DPART STUDY: DIHYDROARTEMISININ-PIPERAQUINE IN THE CONTEXT OF ANTIRETROVIRAL THERAPY

A UCSF/ YALE/ INFECTIOUS DISEASES RESEARCH COLLABORATION (IDRC)

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Table of Contents

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GLOSSARY

ACT	Artemisinin-combination therapy
AE	Adverse event
AL	Artemether-lumefantrine
ALT	Alanine transaminase (SGPT)
ART	Antiretroviral therapy
AS-AQ	Artesunate-amodiaquine
AST	Aspartate aminotransferase (SGOT)
AUC	Area under the curve
BIPAI	Baylor College of Medicine International AIDS Initiative
BID	Twice a day
CBC	Complete blood count
CDC	Center for Disease Control
CI	Confidence Interval
Cmax	Maximum plasma concentration of a drug
Cmin	Trough serum concentration of a drug
CoE	College of Excellence
Cr	Creatinine
CRF	Case report form
CYP	Cytochrome p450
CYP3A4	Cytochrome p450 3A4
DAIDS	Division of AIDS
DHA	Dihydroartemisinin
DMC	Data Management Centre
DP	Dihydroartemisinin-piperaquine
DTG	Dolutegravir
ECG	Electrocardiogram
EFV	Efavirenz
H	Hour
HFA	Height for age
Hg	Hemoglobin
HIV	Human Immunodeficiency Virus
HRPP	Human Research Protection Program
HIC	Human Investigation Committee
HS-RDT	High sensitive rapid diagnostic test
IDRC	Infectious Disease Research Center
IPT	Intermittent preventive therapy (for malaria)
IRB	Institutional review board
ITN	Insecticide treated net
JCRC-REC	Joint Clinical Research Center Research Ethics Committee
LAMP	Loop-mediated isothermal amplification
LBW	Low birth weight (<2500g)
LPV	Lopinavir
LPV/r	Lopinavir/ritonavir
LR	Lumefantrine

MOH	Ministry of Health
MU	Makerere University
NDA	Uganda National Drug Authority
NICHD	National Institute of Child Health and Human Development
NIH	National Institutes of Health
NLME	Non-linear mixed effects
NNRTI	Nonnucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
OR	Odds ratio
PCR	Polymerase chain reaction
PD	Pharmacodynamics
PI	Protease inhibitor
PK	Pharmacokinetics
PO	Oral
PQ	Piperaquine
PROMOTE	Prevention of Malaria and HIV Disease in Tororo
Q	Every
QD	Once every day
RR	Relative Risk
SBSHDREC	School of Biomedical Sciences Higher Degree Regulatory Ethics Committee
SD	Single dose
SP	Sulfadoxine-pyrimethamine
TS	Trimethoprim-sulfamethoxazole
UCSF	University of California, San Francisco
UGT	Uridine diphosphate-glucuronosyltransferase
ULN	Upper limit of normal
UNAIDS	Joint United Nations Program of HIV AIDS
UNCST	Ugandan National Council for Science and Technology
WFA	Weight for age
WFH	Weight for height
WHO	World Health Organization

DPART STUDY SCHEMA

DESIGN:

Open-label prospective intensive pharmacokinetic study of dihydroartemisinin-piperaquine (DP) in HIV-infected children on efavirenz (EFV)-, lopinavir/ritonavir (LPV/r)-, or dolutegravir (DTG)-based antiretroviral therapy (ART) and HIV-uninfected children not on ART. All children will be malaria-uninfected at the time of enrolment.

SAMPLE SIZE:

Total sample size (all arms): up to 190 children, depending on the number of children who are sequentially enrolled in the single dose and 3-dose DP arms.

Intervention groups:

1) HIV-infected children age 3 - 10 years on **LPV/r-based ART**
    - 20 children receiving single dose DP (**Group L1**)
    - 30 children receiving 3-dose DP (**Group L3)**

    Note that the same children from L1 can be re-enrolled into L3 following a washout period of 42 days

2) HIV-infected children age 3 - 10 years on **EFV-based ART**
    - 30 children receiving 3-dose DP (**Group E3**)

3) HIV-infected children age 11 - 17 years on **DTG-based ART**
    - 30 children/adolescents receiving 3-dose DP*¥ (**Group D3**)

Control groups:

1) HIV-uninfected children age 3-10 years
    - 20 children receiving 3-dose DP (with PK taken after the 1st DP dose ONLY) – control for group L1 (**Group C1)**
    - 30 children receiving 3-dose DP – controls for groups E3 and L3 (**Group C3a**)

2) HIV-uninfected children age 11-17 years
    - 30 children receiving 3-dose DP – controls for group D3 **(Group C3b)**

POPULATION:

HIV-infected participants will be enrolled from the Baylor Uganda Center of Excellence on the Mulago Hospital Complex, Kampala, Uganda. HIV-uninfected participants will be enrolled from Masafu General Hospital (MGH) complex in Busia, and other clinics in the surrounding area.

For Group D3, HIV-infected females age 13-17 who are pregnant or of childbearing potential and do not agree to consistent and reliable contraception will be excluded from enrollment due to potential risks of dolutegravir during 1st trimester.

STUDY REGIMENS FOR PARTICIPANTS:

DP weight-based dosing will follow World Health Organization Treatment Guidelines for uncomplicated malaria (April 2015). Three-dose once daily DP has also been extensively studied for chemoprevention in children and pregnant women.

1) ALL HIV-infected participants must be stabilized (i.e. no change in regimen for at least 10 days) on EFV, LPV/r, or DTG + 2 nucleoside reverse transcriptase inhibitors (NRTI).

2) HIV-infected children on LPV/r will be enrolled in two Phases. Phase I results will inform Phase II dosing, as a lower dose of DP over 3 days may be warranted. Phase II will not begin until PK and safety results from Phase I are evaluated. Participants in L1 and L3 will be encouraged to participate sequentially in Phase I and Phase II separated by a minimum 42-day washout period; however different children may be enrolled for the 2 phases.
    - Phase I participants (Group L1) will receive a *single* dose of DP to determine the magnitude (PK and safety) of the interaction before 3 doses are evaluated
    - Phase II participants (Group L3) will receive a 3-dose DP regimen (which consists of 3 days of a once daily DP dose)

3) HIV-infected children on EFV-based or DTG-based ART groups will be studied only in the context of the standard 3-dose DP (Groups E3 and D3).

4) HIV-uninfected children (controls) will only be studied in the context of standard 3-dose DP. However, 20 children will undergo intensive sampling around the 1st dose (Group C1) and 30 children will undergo intensive sampling around the 3rd dose (Groups C3a and C3b).

STUDY DURATION:

- Children in Groups L3, E3, D3, and C3 will be followed for 42 days.
- Children in Group L1 will be followed for 28 days
- Children in Group C1 will be followed for 14 days

HYPOTHESES:

1) Single and 3-dose DP in HIV-infected LPV/r-treated children will provide greater DP PK exposure when compared to single and 3-dose DP in HIV-uninfected children not on ART.

2) 3-dose DP in HIV-infected EFV-treated children will provide lower PK exposure when compared to 3-dose DP in HIV-uninfected children not on ART.

3) 3-dose DP in HIV-infected DTG-treated children will provide comparable PK exposure to 3-dose DP in HIV-uninfected children not on ART.

PRIMARY OBJECTIVES:

1) To evaluate and compare the PK exposure and safety of single dose DP in HIV-infected children on LPV/r-based ART to HIV-uninfected children not on ART (**Group L1 vs C1**)
2) To evaluate and compare the PK exposure and safety of 3-dose DP in HIV-infected children on LPV/r-based ART to HIV-uninfected children not on ART (**Group L3 vs C3a**)
3) To evaluate and compare the PK exposure of 3-dose DP in HIV-infected children on EFV-based ART and HIV-uninfected children not on ART (**Group E3 vs C3a**).
4) To evaluate and compare the PK exposure of 3-dose DP in HIV-infected children on DTG-based ART and HIV-uninfected children not on ART (**Group D3 vs C3b**).

SECONDARY OBJECTIVES:

1) The effects of DP on antiretroviral PK in the above 3-dose arms (EFV, DTG, LPV/r and controls)
2) To evaluate the association of anthropomorphic indicators of malnutrition on PK exposure of DP in HIV-infected and HIV-uninfected children
3) Assess auto-induction of DHA from single dose to 3-dose
4) To assess the prevalence of pharmacogenetic variants in key metabolic enzymes, including UGT1A1, CYP2B6, CYP3A4, and ABCB1, and the impact of these variants on ACT and ART PK.

I.0 INTRODUCTION

1.1 Background

1.1.1 Malaria and artemisinin-combination therapy in sub-Saharan Africa.

Plasmodium falciparum malaria in Africa remains one of the most challenging infectious diseases in the world. According to the latest World Malaria Report, there were 219 million cases of malaria in 2017, up from 217 million cases in 2016. The estimated number of malaria deaths stood at 435 000 in 2017, an improvement from the previous year (451 000)(1). Sub-Saharan Africa has the heaviest burden, bearing >90% of deaths, primarily in young children <5 years of age for whom antimalarial dosing guidelines are not yet optimized.(2) Treatment of uncomplicated malaria in children relies solely upon the artemisinin-combination therapies (ACTs).(2, 3) The artemisinins rapidly reduce parasite load while the long-acting partner drugs eliminate residual parasites and protect against artemisinin resistance and recurrent infection. In 2013 alone, an estimated 392 million ACT courses were procured by malaria-endemic countries(2) with artemether-lumefantrine (AL), artesunate-amodiaquine (AS-AQ), and dihydroartemisinin-piperaquine (DP) stipulated as first-line according to the World Health Organization (WHO).(4) While AL is the most widely utilized ACT, DP use is on the rise as it protects best against recurrent infections, due to the longer half-life of piperaquine (PQ; 1 month) as compared to lumefantrine (3 to 5 days).(4, 5) Thus, DP is being used both for treatment (2, 6-9) and is under study as intermittent prevention therapy for children and pregnant women.(10-13) In addition, given widespread resistance to SP, a commonly used IPT, in parts of Africa, many are considering the use of DHA/PQ, which provides rapid killing of most parasites by DHA and protection for weeks after therapy due to the long half-life of PQ.(14)In Uganda, directly observed monthly DHA/PQ administered to school children 6–14 years of age for 1 year had a protective efficacy of 96 %.(14, 15)[t]

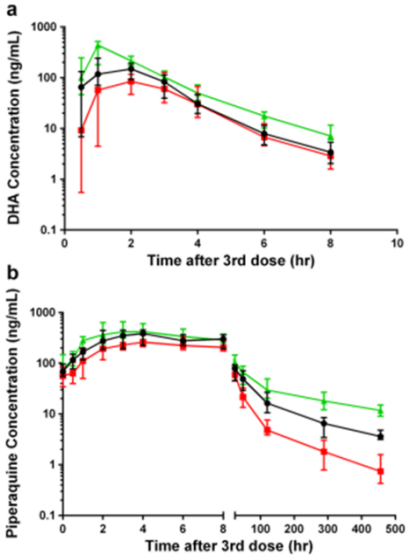
1.1.2 HIV and antiretroviral treatment (ART) in sub-Saharan Africa. Sub-Saharan Africa is also home to 25 million people with HIV; 2.9 million of whom are children <15 years,(16-19) and all of whom are eligible for ART under new WHO guidelines.(20) First-line ART includes lopinavir/ritonavir (LPV/r)-based ART for children <3 years, and efavirenz (EFV)-based ART for children >3 years(20-22) with 86% of 58 WHO focus countries adopting EFV-based ART as their preferred 1st-line regimen. (23) As of 2018, WHO has recommended dolutegravir (DTG), an integrase inhibitor, as alternative first-line therapy for children >6 years weighing at least 15kg (24). The recommended dose for adolescence and adults is 50mg once daily.(23) There have been many initiatives to transition eligible patients from the traditional EFV-based therapy to DTG-based regimen due to its high potency, high genetic resistance barrier, and low toxicity risks.(25) We will study the antimalarial DP, given as single and/or 3 consecutive daily doses in 4 populations: HIV-uninfected children and HIV-infected children stabilized on LPV/r-, EFV-, or DTG-based ART—to further elucidate the PK effects and safety profiles of drug-drug interactions between HIV and anti-malarial medications.(26) Due to reports in May 2018 of a potential increased risk of neural tube defects in women exposed to DTG during the 1st trimester, we will exclude any females on DTG-based

ART, age 13-17 from participation in our study who are pregnant, or of childbearing potential and do not agree to consistent and reliable contraception.(27)

1.1.3 Malaria and HIV in Uganda. Uganda bears a heavy burden of both malaria and HIV. Although malaria prevention and control programs have reduced infections and mortality overall, Uganda still has one of the highest malaria transmission intensities in the world.(23) In Tororo, children experience up to 2 to 6 malaria episodes per year, despite usage of bed nets and trimethoprim-sulfamethoxazole (TS) for those HIV-infected.(2, 28) For HIV, Uganda is lauded for prior success in stemming the HIV epidemic, but new infections are now on the rise.(28-32) It is estimated that 2 million HIV-infected children will reside in sub-Saharan African in 2020.(20) Thus, HIV-malaria co-infection remains common, with treatment complicated by multiple pharmacological factors that require field-based studies.

1.1.4 Significant drug interactions occur between ART and ACT.
Both components of AL and DP undergo metabolism. Dihydroartemisinin (DHA) undergoes metabolism via uridine diphosphate-glucuronosyltransferases (UGT).(20, 33) Our group has also recently reported on the major pathway for PQ metabolism.(34-36) More specifically, our group studied the metabolism of PQ *in vitro* and reported inhibition was highest (83%) with ketoconazole, a selective CYP3A4 inhibitor, suggesting a primary role for CYP3A4 in PQ metabolism. Notably, with regards to ART, ritonavir and EFV cause potent CYP3A4 inhibition and induction, respectively.(37-39) DTG is primarily metabolized by UGT1A1, with a small contribution by CYP3A4.(40)

Figure 1. Plasma concentration–time profile of DHA (a) and piperaquine (b) in HIV-uninfected pregnant women (black line) and HIV-infected pregnant women stabilized on EFV-based ART (red line) and HIV-uninfected postpartum women (green line). Data are represented as median (IQR).



For AL, we have recently demonstrated a marked increase in lumefantrine exposure (defined by the area under the concentration curve, AUC) occurred during LPV/r-based ART co-administration, contrasting with a highly significant decrease in artemether, DHA, and lumefantrine when given with EFV-based ART, resulting in a 10-fold variance in lumefantrine exposure in HIV-infected children with malaria.(41-43) The resulting change in AL exposure with EFV-based ART was associated with a *~4-fold higher risk of recurrent malaria* compared to the change in AL exposure with LPV/r. These results, and our earlier findings of unexpected toxicity with AS-AQ and EFV, have impacted HIV and malaria treatment guidelines; specifically, AQ and EFV co-administration is to be avoided and AL and EFV co-administration should be used with caution.(6, 44-46)

Table 1. Impact of pregnancy and EFV-based ART on the pharmacokinetics of piperaquine

	During pregnancy		After pregnancy	Ratio			
	HIV uninfected (no ART)	HIV infected (EFV)	HIV uninfected	pregnant/non-pregnant (HIV uninfected)		EFV/no ART (all pregnant)	EFV and pregnant/non-pregnant
	n=30***	n=26¥	n=30	Paired (n=27)	All subjects		
C_{max}, ng/mL	391 (323, 474)	342 (285, 411)	499 (393, 633)	0.82 (0.12)	0.78 (0.08)	0.88 (0.13)	0.69 (0.001)
T_{max}, hr	3.11 (3.00, 4.03)	3.99 (2.03, 5.98)	3.06 (2.07, 4.03)	1.01 (0.91)	1.02 (0.62)	1.28 (0.84)	1.30 (0.64)
$t_{1/2}$, hr	161 (143, 183)	124 (104, 149)	208 (187, 232)*	0.79 (0.012)**	0.77 (0.003)	0.77 (0.01)	0.60 (<0.0001)
$AUC_{0\text{-}d21}$, hr·µg/mL	10.6 (8.84, 12.7)	6.60 (5.57, 7.83)	17.6 (15.1, 20.7)	0.61 (0.0001)	0.60 (<0.0001)	0.62 (0.0001)	0.38 (<0.0001)
C_{7d}, ng/mL	30.5 (25.9, 36.0)	15.1 (13.0, 17.6)	39.0 (32.3, 47.2)	0.79 (0.03)	0.78 (0.07)	0.50 (<0.0001)	0.39 (<0.0001)
C_{14d}, ng/mL	15.0 (12.4, 18.1)	6.67(5.44, 8.19)	22.6 (18.7, 27.3)	0.68 (0.004)	0.66 (0.007)	0.45 (<0.0001)	0.30 (<0.0001)
C_{21d}, ng/mL	11.8 (10.2, 13.6)	3.75 (2.77, 5.08)	14.5 (12.2, 17.1)	0.83 (0.05)	0.81 (0.02)	0.32 (<0.0001)	0.26 (<0.0001)

Note: ART, antiretroviral therapy; EFV, efavirenz-based ART; C_{max}, maximal concentration, T_{max}, time to reach maximal concentration, $t_{1/2}$, drug elimination half life, AUC, area under concentration-time curve, AUC was calculated using piperaquine concentrations from venous plasma with conversion of capillary to venous plasma concentrations when necessary; C_{7d}, C_{14d}, and C_{21d} are actual capillary plasma concentrations at day 7, 14, and 21 post the 3rd dose. Data are presented as geometric mean (95% confidence interval) except for T_{max}, which is reported as median (interquartile range). P-value is calculated with Stata® 12.1 using Wilcoxon rank sum test (or signed rank test for paired analysis) Significance level: alpha=0.017(0.05/3); . *n=29. **n=26. Paired represents same HIV uninfected women enrolled antepartum and postpartum.***one subject excluded since missing day 21 PK sample. ¥ one subject excluded since missing day 14 and 21 samples

For DP, data on the interaction with ART *in vivo* is limited. There is one study in malaria uninfected adults (Banda CG et al 2018) that evaluated DP in the context of EFV-, LPV/r-, and nevirapine-based ART regimens. Banda et al. found that there was a significance decrease in DP exposure in the EFV-based ART group and did not find a significant difference in DP exposure between non-HIV infected adults and adults on LPV/r-based ART (albeit this arm only completed the half-dose phase of the study). Our group also recently demonstrated a reduction in DHA and PQ exposure in the setting of EFV-based ART in pregnant women (Figure 1; Table 1).(4, 45, 47-49) No data are available on the interaction of DP and ART in children, nor on the impact of ART on clinical outcomes in the setting of the EFV-, LPV/r-, or DTG-based ART so that optimized treatment guidelines can be developed.

1.1.5 Piperaquine is well tolerated but has been associated with prolongation of the QT interval. Overall, DP is very well tolerated, but concern exists over the potential for QT prolongation.(50) Myint, et al. conducted a systematic review of DP efficacy and safety for treatment of malaria using data from 14 clinical trials involving adults and children.(17, 51, 52) 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. With regards to QT prolongation, a randomized study of a compressed, 2-day regimen of DP for malaria prevention in Cambodian male adults found that 4 of 47 individuals in developed QTcF prolongation of >500 msec, leading to premature study termination.(52) In comparison to individuals receiving placebo, those receiving the compressed regimen had a mean increase in QTcF of 46 msec post-treatment; a change that was correlated with piperaquine peak concentrations. Of note, in our group's recently published study of DP PK in the setting of EFV in pregnant women, there was no significant correlation of PQ PK parameters and QT prolongation, and not QTcF >450 msec were observed, although peaks were lower than those seen in Cambodian adults.(51) Thus, data are conflicting regarding the risk of QT prolongation with PQ, as it remains unclear whether the observed prolongation is due to recovery from malaria (shortening of QT occurs during disease) and/or a direct effect of PQ.(50) Given concerns that higher PQ levels may lead to QT prolongation, and the likelihood of PQ-ART interactions in children, PK data for DP with concomitant ART is urgently needed.

1.1.6 ACT PK exposure is also affected by childhood development. The PK disposition of drugs in children differs substantially from that in adults.(51, 53-57) For metabolism, UGTs mature from 0-6 months of age while CYPs mature over 12 months of age.(58-60) Children >1 year from resource-rich settings often exhibit higher drug clearance compared to adults, which can reduce PK exposure and warrant higher doses (per kg).(61, 62) For DP, Ugandan children <2 years had PQ exposure ~33% lower than children 2-10 yrs,(63-65) and a Worldwide Antimalarial Resistance Network (WWARN) pooled analyses reported that children <5 yrs are at the highest risk of receiving suboptimal DP doses, differences that again may be attributed to higher drug clearance in young children.(10, 66-69) Importantly, as a result of work by our group and others, the WHO recently revised its dosing of DP in young children to improve PK exposure.(19)

1.1.7 Suboptimum dosing of ACTs in the context of EFV-based ART has important implications for the emergence and spread of ACT resistance. Systematic under-dosing, whether due to ART, malnutrition or other factors, is a concerning factor in the development and spread of antimalarial drug resistance.(4) This was suggested for sulfadoxine-pyrimethamine,(70) and recently for DP, where 36% of patients in Cambodia who failed to clear infection had PQ concentrations below *in vitro* target levels.(70, 71) We have now shown that a) EFV-treated children have ~2-fold *reduced* exposure to AL, b) EFV-treated pregnant women have a significantly reduced exposure to DP. For the ~3 million sub-Saharan African children with HIV, EFV is first-line (when >3years).(51) For sub-Saharan African children with malaria, DP use is increasing, both for treatment and for chemoprevention. Thus, with the spread of artemisinin and DP resistance in Southeast Asia,(20) and emerging concerns for Africa,(72-75) there is mounting concern that suboptimal DP dosing in HIV-infected children on EFV-based ART or children will contribute to the emergence and selection of DP-resistant parasites.(76, 77)

1.1.8 Genetic polymorphism in metabolic enzymes can alter both ACT and ART drug exposure. Antiretrovirals and ACT long-acting partner drugs are metabolized by enzymes that display genetic variation. Variants in these enzymes (*CYP2B6, CYP3A4 / CYP3A5, ABCB1,* and *UGT1A1*) have been shown to impact metabolism of drugs that are substrates of these pathways. For example, Maganda et al. found that individuals with a CYP2B6*6 genotype had increased EFV exposure, which was then linked to a significant reduction in lumefantrine exposure compared to individuals who were not taking concomitant EFV (78). As both lumefantrine and PQ are metabolized by CYP3A4, there could be a similar reduction in PQ exposure in CYP2B6*6 children taking concomitant EFV. We will also explore if there are other instances where pharmacogenomics could play a role in drug exposure by investigating the other CYPs and UGTs listed above that are key pathways for drugs studied in this proposal

1.2 Rationale

This study is designed to directly address antimalarial PK and safety objectives in children, primarily HIV-infected children, one of the most vulnerable populations to malaria in the world. This study will focus on the pharmacology of one of the WHO first-line options for antimalarial treatment, DP. DP has also been studied for its use in chemoprevention of malaria, both in children and in pregnant women, and in settings of mass drug administration, although it is not yet been recommended for prevention of malaria in WHO guidelines. Our study will be conducted in healthy HIV-infected children on EFV-, LPV/r- or DTG-based ART, as well as in HIV-uninfected children who will serve as a control group. Children in the study will not have malaria. The primary goal of the study is to assess the PK and safety of DP in the setting of co-administration with first-line ART regimens.

The ACTs are the most important drugs for the treatment of uncomplicated malaria and could have a vital role in the setting of chemoprevention in the upcoming years. Despite their widespread use globally, fundamental questions remain for assuring their optimal

use in our most vulnerable populations, especially for children in the context of interacting medications. Our results on ACT drug interactions are striking and reveal that HIV-infected children treated with AL have a wide range of antimalarial exposure depending on their ART. Compared to no ART, lopinavir/ritonavir (LPV/r)-based ART increased lumefantrine PK exposure >2-fold, while efavirenz (EFV)-based ART, first-line for children >3 years of age, dramatically reduced exposure to artemether, DHA, and lumefantrine. Importantly, these PK changes were associated with a 4-fold higher risk of recurrent malaria after treatment with AL in children receiving EFV compared to those receiving LPV/r.

For DP, our group has demonstrated that PQ is metabolized *in vitro* by CYP3A4, suggesting that it may be subject to similar drug-drug interactions. These findings were supported by a ~38% lower PQ concentration in pregnant women on EFV versus HIV-uninfected pregnant women.(70) Data on the interaction of EFV and DP in children are lacking, and if a similar reduction in DP exposure is seen, may be a risk for reduced efficacy and the selection of resistant parasites. Similarly, no data exists on the interaction of LPV/r-based ART and DP, the concern being that an increase in PQ concentrations may be a risk for QT prolongation. Additionally, there is no data on the interaction of DTG and DP in children and adolescents despite the high potential for DTG usage given the WHO's recent initiative to encourage clinicians to transition eligible patients to DTG due to its high genetic resistance barrier, potency, and relatively low toxicity.

QT prolongation is safety concern with piperaquine. Based on our groups prior work, we know that piperaquine is a substrate of CYP3A4, and therefore piperaquine levels are anticipated to increase with concomitate LPV/r administration, a known CYP3A4 inhibitor. Given concerns for cardiotoxicity, for the LPV/r-based arm ONLY, we will start with a single dose of DP in a group of HIV-infected children on LPV/r-based ART and a control group of single dose DP in HIV-uninfected children, to determine the magnitude and safety of the interaction, as well as to monitor QT intervals via ECGs (Phase I). Following the assessment of the PK and ECG-based safety data from the single dose DP groups, we will proceed with one of three different options: 1) no further study of LPV/r with DP due to safety concerns, 2) a reduced mg/kg dose of 3-dose DP in the context of LPV/r, or 3) standard mg/kg dosing of 3-dose DP in the context of LPV/r-based ART (Phase II). ECGs will be performed in participants in all study groups to ensure safety, and to provide data on QT intervals across the age spectrum.

We will conduct our study in HIV-infected children without malaria living in the urban setting of Kampala, Uganda, where our team has had previous experience in directing PK/PD studies, and where the largest pediatric HIV clinic in Uganda is located, and has long-standing established cohorts. For HIV-uninfected children without malaria, we will conduct our study in the malaria-endemic region of Eastern Uganda in Busia where our team has long standing experience directing field-based PK/PD studies in the most relevant populations. We will utilize state-of-the-art intensive PK designs and drug assay methods to determine drug exposure and safety parameters.

Rationale for this study is summarized below:

- Dosing guidelines for children have historically relied on studies carried out in adults despite knowledge that children exhibit distinct physiological characteristics that impact how drugs are handled by their body
- Insufficient dosing may compromise care of acute infection but more importantly contribute to development of resistance
- Excess PK exposure to PQ may be associated with a risk of QT prolongation
- We have shown that PQ is metabolized by CYP3A4, and that pregnant women on EFV-based ART have significant decreases in DP exposure.
- Administration of DP with LPV/r-based ART is expected to increase PQ exposure, with a concern for a risk of QT prolongation.
- Despite the increase in DTG-usage in recent years, there is limited safety and PK exposure data in the setting of DTG-based ART and ACT co-administration.
- Given the above, the study of drug-drug interactions between DP and ART are best carried out in healthy malaria-uninfected children prior to larger studies to assess PK and PD in the setting of malaria infection.

2.0 STUDY OBJECTIVES

2.1 Primary Objectives:

1) To evaluate and compare the PK exposure and safety of single dose DP in HIV-infected children on LPV/r-based ART to HIV-uninfected children not on ART (**Group L1 vs C1**)
2) To evaluate and compare the PK exposure and safety of 3-dose DP in HIV-infected children on LPV/r-based ART to HIV-uninfected children not on ART (**Group L3 vs C3a**)
3) To evaluate and compare the PK exposure of 3-dose DP in HIV-infected children on EFV-based ART and HIV-uninfected children not on ART (**Group E3 vs C3a**).
4) To evaluate and compare the PK exposure of 3-dose DP in HIV-infected children on DTG-based ART and HIV-uninfected children not on ART (**Group D3 vs C3b**).

2.2 Secondary Objectives:

1) The effects of DP on antiretroviral PK in the above 3-dose arms (EFV, DTG, LPV/r and controls)
2) To evaluate the association of anthropomorphic indicators of malnutrition on PK exposure of DP in HIV-infected and HIV-uninfected children
3) Assess auto-induction of DHA from single dose to 3-dose
4) To assess the prevalence of pharmacogenetic variants in key metabolic enzymes, including UGT1A1, CYP2B6, CYP3A4, and ABCB1, and the impact of these variants on ACT and ART PK.

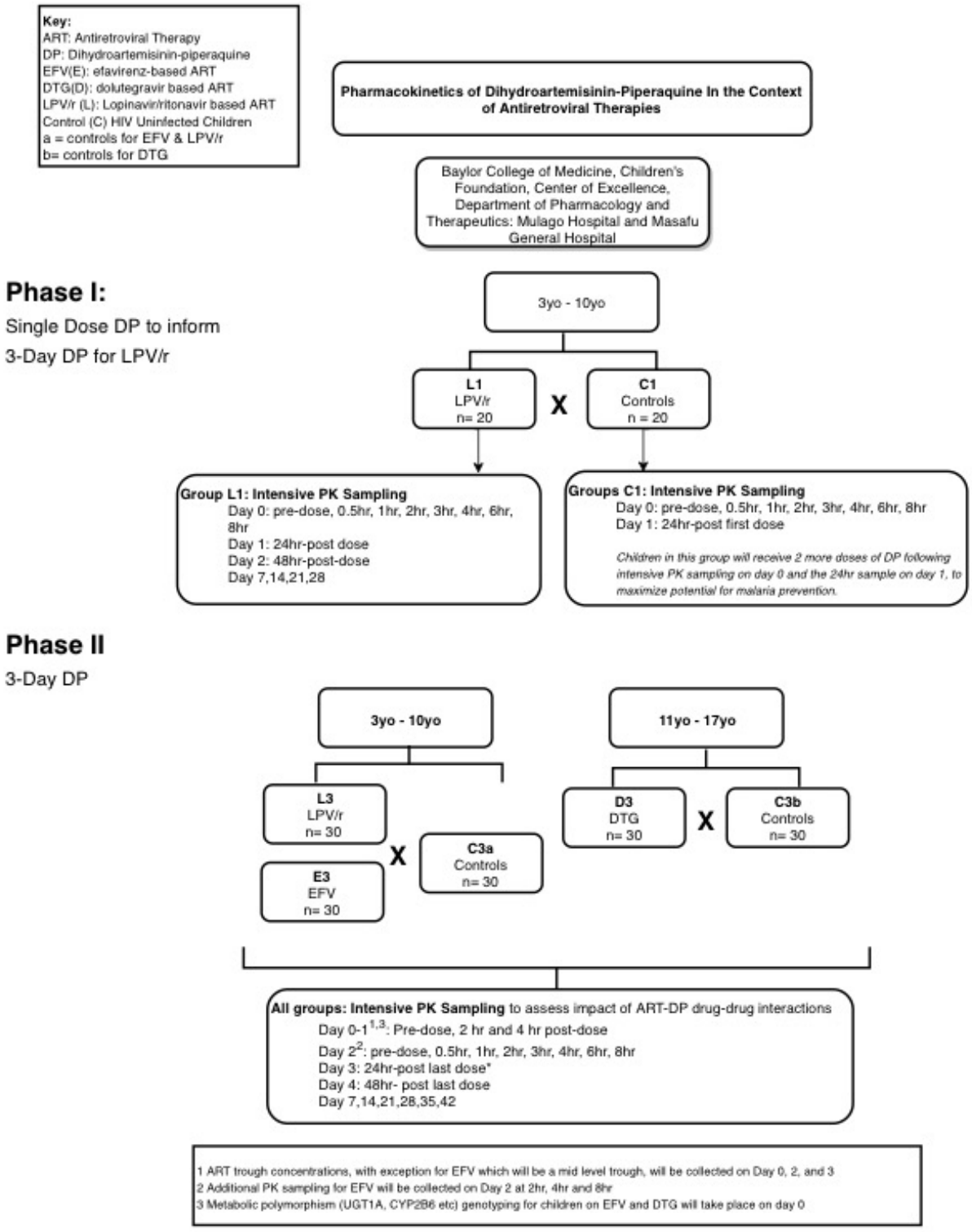
3.0 STUDY DESIGN

This is an open-label prospective pharmacokinetic and safety study of DP and 3 different ART regimens in non-malaria-infected 1) HIV-infected children and 2) HIV-uninfected controls not on ART. DP is a WHO approved first-line treatment for malaria and is increasingly being studied for its use in malaria chemoprevention. No change in standard of care treatment regimens will be made as part of this protocol. Figure 2 summarizes the design. This study will enroll a) HIV-infected children, and b) HIV-uninfected children. HIV-infected participants will be enrolled through the Baylor College of Medicine Children's Foundation Uganda (Baylor-Uganda) Center of Excellence (CoE) in Kampala and the HIV-uninfected participants will be enrolled through the Masafu General Hospital (MGH) in Busia, or other referral centers in the area. Baylor-Uganda is a not for profit child health and development organization affiliated with the Baylor College of Medicine International AIDS Initiative (BIPAI). All HIV care will be managed by the participant's primary HIV providers.

3.1 Overview of Pharmacokinetic Sampling Design

Subjects will undergo an intensive PK study sampling design, which entails multiple venous blood collections in a smaller sample of individuals to accurately estimate drug exposure over time. These studies will be conducted in both HIV-infected and HIV-uninfected participants and will allow us to investigate DP PK exposure in the context of EFV-, LPV/r- and DTG-based ART in HIV-uninfected children. Comparisons will be based on an intensive PK design for DP AUC estimations. A sample size of 20 children/adolescents will be needed in groups L1 and C1. A sample size of 30 will be needed for each of the other arms (D3, E3, L3, C3a, and C3b). Sampling will occur up to day 42 in the 3-dose groups given the long half-life of PQ and for 14 or 28 days in the single dose groups. The generation of an AUC will permit robust comparisons so that results will inform treatment guidelines and policy.

Figure 2—Overview of Study Design


Key:
ART: Antiretroviral Therapy
DP: Dihydroartemisinin-piperaquine
EFV(E): efavirenz-based ART
DTG(D): dolutegravir based ART
LPV/r (L): Lopinavir/ritonavir based ART
Control (C) HIV Uninfected Children
a = controls for EFV & LPV/r
b= controls for DTG
Pharmacokinetics of Dihydroartemisinin-Piperaquine In the Context of Antiretroviral Therapies
Baylor College of Medicine, Children's Foundation, Center of Excellence, Department of Pharmacology and Therapeutics: Mulago Hospital and Masafu General Hospital
Phase I:
Single Dose DP to inform
3-Day DP for LPV/r
3yo - 10yo
L1
LPV/r
n= 20
X
C1
Controls
n = 20
Group L1: Intensive PK Sampling
Day 0: pre-dose, 0.5hr, 1hr, 2hr, 3hr, 4hr, 6hr, 8hr
Day 1: 24hr-post dose
Day 2: 48hr-post-dose
Day 7,14,21,28
Groups C1: Intensive PK Sampling
Day 0: pre-dose, 0.5hr, 1hr, 2hr, 3hr, 4hr, 6hr, 8hr
Day 1: 24hr-post first dose
Children in this group will receive 2 more doses of DP following intensive PK sampling on day 0 and the 24hr sample on day 1, to maximize potential for malaria prevention.
Phase II
3-Day DP
3yo - 10yo
11yo - 17yo
L3
LPV/r
n= 30
X
C3a
Controls
n= 30
E3
EFV
n= 30
D3
DTG
n= 30
X
C3b
Controls
n= 30
All groups: Intensive PK Sampling to assess impact of ART-DP drug-drug interactions
Day 0-1[1,3]: Pre-dose, 2 hr and 4 hr post-dose
Day 2[2]: pre-dose, 0.5hr, 1hr, 2hr, 3hr, 4hr, 6hr, 8hr
Day 3: 24hr-post last dose*
Day 4: 48hr- post last dose
Day 7,14,21,28,35,42
1 ART trough concentrations, with exception for EFV which will be a mid level trough, will be collected on Day 0, 2, and 3
2 Additional PK sampling for EFV will be collected on Day 2 at 2hr, 4hr and 8hr
3 Metabolic polymorphism (UGT1A, CYP2B6 etc) genotyping for children on EFV and DTG will take place on day 0

3.2 Overview of study groups

HIV-infected children will be enrolled primarily through the Baylor-Uganda CoE located on the Mulago Hospital Complex. HIV-uninfected children will be enrolled from Masafu General Hospital in the Busia District and other referral clinics in the area.

Table 2. Summary of Study Groups

Group	HIV status	ART regimen	DP regimen	Age range (years)	Duration follow-up (days)	Sample Size
E3	Infected	EFV-based ART	3-dose	3 - 10	42	30
D3	Infected	DTG-based ART	3-dose	11 - 17	42	30
L1*	Infected	LPV/r-based ART	Single dose	3 - 10	28	20
L3	Infected	LPV/r-based ART	3-dose	3 - 10	42	30
C1**	Uninfected	None	3 -dose	3 - 10	14	20
C3a	Uninfected	None	3-dose	3 - 10	42	30
C3b	Uninfected	None	3-dose	11 - 17	42	30

* Single dose group L1 may be sequentially re-enrolled into 3-dose DP groups L3 provided there is a 42-day washout period in between phases.
** Group C1 will have intensive PK performed after the 1st dose of DP only to minimize blood volumes

All sample sizes refer to “evaluable” PK participants, in other words, those that have completed follow-up sufficiently to obtain all required PK study samples. If a child does not complete PK sampling procedures for the intensive study for any reason (they are not “evaluable”), the child may be re-enrolled in the intensive PK study if they meet eligibility requirements. More than 2 missing PK samples will permit a child to repeat the study after a 42-day washout period from the last dose of DP.

3.2.1 HIV-infected participants

This protocol will study the clinical pharmacology of DP in HIV-infected participants on EFV-, LPV/r- and DTG-based ART regimens. Children and adolescents in the study will be between 3 - 17 years. 30 children will be enrolled for intensive PK studies in each ART regimen and followed for up to 42 days (groups E3, D3, L3). For group L1, 20 children will be enrolled, and PK will be assessed after a single dose, with children followed for 28 days.

3.2.2 HIV-uninfected participants

This protocol will also study the clinical pharmacology of DP in HIV-uninfected children aged 3 - 17 years. Up to 60 HIV-uninfected subjects will be enrolled for intensive PK studies of 3-dose DP and followed for up to 42 days (groups C3a and C3b). For group C1, 20 children will be enrolled, and PK will be assessed after the 1st dose of a 3-dose regimen, with children followed for 14 days. HIV-uninfected children will primarily serve as controls for PK comparison with HIV-infected children.

3.3 Study sites

The primary study site for recruitment of HIV-infected children is the Baylor-Uganda CoE on the Mulago Hospital Complex in Kampala, Uganda. The Baylor College of Medicine Children's Foundation Uganda has been in operation since 2003 and delivers HIV/AIDS care and treatment to over 8500 HIV-infected children and family members, with many additional patients seen at affiliated outreach sites (https://bipai.org/uganda). All HIV-related care will be provided during the study by the child's usual provider at the Baylor clinic.

The primary study site for recruitment of HIV-uninfected children is at the Masafu General Hospital (MGH) campus situated in the Busia District, in Eastern Uganda. The study clinic in Busia will remain open 7 days a week from 8 a.m. to 5 p.m. HIV-uninfected participants will be recruited from the community surrounding the MGH in the Busia District and all study procedures for the HIV-negative children will be conducted at the MGH study site. If non-study related medical care is needed for HIV-uninfected participants, parents/guardians/participants will be instructed to go to the MGH and request that a study physician on-call be contacted.

Similar to our previous PK studies, we will allow for referrals from neighboring health centers in the Busia districts, provided that entry criteria are met.

4.0 SELECTION AND ENROLLMENT OF SUBJECTS

4.1 Inclusion Criteria

4.1.1 All participants:

1) Agreement to come to clinic for all follow-up PK and safety evaluations
2) Provision of informed consent

4.1.2 HIV-infected participants:

1) Residency within 30km of Mulago Hospital
2) Confirmed HIV infection (confirmed positive rapid HIV test or HIV RNA as per Ugandan guidelines)
3) On stable EFV-, LPV/r- or DTG-based ART for at least 10 days prior to enrollment
4) Age 3 - 10 years if on EFV-based ART or LPV/r-based ART
5) Age 11 - 17 years if on DTG-based ART

4.1.3 HIV-uninfected participants:

1) Residency within 30km of Masafu General Hospital
2) Confirmed HIV negative test (confirmed positive rapid HIV test or HIV RNA as per Ugandan guidelines)
3) Age 3 - 17 years

4.2 Exclusion Criteria

1) History of significant comorbidities such as malignancy, active tuberculosis or other active WHO stage 4 disease
2) Receipt of any medications known to affect CYP450 metabolism (except ART) within 14 days of study enrolment (see 4.2.1)
3) Hemoglobin < 7.0 g/dL
4) Current malaria infection or recent treatment with antimalarials within 28 days of enrolment.
5) Asymptomatic parasitemia detected by microscopy or RDT
6) History of side effects with DP
7) Prior history of cardiac disease (personal or family), baseline QTc >450msec, or receipt of any cardiotoxic drugs or those known to prolong QT intervals History of significant comorbidities such as malignancy, active tuberculosis or other WHO stage 4 disease
8) Weight ≤ 6kg
9) HIV-infected females on DTG-based ART and age 13-17 years who are pregnant or of childbearing potential and do not agree to consistent and reliable contraception

4.2.1 Disallowed Medication Guidelines

The following medications are disallowed within 3 weeks prior to receiving study drug:

- Carbamazepine
- Clarithromycin
- Erythromycin (oral)
- Ketoconazole
- Phenobarbital
- Phenytoin
- Rifabutin
- Rifampicin
- Halofantrine
- Any other medication known to significantly affect CYP450 metabolism.
- Grapefruit juice should be avoided during the study due to its potential effects on CYP3A4.

4.3 Identification and recruitment of study participants

HIV-infected children will be enrolled from sites listed above (Section 3.3). Parents/guardians of children will be approached for their willingness to participate in the PK study. Children will undergo up to 42 days of follow-up for which they will have

PK sampling carried out. Participants may be recruited through multiple referral mechanisms including those primary sites described below.

4.4 Screening

HIV-infected and HIV-uninfected children will be screened for study eligibility at the time they present to the Baylor-Uganda CoE, MGH, or other referral sites. Parents/guardians will be asked about their willingness to have their child participate in the PK study involving up to a 42-day follow-up. If the initial verbal screening criteria assessed by interview are met, the child and parent or guardian will be asked to provide informed consent for laboratory screening *and* study participation (see Section 4.5). Consenting for PK procedures and future use of biologic specimens will be on two separate consent forms. Assent will be obtained following guidelines stipulated by the Uganda National Council for Science and Technology. **All laboratory procedures, including those for screening, will only be conducted after informed consent/assent have been obtained**.

4.4.1 Laboratory Screening

1. **Confirmation of negative malaria status.** Will be done by either RDT or thick smear. Blood will be obtained from a finger prick (in very young children, heel sticks may be substituted for finger pricks). Thick smears will be read (and counted if necessary) by the laboratory technicians at the time of presentation. Smears will be considered negative when examination of 100 high-power fields does not reveal parasites. If the thick blood smear is positive, participants will either be managed by study physicians, or referred for care to adjacent clinics, and treated as per standard of care.

2. **Pregnancy testing.** All females, age 13 and above will undergo pregnancy testing. All pregnant females will be excluded from participation.

3. **Hemoglobin testing.** Will be performed by capillary finger-prick. Those with Hb< 7g/dL will be excluded.

4. **HIV testing of HIV-uninfected children.** HIV counseling and testing will be done on all HIV-uninfected children as part of study enrollment screening. Based on results of testing, children will be referred appropriately to study staff and clinical care (in the event they test positive for HIV for the first time).

4.5 Study informed consent and enrollment

Study physicians will conduct the informed consent discussion in the study clinic in the appropriate language for the adult or the parents/guardians; translators will be used if necessary. Children who are on LPV/r-based ART participating in the single dose intensive evaluations may be consented for both the single dose and 3-dose evaluations at the time of enrollment depending on the study enrollment needs. Following the informed consent discussion, parents/guardians/participants will be asked

by the study physicians to sign a research participation informed consent form or assent form (Appendices) approved by the UCSF Human Research Protection Program (UCSF HRPP), Yale Human Investigations Committee (Yale HIC), Baylor College of Medicine IRB, Joint Clinical Research Centre - Research Ethics Committee (JCRC-REC), the National Drug Authority in Uganda and the Uganda National Council for Science and Technology (UNCST) that will be available in 3 languages (Luganda, Samia, English). The informed consent forms will contain information and permission for specimen banking and future use of biological specimens. If the parent/guardian/participant is unable to read or write, their fingerprint will substitute for a signature, and a signature from a witness to the informed consent procedures will be obtained. The witness may be a family member, clinic staff not conducting the informed consent discussions, or a translator. Assent will be obtained for those children 8-17 years, according to Ugandan guidelines.

5.0 MALARIA STUDY DRUG: DIHYDROARTEMISININ-PIPERAQUINE

Participants enrolling in the study will receive DP as oral tablets which will be dosed based on the child's weight and WHO recommendation. DP is a first-line treatment for malaria by the WHO and is being studied for possible use as chemoprevention. Importantly, children with malaria in Uganda may receive DP at no charge by the Ugandan Ministry of Health when presenting for care with malaria. Dosing in children is primarily based on weight-based adjustments to adult dosing. Overall, DP is very well tolerated, but concern exists over the potential for QT prolongation.(50) Myint, et al. conducted a systematic review of DP efficacy and safety for treatment of malaria using data from 14 clinical trials involving adults and children.(17, 51, 52) 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. Of note, the only serious adverse events in these 14 studies included 5 deaths (2 adults, 3 children) that were thought unrelated to DP.

Lwin, et al. conducted a randomized controlled trial of monthly versus bimonthly DP IPT among 961 adults at high risk of malaria at the Northwest border of Thailand.(52) 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 versus bimonthly versus placebo arms. There was an increased risk of joint pain among participants randomized to the placebo arm, but otherwise there was no difference noted in adverse events by study arm. There was only one serious adverse event, which was not related to the use of DP.

5.1 DP and QT prolongation. In 2016, the WHO convened an Evidence Review Group (ERG) to assess the cardiac safety of antimalarials, with particular focus on the QT interval.(17) The QT interval is associated with the risk of drug-induced torsades de pointes (TdP), a potentially lethal arrhythmia. The review followed the "ICH E14: The

clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs", guidelines set forth by the Food and Drug Administration (FDA). According to E14 guidelines, QT intervals should be corrected for heart rate, and Fridericia's correction is considered sufficient ($QTc = QT/RR^{0.5}$). In addition, an increase in QT or QTc > 500ms or >60ms above baseline are considered thresholds of particular concern. Categorical analysis and AE reporting are also recommended with absolute QT/QTc intervals of >450, >480, and >500, or increases of >30ms, and >60ms above baseline.

According to the review, there have been 19 deaths recorded for participants receiving DP in the context of mass drug administration studies, with one case of sudden death and the other 18 deaths not deemed related to cardiac causes. In conclusion, the committee stated that "among ~200 000 treated individuals with close follow-up, one possible sudden cardiac death associated with DP was reported. This finding is consistent with the risk of fatal cardiotoxicity associated with other QT/QTc-prolonging medicines in current use". In addition, "Review of pharmacovigilance, clinical and preclinical data, along with preliminary results of PK/PD modelling, reveals no evidence of a significant difference in the risks of cardiotoxicity following exposure to piperaquine, chloroquine or amodiaquine at the current recommended doses. The risks of cardiotoxicity of piperaquine-containing medicines are probably similar for healthy volunteers and malaria patients."(79)

A randomized study of a compressed, 2-day regimen of DP for malaria prevention in Cambodian male adults found that 4 of 47 individuals in developed QTcF prolongation of >500 msec, leading to premature study termination.(80) In comparison to individuals receiving placebo, those receiving the compressed regimen had a mean increase in QTcF of 46 msec post-treatment; a change that was correlated with piperaquine peak concentrations.

Recently, a large directly relevant study utilizing the previous WHO DP dosing regimen was published, providing additional safety data for DP.(81) A prospective, observational, longitudinal, multi-center study of 10,925 participants with uncomplicated malaria treated with DP, and monitored for adverse events, including a nested cohort of 1002 patients receiving serial ECG's was reported. A total of 797 adverse events were reported. No patients had a QTcF >500msec prior to the last dose, and 3 had post-last dose QTcF>500msec which returned to <500msec by day 7. Pre- vs post-last dose QTcF >60msec occurred in 70 and 89 participants, respectively, but all returned to near baseline levels by day 7.

Relevant to this protocol was an open label randomized clinical trial being conducted by investigators of the Infectious Diseases Research Collaboration (IDRC) evaluating the protective efficacy of 3 different chemoprevention regimens against malaria compared to the current standard of care of no chemoprevention (R. Kajubi, personal communication). This study evaluated the safety of DP when used as chemoprevention in infants. Final results have been generated and show DP 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 arms. In addition, 145 ECGs performed on 19 study participants randomized to DP have documented QTc intervals to be within normal limits.

5.2 Dihydroartemisinin. The artemisinins (dihydroartemisinin in the case of DP) are generally well tolerated in humans. In a clinical safety review of 108 studies including 9241 patients, no serious adverse events or significant toxicities were reported (51). Of minimal concern is the potential for artemisinin associated auditory toxicity. However, in a study based in Thailand, there was no evidence of auditory toxicity reported(82).

5.3 Dosing schedule for DP

Of note, in malaria studies, it is standard practice to refer to the 1st day of treatment as study day 0. This nomenclature is used universally in the clinical care of malaria patients and has been the standard for all our studies in Uganda. We will use the study day nomenclature throughout this protocol.

The primary focus of the intensive PK studies involves PK sampling around the last dose of the DP regimen. Standard DP treatment consists of 3 consecutive once-daily doses. All participants on DTG- and EFV-based ART, and their controls, will receive the standard 3 consecutive once-daily doses. For children in the LPV/r-based ART and respective control group, the study will be conducted in 2 phases. Phase I participants on LPV/r-based ART will receive a single dose of DP (L1) and a control group of HIV uninfected children (C1) will receive 3 consecutive once-daily doses of DP but will undergo intensive sampling ONLY following the first dose. Following PK and safety evaluation of Phase I participants, we will determine if halting or dose adjustment are needed in the before proceeding to Phase II (3 consecutive once-daily dosing in those on LPV/r-based ART (Group L3). Group C3a will serve as controls for L3 and will receive 3 consecutive once-daily doses.

5.4 Drug Administration

Every dose of DP will be administered with only water, three hours before or after food to minimize risk of adverse side effects such as QT prolongation. Doses will be administered observed by a study nurse and time of dosing will be recorded. Due to risk of increase exposure of DP and QT prolongation, especially in the presence of a CYP3A4 inhibitor, children on LPV/r-based regimen will be given a single dose to inform DP dosing for the 3 consecutive once daily regimen. Weight-based dosing will be as below (Table 3 for HIV-infected children on EFV-based and DTG-based ART and HIV-uninfected children.

Table 3. Weight-based daily dosing following 2015 WHO guidelines for DP(4)

Weight (kg)	Dihydroartemisinin + Piperaquine dose (mg)
5 to <8	**20 + 160**
8 to <11	**30 + 240**

11 to <17	**40 + 320**
17 to <25	**60 + 480**
25 to <36	**80 + 640**
36 to <60	**120 + 960**
60 to <80	**160 + 1280**
>80	**200 + 1600**

Efavirenz-, lopinavir/r-, dolutegravir-based ART. This study will not initiate ART treatment. Initiating and management of ART use will be carried out through the study subjects' usual clinic (Baylor CoE). HIV-infected children will receive ART as per prescription through their primary HIV clinic. No changes in ART will be made for the purposes of this protocol. While receiving DP, ART administration in the morning will occur at the same time as DP administration (with the exception of EFV, which is taken at night) and the timing will be documented. In order to meet eligibility, children will need to have been maintained on ART for at least 10 days prior to enrollment.

5.5 Missed Doses of DP

Missed doses due to vomiting:

- If the episode of vomiting occurs ≤30 minutes post-administration of DP, participants will be counseled to repeat the dose. PK sampling will only continue if the subject can be re-dosed with a full dose and vomiting occurred ≤30 minutes post-administration of DP.
- If the vomiting occurs more than 30 minutes but less than 2 hours post-administration of DP, redosing of 50% of the dose should occur. The redosing will be carefully noted on the study case report forms.
- Any patient who vomits repeatedly (> 3 times) will be withdrawn from the study.

Missed or late doses due to other reasons:

- If a DP dose was missed or late for any other reason, the dose needs to be taken as soon as remembered.
- If a dose is deemed too many hours late by the study team, the patient will be withdrawn from the study and PK sampling will not occur.

5.6 Drug supply and distribution

Dihydroartemisinin-piperaquine

DP is a first-line malaria treatment option in Uganda and is readily available in pharmacies and clinics throughout the country. However, to ensure consistency in drug preparation, this study will supply the DP for all participants, and will be purchased through Ugandan suppliers of the Duocotexin product (as per program pharmacist), which is brand name DP. All doses will be provided by and observed in the study clinic.

LPV/r-, EFV-, and DTG based antiretroviral therapy

LPV/r-based ART is the WHO first-line ART regimen for all children under 3 years of age and EFV-based ART is the WHO first-line ART regimen for all children > 3 years of age, adolescents, and adults.(83) DTG-based ART is a new regimen recommended by WHO as alternative first-line therapy for adolescents over 6 years (and weighing at least 15kg) and adults. As of June 2017, DTG has been approved by the FDA for use among children 6 years and older (weighing at least 30 kg), and by the European Medicines Agency for children weighing more than 15 kg (24). ART for HIV-infected participants will be administered and managed through their usual clinic. Those who are not yet followed by a clinic will be referred to the appropriate HIV clinic for follow-up of HIV. This study will maintain a supply of ART, either provided by Baylor Uganda CoE, the Uganda Ministry of Health or purchased by the study, to provide to study participants as necessary.

5.7 Drug Accountability

The study pharmacist will maintain complete records of all study-related medications received in the study pharmacy. Lot number and number of pills given to each participant at each visit will be recorded. Patients will be requested to return all empty drug bottles and to bring any bottles in use to the clinic at follow-up visits. A registry of all medications, 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 medications. Monthly inventory of all medications will be conducted and a record log of medications will be kept at the study clinic. All unused drugs will be returned to the drug dispensaries after the study is completed or terminated.

6.0 CLINICAL AND LABORATORY EVALUATIONS

6.1 Schedule of Evaluations

See Appendix A for Schedule of Evaluation (SOE) Table

6.2 Management of malaria or non-malarial illness

If a participant is diagnosed with uncomplicated malaria he or she will receive treatment with either AL or other antimalarials in the clinic, as per standard of care. Participants will not be treated with DP as it will be assumed that the current infection was not prevented by DP given to the participant earlier in the study. If a patient is diagnosed with severe malaria, he or she will be referred to Mulago or Masafu General Hospital to receive quinine or artesunate following standard MoH treatment guidelines. In both cases, participants will be followed up in our clinic for 7 days to ensure proper response to malaria treatment and to obtain safety follow-up laboratories as part of the study. Participants presenting to clinic for non-malarial illness will managed in clinic or referred as necessary to Mulago or MGH.

6.3 Unscheduled or After-hours Visits

Parents/guardians will be encouraged to visit the study clinic when urgent care is needed for her or his child outside of study clinic hours. Parents/guardians will be instructed to inform hospital personnel of their child's 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 outside of clinic hours, he or she will receive treatment at the local hospital (either Mulago or MGH) and the doctors will be instructed to refer patients to our study clinic when it opens at 8 am the following day. Participants will be instructed to request treatment with antimalarials other than DP when presenting with malaria outside of clinic hours. If a patient is diagnosed with severe malaria, he or she will receive quinine or artesunate following standard MoH treatment guidelines. In both cases, participants will be followed up in our clinic for 7 days to ensure proper response to malaria treatment and to obtain safety follow-up laboratories as part of the study. Patients with non-malarial illnesses will be managed at the discretion of the hospital/clinic staff.

6.4 Missed or late visits.

Parents/guardians/participants will be instructed to return on specified follow-up days in all studies. If a participant fails to return on the appropriate follow-up day, a home visitor will be sent to assist them in returning to the clinic as soon as possible for follow-up.

6.5 Clinical and Laboratory Studies

Scheduled and unscheduled visits will include a detailed history and physical examination, measurement of temperature, height, and weight. Blood will be collected on Study Day 0, 14, 28, and 42 for CBC, differential, and liver function tests (LFTs; AST, ALT) for groups D3, L3, E3, C3a, and C3b. For groups L1 and C1, blood will be collected on Study Day -1, 14, 28 (L1 only) for CBC, differential, and liver function tests (LFTs; AST, ALT). Additional venipunctures may 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 for patient management decision-making. 1 mL of additional blood will be collected on Day 0 for testing of pharmacogenetic variants for groups E3 and D3.

HIV-uninfected children will have HIV serostatus documented through rapid testing, and will follow Ugandan MoH guidelines. All positive test results generated for the purpose of this study will be confirmed through Western Blot or HIV RNA. If needed, HIV counseling and referral will be made. Children will not be notified of HIV testing results. Parents and guardians will be notified, and provided post-test counseling, and referred to the appropriate HIV clinic for care. HIV infected children will be expected to have HIV status confirmed through their clinic and will be required to be stabilized on at least 10 days of ART to meet enrollment criteria.

6.6 ECG studies

The expected peak of piperaquine is 4-6 hours post-last dose. Based on the expected directionality of drug-drug interactions, elevated piperaquine concentrations are ONLY expected in those children receiving lopinavir-ritonavir (LPV/r). However, in an effort to ensure safety for all children, ECGs will be carried out in all participants. This will also allow us to assess the potential impact of age on QT interval changes since we will have data from participants ages 3 to 17 years. All ECGs will be performed in triplicate and central tendency used as the analysis measure. Additional ECGs may be performed for safety follow-up, as needed. All ECG results will be reviewed by two experienced ECG readers. Both ECG readers will be experienced in reading pediatric ECGs, and will be blinded to each other's reading. One of the readers will be a non-study team member. All ECGs with discrepancies between two readers will be re-evaluated.

Timing of ECGs:

1) Group L1 (single dose DP in the context of LPV/r-based ART)
    - Day -1: baseline ECG
    - Day 0: ~ 4 to 6 hours post-dose (corresponding to peak piperaquine concentrations)
    - Day 7
2) Groups E3, D3, L3, C1*, C3a, and C3b (all of these groups receive 3-dose DP).
    - Day -1: baseline ECG
    - Day 2: ~ 4 to 6 hours post-last dose (corresponding to peak piperaquine concentrations in a 3-dose regimen)
    - Day 7
    - Day 42

*__Note__ – for Group C1, ECG will also be performed approximately 4 to 6 hours post-dosing on Day 0 (corresponding to peak piperaquine concentrations) so that single dose QT impacts can be compared to group L1

6.7 Pharmacology Laboratory Studies

6.7.1 PK sampling for 3-dose study groups (E3, D3, L3, C3a, C3b)

DP administration will be on Study Days 0, 1, and 2. PK samples for DHA and PQ will be collected on Study Days 0-4, 7, 14, 21, 28, 35, and 42.

Sampling for DHA Quantitation

Capillary samples (200 µL each*) will be collected by finger stick at approximately 2 and 4 hours post- each morning DP dose (Day 0-1). An additional capillary sample will be collected pre-dosing on Day 1.

Intensive *venous* sampling will occur precisely at the same times as per the single dose PK evaluations except it will occur post-administration of the 3rd dose of DP. Participants will remain in the clinic until completion of the 8-hour blood sampling on

Study Day 2 and discharged home. They will then return to the clinic the next morning for the 24 hours sample (Study Day 3).

Sampling for Piperaquine Quantitation

A pre-1st dose sample will also be collected to confirm no residual antimalarial drug is detectable. Intensive sampling on Day 2 (as for DHA) will also be used for piperaquine analysis. Participants will be asked to return to the clinic on Study Days 3, 4, 7, 14, 21, 28, 35, and 42 (v*enous or capillary* sampling, 200-500 uL each*).

For all evaluations, samples up to 24 hours will be used to measure DHA while all samples collected will be analyzed for PQ. PK sampling will be discontinued in patients who do not meet laboratory eligibility criteria or do not complete DP dosing.

Correlation Sampling

For groups C3a and C3b, a simultaneous venous and capillary sample will be collected on Day 0 (4 hours post-dose), Day 2 (4 hours post-dose), Day 3 (24 hour post last dose), Day 7, and Day 14 to correlate venous and capillary concentrations of artemisinins and piperaquine.

For groups D3, E3, and L3 a simultaneous venous and capillary sample will be collected on Day 14 to correlate venous and capillary concentrations of piperaquine.

Sampling for Antiretrovirals*

A pre-dose *venous* sample on day 0, day 2, and day 3 will be collected to obtain a trough or mid-level of lopinavir/ritonavir, dolutegravir, and efavirenz. In addition, venous samples will be collected on Day 2 in the E3 group at 2-, 4-, and 8-hour post-DP dosing. This will allow for the comparison of trough antiretroviral PK levels in the absence/presence of DP and AUC differences of EFV between pharmacogenetic variants.

*Sample volumes will be as listed above, except for except for day 0, day 2, and day 3 which will be approximately 1 mL (to allow for antiretroviral PK measurement).

6.7.2 PK sampling for single dose study groups (L1 and C1)

For Phase I, intensive sampling will be conducted after a single DP dose only (administered on Day 0). However, C1 participants will get two additional doses of DP (2nd dose on Day 1 and 3rd dose on Day 2).
L1: PK samples will be collected on Study Days 0-2, 7, 14, 21, and 28.
C1: PK samples will be collected on Day 0 and Day 1.

Sampling for DHA Quantitation

Intensive *venous* sampling will occur via an indwelling catheter prior to and 0.5 1, 2, 3, 4, 6 and 8 hours post-administration of the Day 0 dose of DP for the determination of DHA (and PQ) concentrations in plasma (500 µL each. Participants will remain in the clinic until completion of the 8-hour blood sampling on Study Day 0 and be discharged home. They will return to the clinic the next morning for the 24-hour sample (Study Day 1).

Sampling for Piperaquine Quantitation

A pre-1st dose *venous* sample of 500 uL will be collected to confirm no residual piperaquine is detectable. Intensive sampling on Day 0 (as for DHA) will also be used for piperaquine analysis. C1 participants will have their final PK sample collected the next morning for the 24-hour sample (Study Day 1). L1 participants will return on Study Days 1, 2, 7, 14, 21, and 28 (v*enous or capillary* sampling, 200-500 µL each).

For all evaluations, samples up to 24 hours will be used to measure DHA while all samples collected will be analyzed for PQ. PK sampling will be discontinued in patients who do not meet laboratory eligibility criteria or do not complete DP dosing.

Pharmacogenetic studies

1 mL of venous blood will be collected on Day 0 in the E3 and D3 groups to genotype CYP2B6, CYP3A4 / CYP3A5, UGT1A1, ABCB1 and to investigate connections between pharmacogenetic variation and EFV, DTG, and DP PK.

6.8 PK Sample collection and handling

For consistency, blood samples will be obtained from capillary or venous sources, as specified. However, if venous PK samples cannot be obtained at desired times due to technical or other limitations, technicians may obtain blood from a capillary site. 200 µL *capillary* samples will be collected by finger prick and can be used to measure both analytes but without the ability to do repeat analysis if necessary. 500 µL and 1 mL *venous* samples permits repeat analysis as needed. All samples will be immediately placed on dry ice, processed for plasma, transferred to liquid nitrogen, and shipped at a later date on dry ice to our laboratory for analysis. Liquid nitrogen will be obtained from Kampala.

6.8.1 Assays for DHA, Piperaquine and Antiretrovirals

DHA, piperaquine, efavirenz, lopinavir/ritonavir, and dolutegravir will be measured using optimized methods validated in our laboratory. All methods will utilize liquid chromatography tandem mass spectrometry.

7.0 TOXICITY AND DATA AND SAFETY MONITORING PLAN (DSMP)

7.1 Overview and Summary

An adverse event is defined as "unfavorable changes in health, including abnormal laboratory findings that occur in study participants during the study or within a specified period following the study". All of the medications in the study have been approved in Uganda for treatment of HIV and malaria, and dihydroartemisinin-piperaquine is actively being studied around the world for possible use as malaria chemoprevention. DP has been studied for chemoprevention, using standard treatment dosing regimens, with many clinical studies supporting its use, though it is not standard of care. The drugs used in this study are also provided by the Ministry of Health free of charge outside of this study. Data regarding the tolerance of DP will be recorded. Study clinicians will assess patients at each scheduled and unscheduled visit to the clinic according to a standardized clinical record form using scales developed by the NIH Division of AIDS Adult and Pediatric Toxicity Tables version 2.1, March 2017.

7.2 Communication Plan

The study team will have weekly calls on Thursday to discuss the study and review newly enrolled participants, participants' labs, adherence to the protocol, any comments or concerns, etc. In addition, if there are any urgent matters (e.g. adverse events, Grade 3 / 4 lab abnormalities, protocol deviations, etc.) this will be communicated to the team immediately via email or phone communication. Incidents requiring reporting will then be reported as per the guidelines below.

7.3 Reporting

Guidelines for reporting of adverse events due to study participation provided by UCSF HRPP & HIC, Yale HIC, Baylor College of Medicine IRB, NICHD, JCRC-REC, NDA and UNCST will be followed as described below.

Sunil Parikh will be responsible for reporting any adverse events, protocol violations, protocol deviations, etc. to Yale University. Fran Aweeka will be responsible for reporting any adverse events, protocol violations, protocol deviations, etc. to UCSF and NICHD. Norah Mwebaza will be responsible for reporting any adverse events, protocol violations, protocol deviations, etc. to UNCST, NDA, and JCRC-REC. Grace Paul Kisitu will be responsible for reporting any adverse events, protocol violations, protocol deviations, etc. to Baylor College of Medicine.

Per UCSF HRPP & HIC guidelines as found on website updated April 2017: Adverse events which are definitely, probably, or possibly related to study procedures or study participation **AND** serious or unexpected will be reported. AEs which do not meet those criteria will be documented, referenced, and retained in the study files for follow-up. Per UCSF HRPP & HIC guidelines, the following definitions for serious or unexpected adverse events will be followed:

*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 occurring in a gene therapy study
- 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

Per Yale HIC Policy 710, dated April 15, 2017: Any incident, experience or outcome that is unexpected AND related or possibly related to participation in the research, AND unexpected should be reported to the IRB as an "RNI" within 5 calendar days of the Principal Investigator becoming aware of the event.

Events that may require a temporary or permanent interruption of study activities by the Principal Investigator or Sponsor to avoid potential harm to subjects should be reported to the Yale IRB immediately (if possible), followed by a written report to the IRB as an "RNI" in the online IRES regulatory system no more than 5 calendar days after the Yale Principal Investigator becomes aware of the event.

All related internal and external events involving risk but not meeting the prompt reporting requirements should be reported to the IRB in summary form at the time of continuing review.

Per UNCST National guidelines March 2007: "These guidelines give criteria for prompt reporting of certain categories of adverse events to an IRC and the UNCST." "An *adverse event* is any unfavorable and unintended sign, symptom or condition temporally associated with the administration of a health-related intervention, whether or not considered related to the intervention. The requirement to report adverse events to regulatory authorities shall not apply to events that are observed among participants who are in observational studies in which no health-related intervention is being administered." However, for consistency, we will report adverse events which are definitely, probably, or possibly related to study procedures or study participation **AND** serious or unexpected similarly to UNCST and the School of Biomedical Sciences.

Table 4. Guidelines for reporting adverse events related to study participation

Institution	Type of Adverse Events	When to Report
NICHD	• Definitely, Probably, or Possibly related **AND** Serious **or** Unexpected	• Within 10 working days of awareness
UCSF-HRPP&IRB	• Definitely, Probably, or Possibly related **AND** Serious **or** Unexpected	• Within 5 working days of awareness • Internal, related deaths and life-threatening events: report immediately
Yale-HIC	• Definitely, Probably or Possibly related to participation in the research **AND** Serious **AND** Unexpected (in terms of nature, specificity, severity, or frequency)	• Within 5 days of awareness • Related Events not meeting prompt reporting requirements are reportable in summary form at time of continuing review
JCRC-REC	• All Serious and Unexpected events irrespective of relationship;	• All serious adverse events and unexpected events must be reported within 7 calendar days of awareness • All other reportable events should be reported within 14 calendar days
National Drug Authority	• All Serious and Unexpected events irrespective of relationship;	• All serious adverse events and unexpected events must be reported within 7 calendar days of awareness.
UNCST	• All Serious and Unexpected events irrespective of relationship;	• Death and Life-threatening events within 48-hours with written report within 7-calendar days of awareness • All other reportable events within 15-calendar days of awareness

7.4 Antiretroviral Toxicity & Management

For HIV-infected children, ART will be managed through the participant's primary clinic. This study is not designed to initiate ART treatment for study subjects. No changes will be made to the ART regimens during this study, unless directed by the primary HIV provider. ART regimens permitted for this study include EFV-, LPV/r-, DTG-based regimens. Issues related solely to a participant's ART will be managed by the participant's primary HIV clinic. If the clinic is closed, participants will be referred to the local hospital.

Dolutegravir is now part of the WHO options for the management of HIV. A report in May 2018 suggests a possible increased risk of neural tube defects in women exposed to DTG during the 1st trimester. As such, we will not enroll any pregnant women or woman of potential childbearing age on DTG-based ART (age 13-17) who do not agree to consistent and reliable contraception. This is due to unknown interaction of DTG and DP at the time of this study. While DTG has recently been approved by the WHO for children as young as 6 years of age, as of submission of this protocol, the Ugandan MoH has not yet adopted this policy. If the Ugandan MoH guidelines are changed over the course of the study, protocol modifications will be implemented as feasible.

7.5 Dihydroartemisinin-Piperaquine (DP) Toxicity & Management:

DP is a WHO recommended first-line treatment for malaria and is being studied for possible use as chemoprevention. DP given as 3 consecutive once daily doses is the standard of care for treatment of uncomplicated malaria. While not standard of care, this same regimen has also been studied in numerous published clinical trials as chemoprevention for malaria in children and pregnant women. Thus, we will be evaluating this regimen for possible interactions with ART as these medications are often co-administered in clinical practice. We will be assessing and recording data in relation to how participants tolerate DP.

We will be recording participants' tolerance of DP using the NIH Division of AIDS Adult and Pediatric Toxicity Tables (version 2.1, March 2017; https://rsc.tech-res.com/docs/default-source/safety/daids-ae-grading-table-mar2017.pdf). This will be used to screen for eligibility and to evaluate adverse events for children. Study staff will receive extensive training in the identification and management of adverse events. In addition, participants will be informed by the study team about potential adverse events associated with DP and will be encouraged to present to the study clinic for all potential adverse events. The Baylor Uganda CoE study clinic will remain open 5 days a week and the Busia clinic will be open 7 days a week. For events occurring outside clinic hours, HIV-infected participants will be informed to report to the Mulago Hospital, which is on-campus, while HIV-uninfected participants will be told to go to Masafu General Hospital. The participants will be instructed to notify the study clinician/nurse about their admission/presentation. The monitoring, reporting, and management of adverse events will follow guidelines set forth by each respective institution.

7.6 Cardiotoxicity Concerns & ECGs

As noted in Section 1.1.5, PQ is a CYP3A4 substrate and LPV/r is a CYP450-inhibitor, and thus an interaction can raise levels of PQ thus, increasing risk of PQ toxicity. PQ toxicity related to peak PQ concentrations and focuses on QT interval prolongation.

7.6.1 LPV/r-Based ART regimen

Dose Adjustment: Given concerns for cardiotoxicity, for the LPV/r-based arm ONLY, we will start with a single dose of DP in a group of HIV-infected children on LPV/r-based ART and a control group of single dose DP in HIV-uninfected children, to determine the magnitude and safety of the interaction, as well as to monitor QT intervals via ECGs (Phase I). Following the assessment of the PK and ECG-based safety data from the single dose DP groups, we will proceed with one of three different options: 1) no further study of LPV/r with DP due to safety concerns, 2) a reduced mg/kg dose of 3-dose DP in the context of LPV/r, or 3) standard mg/kg dosing of 3-dose DP in the context of LPV/r-based ART (Phase II).

If the magnitude of the effect of LPV/r on the AUC of PQ during Phase I is:

- <40% increase, there will be no adjustment in DP dose for Phase II and Phase II can begin
- 40-75% increase, there will be a 50% reduction in DP dose for Phase II
- >75% increase, further evaluation will be completed for Phase I so that a specific dose for Phase II can be determined to compensate for the magnitude of the change or we will recommend that LPV/r and DP not be taken concomitantly.

7.6.2 All Study Arms

ECGs will be performed in participants in all study groups to ensure safety, and to provide data on QT intervals across the age spectrum. The expected peak of piperaquine is 4-6 hours post-last dose. Based on the expected directionality of drug-drug interactions, elevated piperaquine concentrations are ONLY expected in those children receiving lopinavir-ritonavir (LPV/r). However, in an effort to ensure safety for all children, ECGs will be carried out in all participants. This will also allow us to assess the potential impact of age on QT interval changes since we will have data from participants ages 3 to 17 years.

All ECGs will be performed in triplicate and central tendency used as the analysis measure. Additional ECGs may be performed for safety follow-up, as needed. All ECGs will be read the same day as they are collected. All ECG results will be reviewed by two experienced ECG readers. Both ECG readers will be experienced in reading pediatric ECGs, and will be blinded to each other's reading. All ECGs with discrepancies between two readers will be re-evaluated. One of these ECG readers will be an

individual outside of the main study team and will be a pediatric cardiologist. **At any point, if QTc prolongation of >500 msec or >60 msec from baseline are noted, dosing will be stopped, and the study will be halted until data can be reviewed by the study investigators.**

7.7 Toxicity management by grade

Alternate explanations for clinical and laboratory abnormalities will be sought. Laboratory normal values will be those provided by the clinical laboratory used for this study. For each adverse event identified as grade 3 or higher AND serious or unexpected (See Section 8.0), an adverse event report form will be completed. Adverse events will be followed until they resolve to below a grade 3.

Grade 1 and 2:

It is anticipated that some subjects, particularly HIV-infected children/adolescents, may enter this study with grade 1 or 2 abnormalities already present (e.g. due to ART management). The site physicians will manage the grade 1 or 2 events according to standard practice. An adverse event report form will not be completed for events classified as grade 1 or 2, as these events are common and difficult to distinguish from common childhood illnesses.

Grade 3 or 4 (non-life threatening):

- Notify the study team.
- Repeat observation within 72 hours; notify study team of results.
- Subjects may continue taking DP pending clinic visit or repeat laboratory tests. The clinician has the option of immediately stopping the DP if the subject cannot be examined in clinic, if a repeat laboratory test cannot be performed within 72 hours, or if the clinician determines that continuation of DP is unsafe while awaiting clinic exam or test results.
- Work-up to exclude other causes.
- For all confirmed Grade 3 or 4 toxicities supported by repeat clinical exam or laboratory test results, stop DP until toxicity resolves to < Grade 3.
- If drug is restarted with resolution of toxicity and toxicity recurs on re-challenge, study drugs will be permanently discontinued. If DP is discontinued the participant will be withdrawn from the study.

Grade 4 life threatening:

- Notify study team.
- If still receiving DP, DP should be permanently discontinued.

8.0 STATISTICAL CONSIDERATIONS

8.1 General Design Issues.

This is a prospective study to evaluate the PK of DP in the context of EFV-, LPV/r- and DTG-based ART in HIV-infected children and adolescents compared to the HIV-uninfected children and adolescents. The study will be conducted in Uganda.

The current proposal will study the PK of DP in HIV-infected children and adolescents on EFV-, LPV/r-, and DTG-based ART and matched HIV-uninfected children and adolescents not on ART who will be enrolled through Baylor-Uganda CoE/Busia clinic at the Masafu General Hospital. In addition, we will compare the PK of single dose DP with 3-dose DP in HIV-infected children on LPV/r-based ART and HIV-uninfected children not on ART. Comparisons will be based on an intensive PK design for DP AUC estimations in 20 subjects for groups L1 and C1 and for 30 subjects in each of the other study groups. Study group comparisons will be controlled for age. The proposal will also study the impact of DP on ART pharmacokinetics. Table 5 shows the primary PK comparison groups for the study.

Table 5. Primary PK objectives and comparator groups

Primary Objectives	**Groups**	**Sample sizes and age groups**
Impact of LPV/r on single dose DP PK and safety in HIV-infected children on LPV/r vs "single dose" DP HIV-uninfected children	**L1** **vs** **C1**	L1 (n=20, age 3-10years) vs. C1 (n=20, age 3-10 years)
Impact of LPV/r on 3-dose DP PK and safety in HIV-infected children on LPV/r vs 3-dose DP HIV-uninfected children	**L3** **vs** **C3a**	L3 (n=30, age 3-10years), vs. C3a (n=30, age 3-10 years).
Impact of EFV on DP PK and safety in HIV-infected children on EFV-based ART vs 3-dose DP HIV-uninfected children	**E3** **vs** **C3a**	E3 (n=30, age 3-10years), vs. C3a (n=30, age 3-10 years).
Impact of DTG on DP PK and safety in HIV-infected children on DTG-based ART vs 3-dose DP HIV-uninfected children	**D3** **vs** **C3b**	D3 (n=30, age 11-17 years), vs. C3b (n=30, age 11-17 years).

Safety of DP will be primarily assessed via within-person change in QTc (QT intervals will be corrected for heart rate using Fridericia's correction ($QTc = QT/RR^{0.5}$))

Although certain children (20 HIV-positive children on LPV/r-based ART) are eligible to enroll in both the single-dose DP intensive PK study and the 3-dose DP PK study following a washout period of 42 days, there is no assurance that the same child will contribute to both study components. Thus, it is possible that the total number of subjects for all groups may be as high as 190.

8.2 Outcome Measures

8.2.1 Primary outcomes

1) Area under the plasma concentration versus time curve for all drug analytes
2) Safety of single dose DP in HIV-infected children on LPV/r-based ART determined via assessment of mean change in QT intervals from baseline
3) Safety of 3-dose DP regimens determined via assessment of mean change in QT intervals from baseline

8.2.2 Secondary outcomes

1) The effects of DP on antiretroviral pharmacokinetics in the above 3 dose arms (EFV, DTG, LPV/r and controls)
2) The association of anthropomorphic indicators of malnutrition on PK exposure of DP in HIV-infected and HIV-uninfected children
3) Assess auto-induction of DHA from single dose to 3-doses
4) To assess the prevalence of UGT1A1 pharmacogenetic variants and their impact on DTG PK.
5) To assess prevalence of CYP2B6 pharmacogenetic variants and their impact of EFV PK
6) To assess prevalence of CYP3A4 pharmacogenetic variants and their impact of EFV and piperaquine PK
7) To assess prevalence of ABCB1 pharmacogenetic variants and their impact of EFV PK

8.3 Nutritional Status

Using anthropometric measurements and for a secondary study aim to relate nutritional status to PK and clinical outcomes, children will be characterized as a) “stunted” but not underweight [i.e. height for age (HFA) z-score ≤-2 and weight for age (WFA) z-score>-2); b) underweight, but not stunted (WFA z-score ≤-2 and HFA z-score>-2); or c) of normal nutritional status (WFA and HFA z-scores >-1)

8.4 Intensive PK study

8.4.1 Sample Size

Using measures of mean AUC and its coefficients of variation (CV) from our studies and the literature, the intensive arms of the study are powered to detect differences in ACT AUC between all study groups with n=30 for groups E3, D3, and L3 of HIV-infected children and n=20 for group L1. To assure adequate age-matched HIV-uninfected controls for the single and 3 dose PK evaluations, n=30 in two different age groups each (3-10 years and 11-17 years) is used for the group of HIV-uninfected children and adolescents. Figure 2 and Table 5.

For unpaired comparisons where n=20 in both groups, there will be 80% power to detect a difference in AUC of 40% or more for both the single and 3-dose evaluations,

given an overall Type I error of 0.05, a Bonferroni correction for multiple comparisons, and with assumption that the coefficient of variation (CV) for all AUC's is 35-38%.

For unpaired comparisons where n=30 in both groups, there will be 80% power to detect a difference in AUC of 30-35% or more for both the single and 3 day DP evaluations, given an overall Type I error of 0.05, a Bonferroni correction for multiple comparisons, and with assumption that the coefficient of variation (CV) for all AUC's is 35-38%. Although many children are likely to be studied for both the single dose DP and 3-dose DP regimens, it is not likely that *all* children will provide results for both. Thus, unpaired comparisons will be utilized. In the event the vast majority of children provide results for both single dose and 3-dose DP, a paired analysis will be utilized.

8.4.2 Dose adjustment based on single dose results from Phase I (LPV/r only): 20 children in each "single dose" DP groups (L1 and C1) must be enrolled in Phase I with PK results evaluated before enrollment into 3 dose DP groups (L3 and C3a; Phase II) can be initiated. The results for these 20 children in each single dose group will be compared to one another determine the impact of LPV/r on DP PK exposure prior to any children being given 3 doses of DP in the context of LPV/r.

If the magnitude of the effect of LPV/r on the AUC of PQ during Phase I is:

- <40% increase, there will be no adjustment in DP dose for Phase II and Phase II can begin
- 40-75% increase, there will be a 50% reduction in DP dose for Phase II
- >75% increase, further evaluation will be completed for Phase I so that a specific dose for Phase II can be determined to compensate for the magnitude of the change or we will recommend that LPV/r and DP not be taken concomitantly.

8.4.3 <u>Sample size for ECG studies</u>

Primary outcome: Difference in paired mean QT change (6hrs compared with baseline within person)

Assumptions: two-sided alpha=0.05, sample size =30, paired t-test

Table 6. Power/sample size calculations for ECG studies

Mean of paired differences (Time 6hrs-Baseline) (msec)	SD of the differences (msec)	Power %
10	10	>95%
	15	>95%
	20	>95%
15	10	>95%
	15	>95%
	20	>95%
20	10	>95%
	15	>95%
	20	>95%
25	10	>95%
	15	>95%

	20	>95%
30	10	>95%
	15	>95%
	20	>95%

A sample size of 30 achieves >95% power to detect a mean of paired differences of 10.0 msec between the null hypothesis mean of paired differences of 0.0 and the alternative hypothesis mean of paired differences of 10.0 msec with an estimated standard deviation of the difference 10.0 msec and with a significance level (alpha) of 0.05. In addition, for the group receiving single dose DP (L1) and its comparator (C1), samples sizes of n=20 maintains the same power (>95%) to detect the above differences.

8.4.2 Intensive PK Analysis

PK parameters for each subject will be estimated using non-compartmental analysis (NONCMP) and follow a linear up-log down trapezoidal rule in conjunction with first-order input (WinNonlin). PK parameters, including the elimination rate constant λ_z and $t_{1/2}$ will be estimated, with $t_{1/2}$ calculated as $\ln_2/\lambda_z$. AUC will be estimated as the $AUC_{0\text{-}8h}$ or $AUC_{0\text{-}24h}$ (as results permit) for DHA and the sum of AUC_{last} (AUC to the end of the sampling period) and $AUC_{last\text{-}\infty}$ (from the end of sampling to infinity) for PQ. PK parameters will be compared between groups of interest (Figure 2 and Table 5) using a two-sided unpaired t-test for two-group comparisons, and ANOVA for multi-group comparisons. If PK parameters are found to have skewed distributions and data transformations do not induce symmetry, rank-based tests will be used. The following groups will be compared:

8.5 Exposure-Response Analysis of DP PK and EKG results. Population PK/PD analysis may be employed utilizing data for both HIV infected and HIV uninfected children to evaluate associations between PQ exposure and EKG changes. Covariate analysis may be performed to identify the impact of covariates on the PK of DP with special focus on the impact of markers of malnutrition, as measured by weight, BMI, and various Z scores and mid-arm circumference. Other covariates will include age, body size (weight, height), concomitant medications, hematocrit, and CD4 count (for HIV-infected children). We will test for a significant relationship between drug exposure and the primary safety outcome (QT interval prolongation). We will also investigate if there is any potential impact of indices of malnutrition, HIV status, age and other covariates on safety parameters. We will identify appropriate dosing for DP in the context of ART with the goal of matching the DP exposure seen in non-HIV infected patients. Population PK methods may be used to simulate different DP dosing approaches to identify optimized regimens that ensure concentrations are achieved in the context of ART that match exposure seen in HIV uninfected control participants.

9.0 DATA COLLECTION AND MONITORING

9.1 Record Keeping

All clinical data will be recorded onto standardized case record forms (CRFs) by study physicians. Laboratory data 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. 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 (DMC) for rotating secure storage. In addition, the database from the backup will be placed onto one of the Kampala DMC servers as a data mirror for read-only access in the event that the web-site becomes temporary unavailable. All data will be maintained and kept indefinitely. The medical records for the study will be kept in a locked office and will only be able to be seen by study staff. The online database will be password protected and only the study staff will have access to it. The parent and child's name will not be written in any published reports based on this research and genetic data will be de-identified and will not be put into the permanent medical record to ensure participant privacy.

9.2 Data Quality Assurance and Monitoring

In order to insure data quality, an initial daily QC level 1 will be conducted, followed by a 1 month QC, followed by a quarterly data quality audit by the study Data Manager. 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 the 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.

10.0 HUMAN SUBJECTS

10.1 Risks and Benefits

There are minimal direct benefits to the study subject for participation in the study. These are limited to testing for malaria and anemia in all participants, HIV testing in HIV-infected children, ECG testing, and a month of possible protection from malaria infection. The risks have been reduced to a minimum. For children, we have consciously considered the guidelines set forth by the UCSF Committee on Human Subjects' Research (CHR) of 3ml/kg for blood sample collection and have optimized all assay requirements for accurate quantification of drug concentrations using the lowest amount of blood. We have also consulted other recommendations. The most conservative recommendations suggest no more than 2.5% (~2 mL/kg) of total blood

volume within a 24-hour period or 5% (~4 mL/kg) within a 30-day period. The NIH guidelines state that no more than 3 mL/kg are to be drawn in a single blood draw (24-hour period) and no more than 9.5 mL/kg are to be drawn over any eight-week period for the purposes of research in children. We feel it is unlikely the participant would be harmed by taking the amount of blood required in this study. For the total amount collected over 42 days of follow-up the amount of blood that will be drawn will not exceed 5.4 mL/kg (no more than 30 mLs or 6 teaspoons will be drawn), which are well within these limits.

There is small chance of infection from performing blood draws. Additional risks include anxiety from the blood draws, HIV testing and learning of testing results. We have attempted to minimize the number of needle pokes as much as possible and participants will be counseled regarding test results and referred for medical care if necessary.

10.2 Treatment and Compensation for Injury

If the participant is injured as a result of being in this study, treatment will be available through Baylor College of Medicine Children's Foundation—Uganda, Mulago Hospital, and Masafu General Hospital.

Makerere University, UCSF, Yale, Baylor CoE, and NICHD do not normally provide any other form of compensation for injury.

10.3 Costs to the Subjects

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

10.4 Reimbursement of Subjects

Participants will not be paid for their participation in the study. We will provide all routine medical care, including evaluations, and medications available in our clinic. We will reimburse for transport and compensate caretakers for the time spent (work hours lost) on PK study follow-up days. On certain days, participants will have to be in the clinic for several hours. On those days, we will provide food and drink to participants (breakfast, dinner, and/or snacks) to ensure their well-being. In addition, we will reimburse the cost of consultation for referrals made by study physicians to other clinics and services within Mulago Hospital using available funds when available. However, reimbursement of all diagnostic tests and treatment recommended outside the study clinic cannot be guaranteed in all circumstances.

10.5 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 participating institutions in both the U.S. and in Uganda. This includes the UCSF HRPP/IRB, the Yale HIC, Baylor College of

Medicine IRB, the JCRC-REC, the National Drug Authority and the Uganda National Council of Science and Technology (UNCST).

All consent forms will be translated into the local languages 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. Interviewers will ask parents/guardians/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.

10.6 Study Discontinuation

This study may be discontinued at any time by the NIH, respective IRBs or other Governmental agencies in the United States or Uganda as part of their duties to ensure that research subjects are protected

10.7 Definition of Parent/Guardianship

For this project, we will define a parent as someone who attests that he/she is the biological parent of the potential participant. However, it has been found in Uganda that a high number of the HIV-infected children have lost one or both parents. These children live with caretakers who do not have documented formal guardianship status because there is no formal, legal guardian system in Uganda. In Uganda, orphan children are customarily cared for by one or more relatives; a single individual family member is not usually identified as the sole guardian or custodian of the child. We will define a guardian as someone who identifies him/herself as the primary caregiver who is able to make all health care decisions for the potential participant. A guardian must be at least 18 years of age, however; a parent may be less than 18 years of age. These definitions are currently approved for use in current research projects conducted in Uganda following extensive discussion with the Ugandan Ministry of Justice, the Uganda National Council of Science, the JCRC-REC, and the NIH in 2006.

11.0 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, Yale, Baylor College of Medicine Children's Foundation-Uganda, and JCRC-REC guidelines.

12.0 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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APPENDIX A. SCHEDULE OF EVALUATIONS: **L1 & C1 ARMS**

STUDY DAY	-1[1]	0[2]	1	2	7	14	21	28	Other visit[10]
ENROLLMENT PROCEDURES									
Screening and informed consent	X								
HIV testing, counseling and referral if necessary[3]	50uL								
Rapid Diagnostic Test and Blood Smear	200uL								X[10]
Collect locator information	X								
CLINICAL AND LABORATORY EVALUATIONS									
History and Physical Examination[4]	*Will be performed on each day the child is in clinic (for PK blood draws, CBC, Chemistries, etc.)*								
Hematology and Chemistries[5]	4mL					4mL		4mL	X[10]
Electrocardiogram (ECG)[6]	X	X		X	X				X[10]
DRUG ADMINISTRATION									
DP dosing for all study subjects[7]		X	X[8]	X[8]					
Antiretroviral dosing for HIV- infected subjects[9]	X	X	X	X	X	X	X	X	X
INTENSIVE PK STUDY SAMPLING[9]									
L1: Single dose DP regimen + LPV/r [11]		500 µL (0, 0.5, 1, 2, 3, 4, 6 & 8 hrs)	200µL (24rs)	200µL (48hrs)	200µL (168 hrs)	500µL (336 hrs)	200µL (504 hrs)	500uL (672 hrs)	200uL[10]
APPROXIMATE TOTAL BLOOD VOLUME (mL) [12]	**4.20**	**4**	**0.2**	**0.2**	**0.2**	**4.5**	**0.2**	**4.5**	**0.2**
C1: "Single" dose DP – Intensive sampling after 1st dose DP[11]		500 µL (0, 0.5, 1, 2, 3, 4, 6 & 8 hrs)	200µL (24rs / Pre DP dose)						
APPROXIMATE TOTAL BLOOD VOLUME (mL)[12]	**4.25**	**4**	**0.2**			**4**			**0.2**

Explanation of Intensive PK schedule of events (Appendix A):

1 Study Day -1, will be enrollment day where we will perform baseline ECG, HIV screening, malaria screening and CBC tests on all potential participants

2 As is standard practice in malaria research, study Day 0 refers to the day DP dosing is initiated.

3 Applies only to HIV-negative patients

4 History will include general medical history, diagnosis, medication (including ingestion of herbs), allergies, and current symptoms. Physical examination will include weight, height, and viral signs (temperature, pulse, blood pressure, respiratory rate), lymphadenopathy, hepatomegaly, splenomegaly, infections in ears, mouth, pharynx or skin and pulmonary, cardiac, neurologic or skeletal abnormalities and anthropometric assessments. Mid-upper arm circumference will also be collected at time of first enrollment.

5 Hematology/chemistry draws entail a 4mL venous sample for CBC, differential, ALT/AST, and creatinine. AST is incorporated as part of chemistries at all blood draws.

6 Both groups will receive ECGs. ECGs will be performed at baseline on Day-1 (before the administration of first dose), 4-6 hours post- dose on Day 0. On Day 2, ECGs will be performed for the C1 group only pre-last dose and 2-4 hours post-last dose of DP. On Day 7, ECG will be performed for L1 group only.

7 For L1 and C1, intensive PK sampling will occur following the first dose of DP. However, for C1 group, they will receive additional doses of DP and Days 1 and 2 to complete the 3 day course to maximize benefit of DP.

8 DP dosing will occur in C1 arm only on this date

9 For L1 arm only, we will ask for child to take their morning dose of LPV/r in the clinic.

10 Participants may present for unscheduled visits. Study team may perform these tests at the discretion of the team member.

11 Follow up for L1 will be through Day 28 and follow up for C1 will be through Day 14

12 Total blood volume in either arm is between 5-6 teaspoons for the scheduled follow-up visits of up to 28 days.

APPENDIX A. SCHEDULE OF EVALUATIONS: **D3, E3, L3 & C3 ARMS**

STUDY DAY	-1	0[1]	1	2	3	4	7	14	21	28	35	42	Other visit[15]
ENROLLMENT PROCEDURES													
Screening and informed consent	X												
HIV testing, counseling and referral if necessary[2]	50uL												
Rapid Diagnostic Test and Blood Smear	200uL												X[15]
Collect locator information	X												
CLINICAL & LABORATORY EVALUATIONS													
History and Physical Examination[3]		*Will be performed on each day the child is in clinic (for PK blood draws, CBC, chemistries)*											
Hematology and Chemistries[4]		4mL						4mL		4mL		4mL	X[15]
Pharmacogenomic Sample		1mL[17]											
Electrocardiogram (ECG)[5]	X[5]			X			X					X	X[15]
DRUG ADMINISTRATION													
DP dosing for all study subjects[6]		X	X	X									
Antiretroviral dosing for HIV- infected subjects[7]		X	X	X	X	X	X	X	X	X	X	X	X
INTENSIVE PK STUDY SAMPLING													
3 day DP regimen D3, E3, L3 (HIV+ children)		1.5mL (Pre-DP, Pre ART)[8,9] 200uL (2hr, 4 hrs post-DP dose)	200µL Pre DP, 2 & 4 hrs post-DP	1mL (Pre-ART)[9], 500 µL (0, 0.5, 1, 2, 3, 4, 6 & 8hrs) E3[10]: 500uL (2, 4 & 8 hr)	1mL (Pre-ART)[9] 200µL (24hrs)	200µL (48hrs)	200µL (120 hrs)	500µL (288 hrs) 200µL (288 hrs)	200µL (456 hrs)	500µL (624 hrs)	200µL (792 hrs)	500µL (960 hrs)	200uL[15]
APPROXIMATE TOTAL BLOOD VOLUME (mL)[15]	**0.20**	**L3: 5.9 D3 & E3: 6.9**	**0.6**	**5.0 E3: 6.5**	**1.2**	**0.2**	**0.2**	**4.7**	**0.2**	**4.5**	**0.2**	**4.5**	**0.2**
3 day DP regimen C3a & C3b (HIV uninfected children)		500uL (Pre-DP)[9] 200uL 2hr, 4hr 500uL 4 hrs[12] post-DP dose	200µL Pre DP, 2 & 4 hrs post-DP	500 µL (0, 0.5, 1, 2, 3, 4, 6 & 8hrs) 200uL 4hr [12]	500uL[13] (24hrs) 200µL (24hrs)	200µL (48hrs)	500uL[13] (120hrs) 200µL (120 hrs)	500uL [14] (288 hrs) 200µL (288 hrs)	200µL (456 hrs)	500µL (624 hrs)	200µL (792 hrs)	500µL (960 hrs)	200uL[15]
APPROXIMATE TOTAL BLOOD VOLUME (mL)[16]	**0.25**	**5.4**	**0.6**	**4.2**	**0.7**	**0.2**	**0.7**	**4.7**	**0.2**	**4.5**	**0.2**	**4.5**	**0.2**

Explanation of Intensive PK schedule of events (Appendix A):

1. As is standard practice in malaria research, study Day 0 refers to the day DP dosing is initiated. We will perform baseline ECG, HIV screening, and malaria screening on all potential participants on Day -1. To minimize needle pricks to the child, CBC and chemistries will be drawn on Day 0, along with Pre-DP level and Pre-ART (for D3, E3, L3)
2. Applies only to HIV-negative patients
3. History will include general medical history, diagnosis, medication (including ingestion of herbs), allergies, and current symptoms. Physical examination will include weight, height, and viral signs (temperature, pulse, blood pressure, respiratory rate), lymphadenopathy, hepatomegaly, splenomegaly, infections in ears, mouth, pharynx or skin and pulmonary, cardiac, neurologic or skeletal abnormalities and anthropometric assessments. Mid-upper arm circumference will also be collected at time of first enrollment.
4. Hematology/chemistry draws entail a 4mL venous sample for CBC, differential, ALT/AST, and creatinine. AST is incorporated as part of chemistries at all blood draws.
5. ECGs will be performed at baseline (on Day -1), prior to and 4-6 hours post-last dose, Day 7, and Day 42.
6. All participants will receive a total of 3 doses of DP given over 3 days (Day 0, Day 1, and Day 2)
7. Children on ART will be ask to take their morning doses of LPV/r or DTG in clinic when instructed by study staff. For children on EFV, they can take their doses at home, at bedtime, and record the time of administration.
8. To minimize number of pokes to the child, Pre-ART and Pre-DP levels should be drawn at the same time as CBC, chemistries, and pharmacogenetics biomarker (for a total of 6.5mL venous blood)
9. Pre-ART sample to quantify trough of LPV/r, DTG and mid-level of EFV, sampled collected via venipuncture on Day 0, 2, & 3 to allow for comparisons of ART level
10. For EFV (E3) arm, at 2, 4, and 8 hours during intensive sampling, we will draw additional 500uL samples via venipuncture to characterize EFV PK curves. This will be drawn at the same time as DP, therefore, minimizing number of blood draws.
11. For controls (C3a & C3b) only, at 4 hours on Day 0, an additional 500µL will be drawn via a venipuncture to permit correlation of venous and capillary concentrations.
12. For controls (C3a & C3b) only, at 4 hours on Day 2, an additional 200µL will be drawn via a capillary stick to permit correlation of venous and capillary concentrations.
13. For controls (C3a & C3b) only, on Day 3, and Day 7 an additional 500µL will be drawn via a venipuncture to permit correlation of venous and capillary concentrations.
14. For all 3 day arms, on 14, an additional 500µL will be drawn via venipuncture to permit correlation of venous and capillary concentrations.
15. Participants may present for unscheduled visits. Study team may perform these tests at the discretion of the team member.
16. Total blood volume in either arm is between 5-6 teaspoons over 42-days of follow-up for scheduled visits
17. 1mL of venous blood is collected on day 0 for testing of pharmacogenetic variants for children on DTG and EFV- based regimens.

APPENDIX B. INFORMATION SHEET

Information Sheet

A study on Antimalarial Pharmacology in HIV-infected and HIV-uninfected individuals

Makerere University, University of California, San Francisco, Yale University, and Baylor Center of Excellence are carrying out a study on the malaria medicine, dihydroartemisinin-piperaquine, and how it is taken up by the body in HIV-infected (receiving anti-HIV medications) and HIV-uninfected children.

- Our clinics are located at the Mulago Hospital Complex, Baylor Center of Excellence, and Masafu General Hospital
- We want the following to participate
    - HIV-infected (HIV+) children aged 3 -17 years that are taking either efavirenz, dolutegravir, or lopinavir/ritonavir as part of their HIV regimens
    - HIV-uninfected (HIV-) children aged 3 - 17 years
- Participants in this study will receive medical care during the course of the study at the study clinic
- We will provide transport to and from our clinic if needed
- The Baylor clinic is open every day from Monday to Friday, 8am to 5pm; Makerere College of Pharmacology and Therapeutics Clinic is open 7 days per week from 8am to 5pm; the Masafu General Hospital clinic is open 7 days per week from 8am to 5pm.

For more information, come to Baylor Center of Excellence, Makerere College of Pharmacology and Therapeutics, or Masafu General Hospital and ask for the malaria children's clinic. The doctors will be happy to talk with you.

APPENDIX C. WHO CRITERIA FOR SEVERE MALARIA/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

- 1-2 convulsions over a 24 hour period
- Inability to sit up or stand
- Vomiting everything
- Unable to breastfeed or drink
- Lethargy

APPENDIX D. GUIDELINES FOR ADVERSE EVENT GRADING (DAIDS AE GRADING TABLE)

Estimating Severity Grade

If the need arises to grade a clinical AE that is not identified in the DAIDS AE grading table, use the category "Estimating Severity Grade".

Grading Adult and Pediatric AEs

The DAIDS AE grading table includes parameters for grading both Adult and Pediatric AEs. When a single set of parameters is not appropriate for grading specific types of AEs for both Adult and Pediatric populations, separate sets of parameters for Adult and/or Pediatric populations (with specified respective age ranges) are given in the table. If there is no distinction in the table between Adult and Pediatric values for a type of AE, then the single set of parameters listed is to be used for grading the severity of both Adult and Pediatric events of that type.

Determining Severity Grade

If the severity of an AE could fall under either one of two grades (e.g., the severity of an AE could be either Grade 2 or Grade 3), select the higher of the two grades for the AE.

Definitions

Basic Self-care Functions	Adult Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding. Young Children Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).
LLN	Lower limit of normal
Medical Intervention	Use of pharmacologic or biologic agent(s) for treatment of an AE.
NA	Not Applicable
Operative Intervention	Surgical OR other invasive mechanical procedures.
ULN	Upper limit of normal

APPENDIX E. HIV-INFECTED CHILDREN CONSENT

APPENDIX F. HIV-UNINFECTED CHILDREN CONSENT

APPENDIX G. ASSENT

APPENDIX H. FUTURE USE OF BIOLOGICAL SPECIMENS CONSENT