EXALT TRIAL: EXTENDED DURATION ARTEMETHER-LUMEFANTRINE TREATMENT FOR MALARIA IN CHILDREN

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
BID	Twice a day
CAB	Community Advisory Board
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
Cr	Creatinine
CRF	Case report form
CYP3A4	Cytochrome p450 3A4
CYP2B6	Cytochrome p450 2B6
DAIDS	Division of AIDS
DHA	Dihydroartemisinin
DMC	Data Management Centre
DP	Dihydroartemisinin-piperaquine
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
EFV	Efavirenz
H	Hour
HFA	Height for age
Hg	Hemoglobin
HIV	Human Immunodeficiency Virus
HRPP & IRB	Human Research Protection Program and Institutional Review Board
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
LAMP	Loop-mediated isothermal amplification
LBW	Low birth weight (<2500g)
LPV	Lopinavir
LPV/r	Lopinavir/ritonavir
LR	Lumefantrine
MGH	Masafu General Hospital
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
QTc	Corrected QT interval
QTcB	Corrected QT interval using Bazzett's correction
QTcF	Corrected QT interval using Fridericia's correction
QD	Once every day
RR	Relative Risk
SD	Single dose
SP	Sulfadoxine-pyrimethamine
TASO	The AIDS Support Organization
TDH	Tororo District Hospital
TS	Trimethoprim-sulfamethoxazole
UCSF	University of California, San Francisco
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

EXALT TRIAL SCHEMA

DESIGN:

Randomized open-label prospective pharmacokinetic and pharmacodynamic study of extended duration artemether-lumefantrine in HIV-infected children on EFV-based ART and HIV-uninfected children

SAMPLE SIZE:

1) HIV-infected children with uncomplicated malaria on EFV-based ART
    - Up to 60 children enrolled into an intensive PK study of 3-day vs 5-day AL with 30 needed for each regimen*.
    - Up to 100 children enrolled into a population PK study of 3-day AL and 5-day AL with 50 needed for each regimen*.

2) HIV-uninfected children with uncomplicated malaria
    - Up to 100 children enrolled into an intensive PK study of 3-day vs 5-day AL with 50 needed for each regimen*.
    - Up to 120 children enrolled into a population PK study of 3-day AL and 5-day AL with 60 needed for each regimen*.

** participants will be enrolled from study sites in Uganda, including Tororo District Hospital (TDH) as the primary site, Masafu General Hospital (MGH) in Busia, and other centers.*
** individual participants can be enrolled into the intensive PK study for 2 episodes of malaria and can be enrolled into the population PK studies for 2 episodes to minimize the number of total participants needed.*

POPULATION:

1) HIV-infected children on EFV-based ART aged 3 to 18 years.
2) HIV-uninfected children aged 6 months to 18 years.

STUDY REGIMENS FOR PARTICIPANTS:

1) HIV-infected participants must be on efavirenz (EFV) + 2NRTI
2) All participants will be given either 6-dose or 10-dose AL over the course of the study for individual episodes of uncomplicated malaria. The order of administration will be via randomization.

MALARIA CASE DEFINITION:

Uncomplicated malaria (all of the following)

- Fever (> 38.0ºC tympanic) or history of fever in the previous 24 hours
- Positive thick blood smear
- Absence of complicated malaria

Complicated malaria (any of the following)

- Evidence of severe malaria and parasitemia
- Danger signs present and parasitemia
- Parasite density > 500,000/µl

STUDY DURATION:

Up to 42 days for treatment of each malaria episode.

- Children will be eligible to participate in intensive PK sampling for up to two episodes of malaria. Children will be randomly assigned to either 3-day or 5-day regimens for each episode of malaria for the intensive PK sampling.
- Children will also be eligible to participate in the population PK sampling and again will be randomized to 3-day or 5-day regimens for each subsequent episode of malaria.
- Each child can participate in intensive and/or population PK evaluations for up to a total of 4 malaria episodes

HYPOTHESES:

1) 5-day AL will provide superior PK exposure and improved treatment outcomes compared to 3-day AL in HIV-infected EFV-treated children

2) 5-day AL in HIV-infected EFV-treated children will provide comparable PK exposure and outcomes to 3-day AL in HIV-uninfected children.

3) 5-day AL in HIV-uninfected children will provide superior PK exposure and improved treatment outcomes as compared to 3-day AL in HIV-uninfected children.

PRIMARY OBJECTIVES:

1) To evaluate and compare the PK exposure between 5-day AL treatment and 3-day al treatment in HIV-infected children on EFV-based ART
2) To evaluate and compare the PK exposure of 3-day and 5-day AL treatment in HIV-infected children on EFV-based ART to 3-day AL treatment in HIV-uninfected children not on ART.
3) To evaluate and compare the PK exposure of 3-day and 5-day AL treatment in HIV-uninfected children not on ART.
4) To evaluate the pharmacodynamics of AL in the context of extended dose regimens and HIV infection.

SECONDARY OBJECTIVES:

1) To assess the comparative safety of 5-day vs 3-day AL
2) To assess the impact of 3-day vs 5-day AL on the prevalence of gametocytemia following treatment
3) To evaluate the association of anthropomorphic indicators of malnutrition on PK exposure and outcomes of AL in HIV-infected and HIV-uninfected children.
4) To compare the diagnostic sensitivity of LAMP, HS-RDT, and microscopy for the detection of recurrent parasitemia.
5) To assess the comparative metabolomic profile of HIV-infected and HIV-uninfected children following treatment with 3-day and 5-day AL to assess
6) To assess the relationship between molecular markers of drug resistance and recurrent malaria

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 causing roughly 198 million cases and 584,000 deaths in 2013.[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.[1, 2] Treatment of uncomplicated malaria in children relies solely upon the artemisinin-combination therapies (ACTs).[1] 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[3] with artemether-lumefantrine (AL), artesunate-amodiaquine (AS-AQ), and dihydroartemisinin-piperaquine (DP) stipulated as first-line according to the World Health Organization (WHO).[3, 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).[1, 5-8]

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,[9] and all of whom are eligible for ART under new WHO guidelines.[9-11] First-line ART includes lopinavir/ritonavir (LPV/r)-based ART for children <3 years, and efavirenz (EFV)-based ART for children >3 years[10] with 86% of 58 WHO focus countries adopting EFV-based ART as their preferred 1st-line regimen.[12] We will study AL given as malaria treatment in 3 vs 5-day regimens to HIV-infected children stabilized on EFV-based ART to delineate its impact of previously described drug-drug interactions.[10] Nevirapine (NVP), another non-nucleoside reverse transcriptase inhibitor (NNRTI), has less pharmacokinetic (PK) alterations in the setting of AL, and will not be a subject of this study.[13, 14]

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.[1, 15] In Tororo, the site of this proposal, children experience up to 2 to 6 malaria episodes per year, despite usage of bed nets and trimethoprim-sulfamethoxazole (TS) for those HIV-infected.[15-19] For HIV, Uganda is lauded for prior success in stemming the HIV epidemic, but new infections are now on the rise.[9] It is estimated that 2 million HIV-infected children will reside in sub-Saharan African in 2020.[9, 20] Thus, HIV-malaria co-infection remains common, with treatment complicated by multiple pharmacological factors that require field-based studies, such as those currently proposed in this renewal.[21, 22]

1.1.4 Significant drug interactions occur between ART and ACT. Both components of AL are extensively metabolized.[5] Artemether undergoes rapid demethylation by cytochrome p450 3A4 (CYP3A4) and CYP2B6 to the active metabolite,

dihydroartemisinin (DHA), which undergoes further metabolism via UDP-glucuronosyltransferases (UGT).[23-25] CYP3A4 is also responsible for metabolizing lumefantrine to desbutyl-lumefantrine (desbutyl-LR), and was shown by our group to be the major pathway for PQ metabolism.[26-28] In the setting of HIV-malaria co-infection, ritonavir and EFV cause potent CYP3A4 inhibition and induction, respectively.[29-31] For AL, 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.[5, 32-34]

Table 1 Comparison of AL PK exposure in HIV-infected children on ART compared to HIV-uninfected children (controls)

	HIV-uninfected	HIV-infected	
	AL alone (control)	AL+EFV	
	GM; 95%CI (n=52)	GM; 95%CI (n=31)	GMR, EFV/Control
AR_AUC$_{0-8}$	120 (97.6-147)[a]	48.5 (37.2, 63.1)	0.40***
DHA_AUC$_{0-8}$	212 (176, 256)[a]	62.9 (49.2, 80.3)	0.30***
LR_AUC$_{0-\infty}$	270 (232, 313)	130 (107, 157)	0.48***
LR Half-life[b]	64.3 (52.0, 121)	23.7 (21.8, 46.0)	0.37***
Intensive and sparse PK (n=363 episodes)			
	Median (IQR), n=186	Median (IQR), n=48	MR, EFV/Control
LR C$_{7d}$	340 (257, 531)	111 (63, 192)	0.30***
LR C$_{14d}$	86 (59, 137)	BLQ (BLQ,BLQ)	<1***
LR C$_{21d}$	BLQ (BLQ, 63)	BLQ (BLQ,BLQ)	<1**

1.1.5 ACT-ART interactions significantly alter clinical outcomes. The 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.[3, 33, 35, 36] However, no data are available on alternate ways to administer AL in the setting of the EFV-based ART so that optimized treatment guidelines can be developed.

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.[37-39] For metabolism, UGTs mature from 0-6 months of age while CYPs mature over 12 months of age.[40, 41] 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).[42-44] Our group and others showed that lumefantrine PK is lower in Ugandan children compared to U.S. adults, also suggesting higher clearance in children.[45, 46]

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.[47] This was suggested for sulfadoxine-pyrimethamine,[47, 48] and recently for DP, where 36% of patients in Cambodia who failed to clear infection had PQ concentrations below *in vitro* target levels.[49] We have now shown that a) EFV-treated children have ~2-fold *reduced* exposure to AL with similar changes expected for DP and b) underweight children have *reduced* exposure to

AL and DP compared to children with normal WFA.[17, 28, 34, 50-53] For the ~3 million sub-Saharan African children with HIV, EFV is first-line (when >3years).[9] For sub-Saharan African children with malaria, AL is the most common ACT, with DP use increasing. For all children in sub-Saharan Africa, 28 and 56 million are estimated to be underweight and stunted, respectively.[54] Thus, with the spread of artemisinin and DP resistance in Southeast Asia,[55-58] and emerging concerns for Africa,[59, 60] there is mounting concern that suboptimal AL and DP dosing in HIV-infected children on EFV-based ART or children will contribute to the emergence and selection of ACT resistant parasites.[47]

1.1.8 Lumefantrine exhibits dose-limited absorption. Low exposure to AL is of concern, and various strategies to increase lumefantrine exposure have been proposed. These include an 1) increase in mg/kg dose, 2) increase in dosing frequency (ie. 3 doses per day), and 3) an extended duration of treatment. A notable study by Ashley E, et al. found that doubling of the dose (once to twice daily) yielded only a 30% increase in lumefantrine AUC, and no change in C_{max}.[61] Based on these and other data, absorption of lumefantrine is felt to be dose-limited, and thus, an increase in mg/kg dose or frequency are not felt to be an effective solution. Thus, WWARN and others have suggested that extended dosing regimens be evaluated.[62-65] *In silico* modeling (Tarning J, personal communication) also suggest that an increase in mg/kg twice daily dosing in young children does not reach similar concentrations as adults, whereas three daily doses reaches equivalent concentrations, and a 5-day regimen results in the highest exposure. In addition, with growing concern over the emergence of artemisinin resistance, alternative strategies for ACT use have been proposed in SE Asia, including use of triple ACTs (artemisinin + 2 long-acting partner drugs) and the use of longer regimen durations.[66] An added potential benefit of the extended regimen is the exposure of parasites for 2-complete life cycles (each is 48 hours) to the artemisinin component, as compared to only 1 full cycle with current regimens. Importantly, we have shown that higher exposure to lumefantrine is well tolerated with no increase in adverse events.[17, 67] More specifically, we recently evaluated the impact of another antiretroviral, lopinavir/ritonavir, on levels of lumefantrine in children with malaria.[67] Importantly, in contrast to EFV, lopinavir/ritonavir is a potent inhibitor of CYP3A4, and therefore expected to increase levels of lumefantrine. In our study, day 7 levels in children on lopinavir/ritonavir-based ART had 10-fold higher day 7 levels of lumefantrine and >2-fold higher AUC, as compared to those on EFV-based ART, while exhibiting no increase in toxicity.[17, 67]. Thus, we do not expect an increase in toxicity with a 5-day duration of AL.

1.1.9 Malnutrition is prevalent in African children and may impact ACT pharmacology. Malnutrition can include a) *chronic* protein-calorie malnutrition resulting in slow linear growth (decreased height-for-age: HFA; stunting), b) *acute* protein-calorie malnutrition resulting in weight loss or slow weight gain (decreased weight-for-height: WFH; wasting) and c) micronutrient deficiencies in iron, zinc, and vitamin A that can be difficult to detect clinically.[68] Underweight (decreased weight-for-age: WFA) status reflects the combination of *chronic and acute* protein-calorie malnutrition, and is the most clinically utilized indicator of nutritional status. Grading of malnutrition depends on anthropomorphic measures where z-scores > 2 standard deviations (SD) below the

mean of the reference population denote moderate to severe malnutrition.[69] In Africa, an estimated 36% and 18% of children <5 years are classified as stunted or underweight, respectively (39% and 16% for Uganda, respectively).[54, 70, 71] _Malnutrition can alter drug absorption due to changes in gut integrity and gastric emptying;[72] plasma binding of drugs;[73] drug activation;[74] and drug clearance.[75, 76] The net impact on overall PK exposure depends on both the drug and type of malnutrition. Clear specification of malnutrition indices and their relationship to

Table 1. Impact of factors on ACT PK and outcomes based on Limited Data	ACT	Modifying factor	Mode of modification	Change in PK exposure	Reinfection risk	Selected References
	AL	EFV	Induce CYP/UGT	↓↓↓ AL	↑↑↑ AL	(17, 34, 63, 77, 78)
		Under-weight	↓ F, CYP	↓↓ AL	↑↑ AL	(51, 53, 64, 79)
		Stunted	↓ F, CYP	No data	Likely ↑	(80)
	F: bioavailability, CYP: cytochrome p450, UGT: UDP-glucuronosyltransferases;					

PK evaluations are needed to optimize drug protocols for malnourished children. Dosing of AL in malnourished children using weight-based guidelines that ignore nutritional status and a child's maturational age, may result in mg/kg doses that are too low in malnourished children.[51, 53, 79] and worsen treatment outcomes.

1.1.10 Strategies for completing field-based pharmacology studies. The conduct of antimalarial PK/PD studies in field settings must consider blood volume. Intensive PK studies require serial sampling of blood volumes that must be minimized in children. Small volume methods for venous samples or capillary samples via a finger prick can measure drugs using as little as 100-200 µl of plasma [81-83]. Small volume methods permit easy collection of samples in large populations, permitting optimum population PK designs to investigate relationships between drug exposure, patient and disease covariates, and clinical outcomes [84, 85]. These studies will include utilization of small volume assay methods for ACT and ART to optimize PK/PD study and our group will employ small volume capillary methods that will be easily incorporated into subjects' existing visits/blood draws.

1.2 Rationale

This study is designed to directly address antimalarial PK and PD 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 the most widely prescribed antimalarial treatment regimen, AL, primarily in the context of EFV-based ART but also in HIV uninfected children who will serve as a control group. Therefore, results from this study will inform specific dosing guidelines for treatment of uncomplicated malaria in children, both HIV infected and uninfected. Traditionally, studies have focused on HIV-uninfected, non-pregnant adults, largely ignoring the effects of childhood maturation and ART interactions on drug disposition. This gap in research is specified as a priority by NIAID and leaders of the WorldWide Antimalarial Resistance Network (WWARN), who emphasize the importance of proper PK and PD study in relevant populations to reduce the threat of ACT drug resistance and treatment failure [84-87]. This study will allow optimization of ACT regimens, especially in the setting of HIV co-infection and inform the best use of AL with EFV-based ART, in the setting of malaria, with the goal of improving clinical outcomes.

The ACTs are the most important drugs for the treatment of uncomplicated malaria. 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 or malnutrition. Our results to 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, the active dihydroartemisinin metabolite, 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. These findings suggest that AL dosing should be modified for children receiving EFV. We propose that extending AL dosing from 3 to 5 days will offset these changes. We will study an extended AL regimen, rather than a higher dose, as we know lumefantrine absorption is dose-limited (Section 1.1.8). This strategy should be useful for other conditions causing low exposure, such as pregnancy, and in the setting of emerging resistance.

We will conduct our study in the malaria-endemic region of Eastern Uganda in both Tororo and Busia where our team has long standing experience directing field-based PK/PD studies in the most relevant populations. We will utilize a combination of state-of-the-art intensive and population PK designs and drug assay methods to determine drug exposure and study associations between exposure and specific malaria outcomes including recurrent malaria.

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
- Improper dosing may compromise care of acute infection but more importantly contribute to develop of resistance
- We have shown that interactions between AL and EFV greatly altered levels to BOTH artemether and lumefantrine, and put patients at risk for treatment failure.
- Extended dosing intervals of AL may overcome lower exposure that is found in patients concomitantly taking EFV.
- Higher exposure to AL is not expected to increase toxicity
- Extending dosing intervals in HIV-uninfected children may reduce the likelihood of emergence of artemisinin resistance.
- Intensive PK design resulting in determination of a precise AUC will permit robust comparisons so that results will inform treatment guidelines and policy for HIV-infected children.
- Population PK designs will provide additional data to assess covariates that may impact exposure as well as enhance PK-outcome assessments

2.0 STUDY OBJECTIVES

2.1 Primary Objectives:

1) To evaluate and compare the PK exposure between 5-day AL treatment and 3-day al treatment in HIV-infected children on EFV-based ART
2) To evaluate and compare the PK exposure of 3-day and 5-day AL treatment in HIV-infected children on EFV-based ART to 3-day AL treatment in HIV-uninfected children not on ART.
3) To evaluate and compare the PK exposure of 3-day and 5-day AL treatment in HIV-uninfected children not on ART.
4) To evaluate the pharmacodynamics of AL in the context of extended dose regimens and HIV infection.

2.2 Secondary Objectives:

1) To assess the comparative safety of 5-day vs 3-day AL
2) To assess the impact of 3-day vs 5-day AL on the prevalence of gametocytemia following treatment
3) To evaluate the association of anthropomorphic indicators of malnutrition on PK exposure and outcomes of AL in HIV-infected and HIV-uninfected children.
4) To compare the diagnostic sensitivity of LAMP, HS-RDT, and microscopy for the detection of recurrent parasitemia.
5) To assess the comparative metabolomic profile of HIV-infected and HIV-uninfected children following treatment with 3-day and 5-day AL to assess
6) To assess the relationship between molecular markers of drug resistance and recurrent malaria

3.0 STUDY DESIGN

This is a prospective randomized multi-site study to evaluate the PK/PD of extended duration AL in HIV-infected children on EFV-based ART and HIV-uninfected children not on ART. AL is the first-line treatment for malaria in Uganda. No change in standard of care treatment will be made for the purposes of this study except for the extension of AL to 5-day dosing. Figure 1 summarizes the design. This study will enroll a) HIV-infected children, and b) HIV-uninfected children. All participants may be enrolled through Tororo District Hospital (TDH) or Masafu General Hospital (MGH) in Busia, or other referral centers the area.

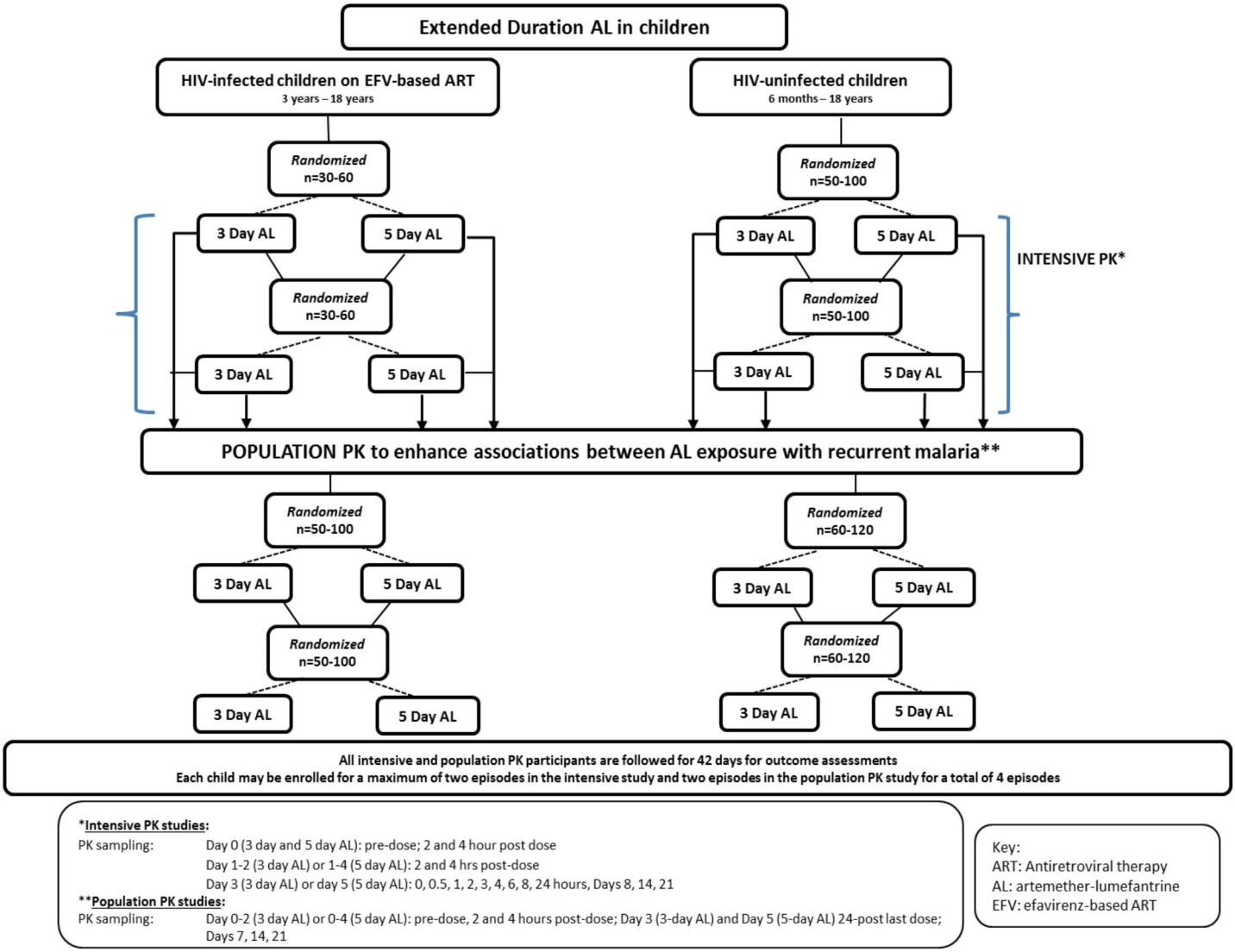
3.1 Overview of Pharmacokinetic Sampling Design

3.1.1 Intensive PK study.

Intensive PK study 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 extended duration AL PK exposure in the context of EFV-based ART and in HIV-uninfected children. This will be accomplished through a design where children will be randomized to either 3-day or 5-day AL and then be re-randomized for subsequent episodes of malaria, should they occur. Comparisons of AL PK exposure will be made among and between a) HIV-infected children with malaria receiving EFV-based ART and b) HIV-uninfected children who are not on ART. Comparisons will be based on an intensive PK design for AL AUC estimations. Conservatively, assuming each enrolled child participates for only a single episode of malaria, up to 60 (30 HIV-infected on 3-day and 30 HIV-infected on 5-day) and 100 (50 HIV-uninfected on 3-day and 50 HIV-uninfected on 5-day) subjects will be enrolled for each of the intensive study groups. The generation of an AUC will permit robust comparisons so that results will inform treatment guidelines and policy.

Figure 1—Overview of Study Design



3.1.2 Population PK study.

Population PK study involves having few PK samples drawn over time which are ideally combined with intensive PK studies to allow for optimal PK-outcomes assessments. These studies will also be conducted in both HIV-infected and HIV-uninfected participants where a randomized design will also be employed where children will be randomized to either 3-day or 5-day AL and then re-randomized for a subsequent episode of malaria, should it occur. The preference will be that children will first have participated in the intensive PK evaluations (for both 3-day and 5-day regimens for two sequential episodes of malaria until the sample size has been reached) before continuing onto the population PK assessments. In total, each child may participate in intensive and/or population PK assessments for a total of 4 malaria episodes.

3.2 Overview of study groups

Both HIV-infected and HIV-uninfected children will be enrolled from Tororo District Hospital (TDH) or other referral centers in Tororo and at Masafu General Hospital in the Busia District. As children can be enrolled for multiple episodes of malaria, these sample sizes represent the maximum numbers of children enrolled in each study arm. Consenting will be done once in each child to cover the potential for enrollment for multiple episodes of malaria and in both intensive and population PK studies.

Conservatively, assuming each enrolled child has only a single episode of malaria, the maximum total number enrolled will be 380 children, as follows:

- HIV-infected 3-day AL regimen (80 total)
    - 30 intensive PK participants
    - 50 population PK participants
- HIV-infected 5-day AL regimen (80 total)
    - 30 intensive PK participants
    - 50 population PK participants
- HIV-uninfected 3-day AL regimen (110 total)
    - 50 intensive PK participants
    - 60 population PK participants
- HIV-uninfected 5-day AL regimen (110 total)
    - 50 intensive PK participants
    - 60 population PK participants

3.2.1 HIV-infected participants

This protocol will study the clinical pharmacology of AL in HIV-infected participants on EFV-based ART regimens, specifically children aged 3 to 18 years. Up to 60 children will be enrolled for intensive PK studies and up to 100 children will be enrolled for population PK studies. All children will be enrolled at the time they present with uncomplicated malaria and will undergo intensive PK sampling during 42 days of follow-up. Some of the same children will 1) re-randomized for a subsequent episode of malaria, thus providing two intensive PK datasets and, potentially, 2) undergo population PK assessments for either or both 3-day and 5-day regimens.

3.2.2 HIV-uninfected participants

This protocol will also study the clinical pharmacology of AL in HIV-uninfected children aged 6 months to 18 years. Up to 100 HIV-uninfected subjects will be enrolled for intensive PK studies and up to 120 children will be enrolled for population PK studies. HIV-uninfected children will primarily serve as controls for comparison with HIV-infected children but will also be assessed for the effects of age on PK (see note). Children will be enrolled at the time they present with uncomplicated malaria and will undergo population PK sampling during 42 days of follow-up. Some of the same children will 1) undergo crossover to the alternate regimen, thus providing intensive PK data for both regimens, and, potentially, 2) undergo population PK assessments for either or both 3-day and 5-day regimens.

Note: the larger sample size of 100 for intensive PK and 120 for population PK in HIV-uninfected children will permit assessment of the impact of age on the PK of AL in these regimens over a range of developmental maturation (6 months to 18 years)

3.2.3 All children

- If a child has multiple episodes of malaria, they will be randomized for each episode into a 3-day or 5-day regimen. For example, a child could be randomized to a 3-day regimen for episode #1 and then randomized to either a 3-day or 5-day regimen for episode #2, or vice-versa.
- 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 (see Note*).
- 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 for subsequent episode(s) of malaria, if they meet eligibility requirements.
- All children can be enrolled into the intensive study for a maximum of two “evaluable” episodes of malaria.
    - *Note: Up to 2 PK samples can be missing to still be considered “evaluable” for intensive PK. More than 2 missing PK samples will permit a child to either repeat a specific intensive study for a subsequent episode or be replaced. They may also be eligible to transition from the intensive study into the population PK sampling study, provided enrolment criteria are met. For any malaria episodes beyond the 4th episode, a child will be referred to the local health center for management.
- At least 28 days must separate each PK study malaria episode.
- All children will preferentially undergo intensive PK studies until the maximum of two episodes in the intensive sampling scheme has been reached

- A child may be enrolled for a maximum of 4 episodes of malaria over the study period whether deemed “evaluable” or not. For any malaria episodes beyond the 4th episode, a child will be referred to the local health center for management.

- If participation in the intensive study is not feasible for any reason or if the desired intensive sampling size has been an achieved, a child can be directly enrolled into the population studies.

3.3 Study sites

The primary study site is the study clinic at the TDH campus situated in Tororo, Eastern Uganda, an area of high malaria transmission. This was the primary study site for the initial RO1. Participants will receive all routine and acute medical care at the TDH study clinic, open 7 days a week from 8 a.m. to 5 p.m. If medical care is needed after hours, parents/guardians/participants will be instructed to go to the TDH premises (where the study clinic is located) and request that a study physician on-call be contacted.

The secondary study site is at the MGH campus situated in the Busia District, also in Eastern Uganda, approximately 50 km south of the TDH site. Busia is currently the site of several active NIH-funded malaria studies by the IDRC and other collaborators. As with TDH, the study clinic in Busia will remain open 7 days a week from 8 a.m. to 5 p.m.

Similar to our previous RO1 PK study, we will allow for referrals from neighboring health centers in the Tororo and Busia districts, provided that entry criteria are met.

4.0 SELECTION AND ENROLLMENT OF SUBJECTS

4.1 Inclusion Criteria

4.1.1 <u>All participants:</u>

1) Residency within 60 km of the study clinics either at TDH or at MGH
2) Agreement to come to clinic for all follow-up clinical and PK evaluations
3) Provision of informed consent
4) Weight ≥6 kg
5) Presentation with uncomplicated *P. falciparum* malaria, or mixed infection with the presence of *P. falciparum* species, as indicated by positive smear for malaria parasites along with clinical evidence of infection (fever or history of fever in the past 24 hours)
6) Willingness to undergo intensive PK sampling and/or population PK sampling during episode(s) of malaria.

4.1.2 <u>HIV-infected participants:</u>

1) Confirmed HIV infection (positive rapid HIV test to be confirmed by Western Blot or HIV RNA after enrollment)

2) On stable EFV-based ART for at least 10 days prior to enrollment
3) Age 3 years to 18 years

4.1.3 HIV-uninfected participants:

1) Confirmed HIV negative test (negative rapid HIV test to be confirmed by Western Blot or HIV RNA after enrollment)
2) Age 6 months to 18 years

4.2 Exclusion Criteria

1) History of significant comorbidities such as malignancy, active tuberculosis or other WHO stage 4 disease
2) Current infection without the presence of *P. falciparum* species
3) Receipt of any medications known to affect CYP450 metabolism (except ART) within 14 days of study enrollment (see 4.2.2)
4) Hemoglobin < 7.0 g/dL
5) Prior treatment for malaria within 28 days of enrollment
6) Signs or evidence of complicated malaria, defined as unarousable coma OR ANY TWO OF THE FOLLOWING SYMPTOMS: Recent febrile convulsions, altered consciousness, lethargy, unable to drink, unable to stand/sit due to weakness, severe anemia (Hb < 5.0 gm/dL), respiratory distress, jaundice (see Appendix D)
7) History of toxicity to AL

4.2.2 Disallowed Medication Guidelines

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

- Carbamazepine
- Clarithromycin
- Erythromycin (oral)
- Ketoconazole
- Phenobarbital
- Phenytoin
- Rifabutin
- Rifampin
- 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

Children will be enrolled from TDH or other referral site or MGH in the Busia District as described below (Section 4.3.1). Parents/guardians of children will be approached for their willingness to participate in intensive PK studies and/or population PK studies for

one of more episodes of malaria. All children will undergo 42 days of follow-up around each malaria episode for which they will have PK sampling carried out. Participants may be recruited through multiple referral mechanisms including those primary sites described below.

4.3.1 Primary Referral Sites

1. Tororo District Hospital (TDH). Tororo District Hospital Pediatric, Antenatal and Adult Clinics and Wards: HIV-infected and HIV-uninfected children presenting with uncomplicated malaria to the TDH clinics or wards.

2. The AIDS Service Organization (TASO, Tororo). TASO has been providing care to HIV-infected children and adults in Tororo since 1989. HIV-infected children presenting with uncomplicated malaria.

3. Masafu General Hospital, Busia District. HIV-infected and HIV-uninfected children presenting with uncomplicated malaria to the clinics or wards.

4.4 Screening

HIV-infected and HIV-uninfected children will be screened for study eligibility at the time they present with uncomplicated malaria at TDH or MGH. Children referred from sites other than TDH or MGH will be screened upon their arrival to the study clinics at either TDH/MGH by study staff. Parents/guardians will be asked about their willingness to have their child participate in intensive and/or population PK studies involving standard 42-day follow-up for up to 4 episodes of uncomplicated malaria. It will be explained to the child and parent or guardian that participation can be during as few as one or as many as four episodes of malaria, depending on their preference. For any malaria episodes beyond the 4th episode, it will be explained that referral/care will be directed to the local health center for management. It will also be explained that children will only be part of the study during the 42-days of malaria follow-up, but not during periods in between episodes of malaria. 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. Consenting for intensive and population PK procedures will be made together in a single consent form, as well as consent for future use of biologic specimens. Assent will be obtained following Ugandan guidelines.

4.4.1 Confirmation of malaria status

All children must have confirmed uncomplicated *P. falciparum* malaria in order to meet criteria for entry into the study. Details on the definition of uncomplicated malaria and how it is diagnosed are detailed in Sections 6.2 and 6.3. All laboratory procedures, including those for screening, will only be conducted after informed consent/assent have been obtained.

4.4.2 HIV Testing of HIV-uninfected children

HIV counseling and testing will be done on all HIV-uninfected children presenting to the clinics or wards in Tororo or Busia as part of study enrolment 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). All children ≥ 6 months will have serology testing performed as per Ugandan policy. Initially, a Determine rapid test is performed. If it is negative, the participant is declared negative. If Determine is positive, the Stat pak rapid test will be done for confirmation. If Stat pak is positive, then the participant is confirmed positive. If Stat pak is negative, then a Unigold rapid test is run. If Unigold is positive, then the participant is positive for HIV. If Unigold test is negative then the participant is negative for HIV. For children who are 6 months to 18 months of age with positive serologic tests, they will be referred to an HIV clinic for confirmatory virologic testing (PCR). HIV testing protocol may be modified to be in-line with Ugandan policy if changes occur over the course of the study.

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. 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 G, H, and I) approved by the UCSF Committee for Human Research (UCSF CHR), Yale Human Investigations Committee, Makerere University College of Health Sciences Ethics Committee, and the Uganda National Council for Science and Technology (UNCST) that will be available in 6 languages (Jopadhola, Teso, Swahili, Luganda, Samia, and English). The informed consent form 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.

Subjects who fulfill verbal eligibility criteria and have provided informed consent, will undergo a series of evaluations including a detailed history and physical examination, measurement of temperature, height, and weight. Blood will then be collected by finger prick for hemoglobin testing, as well as for malaria thick and thin smear, if not already available (see Section 6.2).

5.0 MALARIA AND HIV TREATMENT

5.1 Artemether-lumefantrine (Coartem®)

Participants enrolling in the study will receive AL as dispersible Coartem® (Novartis Pharmaceuticals, Switzerland) tablets treatment for each episode of uncomplicated malaria. Coartem® has been FDA-approved for the treatment of malaria in the U.S since 2009, and is a first-line treatment for malaria by the WHO. Importantly,

participants would have received AL for treatment of malaria if they were not in the study, and it is provided at no charge by the Ugandan Ministry of Health. However, as there may be many generic brands of AL available in Uganda, we will provide AL as dispersible Coartem® for all malaria episodes associated with this study to ensure consistency.

AL is generally considered very effective for treatment of malaria. Dosing in children is primarily based on weight-based adjustments to adult dosing. The artemisinins (artemether in the case of AL) 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 [88]. 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[89]. Longer acting lumefantrine is also generally well tolerated. The drug is chemically similar to halofantrine, a drug associated with cardiac arrhythmogenic potential, but no cardiac issues have been identified in children receiving lumefantrine. In 713 lumefantrine treated patients, serial ECG monitoring indicated no adverse cardiac events[90]. There have been no reports associating elevated AL drug levels with toxicity. There is no evidence of harm in infants exposed to AL. Specifically, in our prior study evaluating AL with lopinavir/ritonavir based ART, AUC estimated for lumefantrine were increased close to 3-fold, without evidence of untoward effects[67]

AL is typically dosed twice daily for 3 days for a total of 6 doses for treatment of uncomplicated malaria. In this study, we will also study when AL is dosed twice daily for 5 days for a total of 10 doses.

5.1.1 Dosing schedule for intensive and population PK studies

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 AL regimen. To ensure that we can begin intensive sampling around the last dose or AL, a modest alteration in the dosing schedule will be made so that the last dose falls in morning. This alteration does not affect treatment efficacy or safety and we have used this approach for our prior intensive PK studies of AL.[67, 91] The dosing schedule specified in Table 4 will be used to assure a) adequate AL treatment and b) the scheduling of intensive PK sampling during the day, rather than during the night.

- AL treatment for the 3-day regimen will be 6 doses of Coartem® administered over 4 days (Study Days 0, 1, 2 and 3) as per weight-based dosing guidelines. Sampling will begin in the morning of day 3. The 6-dose regimen is the standard of care for the treatment of malaria

- AL treatment for the 5-day regimen will be 10 doses of Coartem® administered over 6 days (Study Days 0, 1, 2, 3, 4, and 5) as per weight-based dosing guidelines. Sampling will begin in the morning of day 5.
- Children will be presenting for care at various times of the day. Therefore, the precise timing of the 1st and 2nd dose on Day 0 will be left to the discretion of the medical officer.
- However, spacing between the 1st and 2nd doses of AL should be a minimum of 7 hours and maximum of 10 hours.

Table 4. Coartem® dosing for intensive PK study ONLY

3-day AL regimen			STUDY DAY 1	STUDY DAY 2		STUDY DAY 3			
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6			
	(11AM - 5PM) (C)	(9PM -1AM) (H)	(1PM -3PM) (C)	(8AM - 10AM) (C)	(8PM-10PM) (H)	(8AM-10AM) (C)			

5-day AL regimen	STUDY DAY 0		STUDY DAY 1	STUDY DAY 2		STUDY DAY 3		STUDY DAY 4		STUDY DAY 5
	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8	Dose 9	Dose 10
	(11AM - 5PM) (C)	(9PM -1AM) (H)	(1PM -3PM) (C)	(8AM - 10AM) (C)	(8PM-10PM) (H)	(8AM-10AM) (C)	(8PM-10PM) (H)	8AM-10AM) (C)	(8PM-10PM) (H)	8AM-10AM) (C)

*spacing between the 1st and 2nd doses of AL on study day 0 should be at a minimum of 7 hours, and maximum of 10 hours.

For **population PK studies**, AL will be administered as per standard dosing schedules (twice daily for 3 days) or as the extended regimen (twice daily for 5 days) with the twice daily dosing unaltered during the population PK studies.

5.1.2 Drug Administration

Every dose of AL should be administered with whole milk as specified below since ingestion with fat-containing food and drinks greatly improves lumefantrine absorption.[92] Although risk of treatment failure is very small, subjects who remain averse to food during treatment should be closely monitored. At the time of study entry, subjects and/or subjects' families will be provided with container(s) of whole milk or vouchers to purchase whole milk to be administered with study drug. Adherence of AL will be assured by recording each treatment dose time. Doses will be administered either in the clinic and observed by a study nurse or at the subject's home and observed by the caregiver.

Dosage: Children will receive the dispersible formulation of AL with contains 20 mg artemether, 120 mg of lumefantrine (Coartem® Dispersible). Dispersible tablet(s) composing 1 dose should be completely dispersed in a small amount of water (approximately 10 mL per tablet). Stir gently and administer immediately to the subject. Rinse the glass with an additional small amount of water (approximately 10 mL) and give immediately to the subject. Weight-based dosing will be as below (Table 5).

Table 5. Weight-based dosing guidelines for AL

Weight	Coartem® Dispersible 20mg /120 mg tabs
<15 kg	1

≥15 to <25 kg	2
≥25 to <35 kg	3
≥35 kg	4

Efavirenz-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. HIV infected children will receive EFV-based ART as per prescription through their primary HIV clinic. No changes in ART will be made for the purposes of this protocol. While receiving AL treatment for malaria, EFV-based ART administration in the morning will occur at the same time as AL administration and the timing will be documented. In order to meet eligibility, children will need to have been maintained on EFV-based ART for at least 10 days prior to enrollment.

5.1.3 Missed Doses of AL

Missed doses due to vomiting:

- If the episode of vomiting occurs ≤30 minutes post-administration of AL, 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 AL.
- If the vomiting occurs more than 30 minutes but less than 2 hours post-administration of AL, redosing of 50% of the dose should occur. The redosing will be carefully noted on the study case report forms. Participants may not continue in the intensive study if this occurs, but may be transitioned to the population PK study.
- Any patient who vomits repeatedly (> 3 times) will be recorded as having complicated malaria and treated with quinine or artesunate, as is standard of care.

Missed or late doses due to other reasons:

- If an AL dose was missed or late for any other reason, participants will be counseled that the dose should be taken as soon as remembered.
- Participants in intensive PK studies should have taken the 5th and 6th AL doses (for the 3-day regimen) or the 9th and 10th AL doses (for the 5-day regimen) 12 ± 2 hours apart to participate in the intensive PK sampling.

5.1.4 Drug supply and distribution

Artemether-lumefantrine

AL is the standard of care for malaria treatment in Uganda. However, to ensure consistency in drug preparation, this study will supply the AL for all participants, and will be purchased through Ugandan suppliers of the Novartis product (as per program pharmacist) Coartem®, which is brand name AL. At the time of AL initiation, participants will be provided doses for administration in the afternoon or evening only. All morning doses will be provided by and observed in the study clinic.

Efavirenz-based antiretroviral therapy

Efavirenz-based ART is the WHO first-line ART regimen for all children > 3 years of age, adolescents, and adults.[10, 11, 93] EFV-based ART for HIV-infected participants will be administered and managed through their usual clinic at TDH, MGH, TASO or other referral site. Those who are not yet followed by a clinic will be referred to the appropriate HIV clinic in Tororo or Busia for follow-up of HIV. This study will maintain a supply of EFV-based ART, either provided by the Uganda Ministry of Health or purchased by the study, to provide to study participants as necessary.

5.1.5 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 Tororo District hospital or MGH drug dispensaries after the study is completed or terminated.

6.0 CLINICAL AND LABORATORY EVALUATIONS

6.1 Schedule of Evaluations

See Appendices A and B for Schedule of Evaluation (SOE) Tables

6.2 Malaria Diagnosis

All children will have their malaria diagnosis carried out through their usual clinic in Tororo/Busia or through the study clinic at the time of enrollment in this protocol. At the time of enrollment, parents/guardians/participants will be instructed to come to the study clinic for all medical care over the 42-day follow-up period. For all participants, the study clinics in both Tororo and Busia remain open 7 days a week from 8 a.m. to 5 p.m.

Microscopy. Subjects may be enrolled following diagnosis of uncomplicated malaria if their malaria diagnosis is supported through documentation of thick blood smear results. For those subjects who have not already been diagnosed, but present 1) febrile (tympanic temperature > 38.0˚C) or 2) report history of fever in the past 24 hours, he/she will have blood obtained by finger prick for a thick blood smear (in very young children, heel sticks may be substituted for finger pricks). Thick blood slides will be read and counted by the laboratory technicians at the time of presentation. The parasite density of positive screening thick blood smears will be estimated by counting the

number of asexual parasites per 200 leukocytes, assuming a leukocyte count of 8,000/µl (or per 500 leukocytes, if the count is <10 asexual parasites/200 leukocytes). Smears will be considered negative when examination of 100 high-power fields does not reveal parasites. If the thick blood smear is positive, the patient will be diagnosed with malaria. If the thick blood smear is negative, the patient will be managed by medical officers in their usual clinic or by study staff. Thin blood smears will be evaluated for parasite species. All subjects with a positive smear for *P. falciparum*, and a parasite density of ≤ 500,000/µL will be referred to the PK study medical officers to determine further eligibility for study enrollment which will involve additional screening tests (e.g. HIV test if serostatus not known via appropriate test results, hemoglobin testing) (see section 6.8).

Loop-mediated isothermal amplification (LAMP). LAMP is a recent field-adapted molecular technique to identify malaria parasites with an estimated lower limit of detection of 1-5 parasites/µL, which is below the limit of detection for microscopy (~100 parasites/µL). LAMP will be performed on dried blood spot specimens permitting us to identify parasitemia at lower levels, and perhaps at earlier time points over the course of follow-up (see section 6.8).

Rapid Diagnostic Test (RDT). Alere Inc has recently developed the Alere™ Malaria Ag P.f., a highly sensitive RDT (HS-RDT) for the detection of *P. falciparum*. The RDT can detect the parasite within 20 minutes from capillary whole blood and has a greater than 10-fold higher sensitivity than current RDTs (www.alera.ecom/malaria-POC). The estimated lower limit of detection is anticipated to be similar to LAMP, though head-to-head comparisons have not been performed. In a subset of children, HS-RDT will be performed to compare the sensitivity/specificity to that of LAMP and microscopy (see section 6.8), and to determine the duration of time that the new RDT remains positive after treatment.

6.3 Classification of Uncomplicated Malaria

All episodes of malaria will be classified as uncomplicated or complicated based on the following criteria:

Uncomplicated malaria (all the following must be present)

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

Complicated malaria (any of the following)

1) Evidence of severe malaria (Appendix D) and parasitemia
2) Danger signs present and parasitemia
3) Parasite density > 500,000/µl

6.4 Management and Follow-up of Malaria

6.4.1 3-day AL regimen

Following diagnosis of malaria on Study Day 0, children managed with the 3-day AL regimen will be evaluated clinically on Study Days 1, 2, 3, (4*), 7 (8*), 10, 14, 21, 28, 35, 42 and on any unscheduled day in which the patient is brought to the clinic (Appendix A and B). Children who do not return for a scheduled visit will be visited by a study home visitor and, if necessary, transported to the study clinic. Blood will be obtained by finger prick on Study Days 0, 1, 2, 3, (4*), 7, (8*), 10, 14, 21, 28, 35, 42 and on any unscheduled day in which the patient is brought to the clinic for thick blood smears (for parasite density and gametocytes) and filter paper collection (see section 6.8).

6.4.2 5-day AL regimen

Following diagnosis of malaria on Study Day 0, children managed with the 5-day AL regimen will be evaluated clinically on Study Days 1, 2, 3, 4, 5 (6*), 7 (8*), 10, 14, 21, 28, 35, 42 and on any unscheduled day in which the patient is brought to the clinic (Appendix A and B). Children who do not return for a scheduled visit will be visited by a study home visitor and, if necessary, transported to the study clinic. Blood will be obtained by finger prick on Study Days 0, 1, 2, 3, 4, 5 (6*), 7, (8*), 10, 14, 21, 28, 35, 42, and on any unscheduled day in which the patient is brought to the clinic for thick blood smears (for parasite density and gametocytes) and filter paper collection (see section 6.8).

*participants enrolled in the intensive PK study have F/U visits on Days 4 and 8 (instead of Day 7) if receiving 3-day AL or on Days 6 and 8 if receiving 5-day AL.

6.4.3 Timing of doses

The time of AL and EFV administration will be noted whether at home or in the clinic. It is critical for PK studies that the time of AL and EFV administration and the time of all PK sample collections be recorded on the Case Report Forms.

All PK samples will be immediately placed on dry ice, processed for plasma separation, transferred to liquid nitrogen, and shipped at a later date on dry ice to our laboratory in San Francisco for analysis.

6.4.4 Unscheduled or After-Hours Visits

Blood will be obtained by finger prick on any unscheduled day, only when a fever is documented or reported in the previous 24 hours, for thick blood smears (for parasite density and gametocytes), filter paper collection, and potential additional studies.

Parents/guardians will be encouraged to visit TDH or MGH 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 from a hospital supply of AL and the doctors will be instructed to refer patients to our study clinic when it opens at 8 am the following day. If a patient is diagnosed with severe

malaria, he or she will receive quinine following standard TDH or MGH treatment guidelines. Patients with non-malarial illnesses will be managed at the discretion of the TDH or MGH staff. Study personnel will visit TDH or MGH daily to inquire about visits from study subjects and facilitate follow-up in the study clinic.

6.4.5 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.

6.4.6 Criteria and management of Early/Late Treatment Failure

Treatment outcomes will be based on 42-day treatment outcomes as per standard WHO classification system. Interim 28-day outcomes will also be assessed. The following criteria will be used for all participants participating in intensive and/or population PK studies. Patients treated for uncomplicated malaria but classified as early or late clinical failures within 14 days of treatment will be treated with quinine. Clinical failures between days 15-42 will be treated as a new episode of malaria.

Early Treatment Failure: Study Days 0, 1, 2, and 3

- Development of danger signs or severe malaria on study Days 0-3 in the presence of parasitemia
- Parasitemia on study Day 2 higher than on Day 0, irrespective of temperature
- Parasitemia on study Day 3 with temperature > 38.0º C (tympanic)
- Parasitemia on study Day 3 > 25% of count on Day 0

LPF (Late Parasitological Failure): Days 4 to 42

- Presence of parasitemia on Days 4-42 with temperature < 38.0 º C (tympanic) and no history of fever in past 24 hours, without previously meeting any of the criteria for early treatment failure

LCF (Late Clinical Failure): Days 4 to 42

- Development of danger signs or severe malaria Days 4 to 42 in the presence of parasitemia, without previously meeting any of the criteria of early treatment failure or late parasitological failure
- Temperature > 38.0 º C (tympanic) or history of fever in past 24 hours on Days 4 to 42 in the presence of parasitemia, without previously meeting any of the criteria of early treatment failure or late parasitological failure

Adequate Clinical and Parasitological Response

- Absence of parasitemia on study Day 42, with no prior requirement for additional treatment other than the standard 6 dose regimen received at time of presentation with malaria.

- For subjects who experience treatment failure before study Day 3, PK sampling will be discontinued.

Participants from TDH or MGH who experience recurrent malaria within 42 days will be evaluated for reenrollment eligibility (see Section 4.1 and 4.2). If eligible, they will be re-enrolled into the appropriate study. For those not eligible or not willing to reenroll, effort will be made to follow them for 7 days (or longer if they remain sick) after the diagnosis of recurrent malaria. In addition, the most recent laboratory and clinical tests will be checked to assess for any grade 3 or 4 abnormalities. If present, repeat testing should be done until AE is resolved.

In the case of participant withdrawal (unless a participant cannot be located or refuses follow-up), attempt will be made to follow them until all AEs are resolved to grade 2 or below, AND until Day 7 of follow-up for treatment of current malaria episode. Attempt will also be made to obtain the Day 14 hematology and chemistry blood work for safety follow-up.

6.5 Anthropometric measurements to assess nutritional status.

All children will have nutritional status characterized at enrollment based on assessment of WHO 2009 growth indicators by staff trained in the proper assessment of growth measurements. Specifically, measurements for height, weight, age and mid-upper-arm circumference (as a proxy for "weight for height, WFA") will be collected and recorded at the time of first enrollment (for the first PK assessment around an episode of uncomplicated malaria) and at the time of each subsequent episode of uncomplicated malaria for which children will also undergo PK.

6.6 Clinical Laboratory Studies

Blood will be collected on Study Day 0, 14, and 28 for CBC, differential, 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. HIV-infected children will have baseline CD4 and VL testing performed.

HIV-uninfected children will have HIV serostatus documented through rapid testing. 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 EFV-based ART to meet enrollment criteria.

6.7 Pharmacology Laboratory Studies

6.7.1 Intensive pharmacokinetic study

For children participating in the intensive PK sampling, Coartem® treatment will be on Study Days 0, 1, 2 and 3 (for the 6-dose regimen) or Study Days 0,1,2,3,4 and 5 (for the 10-dose regimen). Intensive PK samples for AL (artemether, DHA, and lumefantrine) will be collected on Study Day 3 or 5, around the 6th or 10th (i.e. last) treatment dose for all subjects enrolled in the study. AL administration of the 6th or 10th dose will be observed in the clinic, as well prior morning doses. In addition, two samples will be collected on Study Days 0, 1 and 2 (for those randomized to 3-day AL) or on Study Days 0, 1, 2, 3 and 4 (for those randomized to 5-day AL).

Sampling for artemether, dihydroartemisinin and lumefantrine.

Capillary samples (200 µL each*) will be collected by finger stick at approximately 2 and 4 hours post- each morning AL dose. A pre-1st dose sample will also be collected to confirm no residual antimalarial drug is detectable. On Study Day 1 (when a single dose is administered mid-day) capillary samples will also be collected at approximately 2 and 4 hours post- this dose.

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 6th or 10th dose (for the 3-day and 5-day AL regimens, respectively) for the determination of artemether, DHA, and lumefantrine concentrations in plasma (500 µL each*). Participants will remain in the clinic until completion of the 8-hour blood sampling on Study Day 3 or 5 (for the 3-day and 5-day AL regimens, respectively) and discharged home. They will then return to the clinic the next morning for the 24-hour sample.

To determine the correlation between capillary and venous artemisinin exposure, a simultaneous capillary sample (200 µL) will be collected at the time of the 2 and 8-hour samples.

Sampling for lumefantrine

Participants will be asked to return to the clinic on Study Days 8, 14, and 21 (v*enous or capillary* sampling, 200 µL each).

PK sampling will be discontinued in patients who do not meet laboratory eligibility criteria or do not complete treatment due to early failure. Subjects with repeat malaria infection during the 42-day follow-up will provide an additional PK sample and blood smear.

> *200 µL *capillary* samples will be collected by finger prick and can be used to measure all 3 analytes but without the ability to do repeat analysis if necessary. 500µL *venous* samples permits repeat analysis as needed.

6.7.2 Population pharmacokinetic study

For children participating in the population PK sampling, AL treatment will be 6 or 10 doses of Coartem® (provided by the study) administered over 3 or 5 days: Study Days 0, 1, and 2 or Study Days 0,1,2,3 and 4, *Capillary* samples for lumefantrine only (200 µL) will be collected on Study Day 0 (prior to AL administration). *Capillary* samples for artemether/DHA and lumefantrine (200 µL*) will be collected by finger prick on Study Days 0-2 (3 day AL) or 0-4 (5 day AL) at approximately 2 and 4 hours post-dose. In a subset of children, *capillary* samples for artemether/DHA and lumefantrine (200 µL*) will be collected by finger prick on Day 2 in the 3-Day regimen and on Day 4 in the 5-Day regimen, prior to AL administration. On Study Days 3 (3 day AL) or 5 (5 day AL) at approximately 24 hours post-last dose, an additional capillary sample will be taken. In addition, c*apillary or venous* samples will be collected for lumefantrine only on Study Days 7, 14, 21. Subjects with repeat infection during the 42-day follow-up will provide an additional PK sample at the time of repeat infection.

> *200 µL *capillary* samples will be collected by finger prick and can be used to measure all 3 analytes but without the ability to do repeat analysis if necessary. 500µL *venous* samples permits repeat analysis as needed.

6.7.3 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. Prior correlation studies between venous and capillary measurements for lumefantrine have confirmed the ratio of concentrations is 1:1. For the artemisininis, correlation studies will be carried out in the context of this protocol.

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.

6.7.4 Assays for artemether, DHA and lumefantrine

Artemether, DHA and lumefantrine and active desbutyl-lumefantrine will be measured using optimized methods validated in our laboratory. All methods will utilize liquid chromatography tandem mass spectrometry.

6.8 Parasitology Laboratory Studies

Thick blood smears. Parasite counts (asexual stage and gametocyte counts) via blood smears will be collected in all participants on each day of clinical follow-up. In addition, in a subset of population PK participants, more frequent thick blood smears will be collected to document the rate of parasite clearance, as we have done in prior studies.[94, 95]

Thick blood smears will be stained with 2% Giemsa and read by experienced laboratory technologists who are not involved in direct patient care. Parasite densities will be calculated by counting the number of asexual parasites per 200 leukocytes (or per 500 leukocytes, if the count is <10 asexual parasites/200 leukocytes), assuming a leukocyte count of 8,000/μL. A blood smear will be considered negative when the examination of 100 high power fields does not reveal asexual parasites. Gametocytemia will also be determined by thick blood smears. At varied time points, blood smears for the determination of parasitemia will be obtained by simultaneous venous and capillary blood draws. Parasitology studies will be performed in the laboratory of Dr. Sunil Parikh at Yale School of Public Health

LAMP will be done using a commercially available DNA amplification kit (such as the Loopamp Plasmodium Kit, Eiken, Japan). LAMP will be performed on dried blood spot specimens during each day of 42-day follow-up (See SOE). All LAMP will be run at our laboratory on the TDH clinic or Yale by those trained in LAMP.[96]

Rapid Diagnostic Test. The Alere™ Malaria Ag P.f., will be prepared and used per manufacturer's instructions (www.alera.ecom/malaria-POC). A drop of capillary whole blood obtained by finger prick will be placed onto the RDT well, along with control buffers, and read according product insert. The total time for preparation and resulting is estimated at 20 minutes.

Molecular studies. Parasite genotyping using polymorphic markers will be conducted to assess genetic diversity using methods routine in our laboratory to determine the multiplicity of infection (MOI).[50, 67] Six polymorphic markers (merozoite surface protein (MSP)-1, MSP-2, and 4 microsatellites) will be typed using capillary electrophoresis. For drug resistance genotyping, extracted DNA will be amplified by PCR, and following amplification, multiplex LDR will be conducted with bead-specific oligonucleotides, followed by hybridization to magnetic beads, and fluorometric assessment of mutation prevalence on a Bio-Plex system (Bio-Rad, Hercules, CA).[97] RNA-based studies will also be performed for more sensitive detection of asexual blood stages and gametocytes. RNA-based studies will allow for the most sensitive quantification of parasite and gametocyte clearance between study regimens, as well as early detection of recurrent parasitemia.

Metabolomics studies. Metabolomics assays will be conducted using plasma and/or RBC pellets. 20uL of plasma will be placed in an ice-cold methanol solution, vortexed, and stored in -80°C. De-identified metabolomics study samples will be shipped on dry ice to the lab of Darren Creek, an expert in malaria metabolomics, at Monash University for analysis.

6.9 Blood volumes

Intensive PK study

The total blood volumes collected from intensive PK study participants during the 42-day follow-up period for single episode of malaria will be no more than 28 mL (~6 teaspoons). (Section 11.1).

Population PK study

The total blood volumes collected from participants in the population PK study during the 42-day follow-up period for each malaria episode will be approximately 21 mL (~4-5 teaspoons). (Section 11.1)

RNA and Metabolomic studies will not be performed in those under 10kg to assure blood volumes are not excessive in this weight range.

6.10 Electrocardiogram (ECG) monitoring

As noted in section 7.2, AL has an excellent safety profile. It was first registered as Coartem® in 1998, and is used in over 90 countries worldwide, including the U.S. In 2016, the WHO convened an Evidence Review Group (ERG) to assess the cardiac safety of antimalarials, with particular focus on the QT interval.[98] Of note, members of th 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, no cases of sudden or unexplained cardiac death have been reported with AL up to the time of the ERG. Despite *in vitro* evidence of inhibition of hERG currents, which are associated with QT interval prolongation, no QTc interval prolongation was seen *in vivo* in dogs with AL, except at doses of 600mg/day, far exceeding the doses being administered in this study. In a thorough QT study, an increase of >10ms was excluded. In clinical trials, no pediatric patients aged <12 years had a post-baseline QTcF interval of >500ms, while 0.2% of the adult patients reported an interval of >500ms. In conclusion, the committee stated that "The small increase in QTc interval associated with AL does not appear to be associated with a significant risk of arrhythmia. The small number of adverse events affecting the cardiovascular system were almost all of mild intensity and resolved without intervention."

In order to confirm the expected safety of an extended dosing regimen of AL, we will conduct ECGs in a sub-set of up to 50 children in each arm (3-Day and 5-Day) prior to the administration of the first dose of Coartem®, at the time of expected Cmax (maximum concentration), and at 2 time-points following recovery from malaria. ECGs will be performed in triplicate, with the average QTc (central tendency) used for analyses. Based on prior data from our group, the time of expected maximum concentration is 4-6 hours following the last dose of AL. ECGs are feasible at this time in the intensive study. However, due to the last dose occurring in the evening of day 2 or 4 in population PK participants, ECGs will only be performed on the morning of day 3 or 5 in the population PK arm, corresponding to an estimated 8-14 hours post-last dose.

Specifically, ECGs will be performed in up to 50 participants in each arm (3-Day and 5-Day) at the following time points:

- **Intensive PK study**
  - 3- day regimen
    - Day 0 – baseline, prior to the 1st dose of AL
    - Day 3 – prior to the last dose, and 4 to 6 hours after the last dose
    - Day 8
    - Day 28 or later
  - 5- day regimen
    - Day 0 – baseline, prior to the 1st dose of AL
    - Day 5 – prior to the last dose, and 4 to 6 hours after the last dose
    - Day 8
    - Day 28 or later
- **Population PK study**
  - 3- day regimen
    - Day 0 – baseline, prior to the 1st dose of AL
    - Day 3 – 8-12 hours after the last dose
    - Day 7
    - Day 28 or later
  - 5- day regimen
    - Day 0 – baseline, prior to the 1st dose of AL
    - Day 5 – 8-12 hours after the last dose
    - Day 7
    - Day 28 or later

7.0 TOXICITY MANAGEMENT

7.1 Antiretroviral toxicity

For HIV-infected children enrolled, ART management for HIV will be managed through the subject's primary clinic. This study is not designed to initiate ART treatment for study subjects. ART regimens permitted for this study include only EFV-based regimens.

7.2 Artemether-lumefantrine toxicity

AL will be used to manage acute uncomplicated malaria. AL is the Ugandan first-line national regimen and would be the preferred treatment for all patients whether enrolled in this study or not. AL as a 3-day regimen is standard of care while AL as a 5-day regimen is being evaluated in this study and is a "study regimen". Therefore, we will be assessing and recording data in relation to how participants tolerate AL. As noted in Section 1.1.8, we evaluated the impact of a cytochrome P450-inhibitor, lopinavir/ritonavir, on levels of lumefantrine in children with malaria.[67] Day 7 levels in children on lopinavir/ritonavir-based ART had 10-fold higher day 7 levels of lumefantrine and >2-fold higher AUC, as compared to those on EFV-based ART, while exhibiting no increase in toxicity.[17, 67]. We will, however, be recording participants tolerance of AL

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 AL and will be encouraged to present to the study clinic for all potential adverse events. Symptomatic screening for auditory AEs will be conducted at each visit. The study clinic will remain open 7 days a week so that participants may present with potential adverse events as they arise. Monitoring, reporting, and management of adverse events is described below, and will follow guidelines set forth by each respective institution.

7.3 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 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 AL pending clinic visit or repeat laboratory tests. The clinician has the option of immediately stopping the AL 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 AL 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 AL 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 AL is discontinued the participant will be withdrawn from the study.

Grade 4 life threatening:

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

8.0 MONITORING OF ADVERSE EVENTS AND MANAGEMENT

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". There are no "study drugs" in this study, and all therapies being studied for PK are the standard of care in Uganda and provided free of charge by the Ministry of Health outside of study participation. This study will be directly observing administration of AL (Coartem®) to participants for the treatment of malaria. Data regarding the tolerance of AL 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. Guidelines for reporting of adverse events due to study participation provided by UCSF Human Research Protection Program and Institutional Review Board, Yale Human Investigations Committee, NICHD, Makerere University School of Medicine Research Ethics Committee (SOMREC), and the Ugandan National Council for Science and Technology will be followed as described below.

Per UCSF HRPP & IRB 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 & IRB 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 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 SOMREC.

Table 7. 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
MU-SOMREC	• 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
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

9.0 STATISTICAL CONSIDERATIONS

9.1 General Design Issues.

This is a prospective study to evaluate the PK and PD of extended dose AL in the context of EFV-based ART and in the context of HIV-uninfected children and to characterize the impact of a 5-day regimen on AL PK/PD as compared to the standard 3-day regimen; the study will be conducted in Uganda.

The current proposal will study the PK/PD of AL in HIV-infected children on EFV-based ART and HIV-uninfected children not on ART either enrolled at TDH or MGH. We will compare the PK of 5-day AL in HIV-infected children on EFV-based ART to 1) 3-day AL in HIV-infected children on EFV-based ART, and 2) 3-day AL in HIV-uninfected children. In addition, we will compare the PK of 5-day AL in HIV-uninfected children to 3-day AL in HIV-uninfected children. Comparisons will be based on an intensive PK design for AL AUC estimations in 30 to 50 subjects for each of the study groups. Study group comparisons will be controlled for age (i.e. results for HIV infected children on EFV-based ART who are 3-10 years of age, will be compared to results for HIV uninfected children who are also 3-10 years of age). The proposal will also study the impact of AL exposure on treatment outcomes and adverse events using a population PK/PD design. This will involve population PK study of up to an additional 100 HIV-infected children and 120 HIV-uninfected children.

Although an individual child is eligible to enroll in both the intensive PK study and the population PK study for separate episodes of malaria, 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 380.

9.2 Outcome Measures

9.2.1 Primary outcomes

1) Area under the plasma concentration versus time curve for all drug analytes.
2) Recurrent malaria following treatment by day 42 (recrudescence or new infection)

9.2.2 Secondary outcomes

1) Safety of 5-day vs 3-day AL regimens
2) Prevalence of gametocytemia following treatment in 3-day vs 5-day AL regimens
3) Weight-for-age and Height-for-age associations with PK
4) Diagnostic sensitivity of LAMP, HS-RDT, and microscopy for the detection of recurrent parasitemia.
5) Metabolomic measurements in HIV infected vs HIV uninfected children
6) Relationship between molecular markers of drug resistance and treatment failure

9.3 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)

9.4 Intensive PK study

9.4.1 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 (3-day AL vs 5-day AL) with n=30 for each group of HIV infected children. To assure adequate age-matched HIV uninfected children, n=50 is used for the group of HIV uninfected children. Figure 1 and Table 8. 50 HIV-uninfected children provides for adequate subjects <3 years and ≥3 years of age as HIV-infected children receiving EFV-based ART is restricted to ≥ 3 years of age. In addition, the same size of 50 will permit PK/PD of the extended regimen in younger children <3 years of age. At least 30 HIV uninfected children must be between 3-10 years of age.

For comparisons where n=30 in both groups, there will be 80% power to detect a difference in AUC of 35% or more, 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 the HIV-uninfected children with n=50, further distinctions based on age can be determined as well as further enhancement of exposure-response analysis.

Although many children are likely to be studied for both the 3-day and 5-day regimens, it is not likely that *all* children will provide results for both. However, all analyses will account for repeated measures/correlated data that occur from participants enrolled into multiple arms.

9.4.2 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 artemether/DHA and the sum of AUC_{last} (AUC to the

end of the sampling period) and $AUC_{last-\infty}$ (from the end of sampling to infinity) for lumefantrine/ desbutyl-lumefantrine. PK parameters will be compared between groups of interest (Figure 1 and Table 8) 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. To enhance comparison between 3-day and 5-day AL, peak concentrations collected on Days 0-4 will be integrated using population methods. The following groups will be compared:

Table 8. Primary objectives and comparator groups for intensive PK study

Primary Objectives	Comparison Groups
Impact of 5-day vs 3-day AL on AL exposure in HIV-infected children on EFV-based ART	HIV-infected children on EFV-based ART 3-day AL (n=30), vs. HIV-infected children on EFV-based ART 5-day AL(n=30).
Impact on AL exposure of 5-day AL in HIV-infected children on EFV-based ART vs 3-day AL in HIV-uninfected children	HIV-infected children on EFV-based ART 3-day AL (n=30), Vs. HIV-uninfected children 3-day AL (n=30, age 3-18years).
Impact of 5-day vs 3-day AL on AL exposure in HIV-uninfected children	HIV-uninfected children 5-day AL (n=50), vs. HIV-uninfected children 3-day AL (n=50).

9.5 Population PK study

9.5.1 Sample Size

160 episodes of malaria in HIV-infected children and 220 episodes of malaria in HIV-uninfected children (up to 4 episodes per child including up to 2 involving intensive sampling) are more than sufficient to obtain population PK parameter estimates for both groups of children with good precision. Outcomes will be assessed in children in both the intensive and population PK studies, as such, sample sizes reflect the sum of children enrolled in each arm for each type of PK sampling. Thus, total sample sizes will be 80 to 110 children per arm. Using WinPOPT®, the standard errors (SE) of all population PK parameters, including variability parameters, will be <20% of their means, except for the interindividual variability of ka, for which the SE is estimated to be 32% of its mean. Based on our recent study from Tororo, where a 42-day recurrence rate of 47% was observed in those on EFV-based ART, we will have 80% power to detect a 20% difference in recurrence rates between 3-day and 5-day arms.[67] Notably, in our previous exposure-outcomes, with 50 to 70 children in each ART arm and 184 children not on ART, a 2.1-fold change in AUC between those on EFV and lopinavir/ritonavir translated to a 2.7-fold increase in risk for reinfection. In other words, the anticipated increase in AUC between 5-day and 3-day AL of 1.7-fold increase in exposure is expected to translate to a clinically relevant change in risk in a similar number of subjects.

9.5.2 Population PK Analysis and Correlation of AL PK with treatment outcomes.

Population PK analysis will utilize both intensive and sparse PK sampling data for both HIV infected and HIV uninfected children. Plasma concentrations will be transformed

into natural logarithms and concentration-time profiles will be analyzed using non-linear mixed effects (NLME). For NLME modeling, PK data will be analyzed using a standard PK model formalism. To analyze population data the PK model is embedded in a two-stage NLME model, accounting for both intra- and inter-subject variability.[99, 100] The estimation of NLME parameters will be accomplished using the software NONMEM (version 7; Icon Development Solutions, Maryland), in particular the first order conditional estimation method with interaction that uses a linear approximation to the likelihood of the data. Diagnostic graphics and post-processing of NONMEM output and simulations will be performed using the statistical software R. Model development will be guided by exploratory analysis of the data, changes in the NONMEM objective function value, and diagnostic plots. Our recent lumefantrine modeling suggest a two- and three-compartment model provide the best fit, respectively, though additional models will be tested[101] Alternative models for intra-subject variability (proportional and constant plus proportional) will be explored as appropriate. To reinforce the primary objectives, base model building will also include quantification and formal test of the impact of EFV on PK of AL. Specifically, the impact of HIV treatment will be tested as potential covariate on clearance and/or bioavailability. Further stepwise covariate analysis will be performed to identify the impact of covariates on the PK of AL 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, parasitemia, hematocrit, and CD4 count (for HIV-infected children). Both linear and nonlinear relationship between continuous covariates and model parameters will be investigated in a stepwise fashion. Subject-specific estimates of artemether, DHA, lumefantrine, and desbutyl-LR exposure will be obtained from individual PK analyses for subjects with intensive PK sampling (AUC) and a posteriori from population PK analysis (as given by dose/(CL/F)). These estimates will be used to test for a significant relationship between drug exposure and the primary treatment outcome (recurrent infection at day 28-42 using standard WHO treatment outcomes[3]) using Cox proportional hazard models with robust sandwich estimators that account for correlated data, as individuals may be enrolled for multiple episodes of malaria. A secondary outcome will be sub-patent infections detectable by LAMP, which will also be analyzed for associations with PK exposure using Cox models. Simultaneous PK/PD analysis will be performed to maximize the entire database. As for PK, we will investigate if there is any potential impact of indices of malnutrition, HIV status, age and other covariates on PD parameters. In other words, we will identify if target concentrations to prevent reinfection might be altered in different patient population due to known covariates. Once targets are identified, population PK models will be used to simulate different AL PK dosing approaches to identify optimized regimens that ensure concentrations are maintained within the concentration range necessary to maximize efficacy in all studied populations.

10.0 DATA COLLECTION AND MONITORING

10.1 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 Tororo web-site becomes temporary unavailable.

10.2 Data Quality Assurance and Monitoring

In order to insure data quality, the study Data Manager will perform a quarterly data quality audit. For this audit a 1% random sample of study forms entered into the data management system from the previous 2 weeks will be selected and compared for accuracy with the original case-report forms and source documents. In addition, the study 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.

11.0 HUMAN SUBJECTS

11.1 Risks and Benefits

There are minimal direct benefits to the study subject for participation in the study. Free medical treatment for malaria is provided in Uganda. In Uganda, typical treatment for malaria involves the provision of medications, but no set schedule of follow-up after treatment. In our study, we will provide an increased level of care, primarily through enhanced follow-up of participants. However, we will not list this as a benefit on our informed consent documents. The risks have been reduced to a minimum. Participants will already be requiring AL for their malaria therapy. For children, we have consciously considered the guidelines set forth by the UCSF HRPP & IRB 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 even children as small as 5 kg, the amount of blood that will be drawn in a 24 hour

period will not exceed 1.2 mL/kg. For the total amount collected over 42 days of follow-up the amount of blood that will be drawn will not exceed 5.3 mL/kg, which are well within these limits.

There is small chance of infection from performing blood draws. Additional risks include anxiety from HIV testing and learning of testing results. Participants will be counseled regarding test results and referred for medical care if necessary.

11.2 Treatment and Compensation for Injury

If the participant is injured as a result of being in this study, treatment will be available through TDH and MGH. Makerere University, UCSF, Yale, and NICHD do not normally provide any other form of compensation for injury.

11.3 Costs to the Subjects

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

11.4 Reimbursement of Subjects

Participants will not be paid for their participation in the study. We will provide all routine medical care, including evaluations, medications available in our clinic, and cost of any transportation free of charge. 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 TDH or MGH using available funds when available. However, reimbursement of all diagnostic tests and treatment recommended outside the study clinic cannot be guaranteed in all circumstances.

11.5 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 Human Research Protection Program & Institutional Review Board (HRPP & IRB), the Yale Human Investigations Committee (HIC), the Makerere University School of Medicine Research Ethics Committee (SOMREC), and the Uganda National Council of Science and Technology (UNCST).

All consent forms will be translated into the local language (Jopadhola, Teso, Swahili, Luganda, Samia, and English) and back-translated into English to ensure correct use of language. Consent forms will be read aloud to parents by trained study interviewers. The informed consent will describe the purpose of the study, all the procedures involved, and the risks and benefits of participation. 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.

11.6 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

11.7 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 Makerere University College of Health Sciences, and the NIH in 2006.

12.0 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, and Makerere University guidelines.

13.0 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: INTENSIVE PK STUDY

	INTENSIVE PK STUDY														
Study Day	**0[1]**	**1**	**2**	**3**	**4**	**5[11]**	**6[11]**	**8[9]**	**10[15]**	**14**	**21**	**28**	**35**	**42**	**Any other day[10]**
ADMINISTRATIVE AND REGULATORY PROCEDURES															
Screening and study participation informed consent	X														
HIV counseling and referral if necessary[2]	X														
Collect locator information	X														
CLINICAL AND LABORATORY EVALUATIONS															
History[3]	X	X	X	X	X	X[11]	X[11]	X		X	X	X	X	X	X
Physical Examination[4]	X	X	X	X	X	X[11]	X[11]	X		X	X	X	X	X	X
HIV testing and documentation[2]	X														
Hematology and Chemistries[5]	4mL									4mL		4mL			
Rapid Diagnostic Test (RDT)	X							X		X	X	X	X	X	X
Thick Blood Smear[13] and Filter Paper collection (finger stick) for LAMP	X	X	X	X	X	X[11]	X[11]	X	X	X	X	X	X	X	X
Thin Blood Smear[6]	X														X
Electrocardiogram (ECG)[17]	X			X		X		X				X			
DRUG ADMINISTRATION															
AL dosing for all study subjects[7]	X	X	X	X	X[11]	X[11]									
Antiretroviral dosing for HIV-infected subjects[8]	X	X	X	X	X	X	X	X		X					
INTENSIVE PHARMACOKINETIC STUDY SAMPLING (VENOUS OR CAPILLARY DRAWS AS PER SECTION 6.7)															
3-day AL regimen Artemether and Lumefantrine	200µL (pre-AL) 2 and 4 hrs post-dose	200µL 2 and 4 hrs post-dose	200µL 2 and 4 hrs post-dose	500 µL (0, 0.5, 1, 2[12], 3, 4, 6 and 8[12] hrs) (additional 200 µL at 2 and 8 hrs)	200µL (24 hrs)			200µL (120 hrs)		200µL (288 hrs)	200µL (456 hrs)				200µL

5-day AL regimen Artemether and Lumefantrine[11]	200µL (pre-AL) 2 and 4 hrs post-dose	200µL 2 and 4 hrs post-dose	200µL 2 and 4 hrs post-dose	200µL 2 and 4 hrs post-dose	200µL 2 and 4 hrs post-dose	500 µL (0, 0.5, 1, 2, 3, 4, 6 and 8 hrs)	200µL (24 hrs)	200µL (120 hrs)		200µL (288 hrs)	200µL (456 hrs)				200µL
Blood for metabolomics and molecular studies	1mL			X[14]				X[14]	X[15]	1mL	X[14]	1mL	X[14]	1mL	1mL
APPROXIMATE TOTAL BLOOD VOLUME (mL)[16]	5.6	0.6	0.6	0.6-4.8	0.4-0.6	0.2-4.2	0.4	0.6	0.3	5.4	0.6	5.2	0.4	1.2	1.4

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

1 As is standard practice, study Day 0 stipulates the time of diagnosis of uncomplicated malaria and initiation of AL treatment.

2 Rapid HIV test will be performed to determine eligibility to enroll as either HIV infected or HIV uninfected participant, although children must be stabilized on EFV-based ART for 10 days prior to enrollment. Positive HIV test results to be confirmed by Western Blot or HIV RNA after enrollment.

3 History will include general medical history, diagnosis, medication (including ingestion of herbs), allergies, and current symptoms.

4 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 for each episode of malaria.

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 A thin smear is performed to assist in species identification.

7 Dosing of AL for all subjects undergoing intensive PK sampling will be scheduled to assure the last dose of a six or 10 dose regimen is given in the morning (Day 3 or 5).

8 On stable ART as per clinical management in TDH or MGH or other referral clinic; ART dosing permitted at home, but time of administration is recorded

9 For subjects participating in intensive PK evaluations, sampling for the 120 PK sample will occur on Day 8 since these subjects receive the 6th or 10th AL dose on Day 3 or 5.

10 Patients presenting on an unscheduled day for a sick visit will undergo sampling for lumefantrine PK if found to have a positive malaria thick

11 Specifically, for children receiving 5-day AL

12 At 2 and 8 hours an additional 200µL will be drawn via a capillary stick to permit correlation of venous and capillary concentrations for the artemisinins.

13 At approximately 0, 24, 48, and 72, 84 and 96 hours post the first dose of AL or until blood smears are consistently negative. In a subset of participants, additional thick blood smears will be done on the days of AL doses to assess parasite clearance.

14 Additional 200uL capillary samples for RNA studies will be done on these days. RNA studies will not be done on participants under 10kg.

15	Day 10 visit can be done in a subset of participants in either the clinic or by home visitor for collection of approximately 250uL for RNA and filter paper. Sampling will note will not be done on participants under 10kg.
16	Total blood volume in either arm is between 5-6 teaspoons over 42-days of follow-up for scheduled visits
17	Perform ECG on subgroup of participants. ECGs will be performed at baseline (before the administration of first dose), prior to and 4-6 hours post-last dose, Day 8, and Day 28. ECG will be performed on Day 3 for 3-day regimen and on Day 5 for 5-day regimen.

APPENDIX B. SCHEDULE OF EVALUATIONS: POPULATION PK STUDY

		POPULATION PK STUDY													
Study Day		**0[1]**	**1**	**2**	**3**	**4**	**5**	**7[9]**	**10[14]**	**14**	**21**	**28**	**35**	**42**	**Any other day[10]**
ADMINISTRATIVE AND REGULATORY PROCEDURES															
Screening and study participation informed consent		X													
HIV counseling and referral if necessary[2]		X													
Collect locator information		X													
CLINICAL AND LABORATORY EVALUATIONS															
History[3]		X	X	X	X	X	X	X		X	X	X	X	X	X
Physical Examination[4]		X	X	X	X	X	X	X		X	X	X	X	X	X
HIV testing and documentation[2]		X													
Hematology and Chemistries[5]		4mL								4mL		4mL			
Rapid Diagnostic Test (RDT)		X						X		X	X	X	X	X	X
Thick Blood Smear[12] and Filter Paper collection (finger stick) for LAMP		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Thin Blood Smear[6]		X													X
Electrocardiogram (ECG)[16]		X			X		X	X				X			
DRUG ADMINISTRATION															
AL dosing for all study subjects[7]		X	X	X	X[11]	X[11]									
Antiretroviral dosing for HIV- infected subjects[8]		X	X	X	X	X	X	X		X					
INTENSIVE PHARMACOKINETIC STUDY SAMPLING (VENOUS OR CAPILLARY DRAWS AS PER SECTION 6.7)															
3-day AL regimen Artemether and Lumefantrine		200µL (pre-AL, 2 and 4 hrs post-dose)	200µL 2 and 4 hrs post-dose	200µL Pre-dose[17], 2 and 4 hrs post-dose	200µL (24 hrs)			200µL (120 hrs)		200µL (288 hrs)	200µL (456 hrs)				200µL
5-day AL regimen Artemether and Lumefantrine[11]		200µL (pre-AL, 2 and 4 hrs post-dose)	200µL 2 and 4 hrs post-dose	200µL 2 and 4 hrs post-dose	200µL[11] 2 and 4 hrs post-dose	200µL[11] Pre-dose[17], 2 and 4 hrs post-dose	200µL[11] (24 hrs)	200µL (120 hrs)		200µL (288 hrs)	200µL (456 hrs)				200µL
Blood for metabolomics and molecular studies		1mL			X[13]			X[13]	X[14]	1mL	X[13]	1mL	X[13]	1mL	1mL
APPROXIMATE TOTAL BLOOD VOLUME (mL)[15]		5.8	0.6	0.8	0.4-0.8	0.2-0.6	0.4	0.6	0.3	5.4	0.6	5.2	0.4	1.2	1.4

Explanation of Population PK schedule of events (Appendix B):

1 As is standard practice, study Day 0 stipulates the time of diagnosis of uncomplicated malaria and initiation of AL treatment.
2 Rapid HIV test will be performed to determine eligibility to enroll as either HIV infected or HIV uninfected participant, although children must be stabilized on EFV-based ART for 10 days prior to enrollment. Positive HIV test results to be confirmed by Western Blot or HIV RNA after enrollment.
3 History will include general medical history, diagnosis, medication (including ingestion of herbs), allergies, and current symptoms.
4 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 for each episode of malaria.
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 A thin smear is performed to assist in species identification.
7 Dosing of AL for all subjects will not be adjusted as done for intensive study so the last dose will be occur in the evening of Day 2 (3 day AL) or 4 (5 day AL)..
8 On stable ART as per clinical management in TDH or MGH or other referral clinic; ART dosing permitted at home, but time of administration is recorded
9 For subjects participating in population PK evaluations, sampling for the 120 PK sample will occur on Day 7 since these subjects will not have adjustments in their dosing
10 Patients presenting on an unscheduled day for a sick visit will undergo sampling for lumefantrine PK if found to have a positive malaria thick
11 Specifically, for children receiving 5-day AL
12 At approximately 0, 24, 48, and 72, 84 and 96 hours post the first dose of AL or until blood smears are consistently negative. In a subset of participants, additional thick blood smears will be done on the days of AL doses to assess parasite clearance.
13 Additional 200uL capillary samples for RNA studies will be done on these days. RNA studies will not be done on participants under 10kg.
14 Day 10 visit can be done in a subset of participants in either the clinic or by home visitor for collection of approximately 250uL for RNA and filter paper. Sampling will note will not be done on participants under 10kg.
15 Total blood volume in either arm is between 4-5 teaspoons over 42-days of follow-up for scheduled visits

16 Perform ECG on subgroup of participants. ECGs will be performed at baseline (before the administration of first dose), the next morning following the last dose, Day 7, and Day 28. ECG will be performed on Day 3 for 3-day regimen and on Day 5 for 5-day regimen.

17 A 200ul capillary draw will be done pre-dose on Day 2 in the 3-Day regimen and on Day 4 in the 5-Day regimen, in a subset of children, to look at AL drug level. This will inform the study team of drug adherence.

APPENDIX C. INFORMATION SHEET

Information Sheet

A study on Antimalarial Pharmacology in HIV infected and uninfected

Makerere University, University of California, San Francisco, Yale University, and Tororo District Hospital are carrying out a study on artemether-lumefantrine (*Coartem*) uptake by the body and response to this malaria treatment in HIV-infected (receiving anti-HIV medications) and HIV-uninfected children.

- Our clinics are located at Tororo District Hospital and Masafu General Hospital
- We are comparing groups receiving *Coartem*, currently recommended uncomplicated malaria treatment by the Uganda Ministry of Health
- We want the following to participate
    - HIV-infected (HIV+) children aged 3 to 10 years
    - HIV-uninfected (HIV-) children aged 6 months to 10 years
- Participants in this malaria 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 clinic is open every day from Monday to Sunday, 8am to 5pm

For more information, come to Tororo District Hospital or Masafu General Hospital and ask for the malaria children's clinic. The doctors will be happy to talk with you.

APPENDIX D. 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 E. WHO MALARIA TREATMENT OUTCOME

ETF (Early Treatment Failure): Days 0, 1, 2, and 3

- Development of danger signs or severe malaria on Days 0-3 in the presence of parasitemia
- Parasitemia on Day 2 higher than on Day 0, irrespective of temperature
- Parasitemia on Day 3 with temperature > 38.0 C (tympanic)
- Parasitemia on Day 3 > 25% of count on Day 0

LPF (Late Parasitological Failure): Days 4 to 42

- Presence of parasitemia on Days 4-42 with temperature < 38.0 C (tympanic) and no history of fever in past 24 hours, without previously meeting any of the criteria for early treatment failure

LCF (Late Clinical Failure): Days 4 To 42

- Development of danger signs or severe malaria Days 4 to 42 in the presence of parasitemia, without previously meeting any of the criteria of early treatment failure or late parasitological failure
- Temperature > 38.0 C (tympanic) or history of fever in past 24 hours on Days 4 to 28 in the presence of parasitemia, without previously meeting any of the criteria of early treatment failure or late parasitological failure

ACPR (Adequate Clinical and Parasitological Response)

- Absence of parasitemia on Day 42, irrespective of temperature, without previously meeting any of the criteria of early treatment failure, late clinical failure, or late parasitological failure

CLASSIFICATION SYSTEM

APPENDIX F. 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 G. HIV-INFECTED CHILDREN CONSENT

APPENDIX H. HIV-UNINFECTED CHILDREN CONSENT

APPENDIX I. ASSENT

APPENDIX J. FUTURE USE OF BIOLOGICAL SPECIMENS CONSENT