenia	Risk of TdP	oral
Vandetanib	Caprelsa®	Anti-cancer	Thyroid cancer	Risk of TdP	oral

Appendix K. List of drugs that potentially inhibit the metabolism of piperaquine

Chloramphenicol
Clarithromycin
Diltiazem
Erythromycin
Fluconazole
Itraconazole
Ketoconazole
Verapamil