Title
Optimization of Malaria Treatment Strategies: A Pharmacokinetic and Pharmacodynamic Analysis of Artemether-Lumefantrine in the Context of Pediatrics and HIV

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Optimization of Malaria Treatment Strategies:
A Pharmacokinetic and Pharmacodynamic Analysis of Artemether-Lumefantrine in the Context of Pediatrics and HIV Infection

by

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DISSERTATION

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Contributions

A chapter in this dissertation contains material that is currently under consideration/review. It does not necessarily represent the final published form and has been edited slightly.

Chapter 2 of this dissertation is a reprint of material currently in review for publication: Whalen ME, Kajubi R, Goodwin J, Orukan F, Colt M, Huang L, Richards K, Wang K, Li F, Mwebaza N, Aweeka FT, Parikh S. “The impact of extended treatment with artemether-lumefantrine on antimalarial exposure and reinfection risks in Ugandan children with uncomplicated malaria: A randomized controlled trial.” M.E.W, J.G, L.H, K. W, F.T.A, and S.P wrote the manuscript; M.E.W, R.K, L.H, N.M, F.T.A, and S.P designed the research; M.E.W, R.K, J.G, F.O, M.C, L.H, N.M, and K.R performed the research; M.E.W, J.G, M.C, K.W, F.L, F.T.A, S.P analyzed the data; L.H contributed new reagents/analytical tools.

Optimization of Malaria Treatment Strategies: A Pharmacokinetic and Pharmacodynamic Analysis of Artemether-Lumefantrine in the Context of Pediatrics and HIV

Meghan Whalen

Abstract

Malaria remains a leading cause of morbidity and mortality worldwide, with young children at particular risk for severe, or complicated disease. The artemisinin-based combination therapies (ACTs) are the first-line treatment for uncomplicated (i.e., non-severe) malaria, but optimal dosing regimens are yet unclear for the most vulnerable populations, such as young children and those with potential drug-drug interactions for treatment of co-morbidities, like HIV. Suboptimal dosing not only diminishes clinical responses, but also exposes parasites to subtherapeutic levels of antimalarials, which may select for resistant parasites. For example, resistance to artemether-lumefantrine (AL), a commonly prescribed ACT, is an emerging problem in Africa. Thus, the goal of this dissertation work was to identify ways to optimize dosing in these populations in order to protect the utility of ACTs.

The specific aims of this work were to evaluate the pharmacologic and clinical benefits of extending AL malaria treatment duration from the standard 3-day (6 dose) regimen to a 5-day regimen (10 dose) in young HIV-uninfected children (Aim 1, Chapter 2) and HIV-infected children on efavirenz (EFV)-based antiretroviral therapy (ART) (Aim 2, Chapter 3). As EFV has potent metabolic inductive effects shown to result in lower AL exposure, in Aim 2 an extended regimen was evaluated as an attempt to compensate for this drug-drug interaction. The overall goal of the study was to determine the impact of extended duration treatment on the

pharmacokinetics (PK) and pharmacodynamics (PD) of AL. Samples collected as part of a prospective, randomized, open-label phase 4 trial of uncomplicated malaria pediatric patients in Uganda with and without HIV co-infection were used to quantify (1) artemether, (2) active metabolite, dihydroartemisinin (DHA), (3) lumefantrine (LF), and (4) active metabolite, desbutyl-lumefantrine (DBL) plasma concentrations. Children received malaria treatment with standard 3-day (6-dose) or extended 5-day (10-dose) AL-based therapy. A combination of intensive and population PK sampling approaches was utilized to determine drug exposure. PK estimates were then linked to clinical outcomes (e.g., recurrent parasitemia) and assessed over a 42-day follow-up period for the PD analyses.

Overall, our analyses demonstrated that extended (5-day) duration of AL-based antimalarial therapy significantly increased AL and AL-metabolite exposure in HIV-uninfected children. These findings included statistically significant higher exposure of the artemisinins and long acting LF and DBL with extended 5-day therapy. In particular, higher LF concentrations were associated with a 46% and 42% lower risk of recurrent parasitemia at 28 and 42 days, respectively. In addition, weight-based associations were observed for LF among HIV-uninfected children; participants in the lowest weight-band had the lowest AL plasma concentrations. Among HIV-infected children, 5 days of AL successfully compensated for the EFV-driven cytochrome P450 (CYP) 3A4 induction effect and resulted in LF exposure that was equivalent to that seen in HIV-uninfected children given 3 days of AL.

Collectively, this dissertation contributes to the limited PK/PD data for AL-based treatment regimens for uncomplicated malaria in children. Here, we studied an underrepresented, pediatric population to highlight the necessity for optimized dosing regimens (for example, among HIV-coinfected and low weight children). Given that this population makes up the

majority of malaria-related morbidity and death, efforts to optimize AL-based treatment regimen dosing in these patients are likely to lead to improved malaria clinical outcomes worldwide.

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Chapter 1: Introduction

Malaria remains a major cause of morbidity and mortality in sub-Saharan Africa, particularly among young children. Despite progress in the past to reduce malaria cases and deaths (e.g. development of more sensitive malaria diagnostic testing, better protective measures with indoor residual spraying and insecticide treated bed nets, and advancements in malaria drug therapy), malaria-related morbidity and mortality are increasing, especially in Africa. The 2021 World Malaria Report estimated that there were, globally, 242 million cases of malaria in 2020, up from 227 million cases in 2019, and 224 million cases in 2015 [1]. Importantly, 95% of these cases occurred in Africa [1]. There were also an estimated 627,000 malaria-related deaths, an increase from 558,000 in 2019 and 562,000 in 2015, with >90% of those deaths occurring in Sub-Saharan Africa [1]. The highest mortality risk group consists of young children, especially those under 5 years of age. Antimalarial dosing for children requires specific recommendations due to physiological changes as they age and the potential for co-treatments for HIV, or other conditions. This is particularly true in Uganda, which has one of the highest malaria transmission intensities in the world and, as of 2020, had ~98,000 children (aged 0-14 years) living with HIV [1-4].

Rapid and effective treatment of malaria is critical in reducing malaria-related morbidity and mortality. Malaria is a disease caused by *Plasmodium* parasites that are transmitted to humans through the bite of a female *Anopheles* mosquito. *Plasmodium falciparum* is both the most common malaria-causing parasite in Africa and the deadliest [1, 5]. Once parasites enter the human body, they travel to the liver where they multiply asexually [6]. The parasites, now in the form of merozoites, enter the bloodstream, invade erythrocytes, and replicate asexually [6]. When the erythrocytes rupture, the parasites are released, invade new red blood cells, and

multiply, a cycle which continues every 48-hours [6, 7]. The rupturing of the red blood cells results in the clinical signs and symptoms associated with uncomplicated malaria: headaches, fevers, chills, muscle aches, nausea, and vomiting [6, 8]. The disease can progress to severe malaria which can result in impaired consciousness, severe anemia, end organ failure, cerebral malaria, or death, thus rapid and effective treatment of malaria is critical [8].

Due to widespread global resistance to older malaria drugs, artemisinin-based combination therapy is now first line. Artemisinin-based combination therapies (ACTs), including artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP), are first-line treatment for uncomplicated *Plasmodium falciparum* malaria due to widespread resistance to older antimalarial medications [8]. The artemisinins rapidly reduce parasite load while the long-acting partner drugs eliminate residual parasites and protect against artemisinin resistance and recurrent infection. In 2020, an estimated 243 million courses of ACTs were used for malaria treatment, with AL being the most widely used [1]. However, ACT efficacy is threatened if specific dosing guidelines for children, the most treated population, are not improved.

Young children make up the majority of worldwide malaria, but proper pediatric dose adjustment of ACTs is not clear. Guidelines have historically been based on pharmacokinetic (PK) and pharmacodynamic (PD) studies in adults. Although studies in children are increasing in frequency, they are hindered by PK design, sample size, variability in childhood age, and analytical limitations [9-11]. This gap in knowledge is of increasing concern due to increasing parasite resistance in Southeast (SE) Asia, and emerging resistance in Africa, to the artemisinins and partner drugs [1, 12]. If optimized pediatric dosing is not established, there is concern that ACT resistance could continue to spread in sub-Saharan Africa and worsen an already challenging situation. The optimization of ACT use is vital in staving off the spread of ACT resistance in Africa.

The development and spread of drug resistance to antimalarial medications remains a major concern and challenge. Sulfadoxine-pyrimethamine (SP), used prior to the expansion of ACTs, was one of the most effective antimalarials until the rise of widespread resistance. The reason for the emergence of resistance is commonly attributed to improper dosing in young children [13]. In the early 1970s, SP replaced chloroquine as the main antimalarial treatment option in Thailand. However, within 5 years of SP being the first-line treatment, the cure rates seen with SP use in Thailand dropped dramatically: from 83% in 1975 to 22% by 1979, a loss attributed to the rise of parasite resistance to SP [13]. SP resistance in Africa remained low until the late 1990s but since then has spread rapidly in malaria endemic regions [14]. During the early years of SP usage, a high proportion of malaria treatment failures occurred among young children, later attributed to the underdosing of SP [15]. A study of Kenyan children younger than 5 years with acute, uncomplicated *P. falciparum* malaria found that when dosing SP based on age (i.e. standard of care in Kenya at the time), three-quarters of the children received less than the current internationally recommended SP target dose of 25 mg of sulfadoxine plus 1.25 mg of pyrimethamine per kg of body weight [15-17]. Underdosing likely resulted in the selection of resistant mutants and is considered a significant factor in the development of SP resistance in Africa [15, 16, 18]. These findings led to a revision of the dose recommendations by the Kenyan Ministry of Health in 1998 [17]. Currently, SP is mainly used for intermittent preventative treatment of malaria in pregnant women, and children in select countries, and is not considered sufficient for treatment of uncomplicated malaria [8]. Underdosing of ACT regimens among children, therefore, should be carefully evaluated and avoided to prevent an analogous situation for these now first-line therapies.

Suboptimal drug concentrations and drug resistance directly impact malaria treatment outcomes. Failure of antimalarial treatment is commonly attributed to two main sources: human

host factors, such as insufficient drug concentrations, and parasite factors, such as drug resistance [19]. These factors can lead to treatment failure in the individual and contribute to the development, intensification, and spread of *P. falciparum* drug resistance by increasing the probability of exposing parasites to suboptimal drug levels [15, 19]. Resistant parasites are selected when the antimalarial drug concentrations are high enough to suppress sensitive parasites but are too low to kill resistant parasites [20-24].

ACT exposure is greatly altered in young, small children and impacted by childhood development. The PK disposition of drugs in young children differs greatly from that in adults [25-27]. Enzymes involved in ACT metabolism, such as uridine diphosphate-glucuronosyltransferases [(UGT), responsible for the metabolism of active dihydroartemisinin (DHA), relevant for both AL and DP, and desbutyl-lumefantrine (DBL), the active metabolite of lumefantrine] and CYP enzymes (responsible for the metabolism of artemether, lumefantrine, and piperaquine), mature during the early stages of life, from 0-6 months of age for UGTs and over 12 months of age for CYP enzymes [28, 29]. For example, studies of DP in Ugandan children <2 years found piperaquine (PQ) exposure to be ~33% lower than in children aged 2-10 years [30-34]. A Worldwide Antimalarial Resistance Network (WWARN) pooled analysis reported that children <5 years are the most likely group to receive suboptimal DP doses, which might be due to higher drug clearance in young children compared to adults, potentially putting these children at an increased risk for recurrence of malaria [35]. Due to these and similar findings, in 2015, the WHO revised its dosing of DP in young children to improve drug exposure [36, 37]. For AL, similar results have been found. Studies have shown documented low lumefantrine exposure and worse outcomes following currently recommended doses in young children [38-42]. In previous research, we showed that lumefantrine exposure, reported as area under the plasma concentration time curve (AUC) post-last dose to infinity ($AUC_{0-\infty}$), in children was reduced by 46% when compared to adults, and that very young children (aged 6 months –

2 years) had lower lumefantrine exposure than older children and adults [40, 42]. In a PK/PD modeling study, researchers found that lumefantrine plasma concentrations 7 days after starting standard AL treatment were 24.2% and 13.4% lower in children weighing <15 kg and 15-25 kg, respectively, compared with adults [38]. Smaller children, due to inadequate AL exposure, could be at an increased risk of recurrent parasitemia and treatment failure. In the past when there was concern for underdosing of antimalarials, the amount of drug given per dose was increased, as was done for SP [15, 16]. However, as AL exhibits dose limited absorption, giving a larger dose is not a viable option [43]. Extending the treatment duration from 3 days to 5 days of AL has been suggested as an alternative [38].

Malaria and HIV co-infection remain common in Africa, complicating the adequate treatment of malaria infections. Sub-Saharan Africa, especially Uganda, continues to bear a heavy burden of both malaria and HIV. In terms of the HIV epidemic, Uganda has been successful in slowing HIV transmission in years past, but there are still over 1.4 million people living in Uganda with HIV [44]. There were 1.5 million new HIV infections and 680,000 deaths from AIDS-related causes that occurred worldwide in 2020 [44]. Currently, 1.7 million children are living with HIV; a majority of these HIV-infected children live in sub-Saharan Africa [44]. Thus, HIV-malaria co-infection remains common in Africa, with treatment for both diseases complicated by multiple pharmacological factors that require further study. Since children may be susceptible to underdosing of ACT regimens already, studying drug-drug interactions between malaria drugs and ART among children with HIV is especially critical.

Serious drug-drug interactions occur between ART and ACT, resulting in significant changes to the PK/PD of ACTs. The antiretroviral therapies (ARTs) ritonavir and efavirenz (EFV) cause potent CYP3A4 inhibition and induction, respectively [45-47]. Artemether undergoes demethylation by CYP3A4 and CYP2B6 to form the active metabolite, DHA, which is

further metabolized via UGTs [48-50]. CYP3A4 is also responsible for metabolizing lumefantrine to DBL, an active metabolite [51, 52]. Studies of AL from our group show that a dramatic increase in lumefantrine exposure occurs when lopinavir/ritonavir-based ART is co-administered [53, 54]. The reverse occurs when EFV-based ART is administered with AL; there is a significant decrease in artemether, DHA, and lumefantrine exposure, which puts patients at an increased risk of malaria recurrence [54-57]. The results highlighted a 10-fold variance in lumefantrine exposure in HIV-infected children with malaria depending on the ART regimen used [54].

ACT resistance is spreading throughout the world, threatening the efficacy of these important first-line therapies. Artemisinin resistance, seen as delayed parasite clearance after therapy and associated with propeller domain mutations in the *P. falciparum* kelch (K13) protein, is well-documented in SE Asia and is emerging in Africa [12, 58-63]. There is also established resistance to many of the partner drugs used in combination with the artemisinins, including mefloquine, amodiaquine, piperaquine, and SP [64-66]. Clinically relevant resistance, identified as treatment failure, occurs when a parasite exhibits resistance to *both* the artemisinin component and long-acting partner drug. Increasing treatment failure rates of DP in Cambodia and artesunate-mefloquine in Thailand, are indicative of this, and it is alarming that artemisinin resistance is now spreading to Africa, specifically to Uganda [12, 61, 63, 65, 67, 68]. Multiple studies now show a rapid increase in the prevalence of mutations associated with artemisinin resistance in *P. falciparum* parasites in northern Uganda. One study found that the incidence of K13 mutations in parasite isolates increased from 3.9% in 2015 to 19.8% in 2019, while another showed a jump in a K13 mutation prevalence from 5.5% in 2017 to ≥15% in 2019 in 3 northern Uganda sites [12, 63]. As seen with SP, there is a major concern that systematic under-dosing of ACTs, specifically in children, could increase the spread of resistance in Africa, since subtherapeutic drug levels can select for resistant parasites [69, 70].

HIV infected children on EFV-based ART are particularly vulnerable to an increased risk for recurrent infection and their underdosing can lead to the selection and spread of ACT resistance. Our group has previously shown that children managed with EFV-based ART have a ~2-fold reduced exposure to AL, increasing the likelihood of suboptimal ACT exposure resulting in higher risk for reinfection [54]. The >1.7 million children with HIV, many of them who are over 3 years old, are still managed with EFV-based ART [44]. Although current guidelines recommend the universal use of the integrase inhibitor, dolutegravir, as first line, switching from EFV to dolutegravir-based ART is a gradual undertaking [71]. Thus, there is a growing concern that suboptimal ACT dosing among HIV-infected children that remain on EFV-based ART will contribute to the selection of ACT-resistant parasites and hasten the spread of ACT-resistance in Africa [58, 60, 69, 72-74].

In order for ACTs to remain effective, it is vital that dosing guidelines be optimized, especially in young children, with human host factors in mind. Specific treatment guidelines, based on carefully designed PK/PD investigations in children, are needed to minimize suboptimal dosing, maintain efficacy, and deter the spread of ACT resistance**.** Therefore, this dissertation aims to fill key gaps in the literature for this most vulnerable population by examining AL PK and PD in the context of pediatrics, HIV-infection, and EFV-based ART.

Extended AL dosing regimens may increase AL exposure and greatly improve clinical outcomes in young children and those with significant drug-drug interactions. This research builds on results from our previous work in pediatric and HIV-infected populations and

addresses the clinically relevant reductions in AL exposure seen in small children and HIV-infected children on EFV-based ART. This research also incorporates findings that AL exhibits dose-limited absorption. We studied an extended AL treatment regimen rather than an increase in mg/kg dosing, since doubling of the dose led to a 30% decrease in lumefantrine exposure (expressed by AUC) with no change in the maximum concentration (C_{max}), suggesting dose-limited absorption of lumefantrine [43]. No previous studies have investigated an extended dosing regimen of AL among both HIV-uninfected and HIV-infected children on EFV. Other studies in the literature suggesting an extended AL treatment duration based on PK/PD modeling, justifies our strategy; these researchers relied on modeling and simulations to show the benefits of an extended 5-day AL dosing regimen [38]. We attempt to demonstrate this strategy using real world patient data from clinical PK studies.

Chapter 2: The pharmacokinetics and pharmacodynamics of extended malaria treatment with artemether-lumefantrine in HIV-uninfected children

2.1 Abstract

Background. Artemether-lumefantrine (AL) is the most widely used artemisinin-based combination therapy (ACT) in sub-Saharan Africa and is threatened by the emergence of artemisinin resistance. Dosing is suboptimal in young children. We hypothesized that extending AL duration will improve exposure and reduce reinfection risks.

Methods. We conducted a prospective, randomized, open-label pharmacokinetic (PK)/ pharmacodynamic (PD) study of extended duration AL (EXALT) in children with malaria in high transmission rural Uganda. Children received 3-day (standard 6-dose) or 5-day (10-dose) AL with sampling for artemether, dihydroartemisinin (DHA), and lumefantrine over a 42-day clinical follow-up. Primary outcomes were 1) comparative pharmacokinetic parameters between regimens, and 2) recurrent parasitemia analyzed as intention-to-treat.

Results. 177 children ages 16 months to 16 years were randomized, contributing 227 total episodes. Terminal median lumefantrine concentrations were significantly increased in the 5-day versus 3-day regimen on days 7, 14, and 21 ($p < 0.001$). A pre-defined day 7 lumefantrine threshold of 280 ng/mL was strongly predictive of recurrence risk at 28 and 42 days ($p<0.001$). Kaplan-Meier estimated 28-day (51% vs 40%) and 42-day recurrence risk (75% vs 68%) did not significantly differ between 3-day and 5-day regimens. No significant toxicity was seen with the extended regimen.

Conclusions. Extending the duration of AL was safe and significantly enhanced overall drug exposure in young children, but there were not significant reductions in recurrent parasitemia

risk compared to the standard 3-day regimen in our high transmission setting. However, day 7 levels were strongly predictive of recurrent parasitemia risk; participants who attained a day 7 lumefantrine concentration > 280 ng/ml had a lower risk of recurrent parasitemia. The 5-day AL group was more successful in achieving this target lumefantrine day 7 concentration than the 3-day AL group. Finally, those in the lowest weight-band were at higher risk of underdosing with the standard 3-day regimen. The implementation of an extended 5-day regimen of AL in this group could prove beneficial in boosting AL PK and improving clinical outcomes, though more research with a larger sample size is needed to confirm.

2.2 Introduction

Plasmodium falciparum malaria remains one of the most devastating infectious diseases, causing roughly 242 million cases and 627,000 deaths in 2020, with improvements stalling since 2015 [75]. Sub-Saharan Africa (SSA) bears >90% of all deaths, primarily in young children for whom antimalarial dosing guidelines are not fully optimized [75]. Treatment currently relies upon artemisinin-based combination therapies (ACTs), all dosed over 3-days, and most effectively targets initial 48-hour blood stage life cycles. Short-acting artemisinins rapidly reduce parasite burden while the long-acting partner drug eliminates residual parasites and protects against resistance and recurrent infection. Unfortunately, ACT resistance is now widespread in Southeast Asia, and recent reports confirm artemisinin-resistance in Uganda and Rwanda [12, 76]. Protecting ACTs in SSA is critical, and leading strategies include optimizing current ACT regimens, the use of triple ACTs, multiple first-line therapies, and cycling of ACTs.[77-80]

Of the six WHO-endorsed ACTs, artemether-lumefantrine (AL) is most widely used [8]. Importantly, all ACTs demonstrate a mismatch in component half-lives. For AL, artemether has a fast onset of action; it is quickly absorbed and cleared as it undergoes demethylation by various cytochrome P450 (CYP) enzymes into dihydroartemisinin (DHA), its active metabolite [48]. DHA then undergoes glucuronidation before it is excreted [50]. Lumefantrine, the long-acting partner drug, has a slow absorption phase and is metabolized primarily by CYP3A4 with a terminal half-life of ~3-4 days [81, 82].

With this pharmacokinetic (PK) mismatch in mind, children in high transmission settings can experience 5 or more episodes of malaria per year, indicating the need for optimized regimens that consider both treatment of the current infection and post-treatment prophylaxis against new or recurrent infections [83, 84]. Consideration of these dynamics can reduce the risk of true

treatment failure (recrudescence), extend the period of post-treatment prophylaxis (in high transmission settings), and mitigate the selection of resistance [85].

However, determining the optimal weight-based ACT dose in children requires consideration of developmental changes, including enzyme maturation, as well as the impact of malnutrition on ACT pharmacokinetics and pharmacodynamics (PD) [27, 29, 41, 42, 86]. For AL, our group and others have documented low lumefantrine exposure and worse outcomes following currently recommended doses in young children [38-42]. Day 7 lumefantrine concentration thresholds from 175-280 ng/mL have been a common target linked to outcomes [38, 81, 87]. Improving lumefantrine exposure, and thereby improving outcomes, requires lengthening the treatment duration (versus increasing mg/kg per dose), as absorption is dose-limited [12, 38, 40, 41, 43, 88-90]. Additionally, artemisinins are cleared within 24 hours, thus extending regimens will expose parasites to the artemisinin component for an additional 48-hour life cycle, which may also mitigate the impact and risk of ACT resistance emergence and spread [90-93].

Ensuring that current ACTs are adequately dosed, both in terms of total PK exposure and duration is critical. We conducted the Extended Duration AL Treatment for Malaria in Children (EXALT) trial and hypothesized that an extended 5-day regimen would be safe, well tolerated, and significantly improve AL PK exposure. We further hypothesized that improved exposure would lower the risk of recurrent parasitemia.

2.3 Methods

Study area and participant enrollment

EXALT is a prospective, randomized, open-label PK/PD study of 3-day (6-dose) versus 5-day (10-dose) AL for the treatment of uncomplicated malaria in Ugandan HIV-uninfected children.

Our study clinic is located on the grounds of Masufu General Hospital in Busia, Uganda, a perennial holoendemic transmission area with an estimated 33.4% *P. falciparum* parasite prevalence in 2–10 year old children [94]. Children ages 6 months to 18 years were recruited at our clinic, open 7 days a week. After informed consent (and assent if ≥7 years), children were randomized 1:1 to 3- or 5-day AL and could be re-enrolled/re-randomized for up to 4 episodes (**Fig 2.1**). All parents or guardians provided informed consent. Assent was also obtained from participants aged 7 years old. Eligibility criteria were the following: (1) living ≤60 km from the study site, (2) confirmed HIV negative status, (3) age 6 months to 18 years, (4) weight ≥6 kg, (5) hemoglobin > 7.0 g/dL, (6) no history of significant comorbidities, (7) no receipt of drugs impacting CYP enzymes ≤14 days (8) no prior malaria treatment ≤28 days, (9) presentation with uncomplicated *P. falciparum* malaria, or mixed infection with the presence of *P. falciparum* species, and (10) agreement to come to clinic for all follow-up clinical and PK evaluations. Children were randomized using a computer-generated randomization list accounting for loss to follow-up and withdrawals. Allocation sequence was concealed from the clinician assessing and enrolling participants through sequentially numbered, opaque, sealed envelopes assembled by a third-party not involved in participant enrollment, treatment, or follow-up. Children could be re-enrolled for up to 2 intensive malaria episodes, and a maximum of 4 total episodes, and re-randomization occurred for each new enrollment. Once the target sample sizes were met for the intensive PK study arm, or if the child had already participated twice in the intensive PK study, randomized enrollment into sparse PK sampling 3-day (6-dose) or 5-day (10-dose) AL groups began.

The study was open-label, with weight-based AL dosing (Coartem® Dispersible 20 mg/120 mg, Novartis, Switzerland). Participants weighing <15 kg, received 1 tablet; ≥15 to <25 kg, 2 tablets; ≥25 to <35 kg, 3 tablets; and ≥35 kg, 4 tablets. Each clinic dose was observed and administered with milk (daytime doses), and milk was provided for non-observed doses at home (evening

doses), to enhance and control for lumefantrine absorption [95]. Powdered milk at approximately 2 scoops (equivalent to 2.8 grams of fat) were given for consumption of each dose to ensure that at least 1.2 grams of fat were taken with each dose. Doses in clinic were readministered for vomiting within 30 minutes.

Primary outcomes were 1) comparative plasma PK parameters for artemether, DHA, and lumefantrine between treatment regimens in the intensive PK sub-cohort, and 2) microscopy-determined recurrent parasitemia in the entire cohort. Secondary outcomes were genotype–unadjusted/adjusted recurrent malaria at 28 and 42 days using standard WHO criteria [8]. Ethical approval was obtained from the Uganda National Council of Science and Technology, the Makerere University School of Medicine Research Ethics Committee, the University of California, San Francisco Committee on Human Research, and the Yale University Human Investigations Committee

Clinical and molecular follow-up

Uncomplicated malaria was confirmed by presence of any parasites on thick smear with a documented or history of fever within 24-hours (≥38.0°C). All 2% Giemsa-stained slides were read by two expert microscopists with third reads conducted for discrepancies. Complete blood count and liver function tests were performed on days 0, 14, and 28. Active and passive follow-up was conducted to day 42 **(Fig 2.1)**. Participants were encouraged to return to clinic on any non-study days for any concerns. The NIAID Division of AIDS criteria (version 2.1, March 2017) were used to assess safety and tolerability, including adverse events. Electrocardiograms were performed in a subset of participants (**Fig 2.1**). Electrocardiograms were completed at baseline (before the administration of the first dose), prior to and 4-6 hours post-last dose (day 3 or 5 for the 6- and 10-dose arms, respectively), day 8 (or day 7 for the sparse sampling arm), and day

28 (**Fig 2.1**). Corrected QT (QTc) intervals were computed using the Fridericia formula (QTcF, $/\sqrt[3]{RR}$). Genotyping was performed using capillary electrophoresis of six loci (msp1, msp2, TA40, TA60, TA81, and TA87), as previously described [54]. Episodes were considered recrudescent (true failures) only if strain genotypes matched at all loci that were successfully genotyped. Recurrences occurring after 14 days were retreated with artemether-lumefantrine, as per standard of care.

Pharmacokinetic sampling and analysis

3-day AL (6-dose regimen): *Intensive PK cohort*, sampling occurred on days 0 to 3 for artemether, DHA, and lumefantrine. Sampling also occurred on days 4 (24 hours post-third dose), 8, 14, and 21 for lumefantrine. *Sparse PK cohort*, sampling occurred on days 0 to 3, 7, 14, and 21. 5-day AL (10-dose regimen): *Intensive PK cohort*, sampling occurred days 0 to 5 for artemether, DHA, and lumefantrine. Additional sampling occurred on day 6, 8, 14, and 21 for lumefantrine. *Sparse PK cohort*, sampling occurred on days 0 to 5, 7, 14, and 21 **(Fig 2.1)**. Concentrations of artemether and DHA were determined using liquid chromatography–tandem mass spectrometry, as previously described [96]. Lumefantrine quantitation was performed on a Waters® UPLC® I class system coupled with Sciex TripleQuad 6500^{+} tandem mass spectrometry system based on a previous method [97]. For the artemisinins, the calibration range was 0.5–200 ng/mL with the lower limit of quantification (LLOQ) at 0.5 ng/mL for both artemether and DHA. For lumefantrine, a previous quantification method was modified as follows: plasma sample volume was reduced from 25 µl to 5 µL, and the instrument time per sample was reduced from 8 min to under 2 min with a newer UPLC column [97]. All sample and PK analysis was completed at the Drug Research Unit, University of California, San Francisco.

For intensive PK studies, PK parameters for each subject around the final dose were determined using non-compartmental analysis and followed a linear up-log down trapezoidal rule in conjunction with first-order input (Phoenix WinNonlin 64). Intensive PK cohort analysis included estimates of the area under the plasma concentration versus time curve post-final dose ($AUC_{0–8\ hours}$ for artemether and DHA; $AUC_{0\text{-}21d}$ for lumefantrine), maximal concentration (C_{max}), time to C_{max} (t_{max}), elimination half-life ($t_{1/2}$), and C_{8h} (artemether and DHA). An estimation of cumulative exposure (i.e. the AUC from the 3rd dose to day 21, AUC_{cum}) was also calculated for lumefantrine for the intensive PK arms of the study. As we only collected pre-dose samples on first day of dosing, the 3rd dose was the earliest post-dose samples that could be utilized to generate the cumulative exposure variable. Specifically, estimates relied on the 2- and 4-hour actual post-dose concentration levels on days 1, 2 (and days 3, 4 for the AL 10-dose regimen), and calculated trough concentrations on those days using an elimination rate constant determined from the post-final dose data. The trapezoidal rule was used to calculate AUCs for the morning, directly observed doses 3 and 4 (6 and 8 for the AL 10-dose regimen), which were then also used as an estimate for lumefantrine exposure for the evening doses 5 (7 and 9 for the AL 10-dose regimen). All dose specific AUCs (i.e. third dose to the ninth dose) were added to the post-final dose estimated $AUC_{0\text{-}21d}$ to generate an estimated cumulative lumefantrine exposure from the third dose to day 21, AUC_{cum}. For the AUC_{cum} calculation, Phoenix WinNonlin 64 was utilized to determine the elimination rate constant and post-last dose AUC. Microsoft Excel version 16.54 was used to calculate the trough values, the dose specific AUCs, and the summation of all AUCs. For sparse PK data, lumefantrine concentrations at day 7, day 14, and day 21 were combined with the concentration data from the intensive PK cohort to compare exposure between the 3-day AL and 5-day AL groups.

Capillary and venous samples collected concurrently at 2- and 8-hours post-final dose on the intensive sampling day (i.e. day 3 for 3-Day AL group) were used to compute capillary-venous

correlations of artemether and DHA concentrations which permitted merging of capillary and venous measurements for PK analysis. Correlation data analysis was performed using STATA® SE14.2 (StataCorp, College Station, TX, USA). The relationship between capillary and venous plasma artemether levels was modeled using linear regression with estimated intercept and slope. The linear least squares regression models were built using natural logarithmic-transformed concentrations and the final models were selected based on maximal coefficient of determination (R^2) and visual check. The same analysis was conducted using DHA venous and capillary sample data to determine the correlation between capillary and venous DHA concentrations.

Statistical Analysis

Analysis included the intention-to-treat population (ITT; all those who were enrolled and randomized) and the per protocol population (PP; those completing 21 days of PK sampling). For the primary PK outcome, mixed effects repeated measures model was used to compare PK parameters between 3-day and 5-day groups and LF concentrations for the combined intensive and population 3-day and 5-day groups, after accounting for correlation within re-randomized subjects. For the primary clinical outcome, the cumulative risk of recurrent parasitemia at days 28 and 42 was assessed using Kaplan-Meier curves and differences between arms were compared using log-rank test. Multivariate Cox regression with robust sandwich estimation to account for within-subject correlation of recurrent enrollment was conducted, controlling for age, weight, baseline parasite density, baseline hemoglobin, sex, lumefantrine mg/kg, lumefantrine PK exposure, and cross-over status (i.e. enrolled in the 3-day arm and re-enrolled with a separate episode in the 5-day arm).

For the intensive PK sub-cohort, a sample size of n=50 in each arm was determined to detect a 35% AUC difference of all analytes (80% power; α=.05) [53, 98]. A similar sample size was determined for the sparse PK cohort. Clinical outcomes were assessed in children in the entire cohort. Based on our recent study in a nearby area with a 42-day recurrence rate of more than 50%, we have 80% power to detect a 20% difference in recurrence rates between the treatment arms (α=.05) [54]. A mixed effects repeated measures model was used to compare artemether and DHA concentrations at 2- and 4-hours post-third and post-last dose (day 3 or 5) between 3-day and 5-day groups. The change in parasite density was compared between regimens from mixed effects repeated measures models, controlling for parasite density on day 0. Secondary clinical outcomes used standard WHO criteria (adequate clinical and parasitological response (ACPR), late clinical failure (LCF), and late parasitological failure (LPF) which were compared using chi-square test. Statistical significance was a 2-sided P value < .05. Stata version SE14.2 (StataCorp, College Station, Texas) and SAS version 9.4 (SAS Institute, Cary, North Carolina) software programs were used for analyses.

2.4 RESULTS

Study Profile

Children were randomized into 3-day or 5-day regimens, first into the intensive cohort, and then into the sparse cohort once sample sizes or maximum enrollment was reached (**Fig 2.1**). Intensive and sparse PK cohorts were combined for all analyses, except for intensive PK parameters. Enrolment and follow-up took place between February 21, 2018, to August 29, 2019. For the intensive PK cohort, 212 episodes were screened, 102 episodes were randomized, and 100 episodes completed the study and were included in the final PK/PD analysis (**Fig 2.2**). For the sparse PK cohort, 276 episodes were screened, 125 episodes met eligibility criteria, and 119 episodes were included in the final PK/PD analysis (**Fig 2.2**). For all

randomized children, the median age (interquartile range, IQR) was 5.8 years (4.1-8.0 years) and median (IQR) weight was 18.4 kg (15.3-22.9) (**Table 2.1**). Baseline geometric mean (95% confidence interval) parasite density was 9542 parasites/μL (7342-12,403 p/μL).

In the PP population, n=169 children participated for a single episode and n=50 were enrolled for more than one episode (two episodes (n=44), three episodes (n=4), and four episodes (n=2)) (**Fig 2.2**). Of the 86 children who participated in the PP intensive PK analysis, n=16 participated for 2 episodes. Overall, children re-enrolled for a subsequent episode (n=50) were marginally younger (median (IQR) age 4.6 years (4.1-7.9) versus 6.2 years (4.6-8.2); p=0.06), weighed less (median (IQR) weight 16.1 kg (13.8-22.4) versus 19.1 (4.6-8.2); p=0.05), and received a lower LUM mg/kg dose in each regimen than those enrolled for only a single episode, respectively. Vomiting post-last dose was rare, with only 5 instances occurring in the trial, all of which were re-dosed.

Correlation of drug concentrations in capillary versus venous plasma

We report, for the first time, on the correlation of simultaneously collected capillary plasma concentrations versus venous plasma concentrations of artemether and DHA, respectively. Briefly, 126 pairs (252 individual data points) of capillary and venous plasma samples collected simultaneously at 2- and 8-hours post-final AL dose were used to complete a linear regression analysis after the data had undergone a natural log transformation. The resulting equation for artemether capillary concentration (C_{cap}) vs venous concentration (C_{ven}) was $\ln[C_{cap}] = 1.03 * \ln[C_{ven}] - 0.08$ (N = 126, $R^2 = 0.924$). The median ratio (IQR) of artemether C_{cap}/C_{ven} was 1.00 (0.94, 1.07). The equation for DHA capillary concentration (C_{cap}) vs venous concentration (C_{ven}) was $\ln[C_{cap}] = 1.08 * \ln[C_{ven}] - 0.21$ (N = 126, $R^2 = 0.967$). The median ratio (IQR) of DHA C_{cap}/C_{ven} was 1.01 (0.98, 1.04). From these results, it was determined that capillary and venous measurements of both artemether and DHA have a 1:1 linear relationship, respectively. Due to this 1:1 linear relationship, artemether and DHA capillary concentration results from the EXALT

study were not converted to predicted venous values using the generated correlation equations. For lumefantrine, we also combined capillary and venous plasma concentrations without conversion as we found a 1:1 correlation previously [99]

PK of artemether and DHA in the 3-day versus 5-day study arms

PK parameters for 3-day and 5-day (n=50 each) episodes with completed intensive PK sampling are summarized in **Table 2.2** and **Figs 2.3.** For children undergoing intensive PK, cumulative artemether and DHA exposure (AUC_{cum}; post-third to 8-hr post-final dose) showed a 1.70- and 1.82-fold increase in artemether and DHA exposure in the 5-day vs 3-day group (p=0.001 and <0.0001, respectively). To investigate artemisinin exposure changes with repeated dosing, post-dose 2-hour concentrations were compared over the course of dosing (**Table 2.3, Fig 2.4).** Artemether concentrations were 68% and 65% lower following the last versus the third dose in the 3-day and 5-day regimens, respectively (p <0.0001 for both); DHA concentrations were 43% and 29% lower following the last versus third dose in the 3-day and 5-day regimens, respectively (p ≤ 0.0039 for all comparisons; **Table 2.3, Fig 2.4**). During analysis, the coefficient of variation (CV%) for artemether was 7.65, 6.73, 7.74% at low, medium, and high concentration quality control samples, respectively, and for DHA was 6.28, 5.95, 6.22%, respectively.

PK of lumefantrine in 3-day versus 5-day study arms

PK parameters for the 3-day and 5-day (n=50 each) intensive episodes are in **Table 2.4** and **Fig 2.3**. An estimate of cumulative lumefantrine exposure (AUC_{cum}; post-third dose to day 21) showed a 1.82-fold increase in lumefantrine exposure in the 5-day vs 3-day group (p=0.0001) (**Table 2.4** and **Fig 2.3)**. Combining data from the intensive and sparse PK cohorts, those receiving the 5-day vs 3-day regimen exhibited markedly higher median lumefantrine concentrations on days 7, 14, and 21 (2.24-, 1.52-, 1.37-fold, respectively; p≤0.001 for all

comparisons; **Table 2.4**). The coefficient of variation (CV%) for lumefantrine was 9.72, 9.27, 8.16% at low, medium, and high quality control samples, respectively.

Treatment outcomes at 28-day and 42-day follow-up

The primary clinical outcome was recurrent parasitemia (with or without fever) detected by microscopy at 28- and 42-days. The Kaplan-Meier estimated cumulative risk of recurrence at day 28 was 51% vs 40%, 3-day vs 5-day AL, respectively (p=0.091), and at day 42 was 75% vs 68%, 3-Day vs 5-Day AL, respectively (p=0.10; **Fig 2.5**). Genotyping was attempted on all children with recurrent parasitemia and was successful for 140/158 children. For the WHO outcomes at 42 days, 24% and 23% of participants had symptomatic (i.e., fever and parasitemia) re-infections in the 3-day and 5-day regimens, respectively (**Table 2.5)**. Overall, 7.1% (n=10/140) of recurrences were recrudescent, and equally proportioned between regimens (n=5 in each). The median multiplicity of infection between 3-day and 5-day AL regimens at enrollment was 4 (range 1-11) and 3 (range 1-14), respectively. There was no difference in the median MOI between regimens upon recurrence.

Multivariate Cox regression was performed to evaluate the risk of recurrent parasitemia after adjusting for the above covariates, as well as sex, baseline parasite density, lumefantrine mg/kg per dose, and whether a participant was re-enrolled and participated in both arms (**Table 2.6**). The adjusted analysis risk differences between 3-day and 5-day regimens were not significantly different at 28 or 42 days.

In an exploratory analysis, when restricting to those that presented with recurrent malaria for re-randomization/re-enrollment (n=50 episodes total), 40.7% and 13.0% of episodes in the 3-day and 5-day developed recurrent parasitemia at 28 days (p=0.030); differences were not significant at 42-days (70.4% and 60.9% of episodes in the 3-day and 5-day (p=0.48). We hypothesize that children who presented for multiple episodes represent a group at higher risk

of malaria, and therefore, the benefit of the extended regimen was more evident in those higher risk children. Parasite densities of recurrent episodes following treatment in each of the treatment duration arms is presented in **Fig 2.6** and **Table 2.7**.

We next examined the relationship between regimen, lumefantrine exposure, and recurrence risk in the intensive cohort (n=100). In this subset, a multivariate Cox model adjusting for the above covariates found that at 28 days, children in the 5-day versus 3-day regimen had a HR of 0.47 (95% CI 0.25-0.88; p=0.019) for recurrence of parasitemia, though differences were not significant at 42 days (HR 0.74; p=0.23) (**Table 2.6**). Lumefantrine $AUC_{0\text{-}21d}$ (AUC post-last dose to 21 days) was significantly associated with malaria risk at both days 28 and 42 (HR 0.54, p=0.028 and HR 0.61, p=0.038; respectively).

Treatment outcomes based on a day 7 lumefantrine level of 280 ng/mL

Associations between recurrent parasitemia and a previously defined day 7 lumefantrine predictive "threshold" of 280 ng/mL for risk of recurrent infection were assessed (**Fig 2.5**) [81]. Approximately 4 times as many children were found to have a day 7 level ≤280 ng/mL in the 3-day versus 5-day regimen (**Table 2.1**). Overall, a lumefantrine level >280 ng/mL on day 7 was associated with a 46% and 42% lower hazard of recurrence at 28 and 42 days, respectively (**Table 2.6**).

In the 3-day arm, height and weight were significantly higher in those achieving targeted day 7 levels (**Table 2.8**). We therefore investigated whether certain dosing weight bins may be associated with higher frequencies of falling below the 280 ng/ml lumefantrine threshold. For those in the 5-14 kg weight bin (1 AL tablet), 64.0% (n=16/25) in the 3-day regimen versus 18.8% (n=3/16) in 5-day regimen, fell below the day 7 threshold.

Safety and tolerability of artemether-lumefantrine

Artemether-lumefantrine was well tolerated throughout the study. There were two serious adverse events (SAEs) documented in the study, both in the 3-day arm. The first was a 5-year-old who was successfully treated and cleared parasitemia by day two, fainted on day 26, and was taken to a nearby healthcare facility where he responded to fluids. The facility deemed that the episode was due to hypoglycemia of unknown etiology. He was seen on day 31 in clinic and was in good health with a negative blood smear. The second SAE that occurred was a 3-year-old boy who responded to 3-day AL, had recurrent parasitemia on day 21, and was found to have grade 4 anemia on day 28 with a negative blood smear (grade 1 on day 0 and 14). He was treated with anthelminthics and iron, was found to have malaria on day 43 and retreated with AL, with hemoglobin returning to grade 1 six days after retreatment. Overall, only a single grade 3 symptom (cough) was noted in a child. All AST, ALT, and creatinine values were grade 2 or below throughout the duration of the study. One child had grade 4 thrombocytopenia at presentation with malaria and rebounded to a normal value at day 14. Graphs of all monitored chemistry and hematology values on days 0, 14, and 28 are presented in **Fig 2.7**. Electrocardiograms conducted in subset of 101 children showed no QTc prolongation greater than 450 ms at any time point. A change in the QTc greater than 60 ms occurred in two children. The first child, in the 5-day arm, had an increase in QTc from 292 ms on day 0 to 386 ms on day 5 (post 10th dose). The second child, in the 3-day arm, had a change in QTc from 420 ms on day 3 (post 6th dose) to 356 ms on day 28 of follow-up (post-treatment baseline).

2.5 Discussion

We conducted EXALT, the first study in children to specifically look at an extended AL duration of 5-day (10-doses) versus the standard 3-day (6-doses) regimen to improve PK exposure and clinical outcomes. We found that the extended regimen was both safe and effective at

increasing AL exposure. Those receiving the 5-day regimen were significantly more likely to attain a previously defined day 7 lumefantrine target concentration (280 ng/ml), with 4-fold more children falling below this level in the 3-day versus 5-day regimen.

In our intense transmission region, true failure after treatment with either 3 days of AL or 5 days of AL remained rare, with 93% of all recurrences attributed to new infections. By 28 and 42 days of follow-up, nearly 50% and 75% of children had recurrent parasitemia. In this setting, the extended 5-day regimen was unable to significantly reduce the risk of recurrent parasitemia, though the risk difference narrowed over follow-up, likely explained by post-treatment prophylactic lumefantrine levels falling below a protective threshold. Indeed, the risks were significantly different when stratifying by regimen and day 7 level, from highest to lowest in those in the 3-day ≤280 ng/ml, 5-day ≤280 ng/mL, 3-day >280 ng/mL, and 5-day >280 ng/mL (**Fig 2.5**).

When limiting our analysis to children in the intensive PK cohort, we saw a significantly reduced 28-day risk of recurrence in the 5-day group. A potential explanation may relate to levels of adherence, as intensive participants spent more time in the clinic, had one additional directly observed dose, and their regimen was extended by an additional 12 hours as compared to children in the sparse cohort (**Fig 2.8)**. Indeed, for those in the 5-day regimen, day 14 and 21 lumefantrine levels were higher in the intensive versus sparse cohort (p=0.0018 and p=0.0004, respectively). It is also notable that parasite densities of recurrent episodes trended towards being lower in the 5-day vs 3-day regimens, perhaps demonstrating a quantitative impact of the additional AL doses on parasite clearance (**Fig 2.6; Table 2.7**).

Our trial builds on a handful of other studies that confirm the safety and efficacy of extended duration of AL in different settings [88, 89, 100]. The first was a trial in Myanmar in 2013-2015,

involving adults and children treated with 3- vs 5-days of AL, all of whom received a dose of primaquine. Both regimens were safe and effective [100]. A similar Tanzanian study involved adults and children treated with 3- vs 6-days of AL plus primaquine [88]. The extended regimen was safe and effective but did not meet superiority specifications. Finally, researchers compared 3-day vs 5-day AL in n=48 pregnant and n=48 nonpregnant Congolese women; again, regimens were safe and effective, and the extended regimen improved PK exposure to a level comparable to nonpregnant adults [89].

An important additional finding in our study was that those children in the lowest weight-band of 3-day AL dosing were 3.4-times more likely to fall below 280 ng/mL than those receiving the 5-day regimen. Previous work by our group showed that children under 2 years were at risk for low AL exposure, which we hypothesized was due to lower bioavailability [40]. A population PK/PD meta-analysis also demonstrated that day 7 lumefantrine concentrations in children weighing <15kg and 15-25 kg were 24.2% and 13.4% lower compared to levels in nonpregnant adults [38]. The first study to demonstrate the potential impact of extending the duration of AL was in Thailand, where six-doses were administered either over 3 or 5 days, with the 5-day dosing interval improving PK exposure and cure rates [90, 101]. Our data now successfully demonstrate the ability of an extended 5-day (10-dose) regimen to improve exposure in the lowest weight children, and we advocate that dosing regimens in the youngest, smallest children be revisited.

Optimizing the dosing of the *artemisinin* component is also critical, particularly considering the recent emergence of artemisinin resistance in SSA [12, 76]. Five-days of AL exposes the parasite to the artemisinin component for an additional 48-hour trophozoite cycle where artemisinins are most active [90]. This additional exposure may leave fewer parasites for

lumefantrine and/or the immune system to clear, reducing the risk of emergence and spread of artemisinin resistance [91, 93]. We also observed a notable decrease in artemisinin exposure (post-dose 2-hour concentrations) with repeated dosing. This aligns with previous studies and has been thought to be caused by CYP3A4 autoinduction (likely an intestinal first-pass effect) by artemether and/or recovery from malaria leading to improved bioavailability and absorption [102-107]. The clinical impact on declining artemisinin exposure with each dose is unclear, however, any impacts are more likely to be seen in the 3-day regimen, as the extended regimen significantly enhanced artemisinin exposure.

Our study is subject to a few limitations. Evening doses were not observed. In addition, while active sampling occurred on up to 13 visits, alongside passive follow-up available daily, we are unable to comment on parasitemia occurring on intervening days, or submicroscopic parasitemia occurring during follow-up. In addition, we are unable to comment on regimen effectiveness if deployed outside of a controlled study where adherence to an extended regimen may be more problematic.

In summary, our data demonstrate that extended duration 5-day (10-dose) AL treatment regimen is safe and efficacious in HIV-uninfected children living in a high transmission setting. Specifically, children in the lowest weight category appeared to be at highest risk of underdosing, a deficit that was largely overcome with additional days of dosing. In addition, children in the 5-day regimen were more likely to attain the 280 ng/mL threshold, and those achieving this threshold had the lowest recurrence risk. In our setting, the increased exposure led to marginal reductions in the overall 28-day recurrence risk, which was no longer evident at 42-days, likely due to new parasites emerging from the liver or newly inoculated over time entering the blood when lumefantrine levels were no longer protective. It is critical that we

explore multiple potential options to preserving the utility of current ACTs. Extending AL regimen duration should be considered as a potential option, and additional study in lower transmission settings, or in areas where artemisinin resistance is emerging in Africa should be considered to mitigate the emergence and spread of ACT resistance in SSA.

2.6 Figures

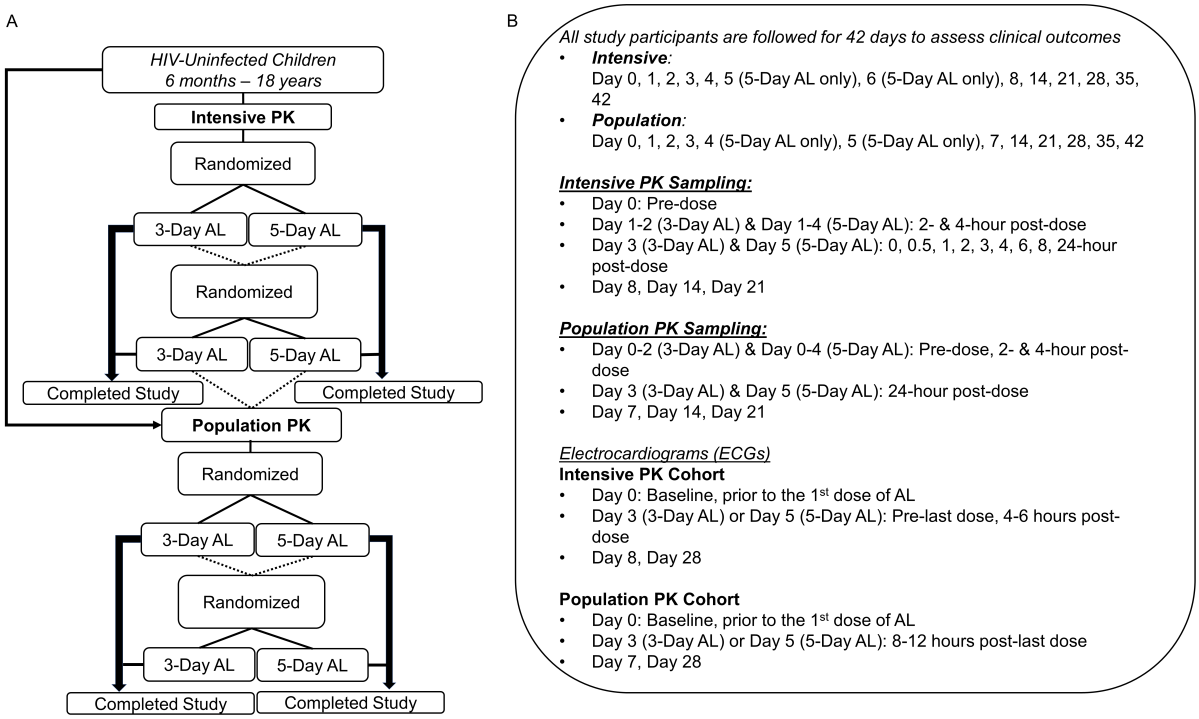

Figure 2.1 (A) Randomization schema and **(B)** PK/PD study follow-up and sampling design. Children were initially randomized into 3-day or 5-day intensive study arms, and re-randomized upon presentation for a subsequent episode ocurring during follow-up or at a later date, provided eligibility criteria were met, and sample sizes had not been met for a specified study arm PK study sampling design and malaria follow-up schedule.

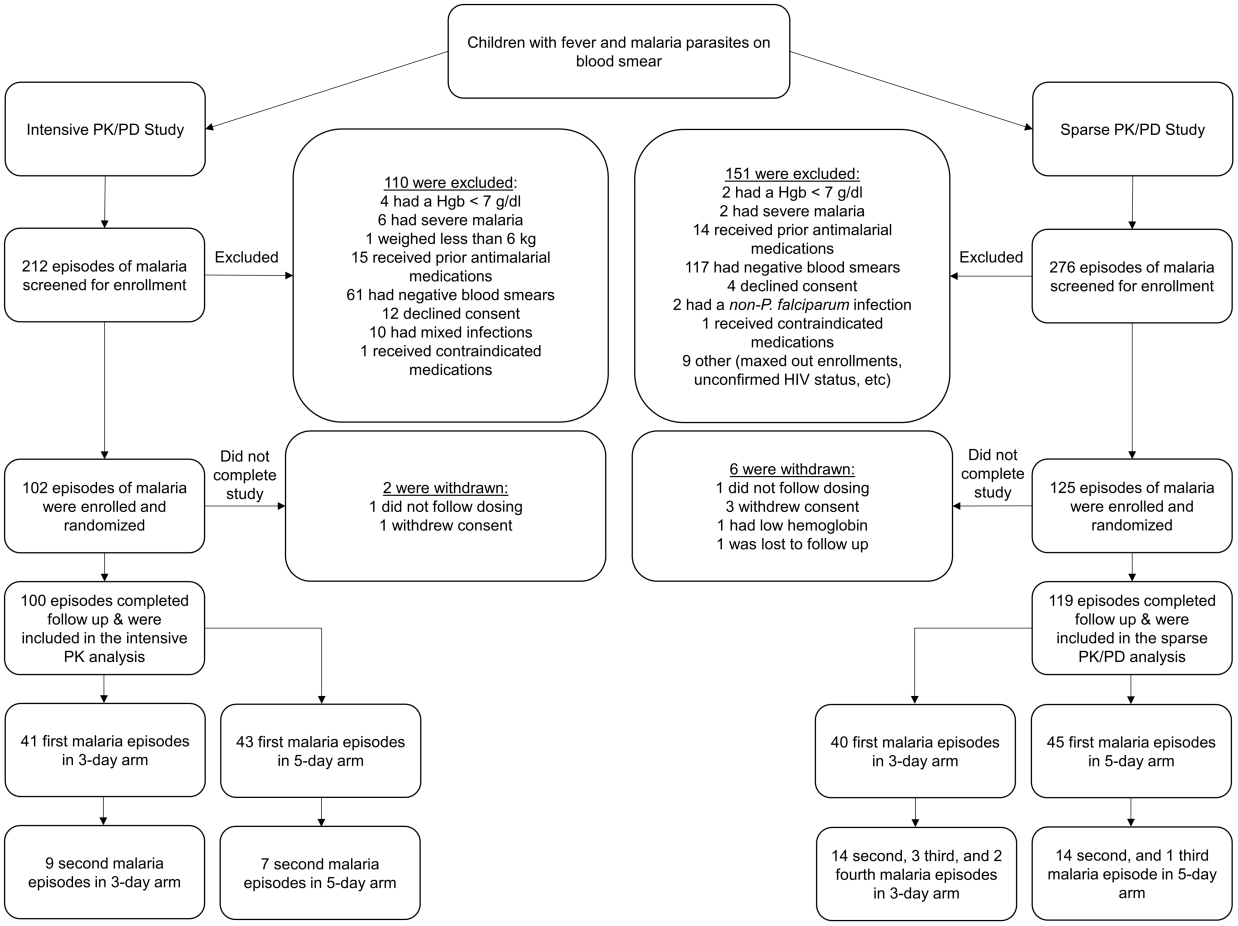

Figure 2.2 Trial Profile. Study screening and enrolment flowchart for the intensive and sparse PK sampling arms showing intention to treat (ITT) and per protocol cohorts (Consort diagram).

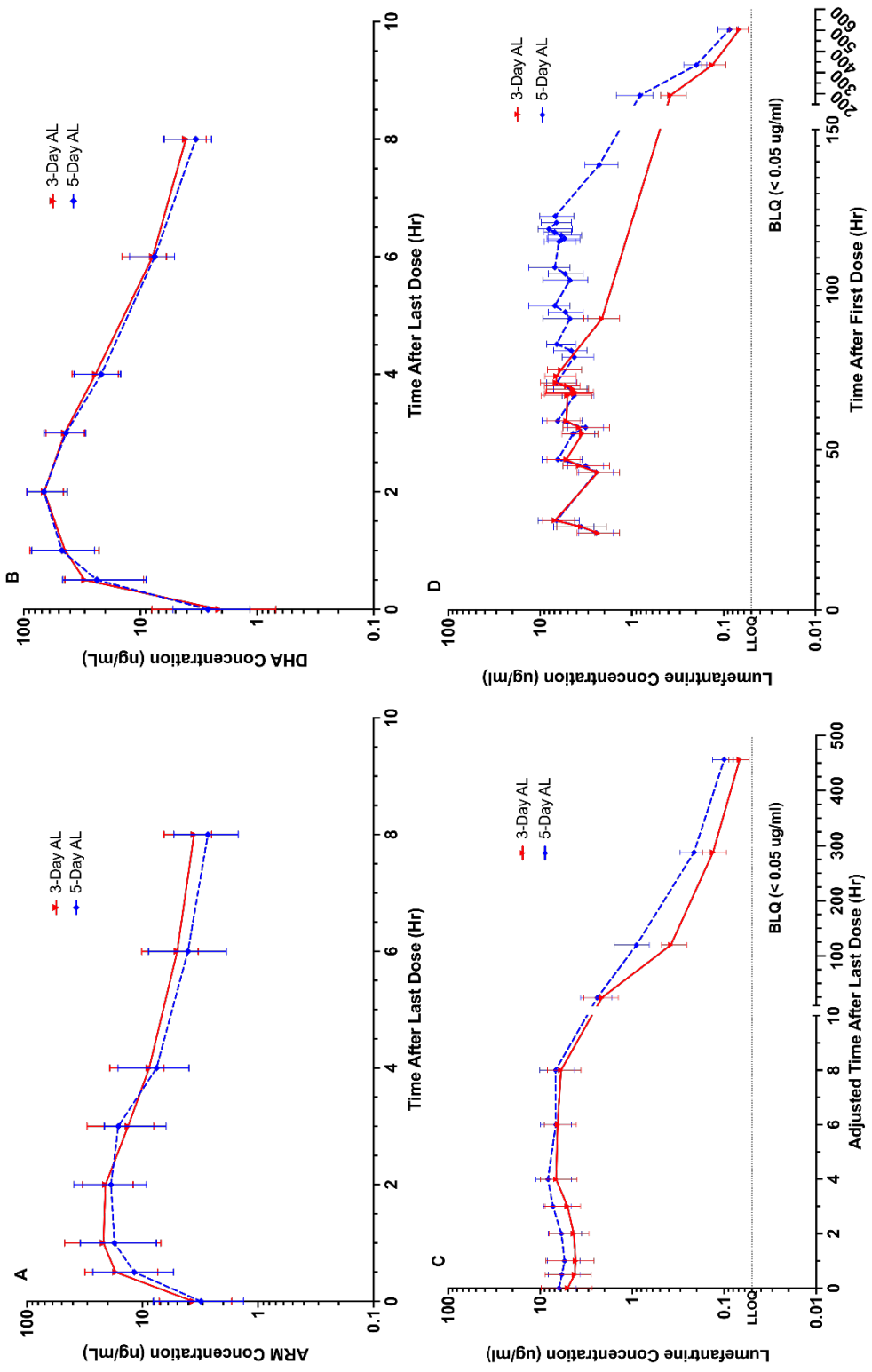

Figure 2.3 (A) Plasma concentration-time profiles of artemether, **(B)** dihydroartemisinin (DHA), and **(C)** lumefantrine in children treated with three days of AL and children treated with five days of AL, and **(D)** estimated cumulative AUC (AUC from the 3rd dose to day 21; AUC_{cum}). Data are

represented as median, and values below the limit of quantitation (BLQ) are shown. Note that lumefantrine concentrations are shown in µg/mL.

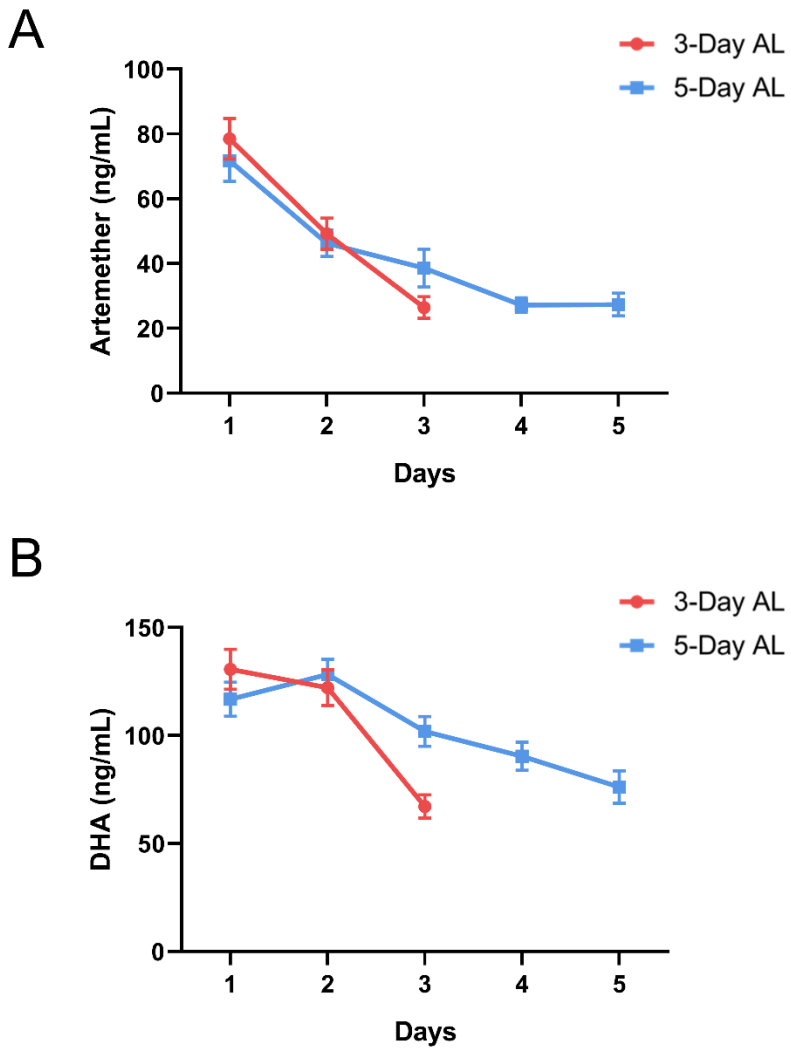

Figure 2.4. **(A)** Artemether and **(B)** DHA concentrations 2 hours following each morning dose in 3-day and 5-day regimens.

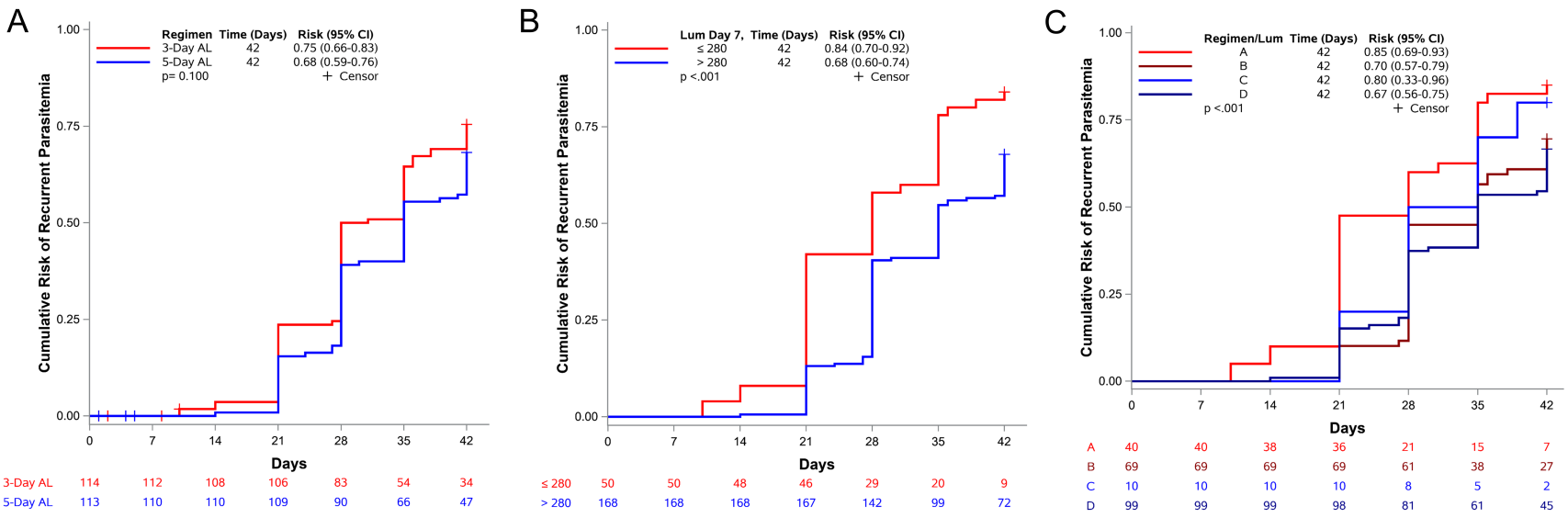
A
Regimen Time (Days) Risk (95% CI)
3-Day AL 42 0.75 (0.66-0.83)
5-Day AL 42 0.68 (0.59-0.76)
p= 0.100
+ Censor
Cumulative Risk of Recurrent Parasitemia
1.00
0.75
0.50
0.25
0.00
0 7 14 21 28 35 42
Days
3-Day AL 114 112 108 106 83 54 34
5-Day AL 113 110 110 109 90 66 47
B
Lum Day 7, Time (Days) Risk (95% CI)
≤ 280 42 0.84 (0.70-0.92)
> 280 42 0.68 (0.60-0.74)
p <.001
+ Censor
Cumulative Risk of Recurrent Parasitemia
1.00
0.75
0.50
0.25
0.00
0 7 14 21 28 35 42
Days
≤ 280 50 50 48 46 29 20 9
> 280 168 168 168 167 142 99 72
C
Regimen/Lum Time (Days) Risk (95% CI)
A 42 0.85 (0.69-0.93)
B 42 0.70 (0.57-0.79)
C 42 0.80 (0.33-0.96)
D 42 0.67 (0.56-0.75)
p <.001
+ Censor
Cumulative Risk of Recurrent Parasitemia
1.00
0.75
0.50
0.25
0.00
0 7 14 21 28 35 42
Days
A 40 40 38 36 21 15 7
B 69 69 69 69 61 38 27
C 10 10 10 10 8 5 2
D 99 99 99 98 81 61 45

Figure 2.5. Kaplan-Maier estimate of time to microscopically-determined recurrent parasitemia over 42-day follow-up for ITT cohort in **(A)** children randomized to the 3-day versus 5-day regimen, **(B)** children attaining a day 7 lumefantrine levels > 280 ng/mL and ≤ 280 ng/L, and **(C)** children stratified by treatment regimen duration and day 7 lumefantrine level > 280 ng/mL and ≤ 280 ng/L. A represents 3-day AL, with a lumefantrine day 7 ≤ 280 ng/mL (red); B: 3-day AL, lumefantrine day 7 > 280 ng/mL (dark red); C: 5-day AL, lumefantrine day 7 ≤ 280 ng/mL (blue); D: 5-day AL, lumefantrine day 7 > 280 ng/mL (dark blue)

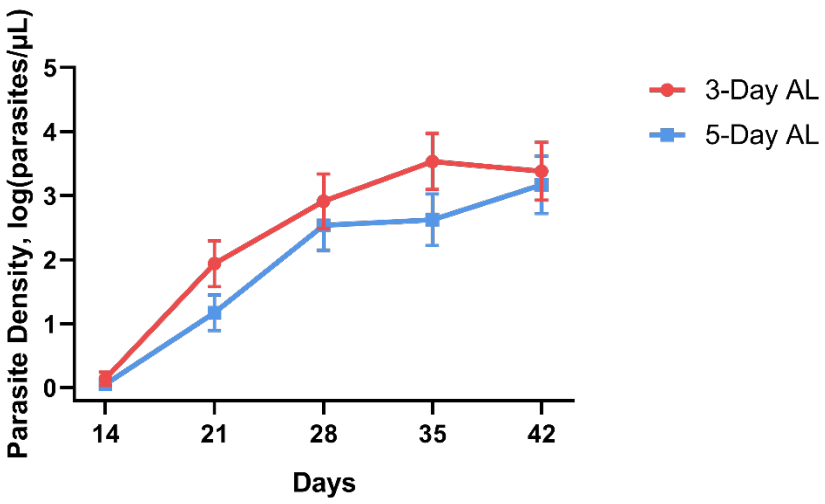

Figure 2.6 Parasite densities of children during recurrent episodes of parasitemia by treatment duration arm.

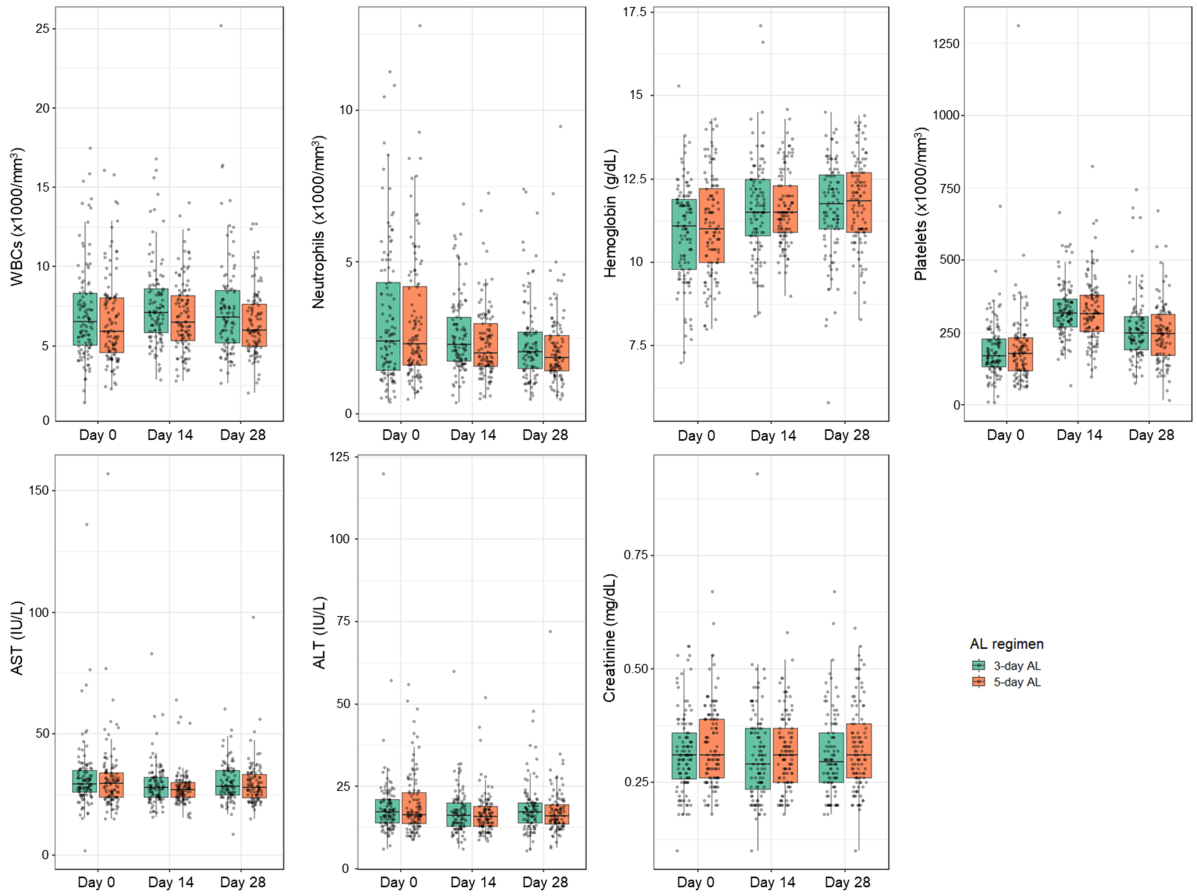

Figure 2.7 Laboratory values on days 0, 14, and 28 by AL regimen duration. Day 14 values include six children who followed up on day 15 (n=5) or day 17 (n=1). Day 28 values include twelve children who followed up on day 29 (n=2), day 30 (n=6), or day 31 (n=4). Data are presented for total white blood cells (x1000/per mm^3), neutrophils (x1000/per mm^3), hemoglobin (g/dL), platelets (x1000/per mm^3), aspartate aminotransferase (IU/L), alanine aminotransferase (IU/L), and creatinine (mg/dL).

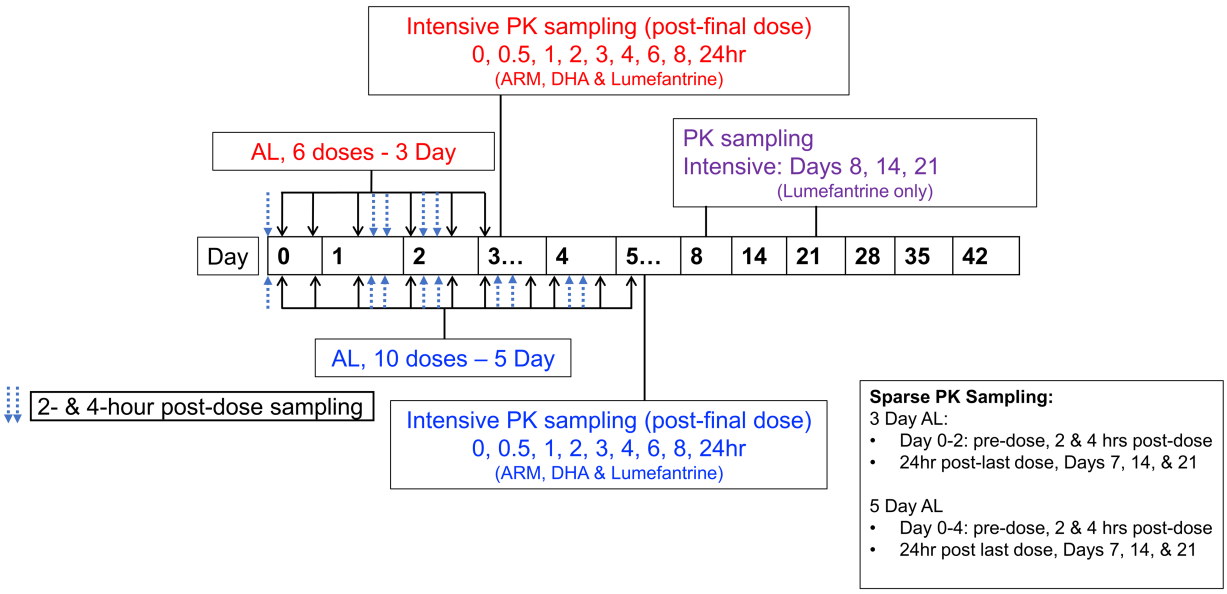

Figure 2.8 PK sampling and clinical follow-up schedule.

Tables 2.7

Table 2.1 Demographics of Study Participants in the ITT cohort

	AL Dosing Regimen group			
	3-Day AL (N = 114 episodes /87subjects)	*5-Day AL (N = 113 episodes/90 subjects)*	*Total (N = 227)*	*P Value*
Malaria episodes, per enrolled child				
One	87 (76.3%)	90 (79.6%)	177 (78.0%)	0.69
Two	22 (19.3%)	21 (18.6%)	43 (18.9%)	
Three	3 (2.6%)	2 (1.8%)	5 (2.2%)	
Four	2 (1.8%)		2 (0.9%)	
PK study arm				
Intensive	51 (44.7%)	51 (45.1%)	102 (44.9%)	0.95
Sparse	63 (55.3%)	62 (54.9%)	125 (55.1%)	
Age (Years)				
Median (IQR)	5.3 (4.1 – 7.9)	5.9 (4.1 – 8.0)	5.8 (4.1 – 8.0)	0.24
Sex				
Female	64 (56.1%)	60 (53.1%)	124 (54.6%)	0.65
Height (cm)				
Median (IQR)	105.0 (93.0 – 118.0)	108.0 (96.0 – 124.0)	107.0 (95.0 – 122.0)	0.26
Weight (kg)				
Median (IQR)	17.3 (15.1 – 23.0)	19.1 (15.4 – 22.6)	18.3 (15.3 – 22.9)	0.26
Parasite density at diagnosis				
Geometric mean (95% CI)	8552 (6112-12821)	10293 (7065-14995)	9542 (7342-12403)	0.50
Gametocytes detected by microscopy on the day of diagnosis				
Yes	34 (29.8%)	30 (26.5%)	64 (28.2%)	0.58
No	80 (70.2%)	83 (73.5%)	163 (71.8%)	
Artemether dosing (mg/kg) per each dose				
Median (IQR)	2.0 (1.7 – 2.3)	2.1 (1.8 – 2.4)	2.0 (1.8 – 2.4)	0.37
Lumefantrine dosing (mg/kg) per each dose				
Median (IQR)	12.2 (10.4 – 14.0)	12.3 (11.0 – 14.1)	12.3 (10.7 – 14.1)	0.37

Table 2.2 Artemisinin pharmacokinetics following a 6-dose regimen or 10-dose regimen of AL in children undergoing intensive PK sampling

Pharmacokinetic Parameter	3-Day AL n=50	5-Day AL n=50	Ratio (p-value) 5-Day AL / 3-Day AL
Artemether			
C_{max}, ng/mL, geometric mean (95% CI)	32.5 (25.4, 41.5)	27.3 (20.5, 36.3)	0.84 (0.34)
t_{max}, hr	1.10 (0.98, 2.03)	1.08 (0.97, 2.02)	0.98 (0.74)
$AUC_{0\text{-}8hr}$, hr·ng/mL, geometric mean (95% CI)	95.8 (77.5, 118)	78.6 (61.3, 101)	0.82 (0.25)
C_{8hr}, ng/mL	3.6 (2.58, 6.33)	2.72 (1.51, 5.33)	0.75 (0.07)
AUC_{cum}, hr·ng/mL, geometric mean (95% CI)[a]	792 (645, 974)	1344 (1090, 1656)	1.70 (0.001)
Dihydroartemisinin			
C_{max}, ng/mL, geometric mean (95% CI)	89.0 (77.4, 102)	87.9 (75.8, 102)	0.99 (0.83)
t_{max}, hr	2.00 (1.00, 2.03)	2.00 (1.08, 2.08)	1.00 (0.68)
$AUC_{0\text{-}8hr}$, hr·ng/mL, geometric mean (95% CI)	241 (216, 269)	229 (202, 261)	0.95 (0.52)
C_{8hr}, ng/mL	4.09 (2.73, 6.32)	3.36 (2.48, 6.11)	0.82 (0.23)
AUC_{cum}, hr·ng/mL, geometric mean (95% CI)[b]	1670 (1467, 1901)	3038 (2629, 3510)	1.82 (<0.0001)

Abbreviations: AL, artemether-lumefantrine; $AUC_{0\text{-}8hr}$, area under the concentration-time curve post-last dose; AUC_{cum}, area under the concentration-time curve post-third dose to Day 21; C_{8hr}, concentration 8 hours post-last dose; CI, confidence interval; C_{max}, maximal concentration post-last dose; IQR, interquartile range; t_{max}, time to maximal concentration post-last dose. Per protocol cohort.

[a]Artemether AUC_{cum}: N = 48 in 3-day AL group, N = 45 in 5-day AL group
[b]Dihydroartemisinin AUC_{cum}: N = 50 in 3-day AL group, N = 45 in 5-day AL group

Table 2.3 Comparing Artemether and DHA post-third dose exposure to post-last dose exposure in children receiving 3- or 5-day AL in the intensive PK sampling study arm

	Post-3rd Dose of AL GM; 95%CI	Post-Last Dose of AL (Dose 6 or 10) GM; 95%CI	GMR Ratio (pre-/post-last dose) (p-value)
	Artemether, C_{2hr}, ng/mL		
3-Day AL Regimen[a]	60.1 (47.3, 76.4)	19.4 (15.3, 24.7)	0.32 (<0.0001)
5-Day AL Regimen[b]	51.5 (39.9, 66.5)	18.0 (13.6, 23.8)	0.35 (<0.0001)
	DHA, C_{2hr}, ng/mL		
3-Day AL Regimen[c]	105 (83.6, 132)	59.2 (49.4, 71.0)	0.57 (<0.0001)
5-Day AL Regimen[d]	89.3 (72.5, 110)	63.8 (53.8, 75.5)	0.71 (0.0039)

Wilcoxon signed-rank test used for all; GM (95% CI). Per protocol cohort. Abbreviations: AL, artemether-lumefantrine; C_{2hr}, concentration 2-hours post-dose; CI, confidence interval; DHA, dihydroartemisinin; GM, geometric mean; GMR, geometric mean ratio

[a] 3-Day AL: post-3rd dose, n=49; post-last dose AL, n=50

[b] 5-Day AL: post-3rd dose, n=49; post-last dose AL, n=50

[c] 3-Day AL: post-3rd dose, n=50; post-last dose AL, n=50

[d] 5-Day AL: post-3rd dose, n=49; post-last dose AL, n=50

Table 2.4 Lumefantrine Pharmacokinetics Following a 6-Dose Regimen or 10-Dose Regimen of Artemether-Lumefantrine

	3-Day AL	5-Day AL	Ratio (p-value)
Pharmacokinetic Parameter	Median (IQR)	Median (IQR)	
Intensive PK arm	n=50	n=50	5 Day AL / 3 Day AL
C_{max}, ng/mL, GM (95% CI)	7236 (6023, 8692)	8450 (7085, 10079)	1.16 (0.39)
t_{max}, hr	4.00 (0.00, 6.00)	4.00 (1.00, 6.00)	1.00 (0.69)
$t_{1/2}$, hr [a]	120 (91.4, 158)	97.4 (81.6, 119.1)	0.81 (0.007)
$AUC_{0\text{-}21d}$, hr·µg/mL, GM (95% CI)	259 (222, 302)	318 (274, 370)	1.22 (0.12)
AUC_{cum}, hr·ug/mL, GM (95% CI) [b]	468 (410, 534)	852 (746, 974)	1.82 (< 0.0001)
Intensive + sparse sampling PK episodes	n=109	n=110	
C_{7d}, ng/mL [c]	363 (188, 478)	816 (524, 1290)	2.25 (< 0.0001)
Day 7 > 280 ng/mL	69 (63.3%)	99 (90.8%)	< 0.001
C_{14d}, ng/mL [d]	122 (86.7, 171)	186 (122, 269.5)	1.52 (< 0.0001)
C_{21d}, ng/mL [e]	65.0 (BLQ, 85.2)	89.1 (63.5, 116)	1.37 (< 0.0001)

Data are from the per protocol analysis and are presented as frequency (percentage) or median (interquartile range) unless otherwise specified. C_{max}, t_{max}, $t_{1/2}$, & $AUC_{0\text{-}21d}$ data all refer to post-last dose values.

Abbreviations: AL, artemether-lumefantrine; AUC, area under the concentration-time curve; AUC_{cum}, area under the concentration-time curve from post-3rd dose until day 21; BLQ, below the limit of quantitation; CI, confidence interval; C_{max}, maximal concentration; C_{7d}, concentration at day 7; C_{14d}, concentration at day 14; C_{21d}, concentration at day 21; GM, geometric mean; PK, pharmacokinetics; $t_{1/2}$, elimination half-life; t_{max}, time to maximal concentration.

[a] Due to the additional dosing days and set terminal concentration sampling times, the 5-Day AL group has a shorter window between the end of AL dosing and the C_{7d} sampling time than the 3-Day AL group. This caused the $t_{1/2}$ in the 5-Day AL regimen to appear overly short when compared to the 3-Day group. N= 50 for $t_{1/2}$ in 3-Day AL group; 49 for $t_{1/2}$ in 5-Day AL group
[b] N= 50 for AUC_{cum} in 3-Day AL group; 45 for AUC_{cum} in 5-Day AL group
[c] N= 109 for C_{7d} in 3-Day AL group; 109 for C_{7d} in 5-Day AL group
[d] N= 106 for C_{14d} in 3-Day AL group; 108 for C_{14d} in 5-Day AL group
[e] N= 102 for C_{21d} in 3-Day AL group; 108 for C_{21d} in 5-Day AL group

Table 2.5 28-Day & 42-Day Treatment Outcomes Stratified by Treatment Regimen

	3-day AL (n, %)	5-day AL (n, %)	p-value
Recurrent parasitemia by day 28	55/114 (48.2%)	44/113 (38.9%)	0.12
Recurrent parasitemia by day 42	83/114 (72.8%)	75/113 (66.4%)	0.29
Day 28 WHO Outcomes (uncorrected)			
Adequate clinical and parasitological response (ACPR)	54 (49.5%)	66 (60.0%)	
Late Clinical Failure (LCF)	12 (11.0%)	9 (8.2%)	0.29
Late Parasitological Failure (LPF)	43 (39.4%)	35 (31.8%)	
Day 42 WHO Outcomes (uncorrected)			
Adequate clinical and parasitological response (ACPR)	27 (24.5%)	35 (31.8%)	
Late Clinical Failure (LCF)	26 (23.6%)	25 (22.7%)	0.47
Late Parasitological Failure (LPF)	57(51.8%)	50 (45.5%)	

*Data are presented as number (percentage) with polymerase chain reaction–unadjusted treatment outcome.

Table 2.6 Multivariate Cox regression analysis of PK exposure and 28-day and 42-day outcomes of recurrent parasitemia in the ITT cohort

	Day 28 Outcome		Day 42 Outcome	
	HR (95% CI)	P value	HR (95% CI)	P value
Overall cohort	n = 217		n = 217	
AL 5 Day vs. 3 Day	0.95 (0.62, 1.46)	0.82	0.92 (0.66, 1.27)	0.614
Lumefantrine at Day 7, >280 vs. ≤ 280	0.54 (0.32, 0.91)	0.021	0.58 (0.38, 0.88)	0.010
Intensive PK participants only	n = 98		n = 98	
AL 5 Day vs. 3 Day	0.47 (0.25, 0.88)	0.019	0.74 (0.46, 1.21)	0.23
Lumefantrine $AUC_{0\text{-}21d}$	0.54 (0.31, 0.93)	0.028	0.61 (0.38, 0.97)	0.038

Cox regression models with robust sandwich estimation on the risk of recurrent parasitemia by AL arms, adjusted with age, sex, weight, baseline HGB, baseline parasite density, patient indicator for trial arm crossover, patient indication for multiple episodes, lumefantrine mg/kg

Table 2.7 LS Mean estimated Parasite Density (log) from Repeated Measurement Models

All Episodes	**AL 3-Day**	**AL 5-Day**	**Δ 5-Day vs. 3 Day**	**P_{Δ}**
Day 14 (n= 217)	0.03 (0.34)	0.13 (0.33)	-0.10 (0.47)	0.84
Day 21 (n= 217)	2.57 (0.34)	2.97 (0.36)	-0.77 (0.47)	0.10
Day 28 (n= 200)	2.70 (0.35)	3.67 (0.36)	-0.40 (0.49)	0.42
Day 35 (n= 189)	3.31 (0.37)	3.56 (0.38)	-0.97 (0.51)	0.055
Day 42 (n= 171)	1.16 (0.34)	1.94 (0.33)	-0.25 (0.53)	0.64
Recurrent Episodes	**AL 3-Day**	**AL 5-Day**	**Δ 5-Day vs. 3 Day**	**P_{Δ}**
Day 14 (n= 48)	0 (0.68)	-0.04 (0.63)	0.04 (0.93)	0.97
Day 21 (n= 49)	0.70 (0.68)	1.89 (0.62)	-1.19 (0.92)	0.20
Day 28 (n= 47)	0.68 (0.68)	2.39 (0.64)	-1.72 (0.94)	0.07
Day 35 (n= 45)	2.17 (0.70)	3.75 (0.65)	-1.59 (0.96)	0.10
Day 42 (n= 40)	2.93 (0.76)	2.60 (0.68)	0.34 (1.02)	0.74

Parasite density was log transformed, and measures were grouped into 5 timepoints: 14±1, 21±1, 28±1, 35±1, 42±1 days. Time was treated as categorical variable. Model adjusted with parasite density at day 0, and an interaction term between AL regimen and time.

Earliest recurrent parasitemia is at day 10 among all episodes.

Table 2.8 Demographics and PK data for children in the 3-day regimen by day 7 lumefantrine levels

	Day 7 Lumefantrine level			
	≤ 280 ng/ml (N = 40)	***> 280 ng/ml (N = 69)***	***Total (N = 109)***	***P Value***
PK Arms				
Intensive PK	14 (35.0%)	36 (52.2%)	50 (45.9%)	0.08
Population PK	26 (65.0%)	33 (47.8%)	59 (54.1%)	
Episodes				
First	31 (77.5%)	51 (73.9%)	82 (75.2%)	0.81
Recur, Early Enroll	1 (2.5%)	4 (5.8%)	5 (4.6%)	
Recur, Late Enroll	8 (20.0%)	14 (20.3%)	22 (20.2%)	
Age (Year)				
Median (IQR)	4.6 (3.5 – 6.9)	5.8 (4.2 – 8.4)	5.3 (4.1 – 8.0)	0.07
Sex:				
Male	17 (42.5%)	32 (46.4%)	49 (45.0%)	0.69
Female	23 (57.5%)	37 (53.6%)	60 (55.0%)	
Height				
Median (IQR)	97.5 (90.1 – 109.0)	109.0 (95.6 – 126.0)	104.0 (93.0 – 118.0)	0.048*
Weight				
Median (IQR)	15.6 (13.6 – 19.3)	19.1 (16.0 – 23.9)	17.4 (15.1 – 23.1)	0.001**
Parasite Density at Day 0				
Geometric mean (95% CI)	9512 (3108-4911)	8845 (5523-14166)	9084 (6225-13257)	0.76
Gametocytes at Day 0				
Yes	9 (22.5%)	22 (31.9%)	31 (28.4%)	0.30
No	31 (77.5%)	47 (68.1%)	78 (71.6%)	
Artemether dosing (mg/kg)				
Median (IQR)	1.9 (1.6 – 2.3)	2.1 (1.8 – 2.4)	2.0 (1.7 – 2.3)	0.23
Lumefantrine dosing (mg/kg)				
Median (IQR)	11.5 (9.8 – 14.0)	12.4 (10.7 – 14.1)	12.2 (10.4 – 14.0)	0.23
Lumefantrine mg per dose				
120	16 (40.0%)	9 (13.0%)	25 (22.9%)	0.009**
240	19 (47.5%)	45 (65.2%)	64 (58.7%)	
360	5 (12.5%)	12 (17.4%)	17 (15.6%)	
480	0 (0.0%)	3 (4.3%)	3 (2.8%)	

Chapter 3: Impact of extended duration treatment on the pharmacokinetics and pharmacodynamics of artemether-lumefantrine in HIV-infected children on efavirenz-based antiretroviral therapy

3.1 Introduction

Malaria and human immunodeficiency virus (HIV) infection continue to cause high rates of morbidity and mortality in vulnerable populations, including children in sub-Saharan Africa. For malaria, despite previous progress in driving down cases and deaths, this progress has stalled, and malaria cases have begun to increase. In 2020 alone, there were roughly 242 million cases, up from 227 million cases in 2019, and 627,000 deaths [1]. Sub-Saharan Africa continues to account for > 90% of deaths due to malaria, primarily in children, with Uganda, specifically, having the 3rd highest number of malaria cases in 2020 [1].

In addition to the heavy load of malaria cases, sub-Saharan Africa also bears the highest burden of HIV infections with the risk of malaria-HIV co-infection remaining prevalent in children and adolescents**.** There are currently 25 million people with HIV who reside in sub-Saharan Africa, and 2.9 million are children <15 years [108-110]. Eastern and southern Africa are the places most heavily affected, accounting for approximately 55% of all people living with HIV, and home to two thirds of all HIV-infected children [109]. Uganda, specifically, has 1.4 million adults and children living with HIV [44]. These high rates of both malaria and HIV increase the likelihood of co-infection, raising the potential for highly significant drug-drug interactions between antimalarial medications and HIV anti-retroviral therapy (ART).

Artemisinin-based combination therapies (ACTs) are the standard of care for treating uncomplicated *Plasmodium falciparum* malaria [111] and include a short-acting artemisinin component [e.g. artemether and dihydroartemisinin (DHA)] that rapidly reduces parasite burden and a long-acting partner drug (e.g. lumefantrine) that eliminates residual parasites and protects against recurrent infection. Currently, artemether-lumefantrine (AL) is the most widely used ACT worldwide [111, 112]. Artemether is metabolized to DHA via cytochrome P450 3A4 (CYP3A4); both compounds are active against malaria [48, 113, 114]. Once formed, DHA undergoes glucuronidation via uridine diphosphate glucuronosyltransferase (UGT) before excretion [50]. Both artemether and DHA have relatively short half-lives, estimated as ~1 hour for each [90]. Long-acting lumefantrine (LF), is characterized by a much longer half-life estimated as 3-5 days [90]. It is converted by CYP3A4 to desbutyl-lumefantrine (DBL), an active metabolite that is 4-8 times more potent than LF, although due to low measurable levels, it remains unclear how much DBL contributes to overall antimalarial activity. Specifically, the ratio of systemic exposure of DBL to LF has been estimated to be 1/100 [38, 39, 48, 81, 82, 115].

Maintaining sufficient levels of drug exposure for all ACT drug components is key to ensuring adequate clinical response, and it has been confirmed that administering AL with high fat foods (such as milk) significantly increases the bioavailability of artemether and LF; thus, concomitant food intake is recommended [43, 82]. Indeed, low LF concentrations (i.e. concentrations on day 7, 14, and 21 post-treatment initiation) have been associated with malaria treatment failure and increased risk of exposing malaria parasites to subtherapeutic drug concentrations, which may select for drug resistance [54, 69, 90]. With this in mind, we believe optimized treatment with ACTs in children must have a three-pronged approach: effectively treating the current infection, maximizing the post-treatment prevention recurrent infections, and minimizing the selection of resistant malaria parasites by ensuring appropriate drug plasma concentrations [83, 85].

Per the World Health Organization (WHO) clinical guidelines for HIV, all HIV-infected children, regardless of disease status, should receive ART [116]. A high percentage of these children over 3 years of age receive efavirenz (EFV)-based ART, despite the WHO recently recommending dolutegravir-based ART as first line in all populations, including children [116]. A recent report estimated that, in spite of new guidelines, dolutegravir-based regimens accounted for only ~18% of pediatric ART in low and middle income countries in 2020, while lopinavir/ritonavir-, EFV-, and nevirapine-based ART accounted for 53%, 19%, and 10%, respectively, a distribution that is expected to shift over time. [117] As the transition to dolutegravir slowly continues and is unclear when (and if) all countries will complete this transition, children will continue to receive EFV-based ART. Additionally, if any children are unable to tolerate dolutegravir, EFV is a likely alternative therapy. The continued use of EFV underscores the importance of investigating the impact of EFV on concomitant therapies, including antimalarials, to best inform guidelines specifying appropriate dosing regimens.

Multiple studies have shown that ART choice, such as EFV, influences AL pharmacokinetics (PK) as well as malaria treatment outcomes due to pronounced drug-drug interactions [54, 56, 118, 119]. EFV is metabolized mainly by CYP2B6 and CYP3A4 and is a known autoinducer of CYP3A4 [120, 121]. Due to the shared metabolism pathway between AL and EFV, and the autoinduction effect of EFV, AL PK is significantly altered in the context of EFV leading to exposure reductions in all AL drug components from 30-70% [54, 56, 57]. Our group and others have shown that EFV leads to a significant reduction in artemisinin and LF exposure and could leave children on EFV-based ART vulnerable to treatment failure when compared to children on other ART [54, 119]. As the absorption of LF is dose limited, an increase in mg/kg dosing is likely ineffective [43]. Thus, an extended treatment duration has been suggested to improve AL exposure [38, 43, 54, 122]. Extending the AL treatment regimen from 3 days to 5 days is

hypothesized to compensate for the EFV effect and remedy the differences in LF exposure, a compensation expected to protect against poor treatment outcomes.

This dissertation chapter details our PK and exposure-outcomes investigation of the AL-EFV interaction in HIV infected children who present with uncomplicated malaria. We evaluated the use of a 5-day AL treatment regimen, in comparison to the standard 3-day AL treatment, in these children to compensate for the AL-EFV interaction. In addition, we compared results for both groups to results in a group of HIV-uninfected children not on EFV and receiving standard 3-day AL treatment. We conducted the Extended Duration AL Treatment for Malaria in Children (EXALT) trial and hypothesized that an extended 5-day AL regimen would significantly improve PK exposure to both the artemisinin and LF components of AL and that the improved drug exposure of all ACT drug components would lower the odds of 28- and 42-day recurrent parasitemia.

3.2 Methods

EXALT (Extended Duration AL Treatment for Malaria in Children), a randomized, open-label prospective pharmacokinetic/pharmacodynamic (PK/PD) study, compared the standard 3-day (6-dose) regimen of AL to an extended 5-day (10-dose) AL regimen for the treatment of uncomplicated malaria in both HIV-uninfected and HIV-infected children (Figure 3.1). From August 2018 to January 2020, eligible participants (HIV-infected children on EFV-based ART and HIV-uninfected children) were recruited in Busia, Uganda at our study clinic, located on the campus of the Masufu General Hospital. Children presented with uncomplicated *P. falciparum* (mono- or mixed infection) malaria; eligible participants had a fever or history of fever within the last 24 hours (tympanic temperature of ≥ 38°C) and a positive thick blood smear. Participants could not enroll if they had been treated for malaria within the past 28 days. Children were aged

6 months to 18 years for HIV-uninfected individuals or 3 to 18 years for HIV-infected children; clinical outcomes analysis included all children, but also explored limiting the analysis to children only 3 to 18 years of age in both groups to ensure ages were matched appropriately and younger (< 3 years of age) children did not impact the clinical results. Additionally, eligible children had a hemoglobin ≥ 7 g/dL; weighed ≥ 6 kg; lived within 60 km of the study clinic; and had not taken medications (other than the study drugs) known to affect CYP3A4 metabolism, such as antituberculosis medications (i.e., rifampin) or antifungals (i.e., itraconazole and ketoconazole). HIV status was confirmed positive (via rapid HIV test + Western Blot or HIV RNA after enrollment) for the HIV-infected individuals. HIV-infected children must have initiated EFV-based ART for at least 10 days prior to enrolling in the study. All HIV-infected children were on standard weight-based dosing of EFV and daily trimethoprim-sulfamethoxazole prophylaxis [116]. All children, both HIV-infected and uninfected children, were randomized 1:1 to the 3-day (6-dose) or 5-day (10-dose) AL arm. In this chapter, however, only HIV-uninfected children treated with the standard-of-care, 3-day AL regimen are reported; the HIV-uninfected 5-day AL group has previously been discussed (Chapter 2). For the HIV-infected children on EFV-based ART, the 3-day AL and 5-day AL arms are assessed. All children could be re-enrolled for up to 2 times in the intensive sampling cohort and 2 times in the sparse sampling cohort, for a maximum of 4 total episodes. Re-randomization occurred for each new enrollment. Participants were enrolled in the sparse PK sampling cohorts only when target sample sizes were met for the intensive PK study arm or if the child had already been enrolled twice in the intensive PK study arm.

The trial was registered at http://ClinicalTrials.gov. Protocols and procedures were approved by the Uganda National Council of Science and Technology, the Makerere University School of Medicine Research Ethics Committee, the University of California, San Francisco Committee on Human Research, and the Yale University Human Investigations Committee. Written informed

consent was obtained from the parents or guardians of the children, and children ≥7 years of age were also assented.

Clinical Management and Pharmacokinetic Methods

Children enrolled had uncomplicated *P. falciparum* malaria confirmed by thick blood smear (regardless of parasite density) and either a documented or 24-hour fever history (≥ 38.0°C). For the intensive sampling cohort, follow-up occurred on days 0 (diagnosis), 1, 2, 3, 4, (5, 6 for the 5-day AL group), 8, 14, 21, 28, 35 and 42. For the sparse sampling group, follow-up occurred on days 0 (diagnosis), 1, 2, 3, (4, 5 for the 5-day AL group), 7, 14, 21, 28, 35 and 42.

HIV-infected children on EFV-based ART were randomized to either a standard 6-dose treatment of weight-based AL (Coartem® Dispersible 20 mg/120 mg, Novartis Pharma AG, Basel, Switzerland) over the course of 3 days or an extended 10-dose treatment over 5 days (Table 3.1). All HIV-uninfected children evaluated in this manuscript received the standard 6-dose of weight-based AL (3-day AL treatment). Doses were administered with milk in the clinic or at home, to enhance and control for LF absorption [95]. For the intensive sampling arm, the first, third, fourth and sixth doses in all groups were observed in the clinic; the additional eighth and tenth doses were also directly observed for those in the 5-Day AL group. The second and fifth doses (in addition to the seventh and ninth in the 5-Day AL arm) were taken at home and the time of administration recorded. The dosing schedule in the intensive arm was slightly extended so that the last dose was administered in the morning to facilitate intensive PK sampling. For the sparse sampling arm, the first, third, and fifth doses in all groups were observed in the clinic, with the seventh and ninth doses also directly observed for those in the 5-Day AL group.

The primary outcome was to evaluate PK parameters of LF, ARM, and DHA in the three cohorts. Secondary outcomes included examining differences in DBL PK parameters and comparing incidence of recurrent parasitemia, as determined by microscopy obtained on every clinic visit day, between groups.

Pharmacokinetic sampling and processing

3-day AL (6-dose regimen): For children in the *intensive PK cohort*, sampling occurred on day 0 (pre-dose), 2-and 4-hour post-dose on days 1 and 2, and serially (0 hr, 0.5 hr, 1 hr, 2 hr, 3 hr, 4 hr, 6 hr, 8 hr) on day 3 to determine artemether, DHA, and LF concentrations (Figure 3.2). Sampling also occurred on days 4 (i.e. 24 hr post-third dose), 8, 14, and 21 to generate LF concentrations. For children in the *sparse PK cohort*, a pre-dose sample was collected on day 0 and samples were collected 2- and 4-hours post-dose on days 1, 2, and 3. Additional samples were collected on days 7, 14, and 21.

5-day AL (10-dose regimen): For children in the *intensive PK cohort*, samples were collected on day 0 (pre-dose), 2- and 4-hours post dose on days 1, 2, 3, and 4, and serial sampling occurred on day 5 (0 hr, 0.5 hr, 1 hr, 2 hr, 3 hr, 4 hr, 6 hr, 8 hr) to measure artemether, DHA, and LF concentration data (Figure 3.2). Additional sampling occurred on days 6, 8, 14, and 21 for the determination of LF concentrations. In the *sparse PK cohort*, sampling occurred on days 0, 1, 2, 3, 4, 5, 7, 14, and 21.

Concentrations of artemether and DHA were determined using liquid chromatography–tandem mass spectrometry (LCMS/MS), as previously described [96]. The calibration range was 0.5–200 ng/mL with the lower limit of quantification (LLOQ) at 0.5 ng/mL for both artemether and DHA. LF quantitation was performed on a Waters® UPLC® I class system coupled with Sciex

TripleQuad 6500^{+} tandem mass spectrometry system based on a previous method with modifications [97]. The changes were as follows: plasma sample volume was reduced from 25 µl to 5 µL, and the instrument time per sample was reduced from 8 min to <2 min with a newer UPLC column. The calibration range was 50–20,000 ng/ml with the LLOQ at 50 ng/mL. DBL quantitation was also performed on a Waters® UPLC® I class system coupled with a Sciex TripleQuad 6500^{+} tandem mass spectrometry system. Briefly, 100 uL of 5% formic acid was added to 1.5 mL Eppendorf tubes followed by 10 µL of the internal standard working solution (1/100 ng/mL DBL/LF in 50% acetonitrile + 0.5% formic acid). To the double blank, 10 µL of 50% acetonitrile + 0.5% formic acid was added. Using a micropipette, 5 µL of each plasma sample, or 5 µL of blank plasma for the blank and double blank, was added into the Eppendorf tubes and triturated briefly. After trituration, 900 µL of ethyl acetate was added to each tube, vortexed briefly, rotated for 30 minutes, followed with a 1-minute centrifugation at 20,000 *g*. The organic phase was then transferred into clean test tubes, dried under a gentle stream of nitrogen gas, reconstituted with 200 µL of 50% acetonitrile + 0.5% formic acid, and vortexed. The reconstituted solutions were transferred to a 96-well plate and 5 µL injected into the Waters® UPLC® I class system coupled with Sciex TripleQuad 6500^{+} tandem mass spectrometry system. Sample analysis took ~2 min on the LCMS/MS machine. The calibration range was 0.5-500 ng/mL for DBL with an LLOQ of 0.5 ng/ml.

Non-compartmental PK analyses

Intensive PK cohort analysis for both 3-day and 5-day AL included estimates of the area under the plasma concentration-time curve post-final dose (AUC_{0-8h} for artemether and DHA; AUC_{0-21d} for LF and DBL), maximal concentration (C_{max}), time to C_{max} (t_{max}), elimination half-life ($t_{1/2}$), and C_{8h} (artemether and DHA). AUC_{0-8h} post-last dose was utilized for artemether and DHA instead of an AUC_{0-24h} post-dose to ensure that most participants had a measurable drug

concentration for the last time point, as concentrations are often below the LLOQ by 24 hours post-dose. For intensive PK studies, PK parameters for each subject around the final dose were determined using non-compartmental analysis and followed a linear up-log down trapezoidal rule in conjunction with first-order input (Phoenix WinNonlin 64). In addition, an estimation of cumulative exposure [i.e. the AUC from the 3rd dose (of either regimen) to 8 hours post-last dose (AUC_{cum} for artemether and DHA; the AUC from the 3rd dose to day 21 (AUC_{cum}) for LF and DBL] was also calculated for ARM, DHA, LF, and DBL for individuals with complete data and who had participated in the intensive PK arms of the study. As we only collected pre-dose samples on first day of dosing, the 3rd dose was the earliest post-dose sample that could be utilized for either regimen to generate the cumulative exposure variable. Specifically, estimates relied on the 2- and 4-hour actual post-dose concentration levels on days 1, 2 (and days 3, 4 for the AL 10-dose regimen), and calculated trough concentrations on those days using an elimination rate constant determined from the post-final dose data. The trapezoidal rule was used to calculate AUCs for the morning, directly observed doses 3 and 4 (and 6, 8 for the AL 10-dose regimen), which were then also used as an estimate for artemether, DHA, LF, and DBL exposure for the evening doses 5 (and 7, 9 for the AL 10-dose regimen). All dose specific AUCs (i.e. third dose to the fifth or ninth dose) were added to the post-final dose estimated AUC ($AUC_{0-8\,h}$ for artemether and DHA; $AUC_{0\text{-}21d}$ for LF and DBL) to generate an estimated cumulative AUC for each component of AL (artemether or DHA AUC_{cum}: exposure from the third dose to 8 hours post-last dose; LF or DBL AUC_{cum}; exposure from the third dose to day 21). Phoenix Winonlin 64 and Microsoft Excel version 16.54 were used to determine cumulative exposure. Additionally, AUC_{DBL+LF}, a composite $AUC_{0\text{-}21d}$, was calculated for each individual in the intensive sampling arm who had both DBL and LF $AUC_{0\text{-}21d}$ data. As both DBL and LF are active compounds against malaria, we wanted to investigate their combined efficacy. DBL $AUC_{0\text{-}21d}$ was multiplied by 4 (i.e. weighted) to conservatively account for the increased potency

compared to LF and then combined with the LF $AUC_{0\text{-}21d}$ to generate the composite AUC_{DBL+LF} [39, 52, 123-125].

Capillary and venous samples collected concurrently at 2- and 8-hours post-dose on the intensive sampling day (i.e. day 3 for 3-Day AL group) in the intensive PK cohorts were used to compute capillary-venous correlations of DBL concentrations which permitted merging of capillary and venous measurements for PK analysis. Specifically, linear regression was used to determine the correlation between capillary and venous plasma DBL concentration results before, and after, natural log transformation of the data using STATA SE 14.2 (StataCorp, College Station, TX, USA). For sparse PK cohort data, LF concentrations at day 7, day 14, and day 21 were combined with the LF concentration data from the intensive PK cohort to compare exposure between the 3-day AL and 5-day AL groups. This was also done for day 7, 14, and 21 concentrations of DBL. All sample and PK analysis was completed within the Drug Research Unit, University of California, San Francisco.

Treatment Outcomes

Parasitemia and gametocytemia were assessed on day 0. Blood smears were also collected at each day of follow-up to monitor for parasitemia throughout the study.

Statistical Analysis

All children enrolled in the study, regardless of age, were included in the main analysis. An additional exploratory analysis was conducted in participants aged 3-18 years for all groups to ensure age differences between groups did not impact overall study results. For intensive PK, it was necessary to use at least 30 subjects for each arm to detect a $\geq$35% AUC difference of all analytes (80% power; $\alpha = 0.05$). This is based on a conservative estimated coefficient of

variation of 59% for exposure estimates of all analytes [82, 126]. Odds of recurrent parasitemia were assessed using generalized estimating equations (GEEs) with robust standard errors to account for repeated measures. Univariate GEE analysis was utilized to evaluate regimen, HIV status, age, baseline parasite density, baseline hemoglobin, sex, DBL exposure, and LF exposure before moving forward with selected covariates to the multivariate GEE analysis. A second analysis, evaluating only HIV-infected participants, also implemented univariate and multivariate GEEs with robust standard errors to assess odds of recurrent parasitemia. Covariates tested for the HIV-infected cohort univariate GEE analysis included regimen, age, baseline parasite density, baseline hemoglobin, sex, DBL exposure, and LF exposure. Wilcoxon rank sum tests were used to compare PK parameters between the three groups. Statistical significance was a two-sided p-value < 0.05, except for the PK parameter evaluations where a Bonferroni correction was used with significance set at <0.017 to account for comparison between 3 groups. Geometric means (GM) or medians were reported as appropriate. Data analysis was completed using STATA® version SE12.1 (StataCorp, College Station, TX, USA). Power calculations were based on observed mean AUC and standard deviations from our previous studies.

3.3 Results

Study Profile

Participants were enrolled into intensive or sparse PK arms (Table 3.2; Figure 3.3). Intensive and sparse cohorts were combined for analyses except for intensive PK parameters, as specified. This chapter focuses on three arms of the EXALT study (3-day AL HIV-uninfected, 3-day AL HIV-infected, and 5-day AL HIV-infected), while Chapter 2 evaluated the additional 5-day AL HIV-uninfected cohort. Children were screened for enrollment over the course of 764

episodes of malaria; 305 met enrollment criteria for the full study. Focusing on the three main arms of interest, and combining both the intensive and sparse cohorts, 181 episodes were included in the final PK/PD analysis (3-day AL HIV-uninfected: n = 110; 3-day AL HIV-infected: n = 36; 5-day AL HIV-infected: n = 35) For the PK/PD sub-analysis with only the intensive cohort, 110 episodes were included (50 HIV-uninfected, 3-day AL; 30 HIV-infected, 3-day AL; 30 HIV-infected, 5-day AL).

When compared to the 3-day AL HIV-uninfected cohort, parasite densities were comparable with the 3-day AL HIV-infected group and slightly lower in the 5-day AL HIV-infected group (Table 3.2). Parasite densities were comparable between HIV-infected groups (Table 3.2). Compared to the 3-day AL HIV-uninfected children who had a median age of 5.33 years, both 3-day AL and 5-day AL HIV-infected groups were older (median age, 11.5 and 10.4 years, respectively). Even after removing the eight children who were < 3 years of age, the median age of the 3-day AL HIV-uninfected group only shifted from 5.33 years to 5.55 years old and the 3-day AL HIV-uninfected cohort was still significantly younger than the children in the HIV-infected arms. Examining the HIV-infected arms, only three individuals were < 3 years of age (versus the 45 individuals in the 3-day AL HIV-uninfected arm). Due to this age difference, when compared to the 3-day AL HIV-uninfected group, the children in both HIV-infected arms weighed more: 60% more in the 3-day AL HIV-infected arm and 45% more in the 5-day AL HIV-infected group ($p < 0.0001$ for both). There were no differences in age or weight between the two HIV-infected arms. All other baseline characteristics were comparable between all three groups.

Pharmacokinetics of Artemether

Pharmacokinetic parameters for artemether in the intensive cohort are summarized in Table 3.3 and Figure 3.4. Compared to results from the 3-day AL HIV-uninfected arm, artemether AUC_{cum} was 38% lower in children in the 3-day AL HIV-infected group on EFV-based ART ($p=0.0104$).

Additionally, there was a trend towards a lower $AUC_{0\text{-}8\ hr}$ and lower C_{8hr} in the 3-day AL HIV-infected children when compared to HIV-uninfected children, though this did not reach statistical significance. There were no statistically significant differences in artemether PK parameters between the 5-day AL HIV-infected children and the 3-day AL HIV-uninfected group, with estimates of $AUC_{0–8h}$, (71.7 vs 95.8 h ×ng/mL, respectively) and AUC_{cum} (1021 vs 792 h × ng/mL) being similar between the two groups. Evaluating the two HIV-infected arms (5-day AL vs 3-day AL), AUC_{cum} was significantly higher (2.09-fold) in the 5-day AL group than in the 3-day AL group ($p = 0.0010$). There were no other significant differences in artemether PK parameters between the two arms.

Pharmacokinetics of DHA

Pharmacokinetic parameters for DHA in the intensive cohort are summarized in Table 3.3 and Figure 3.4. Compared to 3-day AL HIV-uninfected children, those in the 3-day AL HIV-infected, cohort had significantly lower C_{max} and $AUC_{0\text{-}8hr}$ (51% and 55% lower, respectively; $p < 0.0001$ for both comparisons). Similarly, AUC_{cum} and C_{8hr} were lower in 3-day AL HIV-infected children compared to those in the 3-day AL HIV-uninfected group (62% and 70% lower, respectively; $p < 0.0001$ for both comparisons).

Notably, there were also multiple differences in DHA PK parameters between the 3-day HIV-uninfected cohort and the 5-day AL HIV-infected children. The 5-day AL HIV-infected children had a 61% lower C_{max} and 63% lower C_{8hr} when compared to 3-day AL HIV-uninfected participants ($p < 0.0001$ for each). Additionally, DHA exposure post-final dose ($AUC_{0\text{-}8hr}$) was markedly reduced in the 5-day AL HIV-infected group when compared to the HIV-uninfected participants; 5-day AL participants had a 60% lower $AUC_{0\text{-}8hr}$ ($p < 0.0001$). In contrast, cumulative DHA exposure from the third dose until 8 hours post-final dose (AUC_{cum}) was

comparable between the 3-day AL HIV-uninfected children treated and the 5-day AL HIV-infected children on EFV-based ART (1670 vs 1486 h x ng/ml, respectively; $p < 0.3931$).

There were no differences in DHA PK parameters seen between the HIV-infected groups (3-day AL versus 5-day AL), except that AUC_{cum} was 2.31-fold higher in the 5-day AL group ($p=0.0001$).

Pharmacokinetics of Lumefantrine

Pharmacokinetic parameters of LF are summarized in Table 3.4 and Figure 3.5. Compared with the 3-day AL HIV-uninfected cohort, 3-day AL HIV-infected children demonstrated significant changes in LF $AUC_{0\text{-}21d}$ and AUC_{cum}: a 44% ($p = 0.0001$) and 37% ($p = 0.0001$) decrease, respectively. Additionally, C_{max} and $t_{1/2}$ appeared to be reduced in the 3-day HIV-infected children when compared to the HIV-uninfected cohort, though this did not reach statistical significance with the utilization of a Bonferroni correction ($p = 0.0267$ and 0.0288, respectively).

Evaluations between HIV-uninfected children and 5-day AL HIV-infected children resulted in a 40% lower LF $t_{1/2}$ in the 5-day AL HIV-infected cohort. Notably, with the aim of the 5-day AL treatment being to compensate for the reduction in LF exposure due to the drug-drug interaction with efavirenz, all other comparisons of LF PK parameters ($AUC_{0\text{-}21d}$, AUC_{cum}, C_{max}, t_{max}) revealed no statistically significant differences between the two groups.

Comparisons between the two HIV-infected arms revealed that cumulative LF exposure from the 3rd dose until day 21 (AUC_{cum}) was 90% higher in the 5-day AL group than the 3-day AL group (561 vs 296 h x μg/ml, $p = 0.0001$). Additionally, there was a trend towards significance for LF $AUC_{0\text{-}21d}$ with values 42% higher in the 5-day AL HIV-infected group than the 3-day AL

HIV-infected cohort (p = 0.0428). There were no significant differences in LF exposure found between the HIV-infected arms for $C_{max,}$ $t_{max,}$ or $t_{1/2}$.

Lumefantrine Day 7 Concentrations

Day 7 LF levels are commonly used as a rough estimate of AUC to predict therapeutic outcomes, and a threshold of > 280 ng/ml has been shown to be protective [38, 81, 127-130]. LF plasma concentrations were significantly lower in the 3-day AL HIV-infected children than in the 3-day AL HIV-uninfected children; median LF concentrations were 61% lower on study day 7 when compared to the HIV-uninfected arm (141 ng/ml vs 364 ng/ml, p < 0.0001). In contrast, compared to the 3-day AL HIV-uninfected group, 5-day AL HIV-infected children had 51% *higher* median day 7 LF concentrations (363 ng/ml vs 550 ng/ml, p = 0.0023). Comparing the two HIV-infected arms, the 5-day AL group had 3.9-fold higher median LF day 7 concentrations than the 3-day AL HIV-infected cohort (p < 0.0001).

Overall, HIV-infected 3-day AL-treated children had the lowest day 7 LF concentrations. Only 14% of HIV-infected 3-day AL-treated children met or exceeded the day 7 protective LF concentration threshold of 280 ng/mL, while 78% of 5-day AL-treated HIV-infected children and 64% of 3-day AL-treated HIV-uninfected children attained day 7 LF concentrations above 280 ng/ml.

Pharmacokinetics of Desbutyl-lumefantrine

Pharmacokinetic parameters of DBL are summarized in Table 3.4 and Figure 3.6 for this exploratory analysis in a sub-set of participants from the study. Children in the 3-day AL HIV-infected cohort had 29% lower DBL exposure post-final dose ($AUC_{0\text{-}21d}$) when compared to HIV-uninfected children (p = 0.0102). Likewise, the weighted DBL and LF composite AUC (AUC_{DBL+LF}) was also lower in children in the 3-day AL HIV-infected group than those in the HIV-

uninfected cohort (AUC_{DBL+LF}: 169 vs 288 h x μg/mL, p = 0.0049). All other PK parameters, including DBL concentrations on day 7, 14, and 21, AUC_{cum}, and C_{max}, were not significantly different between groups.

Comparisons of DBL PK parameters between 3-day AL HIV-uninfected children and 5-day AL HIV-infected children revealed that those in the 5-day AL HIV-infected cohort had a 14% shorter $t_{1/2}$ (p = 0.0098). Additionally, the HIV-infected 5-day AL group had significantly higher DBL concentrations on day 7, 14, and 21 (74%, 65%, and 46% higher, respectively; $p \leq 0.0118$ for all comparisons). Finally, no additional differences were seen between the 3-day AL HIV-uninfected group and the 5-day AL HIV-infected in the other PK parameters, including DBL C_{max}, $AUC_{0\text{-}21d}$, AUC_{cum}, and AUC_{DBL+LF}.

Comparing the two HIV-infected cohorts, both $AUC_{0\text{-}21d}$ and AUC_{cum} were higher in the 5-day AL HIV-infected children than those in the 3-day AL HIV-infected group (45% and 65% higher, respectively; $p \leq 0.0031$ for both comparisons). Children in the 5-day AL HIV-infected group had markedly higher LF concentrations than those in the 3-day AL HIV-infected group: 2.91-, 2.16-, 2.07-fold higher on days 7,14, and 21 respectively ($p < 0.0001$ for all comparisons). In contrast, those in the 5-day AL HIV-infected group had a shorter half-life than those in the 3-day AL HIV-infected cohort (Geometric Mean, 109 vs 130 hr, p = 0.0002).

Overall, in all three study arms, LF exposure was much higher than DBL exposure; LF $AUC_{0\text{-}21d}$ was $\geq$ 25x higher than DBL $AUC_{0\text{-}21d}$ for all treatment arms.

Correlation of capillary versus venous plasma DBL concentrations

Here we report, for the first time, on the correlation of simultaneously collected capillary plasma concentrations versus venous plasma concentrations of DBL, respectively (Figure 3.7). Briefly,

91 pairs of capillary and venous plasma samples collected simultaneously at 2- and 8-hours post-final AL dose were used to complete a linear regression analysis before, and after, the data had undergone a natural log transformation. The resulting equation for DBL capillary concentration (C_{cap}) vs venous concentration (C_{ven}) using untransformed data was $[C_{cap}] = 0.94*[C_{ven}] – 4.39$ (n = 91, $R^2 = 0.91$). The median ratio (interquartile range [IQR]) of artemether C_{cap}/C_{ven} was 1.01 (0.88, 1.10). Using log transformed data the equation for DBL capillary concentration (C_{cap}) vs venous concentration (C_{ven}) was $\ln[C_{cap}] = 0.95 * \ln[C_{ven}] – 0.22$ (n = 91, $R^2 = 0.92$). The median ratio (IQR) of log transformed DBL C_{cap}/C_{ven} was 1.00 (0.97, 1.02). From these results, it was determined that capillary and venous measurements of DBL have a 1:1 linear relationship. Due to this 1:1 linear relationship, DBL capillary concentration results from the EXALT study were not converted to predicted venous values using the generated correlation equations. For DHA and artemether, capillary plasma concentrations were used without conversion as the correlation was 1:1 (Chapter 2). Additionally, for LF, capillary and venous plasma concentrations were used interchangeably as a previous correlation was 1:1 [99].

Treatment Outcomes Analysis

Univariate analysis using generalized estimating equations (GEEs) with the combined population and intensive cohorts showed that HIV-infection, age, and baseline hemoglobin all were significant predictors for odds of recurrent parasitemia by day 28 ($p \leq 0.035$ for all) (Table 3.5). Additionally, AL treatment regimen (3-day vs 5-day) trended towards significance ($p = 0.051$) as a predictor for odds of recurrent parasitemia by day 28. When examining a combined variable of HIV-infection status and AL treatment regimen, 5-day AL HIV-infected children had a significantly lower odds of recurrent malaria than 3-day AL HIV-uninfected children (OR = 0.37, $p = 0.027$). These differences were not seen by day 42 (Table 3.6). Using GEEs adjusted for covariates (multivariate analysis), statistical significance was lost for all predictors described in the univariate analysis, and no relationship was observed between HIV-infection status, age,

baseline hemoglobin or AL-treatment regimen and clinical outcomes (recurrent parasitemia) both at day 28 and day 42. Additionally, associations between day 28 and day 42 treatment outcomes and AL exposure were explored using GEEs in both univariate and multivariate analysis. LF concentrations on day 7, 14, and 21, and DBL concentrations on day 7 and 14 had no observed relationship to odds of recurrent parasitemia during the univariate or multivariate analysis. The day 7 LF concentration threshold of 280 ng/mL was also not a significant predictor in odds of recurrent parasitemia by day 28 or day 42. During the univariate analysis, DBL day 21 concentration was a statistically significant predictor for the odds of recurrent parasitemia by day 28 ($p = 0.038$), but lost significance in the multivariate analysis. For the GEE analysis investigating odds of recurrent parasitemia by day 42, no LF or DBL exposure parameters were significant predictors.

Interestingly, in a sub-analysis evaluating only those in the intensive sampling cohorts, day 14 LF concentration and the HIV-infection status + AL regimen variable were both significant predictors of odds of recurrent parasitemia by day 28 (Table 3.7). In the univariate analysis, the combination variable of HIV status + AL regimen was a significant predictor for odds of recurrent parasitemia by day 28, with day 14 LF concentration, age and baseline hemoglobin trending towards significance ($p = 0.080$, 0.075, and 0.081, respectively). There was no significant relationship between day 7 LF concentration, day 21 LF concentration, LF $AUC_{0\text{-}21d}$, LF AUC_{cum}, DBL $AUC_{0\text{-}21d}$, DBL AUC_{cum}, or DBL concentrations on day 7, 14, or 21 and treatment outcomes (Table 3.7). The composite weighted DBL-LF AUC (AUC_{DBL+LF}) was also not a significant predictor for odds of recurrent parasitemia by day 28. For the multivariate analysis, when adjusted for age, sex, baseline parasitemia, and baseline hemoglobin, day 14 LF concentration was associated with a 55% lower odds of recurrent parasitemia ($p = 0.029$), while 3-day AL HIV-uninfected children had a higher odds of recurrent parasitemia compared to the 3-day AL HIV-infected children (74%, $p = 0.055$) and 5-day AL HIV-infected children (76%, $p = 0.032$). By day

42, there was a loss of significance for these covariates, and no patient or treatment characteristics were significant predictors by day 42 (Table 3.8).

Our GEE analysis focusing on only HIV-infected participants found that no disease or treatment characteristics were significant predictors of odds of recurrent parasitemia by day 28 or 42 (Table 3.9 and 3.10). AL regimen, age, hemoglobin, sex, and baseline parasitemia did not significantly impact the odds of recurrent parasitemia by either day 28 or day 42. Similar to the results of the GEE analysis that included the sparse and intensive data from all three study arms, LF and DBL concentrations on day 7, 14, and 21, and LF day 7 values > 280 ng/ml vs. ≤ 280, were not significant predictors of recurrent parasitemia. Additionally, when limited to the HIV-infected intensive sampling cohorts, none of the variables listed previously nor LF $AUC_{0\text{-}21d}$, LF AUC_{cum}, DBL $AUC_{0\text{-}21d}$, DBL AUC_{cum}, or AUC_{DBL+LF} were significant predictors ($p<0.05$) of recurrent parasitemia by day 28 or 42 for the univariate analysis.

3.4 DISCUSSION

Prior studies suggest that the standard of care 3-day six-dose AL treatment regimen under current weight-based dosing categories is associated with suboptimum exposure in children who are HIV-infected and managed with EFV-based ART [38]. We had previously reported a 4-fold higher risk of recurrent malaria in children managed with EFV and treated with 3-days of AL compared to other HIV-infected children on different ART regimens, differences attributed to a 10-fold reduction in AL exposure in the EFV-based ART group compared to children receiving a lopinavir/ritonavir combination known to have an opposing effect on CYP metabolism [54]. Knowing that LF absorption is dose limited, we hypothesized that this reduction in exposure could potentially be remedied with an extended treatment duration and demonstrated that such

an extension (as opposed to an increase in mg/kg given for each dose), increased AL exposure [38-40, 42, 54, 131]. To our knowledge, EXALT is the first study to evaluate an extended AL 5-day (10-doses) treatment in HIV-infected children on EFV-based ART versus the standard 3-day (6-doses) regimen in both HIV-uninfected and HIV-infected children (also on EFV-based ART), and assess the impact on clinical outcomes and PK parameters.

Extended duration AL significantly increased LF exposure and compensated for the EFV-induction effect. Data on LF PK from this study coincide with previous literature citing lower LF exposure in HIV-infected children treated with the standard 3 days of AL when compared to HIV-uninfected children [54, 56, 128, 132]. We saw a significant reduction in LF $AUC_{0\text{-}21d}$ (44% decrease) and concentrations on day 7, 14, and 21 between HIV-uninfected children and HIV-infected children on EFV-based ART, both treated with 3 days of AL. In contrast, when comparing the 5-day AL HIV-infected to 3-day AL HIV-uninfected children, there were no differences in LF $AUC_{0\text{-}21d}$ or AUC_{cum} between groups. This shows that 5-days of AL in HIV-infected children on EFV-based ART generates LF exposure that is equivalent to 3 days of AL in HIV-uninfected children and compensates for EFV-driven CYP3A4 induction, a result consistent with our overall goal for this study.

When comparing HIV-infected children on EFV-based ART, we found that the 5-day AL regimen significantly increased PK exposure of LF with a 1.9-fold higher estimated cumulative AUC and significantly higher LF concentrations on days 7 and 14, as compared to HIV-infected children receiving the 3-day AL regimen (3.90- and 2.01-fold, respectively; $p \leq 0.0002$ for all comparisons). Indeed, multiple PK/PD models and studies in the literature hypothesized that extending AL treatment to 5 or 7 days (as we have done) would compensate for the EFV-induction effect and restore LF exposure to levels seen in HIV-uninfected individuals receiving 3

days of AL [54, 56, 122, 131, 133]. The results from this study confirm what was hypothesized in the literature: 5 days of AL in HIV-infected study participants on EFV-based ART increases LF exposure to that of HIV-uninfected individuals given the standard 3 days of AL.

Our exploratory PK analysis of DBL, the active metabolite of LF, confirms the lower exposure of DBL when compared to LF; DBL exposure was only 1.85 – 3.89% of parent LF exposure, for all three groups. Interestingly, its exposure is reduced in the context of EFV-based ART, similar to LF. Based on our analysis in a sub-set of 76 participants, we found that DBL has a longer $t_{1/2}$ and significantly lower plasma concentrations than LF in all three arms of the study, with this finding consistent with what is seen in the literature [52, 134, 135]. Our median LF-to-DBL ratios of 33.1 (HIV-uninfected children), 26.3 (3-day AL HIV-infected children), and 25.4 (5-day AL HIV-infected children) coincide with the mean *in vivo* LF-to-DBL exposure ratio of 27.4 seen in Papua New Guinean children, and similar findings in Thai and Colombian patients [52, 134, 135]. Notably, there appears to be a similar theme of reduced DBL exposure in the context of HIV-infection and EFV-based ART. Children in the 3-day AL HIV-infected group had a 29% lower DBL $AUC_{0\text{-}21d}$ than children in the 3-day AL HIV-uninfected group. Additionally, though not statistically significant, DBL C_{max} and day 7, 14, and 21 concentrations appeared to be lower in the 3-day HIV-infected children when compared to the 3-day AL HIV-uninfected children. This could be due to the lower amount of lumefantrine in the 3-day AL HIV-infected children on EFV-based ART, resulting in less conversion to DBL, or possibly due to a direct effect of EFV on DBL PK. Data on the specific pharmacology of DBL is scarce. It is known that DBL undergoes glucuronidation via UGT, though the specific UGT isoform is unknown [136]. DBL is eliminated in the bile, likely via a transporter, similar to LF [82, 137]. Studies show that LF is excreted in the bile via efflux transporters, such as multidrug resistance–associated protein 2 (MRP2) and P-glycoprotein (P-gp); DBL may also be excreted via these pathways [138-140]. Data on the

impact of EFV on UGTs and drug efflux transporters is unclear, though one study shows EFV induces UGT1A1 [141]. Additional studies show that EFV induces both MRP2 and P-gp [142, 143]. Therefore, the lower DBL exposure could be due to EFV-driven induction of UGTs and drug transporters. The higher DBL concentrations on day 7, 14, and 21 and the shorter $t_{1/2}$ in the 5-day AL HIV-infected group when compared to the HIV-uninfected 3-day AL group is most likely due to additional days of dosing and there being less time from the final dose to the day 7, 14, and 21 sampling than in the 3-day AL HIV-uninfected group. Notably, the small sample sizes could have hindered a more complete understanding of DBL PK, and further research with more participants may be required to elucidate the full PK/PD of DBL in malaria infected children treated with AL.

Day 7 LF concentrations have been shown to be a surrogate marker of LF AUC and have been used to predict AL treatment efficacy by days 28 or 42 of follow-up, with concentrations that fall below the 280 ng/mL threshold being associated with increased risk of malaria recurrence or recurrent parasitemia [38, 54, 81, 129, 132]. Though we did see a greater number of participants meeting the protective 280 ng/mL cutoff on day 7 in the HIV-infected 5-day AL group (78%, versus 64% in the HIV-uninfected group and 14% in the 3-day HIV-infected group), overall, we did not see a significant difference in rates of recurrent parasitemia between the participants who achieved day 7 LF concentrations ≥ 280 ng/mL and those who did not. These results contradict those from a previous study in Tanzania, which evaluated HIV-infected adults on EFV-based ART with uncomplicated malaria and found that participants who had recurrent parasitemia by day 28, after treatment with AL, had lower median LF concentrations than those who did not have parasites. The Tanzanian study also found that the proportion of participants who achieved day 7 LF concentrations ≥ 280 ng/mL was higher in those who remained parasite-free [128]. Notably, our results also do not match our own findings from a previous study with

HIV-infected children on EFV-based ART, where day 7 concentrations were directly related to clinical outcomes [54]. However, the results from the EXALT study do coincide with those from a study in HIV-infected adults on EFV-based ART with uncomplicated malaria in Zambia, which did not find a relationship between day 7 LF concentrations and clinical outcomes [132]. These contradictory results are likely due, in part, to our small sample size and being underpowered to detect differences in clinical outcomes. Additionally, the Zambia study attempted to link day 7 LF concentrations to clinical outcomes at day 42, though our results show most of LF has been cleared by the body by day 42, so drug concentrations would likely have a limited impact on clinical outcomes. Importantly, in this analysis of all three arms with sparse and intensive sampling data combined, none of the other PK measurements for AL exposure significantly impacted clinical outcomes, including LF concentrations on day 14 and 21, LF $AUC_{0\text{-}21d}$, LF AUC_{cum}, DBL concentrations (on day 7, 14, and 21), DBL $AUC_{0\text{-}21d}$, DBL AUC_{cum}, and a composite AUC of DBL and LF (AUC_{DBL+LF}). None of these were significant predictors in the odds of recurrent parasitemia in the GEE multivariate analysis that included all three arms nor in the GEE analysis with only the HIV-infected participants.

In addition to LF and DBL exposure, other factors, such as AL regimen, were evaluated to determine if they impacted the odds of recurrent parasitemia. In the univariate GEE analysis for all three arms, HIV-status, baseline hemoglobin, and age were all statistically significant predictors ($p < 0.05$) of the odds of recurrent malaria by day 28, with AL regimen close to statistical significance ($p = 0.051$). When we combined HIV status and AL regimen into a new variable, this combination variable showed that the 3-day AL HIV-infected group did not have a significantly different odds of recurrent parasitemia by day 28 compared to the HIV-uninfected children and that the HIV-infected 5-day AL group had *lower* odds of recurrent parasitemia by day 28 than the HIV-uninfected group (OR 0.37, $p = 0.027$). However, when comparing the two

HIV-infected arms, AL regimen was not a significant predictor for the odds of recurrent parasitemia. Overall, the multivariate GEE analysis showed that none of the participants' disease or treatment characteristics, including AL PK parameters, predicted the odds of recurrent parasitemia by day 28 or day 42. This differs from our previous study that showed AL exposure, HIV-status, and baseline hemoglobin significantly impacted clinical outcomes [54]. Due to the difficulty in enrolling HIV-infected children on EFV-based ART, and our resulting small sample size, the study may not have been powered to identify predictors of recurrent parasitemia and evaluate trends in clinical outcomes in this study population.

Notably, in the analysis limited to children in the intensive PK cohorts, we saw a significantly reduced 28-day odds of recurrent parasitemia in both HIV-infected groups when compared to the HIV-uninfected children. Additionally, day 14 LF concentration also was associated with lower odds of recurrent parasitemia (OR 0.45, $p = 0.027$). A potential explanation for this contradiction with the larger analysis of sparse and intensive sampling data, may relate to treatment adherence, as children in the intensive sampling cohorts spent more time in the clinic, had an additional directly observed dose, and, in order to have final dose and intensive post-final dose sampling occur in the morning, their AL dosing regimen was extended by ~12 hours as compared to children in the sparse sampling cohorts. It is important to note, however, that when the analysis was limited to solely HIV-infected participants in the intensive PK cohorts, AL regimen and drug exposure (either LF or DBL) did not impact the odds of recurrent parasitemia.

There are multiple explanations for the conflicting results and lack of association between AL exposure and clinical outcomes, with the most likely being our small sample size and the study not being powered appropriately to detect small changes in clinical outcomes. This is especially true for the analysis limited to the HIV-infected cohorts (3-day AL vs 5-day AL) since we were

not able to recruit as many children (total HIV-infected episodes, n = 71 vs the desired 160). Additionally, for the analysis of all three arms, there are major differences in age and weight between the HIV-uninfected and the HIV-infected children, with both groups of the HIV-infected children (3-day AL and 5-day AL) weighing more and being older that the 3-day AL HIV-uninfected children. This inherent difference between groups could confound our results, as older children have more immunity to malaria and previous studies have shown that young (smaller) children are more often underdosed [38, 40]. Efforts were made during the analysis to minimize the differences in age; we tested the impact on clinical outcomes of including and excluding the eight children in the HIV-uninfected arm who were < 3 years old. Even after excluding the eight children, the HIV-uninfected cohort was still younger (and smaller) than the two HIV-infected arms and there were no changes in clinical outcomes from when they were included in the analysis. Finally, HIV-infected children in this study were receiving TS for bacterial prophylaxis, as recommended by the Ugandan HIV-treatment guidelines [144]. TS has been shown to have antimalarial activity and could have impacted treatment outcomes in our study [145, 146]. Despite the lower AL exposure in the 3-day HIV-infected group, the odds of recurrent parasitemia by day 28 were similar to those in HIV-uninfected children. Additionally, as the 3-day HIV-uninfected children had *higher* odds of recurrent parasitemia than the 5-day HIV-infected children, even though their AL exposure was similar, the TS effect, coupled with the increased age of participants, could have impacted the clinical outcomes separate from AL exposure. Our results coincide with what we observed in our previous study where HIV-infected children on EFV-based ART had a lower overall risk of recurrent malaria compared with HIV-uninfected children even though their AL exposure was reduced [54].

In the context of rising resistance to artemisinin compounds in Uganda and Rwanda, optimizing the dosing and exposure of the artemisinin components of ACT is vital to maximizing the

viability and usage of the ACTs to treat malaria [12, 76]. Artemisinin resistance, largely defined as delayed parasite clearance, could potentially be overcome by extending AL treatment duration. Extension of AL dosing from 3 days to 5 days allows for the exposure of the *Plasmodium* parasite to the artemisinin for an additional 48-hour life cycle, and in particular, an additional trophozoite cycle where artemisinin drugs exhibit a majority of their antimalarial activity [90]. This additional cycle would clear out lingering parasites exhibiting delayed parasite clearance and would also kill any newly emerging parasites from the liver. Indeed, comparing the cumulative DHA AUC and cumulative ARM AUC (post-third dose to 8-hour post-last dose, AUC_{cum}) in the 3-day AL HIV-infected 3-day AL group to the 5-day AL HIV-infected group, the two additional days of dosing resulted in an overall 2.09-fold and 2.32-fold increase in artemether and DHA exposure in 5-day AL regimen, respectively ($p \leq 0.001$, for all comparisons). This additional artemisinin exposure could significantly reduce parasite burden, leaving fewer parasites for LF/DBL and the immune system to clear, as well as potentially reducing the spread of artemisinin resistance [91].

The EXALT study is subject to a few limitations that require consideration. Not all doses were directly observed; while all morning doses of AL were observed in clinic by a study staff member, evening doses were given to each participant to take at home and were not witnessed. Adherence for these evening doses was only assessed by questionnaires when the participant returned each subsequent morning, and timing of the evening dose was recorded based solely on the dosing times reported by the participant and the family. Another limitation stems from the difficulty in finding and recruiting HIV-infected participants on EFV-based ART near Busia, Uganda. Due to this difficulty, the age range for HIV-infected children was adjusted from the desired 3 – 10 years of age to 3 – 18 years of age. Unfortunately, this change occurred after much of the HIV-uninfected cohort had already been enrolled in the study. This change in

enrollment criteria resulted in the HIV-uninfected group being younger than the HIV-infected cohorts. The difficulty in recruiting HIV-infected children on EFV-based ART also resulted in the early termination of recruitment for the HIV-infected sparse sampling arms and the full number of desired sparse sampling HIV-infected children were not enrolled in the study. This greatly limited the sample size of the study, and, because of this, we may have been underpowered to detect meaningful differences in study arms for the clinical outcomes analysis. Finally, while we have demonstrated that 5-days of AL is effective for the treatment of malaria, we are unable to comment on the effectiveness of the regimen if deployed outside of a controlled study and in a different population (i.e. HIV-infected adults, pregnant women, etc.).

In summary, our data demonstrate that extended duration 5-day (10-dose) AL treatment regimen successfully compensates for the EFV-driven CYP3A4 induction effect and results in AL exposure that is similar to that of HIV-uninfected children treated with the standard 3-day AL regimen. The 5-day AL regimen is an efficacious treatment for uncomplicated malaria in HIV-infected children on EFV-based ART living in a high transmission setting and could be a useful regimen in the context of emerging ACT resistance. Despite the increased exposure of LF in the 5-day AL group, there were no statically significant differences for odds of recurrent parasitemia at 28 days or 42 days between any of the study arms in the combined sparse and intensive multivariate analysis. This is possibly due to issues from our smaller sample size not being powered, children being older in the HIV-infected arms than the HIV-uninfected group, or the antimalarial activity of TS that the HIV-infected children were taking for bacterial prophylaxis. More research is needed to determine the future utility of extended ACT regimens, such as AL, and if these extended regimens may be an effective measure in children on non-EFV-based ART, pregnant women, in lower transmission settings, or in areas where artemisinin resistance is already emerging.

3.5 Figures

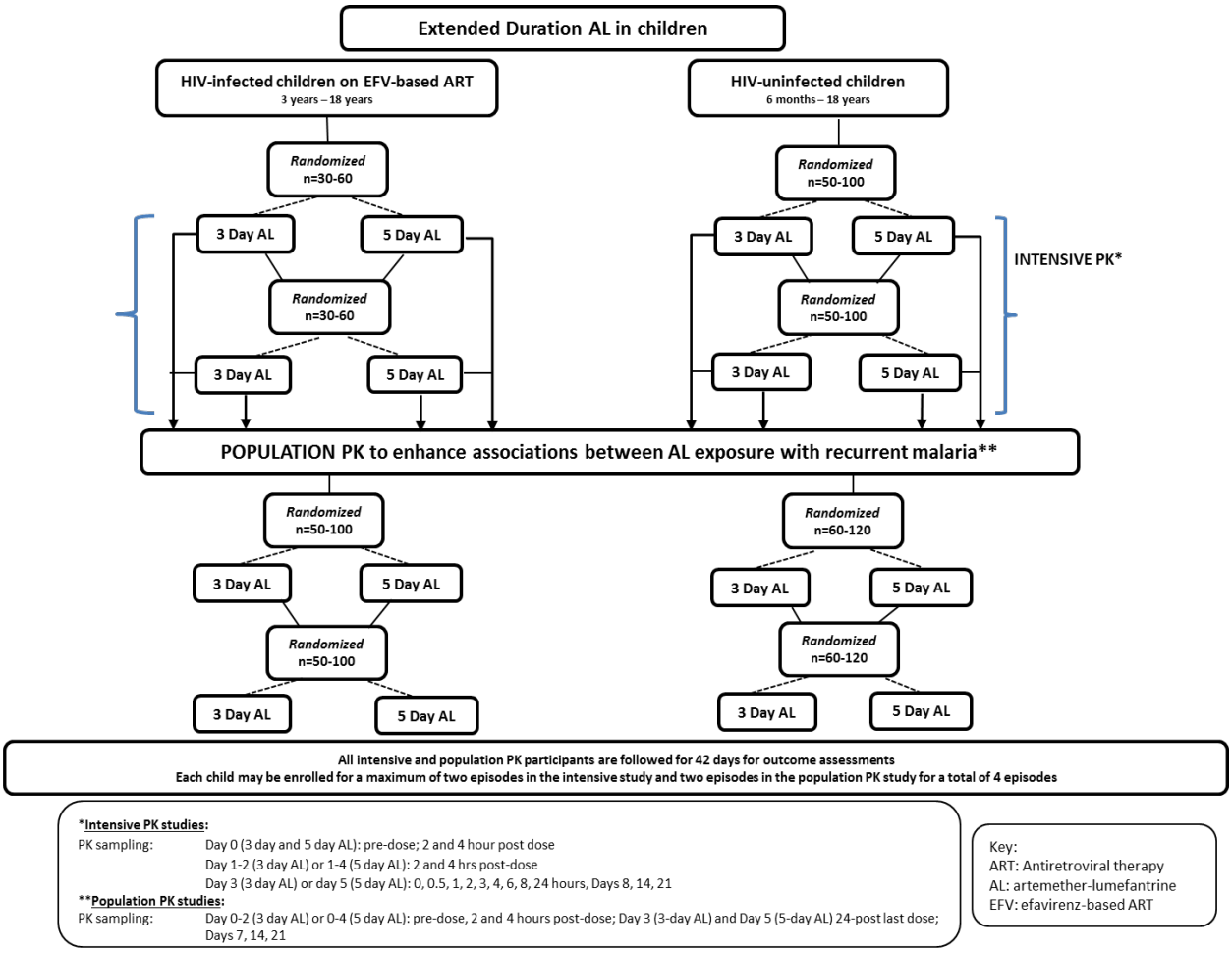

Figure 3.1 Overview of EXALT Study Design

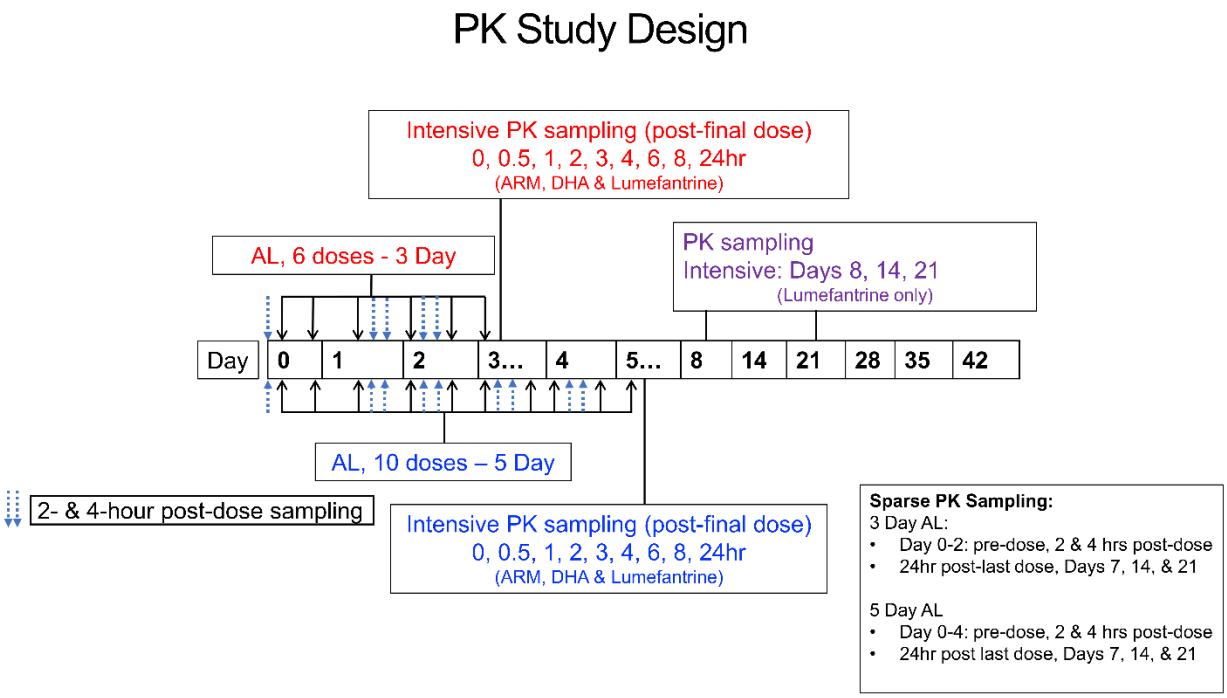

Figure 3.2 Treatment and PK sampling schedule. Following malaria diagnosis on study day 0, subjects received six doses of AL or 10 doses of AL. For the six dose AL group, doses were administered on study days 0 to 3 (Blue) Plasma PK samples were collected on day 0 prior to treatment, 2- and 4-hr post-dose on day 1, 2- and 4-hr post-dose on day 2, and on day 3 before (0 hr) and at 0.5, 1, 2, 3, 4, 8, 24, 120 (day 8*), 264 (day 14), and 432 (day 21) hr post sixth dose (blue arrows).
AL, artemether-lumefantrine; ARM, artemether; DHA, dihydroartemisinin; DBL, desbutyl-lumefantrine; PK, pharmacokinetics.

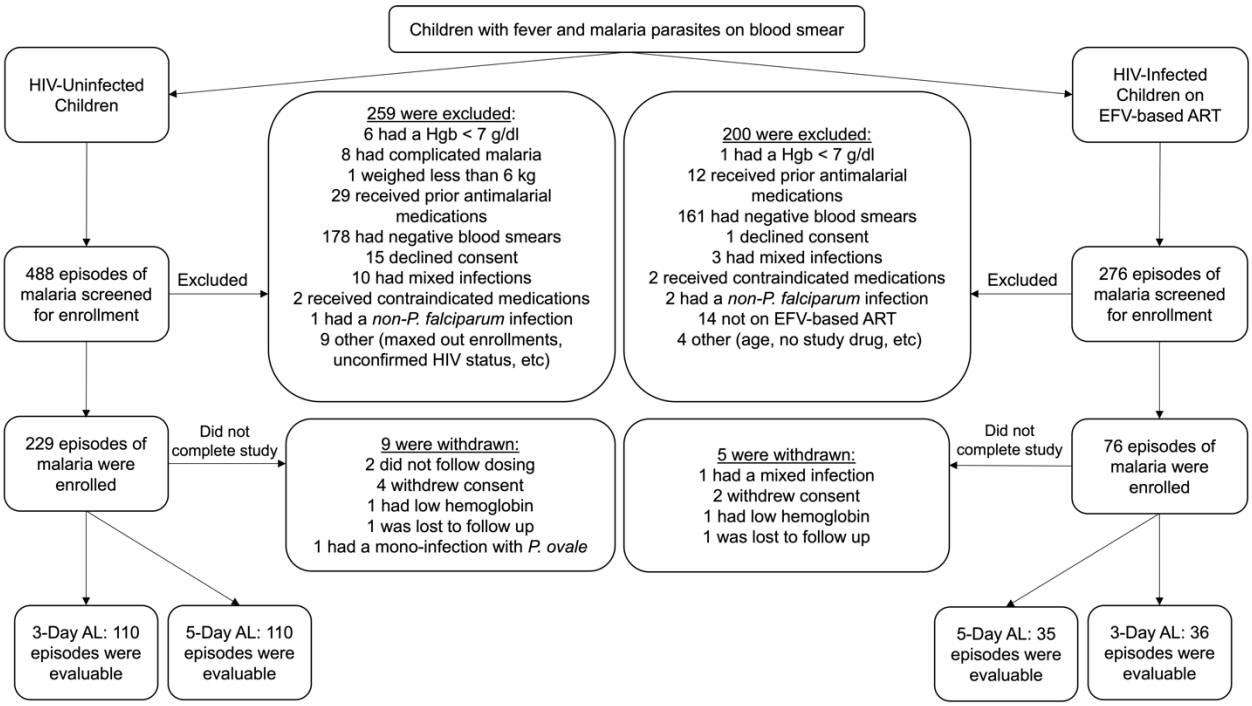

Figure 3.3 Enrollment and completion of PK studies from study evaluating an extended AL treatment regimen for uncomplicated malaria in HIV-uninfected and HIV-infected children on EFV-based ART. AL denotes artemether-lumefantrine; ART, antiretroviral therapy; Hgb, hemoglobin; HIV, human immunodeficiency virus; kg, kilogram; PK, pharmacokinetics.

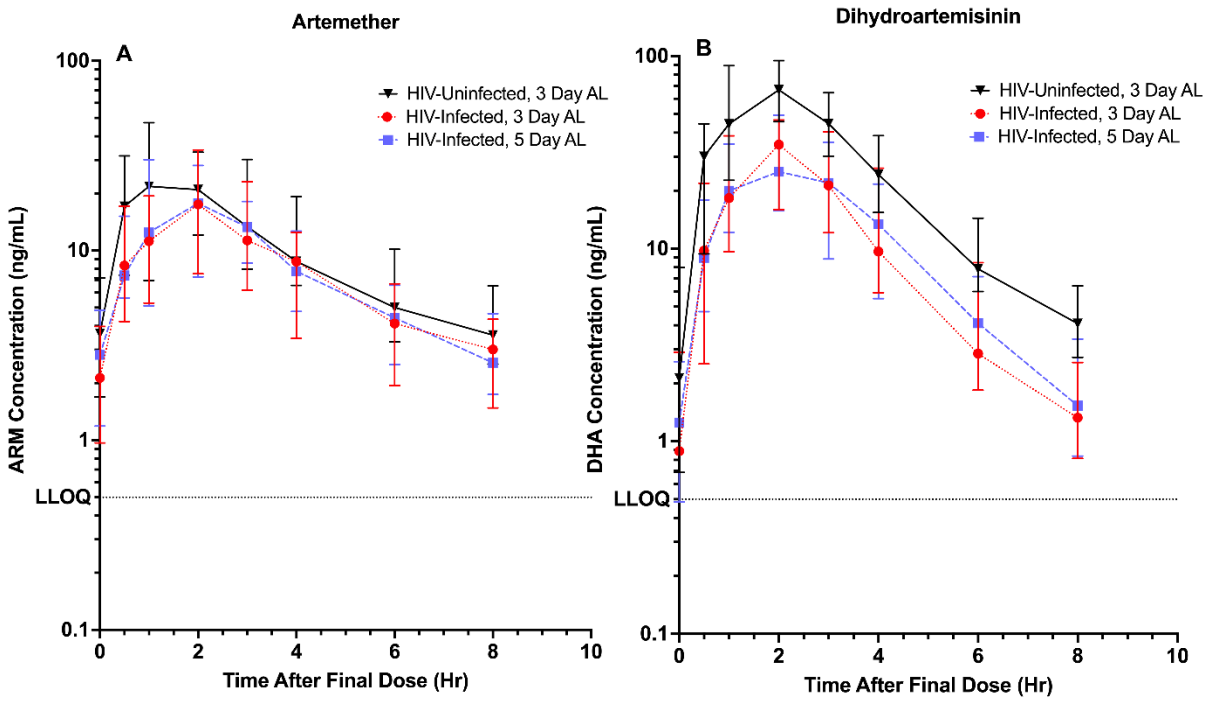

Figure 3.4 Plasma concentration-time profiles of artemether (ARM) (A), dihydroartemisinin (DHA) (B), in HIV-uninfected children treated with 3 days of artemether-lumefantrine (AL) therapy (black line) and HIV-infected children (stabilized on efavirenz-based ART) treated with either 3 days of AL (red line) or 5 days of AL (purple line). Data are represented as median with interquartile range, and values below the limit of quantitation are shown. Lower limit of quantitation (LLOQ) is 0.5 ng /ml.

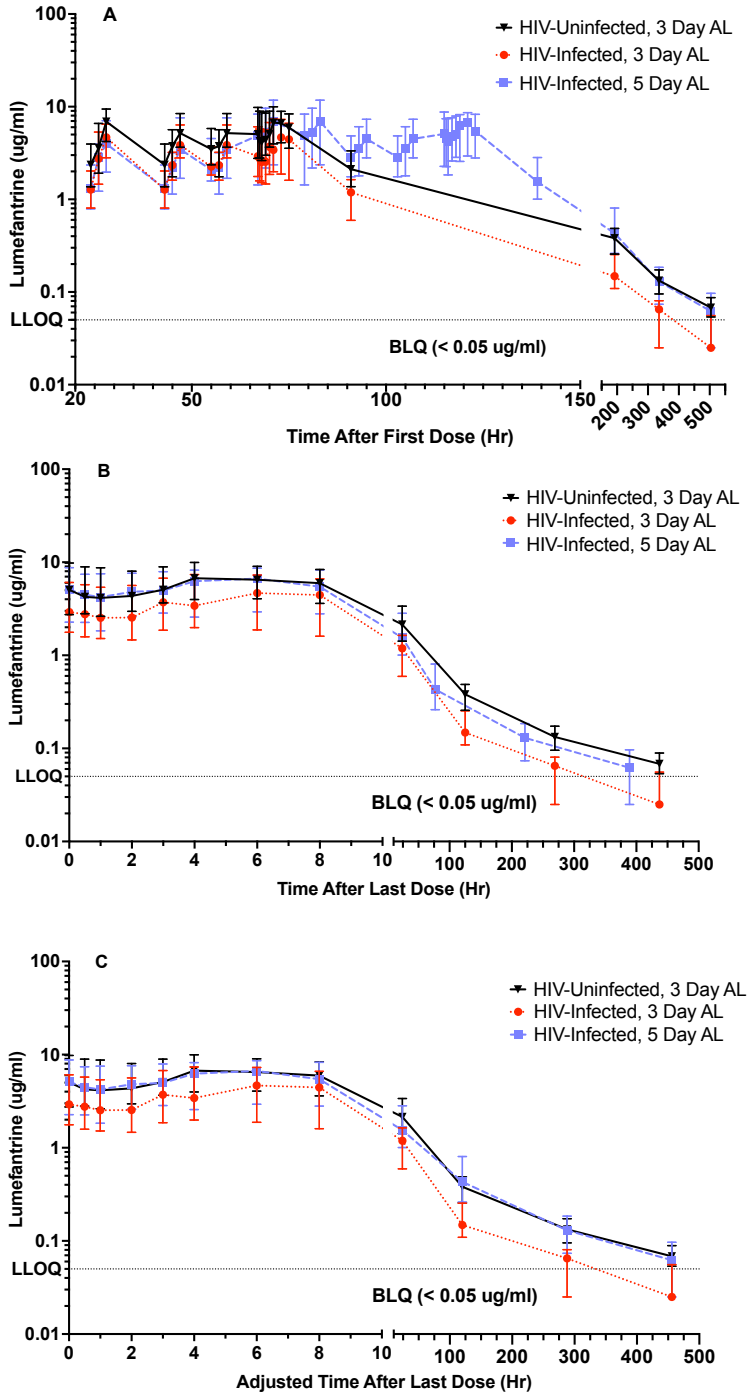

Figure 3.5 Plasma concentration-time profiles of lumefantrine in HIV-uninfected children treated with 3 days of artemether-lumefantrine (AL) therapy (black line) and HIV-infected children (stabilized on efavirenz-based ART) treated with either 3 days of AL (red line) or 5 days of AL (purple line) post-3rd dose (A) or post-final dose (B, C). Data are shown with real post-final dose timing (B) or adjusted to line up the terminal sampling days (C). Data are represented as median with interquartile range, and values below the limit of quantitation are shown. Lower limit of quantitation (LLOQ) is 0.05 ug/ml.

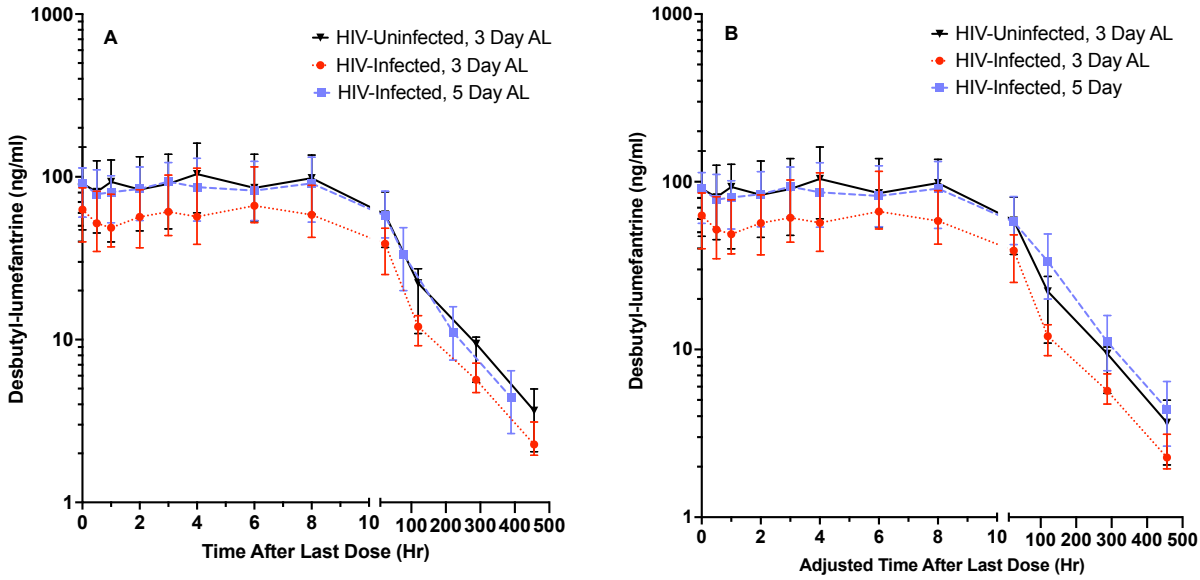

Figure 3.6 Plasma concentration-time profiles of DBL in HIV-uninfected children treated with 3 days of artemether-lumefantrine (AL) therapy (black line) and HIV-infected children (stabilized on efavirenz-based ART) treated with either 3 days of AL (red line) or 5 days of AL (purple line) post-final dose (A, B). Data are shown with real post-final dose timing (A) or adjusted to align terminal sampling days 7, 14, and 21 (B). Data are represented as median with interquartile range, and values below the limit of quantitation are shown. Lower limit of quantitation (LLOQ) is 0.5 ng/ml.

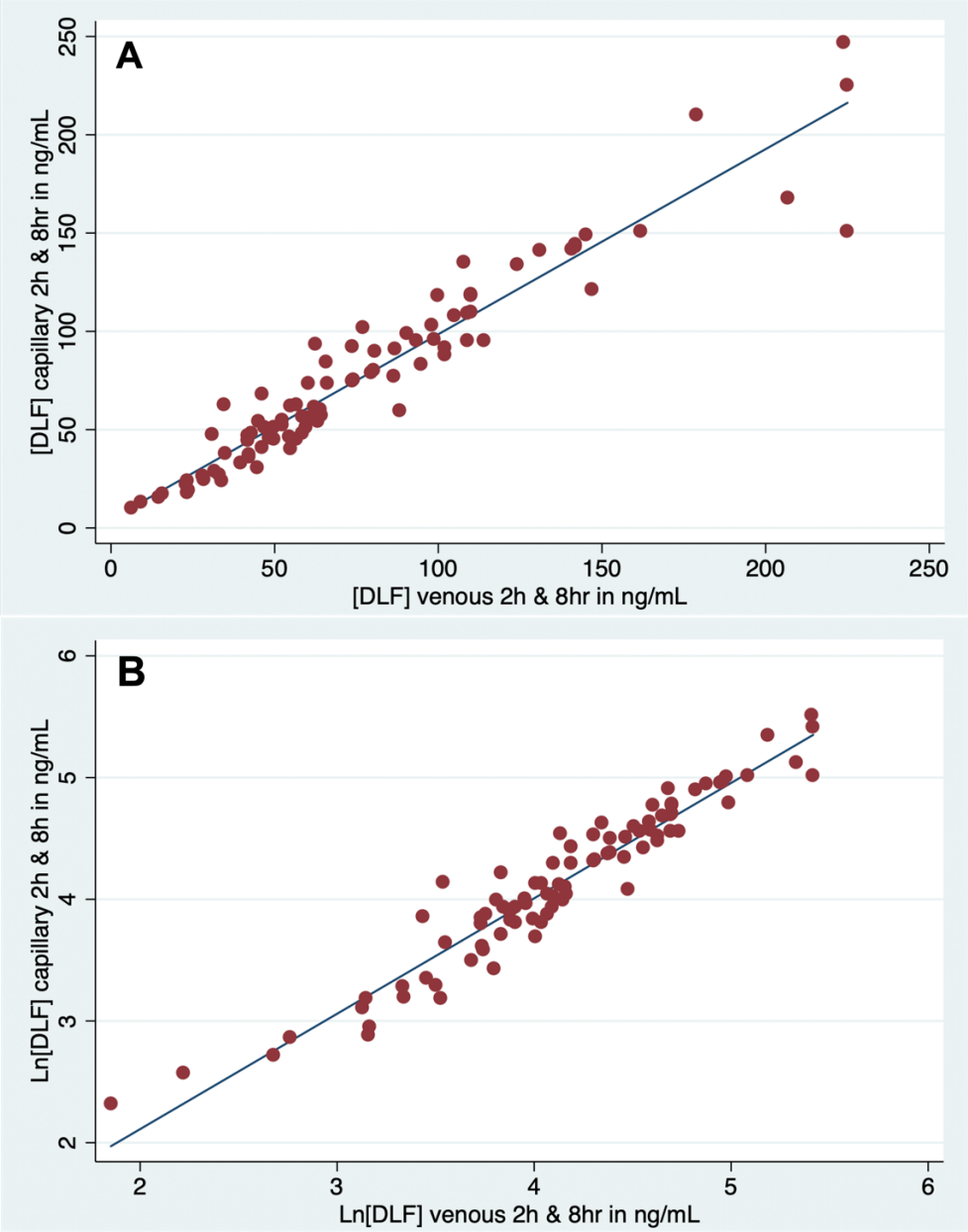

Figure 3.7 Linear regression of capillary versus venous plasma desbutyl-lumefantrine in children at 2 hr and 8 hr post-last dose. Concentrations are untransformed (A) and natural log transformed (B).

3.6 Tables

Table 3.1 Weight-based dosing for artemether-lumefantrine

Weight	Coartem® Dispersible 20 mg /120 mg tabs
< 15 kg	1
≥ 15 to < 25 kg	2
≥ 25 to < 35 kg	3
≥ 35 kg	4

Table 3.2 Demographics of Study Participants

Variable	HIV-Uninfected **3-Day AL, No ART (n = 110)**	HIV-Infected **3-Day AL, EFV-Based ART (n = 35)**	HIV-Infected **5-Day AL, EFV-Based ART (n = 36)**
Malaria episodes, intensive sampling, n	50	30	30
Malaria episodes, sparse sampling, n	60	5	6
Sex, female, n (%)	61 (55)	21 (60)	15 (42)
Age, y, median (range)[a]	5.33 (1.43 - 13.9)	11.5 (4.26 - 17.05)	10.4 (3.38 - 15.9)
Weight, kg, median (range)[b]	17.6 (8.7 - 39.1)	28.6 (15.2 - 54.5)	25.8 (14.6 - 54.5)
Parasite density at diagnosis, μL^{-1}, median (IQR)[c]	11760 (2400 - 41920)	6660 (720 - 28800)	2040 (880 - 14160)
Gametocytes present at diagnosis, n (%)	32 (29)	10 (29)	9 (25)
Hemoglobin at diagnosis, g/dL, median (IQR)	11.1 (9.8 - 11.9)	11.3 (10.3 - 11.8)	11.2 (10.4 - 12.3)
Total lumefantrine dose, mg/kg, median (range)	12.3 (8.16 - 16)	12.5 (8.81 - 15.8)	11.9 (8.22 - 15.7)
Total artemether dose, mg/kg, median (range)	2.04 (1.36 - 2.67)	2.08 (1.47 - 2.63)	1.99 (1.37 - 2.61)

There were no differences in demographic parameters between the intensive and sparse PK sampling cohorts.
Abbreviations: ART, antiretroviral therapy; EFV, efavirenz; HIV, human immunodeficiency virus; IQR, interquartile range, PK, pharmacokinetics.

[a]Age was significantly different in 3-day AL HIV-infected vs HIV-uninfected children ($p < 0.0001$) and 5-day AL HIV-infected vs HIV-uninfected children ($p < 0.0001$); differences not significant between the two HIV-infected arms.

[b]Weight was significantly different in 3-day AL HIV-infected vs HIV-uninfected children ($p < 0.0001$) and 5-day AL HIV-infected vs HIV-uninfected children ($p < 0.0001$); differences not significant between the two HIV-infected arms

[c]Parasite density at diagnosis was lower in the 5-day AL HIV-infected group when compared to 3-day AL HIV-uninfected children ($p = 0.0109$)

Table 3.3 Artemisinin Pharmacokinetics Following a 6-Dose or 10-Dose Regimen of Artemether-Lumefantrine in HIV-Uninfected and HIV-Infected Children

	HIV-Uninfected	HIV-Infected on EFV-based ART		Ratio (*P* Value)		
PK Parameter	3-Day AL (n = 50)[a]	3-Day AL (n = 30)[b]	5-Day AL (n = 30)[c]	3-Day AL + EFV/ 3-Day AL No ART	5-Day AL + EFV/ 3-Day AL No ART	5-Day AL + EFV/ 3-Day AL + EFV
Artemether						
C_{max}, ng/mL	32.5 (25.4, 41.5)	22.4 (15.3, 32.8)	23.0 (16.4, 32.3)	0.69 (0.0892)	0.71 (0.0990)	1.03 (0.9882)
t_{max}, h	1.10 (0.98, 2.03)	1.55 (0.57, 2.00)	1.91 (1.00, 2.03)	1.40 (0.8025)	1.71 (0.6314)	1.23 (0.5607)
$AUC_{0–8h}$, h × ng/mL	95.8 (77.5, 118)	64.0 (45.5, 90.0)	71.7 (54.8, 93.8)	0.67 (0.0406)	0.74 (0.1271)	1.12 (0.6168)
AUC_{cum}, h × ng/mL	792 (645, 974)	488 (372, 642)	1021 (766, 1361)	0.62 (0.0104)	1.28 (0.1643)	2.09 (0.0010)
C_{8h}, ng/mL	3.59 (2.58, 6.33)	2.81 (1.32, 4.46)	2.56 (1.75, 4.61)	0.78 (0.0624)	0.71 (0.0736)	0.91 (0.7618)
DHA						
C_{max}, ng/mL	89.0 (77.4, 102)	43.8 (33.5, 57.2)	34.9 (24.6, 49.4)	0.49 (< 0.0001)	0.39 (<0.0001)	0.80 (0.2838)
t_{max}, h	2.00 (1.00, 2.03)	2.00 (1.98, 2.03)	2.00 (1.00, 3.00)	1.00 (0.5203)	1.00 (0.3315)	1.00 (0.7080)
$AUC_{0–8h}$, h × ng/mL	241 (216, 269)	109 (83.9, 141.6)	95.8 (69.7, 132)	0.45 (< 0.0001)	0.40 (<0.0001)	0.88 (0.6823)
AUC_{cum}, h × ng/mL	1670 (1467, 1901)	641 (480, 855)	1486 (1087, 2031)	0.38 (< 0.0001)	0.89 (0.3931)	2.32 (0.0001)
C_{8h}, ng/mL,	4.09 (2.73, 6.32)	1.24 (0.815, 2.51)	1.53 (0.88, 3.32)	0.30 (< 0.0001)	0.37 (<0.0001)	1.23 (0.4329)

Data are presented as geometric mean (90% confidence interval) unless otherwise specified. Significance level: α = 0.0167 (0.05/3); t_{max} and C_{8h} reported as median (interquartile range); Wilcoxon rank sum tests were used to compare between all groups.
AUC_{cum} is the cumulative drug exposure of the third dose to 8 hr post last dose (either 6th dose or 10th dose).

Abbreviations: AL, artemether-lumefantrine; ARM, artemether; ART, antiretroviral therapy; AUC, area under the concentration-time curve; C_{max}, maximal concentration; DHA, dihydroartemisinin; EFV, efavirenz; HIV, human immunodeficiency virus; t_{max}, time to maximal concentration.

[a]n =48 for ARM AUC_{cum}
[b]n =28 for ARM & DHA AUC_{cum}
[c]n =29 for ARM & DHA AUC_{0-8h}; n = 27 for ARM & DHA AUC_{cum}

Table 3.4 Lumefantrine and Desbutyl-lumefantrine Pharmacokinetics Following a 6-Dose or 10-Dose Regimen of Artemether-Lumefantrine in HIV-Uninfected and HIV-Infected Children

Pharmacokinetic Parameter	HIV-Uninfected	HIV-Infected on EFV-based ART		Ratio (*P* Value)		
	3-Day AL (n = 50)[a]	3-Day AL (n = 30)[b]	5-Day AL (n = 30)[c]	3-Day AL+EFV/ No ART	5-Day AL+EFV/ No ART	5-Day AL+EFV/ 3-Day AL+EFV
Lumefantrine						
C_{max}, ng/mL,	7236 (6023, 8692)	5065 (3894, 6589)	6027 (4253, 8543)	0.70 (0.0267)	0.83 (0.5812)	1.19 (0.2062)
t_{max}, h	4.00 (0.00, 6.00)	3.99 (0.57, 5.50)	4.00 (0.50, 6.00)	1.00 (0.8568)	1.00 (0.6879)	1.00 (0.6281)
$t_{1/2}$, h	119 (107, 133)	87.9 (69.4, 111)	71.1 (61.4, 82.3)	0.74 (0.0288)	0.60 (<0.0001)	0.81 (0.2466)
$AUC_{0–21d}$, h × ug/mL	259 (222, 302)	144 (114, 182)	205 (151, 279)	0.56 (0.0001)	0.79 (0.1797)	1.42 (0.0428)
AUC_{cum}, h × ug/mL	468 (410, 534)	296 (251, 348)	561 (423, 743)	0.63 (0.0001)	1.20 (0.1746)	1.90 (0.0001)
LF C_{D7}, ng/mL	364 (191, 480)	141 (106, 252)	550 (326, 898)	0.39 (<0.0001)	1.51 (0.0023)	3.90 (<0.0001)
LF C_{D14}, ng/mL	119 (86.7, 170)	67.5 (42.4, 98.3)	136 (79.4, 220)	0.57 (<0.0001)	1.14 (0.4055)	2.01 (0.0002)
LF C_{D21}, ng/mL	64.1 (BLQ, 83.3)	BLQ (BLQ, 52.1)	62 (BLQ, 94.1)	<1 (0.0006)	0.96 (0.8736)	>1 (0.0249)
DBL	**n = 17**	**n = 30**	**n = 29**			
C_{max}, ng/mL	106 (77.3, 146)	78.3 (62.6, 97.9)	98.2 (74.6, 129)	0.74 (0.0803)	0.93 (0.7500)	1.25 (0.0895)
t_{max}, h	4.00 (3.02, 6.00)	4.05 (3.00, 6.00)	4.00 (3.00, 6.03)	1.01 (0.8498)	1.00 (0.7495)	0.99 (0.7028)
$t_{1/2}$, h	127 (110, 147)	130 (120, 141)	109 (99.0, 120)	1.02 (0.6902)	0.86 (0.0098)	0.84 (0.0002)
$AUC_{0–21d}$, h × ng/mL	7718 (5931, 10045)	5491 (4658, 6472)	7982 (6216, 10250)	0.71 (0.0102)	1.03 (0.7935)	1.45 (0.0031)
AUC_{cum}, h × ng/mL	8261 (4307, 15846)	7399 (6168, 8875)	12229 (9320, 16045)	0.90 (0.3865)	1.48 (0.1588)	1.65 (0.0007)
AUC_{DBL+LF}, h × ug/mL	288 (215, 386)	169 (137, 210)	239 (177, 323)	0.59 (0.0049)	0.83 (0.4062)	1.41 (0.0378
AUC ratio (LF/DBL)	33.1 (27.7, 39.5)	26.3 (20.9, 33.1)	25.4 (19.9, 32.4)	0.79 (0.0354)	0.77 (0.0080)	0.97 (0.7733)
DBL C_{D7}, ng/mL	19.4 (8.23, 26.2)	11.6 (9.14, 13.9)	33.7 (20.9, 58.6)	0.60 (0.0305)	1.74 (0.0001)	2.91 (<0.0001)
DBL C_{D14}, ng/mL	7.16 (4.03, 9.60)	5.46 (4.14, 6.46)	11.8 (8.47, 16.3)	0.76 (0.0959)	1.65 (0.0003)	2.16 (<0.0001)
DBL C_{D21}, ng/mL	3.09 (1.72, 4.87)	2.18 (1.84, 3.11)	4.51 (3.08, 6.95)	0.70 (0.0559)	1.46 (0.0118)	2.07 (<0.0001)

Data are presented as geometric mean (90% confidence interval) unless otherwise specified. Significance level: α = 0.0167 (0.05/3); t_{max} (for both LF & DBL) and Day 7, 14, and 21 concentrations (for both LF and DBL) reported as median (interquartile range); Wilcoxon rank sum tests were used to compare between all groups.
AUC_{cum} is the cumulative drug exposure of the third dose to day 21. AUC_{DBL+LF} is a composite AUC post-last dose to 21 days with DBL weighted 4x as much as LF.

Abbreviations: AL, artemether-lumefantrine; ART, antiretroviral therapy; AUC, area under the concentration-time curve; C_{max}, maximal concentration; DBL, desbutyl-lumefantrine; EFV, efavirenz; HIV, human immunodeficiency virus; LF, lumefantrine; t_{max}, time to maximal concentration.

[a]n=48 for LF $t_{1/2}$; n= 108 LF C_{D7}; n= 102 LF C_{D14}; n= 96 LF C_{D21}; n= 7 DBL AUC_{cum}; n= 33 DBL C_{D7}; n= 30 DBL C_{D14}; n= 30 DBL C_{D21}
[b]n=28 for LF $t_{1/2}$; n = 28 LF AUC_{cum}; n= 35 LF C_{D7}; n= 31 LF C_{D14}; n= 33 LF C_{D21}; n= 28 DBL AUC_{cum}; n= 34 DBL C_{D7}; n= 38 DBL C_{D14}; n= 31 DBL C_{D21}
[c]n=28 for LF $t_{1/2}$; n = 26 LF AUC_{cum}; n = 32 LF C_{D7}; n= 36 LF C_{D14}; n= 33 LF C_{D21}; n= 26 DBL AUC_{cum}; n= 31 DBL C_{D7}; n= 33 DBL C_{D14}; n= 31 DBL C_{D21}

Table 3.5 Generalized Estimating Equation Logistic Regression on Recurrent Parasitemia by Day 28 – HIV-Infected and HIV-Uninfected, with Combined Sparse and Intensive Data

Variables	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	P-value	OR (95% CI)	P-value
AL Regimen[a]				
3-Day AL	Ref			
5-Day AL	0.43 (0.18, 1.00)	0.051		
HIV Status[a]				
HIV-Uninfected	Ref			
HIV-Infected on EFV-based ART	0.47 (0.24, 0.89)	0.022		
AL Regimen & HIV Status				
3 Day, HIV-Uninfected	Ref		Ref	
3 Day, HIV-Infected	0.57 (0.27, 1.23)	0.153	0.51 (0.19, 1.41)	0.195
5 Day, HIV-Infected	0.37 (0.15, 0.89	0.027	0.42 (0.16, 1.10)	0.078
Age (Years)	0.91 (0.84, 0.99)	0.031	1.00 (0.88, 1.12)	0.937
Sex				
Male	Ref		Ref	
Female	1.02 (0.56, 1.85)	0.955	1.04 (0.54, 1.99)	0.904
Hemoglobin at Day 0	0.81 (0.67, 0.99)	0.035	0.82 (0.63, 1.08)	0.153
Parasite Density at Day 0 (log)	1.11 (0.97, 1.28)	0.144	1.04 (0.88, 1.23)	0.620
LF at Day 7 (log)	0.83 (0.60, 1.15)	0.263		
LF D7 > 280 ng/ml vs. ≤ 280				
LF D7 > 280 ng/ml	Ref			
LF D7 ≤ 280 ng/ml	0.69 (0.37, 1.26)	0.222		
LF at Day 14 (log)*	0.71 (0.48, 1.05)	0.086	0.68 (0.43, 1.08)	0.101
LF at Day 21 (log)	0.74 (0.48, 1.13)	0.165		
DBL at Day 7 (log)	0.70 (0.42, 1.16)	0.166		
DBL at Day 14 (log)	0.64 (0.38, 1.09)	0.101		
DBL at Day 21 (log)	0.56 (0.33, 0.97)	0.038		

Abbreviations: AL, artemether-lumefantrine; ART, antiretroviral therapy; CI, confidence interval; DBL, desbutyl-lumefantrine; EFV, efavirenz; HIV, human immunodeficiency virus; LF, lumefantrine; OR, odds ratio.
[a]When HIV status and AL regimen are included in the multivariate model as separate variables, statistical significance is lost.
*All LF and DBL parameters were tested in the multivariate model, and no variable was statistically significant ($p < 0.05$).

Table 3.6 Generalized Estimating Equation Logistic Regression on Recurrent Parasitemia by Day 42 – HIV-Infected and HIV-Uninfected, with Combined Sparse and Intensive Data

Variables	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	P-value	OR (95% CI)	P-value
AL Regimen				
3-Day AL	Ref			
5-Day AL	0.62 (0.28, 1.37)	0.234		
HIV Status				
HIV-Uninfected	Ref			
HIV-Infected on EFV-based ART	0.65 (0.35, 1.22)	0.178		
AL Regimen & HIV Status				
3 Day, HIV-Uninfected	Ref		Ref	
3 Day, HIV-Infected	0.74 (0.33, 1.67)	0.466	0.58 (0.22, 1.57)	0.287
5 Day, HIV-Infected	0.58 (0.26, 1.30)	0.188	0.49 (0.21, 1.18)	0.114
Age (Years)	0.99 (0.92, 1.07)	0.807	1.04 (0.93, 1.17)	0.477
Sex				
Male	Ref		Ref	
Female	1.29 (0.69, 2.41)	0.430	1.04 (0.53, 2.03)	0.905
Hemoglobin at Day 0	1.03 (0.83, 1.27)	0.815	1.01 (0.77, 1.34)	0.922
Parasite Density at Day 0 (log)	1.02 (0.87, 1.20)	0.794	1.01 (0.85, 1.21)	0.910
LF at Day 7 (log)	0.86 (0.60, 1.22)	0.402		
LF D7 > 280 ng/ml vs. ≤ 280				
LF D7 > 280 ng/ml	Ref			
LF D7 ≤ 280 ng/ml	0.66 (0.33, 1.33)	0.245		
LF at Day 14 (log)*	0.85 (0.58, 1.26)	0.422	0.85 (0.59, 1.25)	0.411
LF at Day 21 (log)	0.69 (0.45, 1.06)	0.093		
DBL at Day 7 (log)	0.88 (0.52, 1.49)	0.633		
DBL at Day 14 (log)	0.90 (0.49, 1.67)	0.744		
DBL at Day 21 (log)	0.76 (0.43, 1.34)	0.343		

Abbreviations: AL, artemether-lumefantrine; ART, antiretroviral therapy; CI, confidence interval; DBL, desbutyl-lumefantrine; EFV, efavirenz; HIV, human immunodeficiency virus; LF, lumefantrine; OR, odds ratio.
*All LF and DBL parameters were tested in the multivariate model, and no variable was statistically significant ($p < 0.05$).
LF at Day 14 is shown to match the day 28 analysis.

Table 3.7 Generalized Estimating Equation Logistic Regression on Recurrent Parasitemia by Day 28 – HIV-Infected and HIV-Uninfected, with Only Intensive Data

Variables	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	P-value	OR (95% CI)	P-value
AL Regimen[a]				
3-Day AL	Ref			
5-Day AL	0.39 (0.17, 0.93)	0.034		
HIV Status[a]				
HIV-Uninfected	Ref			
HIV-Infected on EFV-based ART	0.39 (0.17, 0.87)	0.021		
AL Regimen & HIV Status				
3 Day, HIV-Uninfected	Ref		Ref	
3 Day, HIV-Infected	0.50 (0.20, 1.23)	0.132	0.26 (0.68, 1.03)	0.055
5 Day, HIV-Infected	0.29 (0.11, 0.78)	0.014	0.24 (0.07, 0.89)	0.032
Age (Years)	0.92 (0.83, 1.01)	0.075	1.03 (0.89, 1.20)	0.687
Sex				
Male	Ref		Ref	
Female	1.33 (0.61, 2.92)	0.476	1.29 (0.54, 3.06)	0.571
Hemoglobin at Day 0	0.79 (0.61, 1.03)	0.081	0.82 (0.58, 1.17)	0.275
Parasite Density at Day 0 (log)	1.08 (0.91, 1.28)	0.405	1.01 (0.82, 1.25)	0.899
LF at Day 7 (log)	0.87 (0.56, 1.36)	0.550		
LF D7 > 280 ng/ml vs. ≤ 280				
LF D7 > 280 ng/ml	Ref			
LF D7 ≤ 280 ng/ml	0.70 (0.32, 1.55)	0.384		
LF at Day 14 (log)*	0.62 (0.36, 1.06)	0.080	0.45 (0.22, 0.92)	0.029
LF at Day 21 (log)	0.80 (0.44, 1.45)	0.465		
LF $AUC_{0\text{-}21d}$ (log)	0.85 (0.49, 1.48)	0.566		
LF AUC_{cum} (log)	0.63 (0.31, 1.27)	0.198		
DBL at Day 7 (log)	0.80 (0.43, 1.47)	0.466		
DBL at Day 14 (log)	0.77 (0.37, 1.61)	0.486		
DBL at Day 21 (log)	0.65 (0.35, 1.22)	0.180		
DBL $AUC_{0\text{-}21d}$ (log)	0.80 (0.37, 1.71)	0.567		
DBL AUC_{cum} (log)	0.60 (0.30, 1.19)	0.143		
DBL + LF AUC_{DBL+LF} (log)	0.70 (0.35, 1.34)	0.275		

Abbreviations: AL, artemether-lumefantrine; ART, antiretroviral therapy; AUC, area under the concentration-time curve; CI, confidence interval; DBL, desbutyl-lumefantrine; EFV, efavirenz; HIV, human immunodeficiency virus; LF, lumefantrine; OR, odds ratio.
[a]When HIV status and AL regimen are included in the multivariate model as separate variables, statistical significance is lost.
*All LF and DBL parameters were tested in the multivariate model, and day 14 LF concentration was selected for the final multivariate analysis as it was the only parameter close to statistical significance.

Table 3.8 Generalized Estimating Equation Logistic Regression on Recurrent Parasitemia by Day 42 – HIV-Infected and HIV-Uninfected, with Only Intensive Data

Variables	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	P-value	OR (95% CI)	P-value
AL Regimen				
3-Day AL	Ref			
5-Day AL	0.79 (0.34, 1.88)	0.598		
HIV Status				
HIV-Uninfected	Ref			
HIV-Infected on EFV-based ART	0.99 (0.44, 2.19)	0.973		
AL Regimen & HIV Status				
3 Day, HIV-Uninfected	Ref		Ref	
3 Day, HIV-Infected	1.17 (0.43, 3.17)	0.752	0.90 (0.23, 3.47)	0.880
5 Day, HIV-Infected	0.84 (0.33, 2.13)	0.716	0.83 (0.28, 2.43)	0.730
Age (Years)	1.00 (0.91, 1.09)	0.984	1.01 (0.88, 1.15)	0.886
Sex				
Male	Ref		Ref	
Female	1.95 (0.89, 4.27)	0.095	1.71 (0.75, 3.94)	0.204
Hemoglobin at Day 0	1.00 (0.75, 1.35)	0.953	1.02 (0.73, 1.43)	0.904
Parasite Density at Day 0 (log)	1.04 (0.85, 1.27)	0.687	1.01 (0.83, 1.23)	0.910
LF at Day 7 (log)	0.85 (0.53, 1.36)	0.495		
LF D7 > 280 ng/ml vs. ≤ 280				
LF D7 > 280 ng/ml	Ref			
LF D7 ≤ 280 ng/ml	0.61 (0.27, 1.41)	0.247		
LF at Day 14 (log)*	0.80 (0.45, 1.42)	0.443	0.80 (0.44, 1.45)	0.466
LF at Day 21 (log)	0.77 (0.41, 1.44)	0.405		
LF $AUC_{0\text{-}21d}$ (log)	0.81 (0.44, 1.51)	0.508		
LF AUC_{cum} (log)	0.87 (0.42, 1.78)	0.697		
DBL at Day 7 (log)	1.12 (0.57, 2.18)	0.741		
DBL at Day 14 (log)	1.44 (0.66, 3.14)	0.365		
DBL at Day 21 (log)	1.01 (0.52, 1.97)	0.980		
DBL $AUC_{0\text{-}21d}$ (log)	1.35 (0.55, 3.29)	0.514		
DBL AUC_{cum} (log)	0.93 (0.43, 1.99)	0.852		
DBL + LF AUC_{DBL+LF} (log)	0.85 (0.40, 1.82)	0.680		

Abbreviations: AL, artemether-lumefantrine; ART, antiretroviral therapy; AUC, area under the concentration-time curve; CI, confidence interval; DBL, desbutyl-lumefantrine; EFV, efavirenz; HIV human immunodeficiency virus; LF, lumefantrine; OR, odds ratio.
*All LF and DBL parameters were tested in the multivariate model. All PK parameters were not statically significant and day 14 LF concentration was selected for the final multivariate analysis to match the Day 28 analysis.

Table 3.9 Generalized Estimating Equation Logistic Regression on Recurrent Parasitemia by Day 28 – HIV-Infected, with Combined Sparse and Intensive Data

Variables	Univariate Analysis	
	OR (95% CI)	**P-value**
AL Regimen		
3-Day AL	Ref	
5-Day AL	0.62 (0.22, 1.75)	0.366
Age (Years)	0.99 (0.92, 1.07)	0.807
Sex		
Male	Ref	
Female	1.06 (0.35, 3.18)	0.918
Hemoglobin at Day 0	0.79 (0.57, 1.11)	0.178
Parasite Density at Day 0 (log)	1.26 (0.96, 1.64)	0.091
LF at Day 7 (log)	0.91 (0.57, 1.43)	0.677
LF D7 > 280 ng/ml vs. ≤ 280		
LF D7 > 280 ng/ml	Ref	
LF D7 ≤ 280 ng/ml	0.56 (0.16, 1.95)	0.364
LF at Day 14 (log)*	0.65 (0.32, 1.35)	0.251
LF at Day 21 (log)	0.56 (0.20, 1.56)	0.270
DBL at Day 7 (log)	0.98 (0.54, 1.76)	0.946
DBL at Day 14 (log)	0.99 (0.53, 1.85)	0.972
DBL at Day 21 (log)	0.68 (0.33, 1.41)	0.299

Abbreviations: AL, artemether-lumefantrine; CI, confidence interval; DBL, desbutyl-lumefantrine; LF, lumefantrine; OR, odds ratio.
All covariates were tested in multivariate models. None were statistically significant.

Table 3.10 Generalized Estimating Equation Logistic Regression on Recurrent Parasitemia by Day 42 – HIV-Infected, with Combined Sparse and Intensive Data

Variables	Univariate Analysis	
	OR (95% CI)	**P-value**
AL Regimen		
3-Day AL	Ref	
5-Day AL	0.78 (0.27, 2.22)	0.639
Age (Years)	1.05 (0.94, 1.17)	0.423
Sex		
Male	Ref	
Female	1.02 (0.41, 2.51)	0.972
Hemoglobin at Day 0	0.92 (0.67, 1.27)	0.625
Parasite Density at Day 0 (log)	1.22 (0.93, 1.60)	0.143
LF at Day 7 (log)	0.96 (0.61, 1.51)	0.862
LF D7 > 280 ng/ml vs. ≤ 280		
LF D7 > 280 ng/ml	Ref	
LF D7 ≤ 280 ng/ml	0.81 (0.29, 2.24)	0.679
LF at Day 14 (log)*	0.82 (0.52, 1.28)	0.376
LF at Day 21 (log)	0.62 (0.37, 1.04)	0.071
DBL at Day 7 (log)	1.15 (0.58, 2.29)	0.690
DBL at Day 14 (log)	1.22 (0.53, 2.78)	0.640
DBL at Day 21 (log)	0.91 (0.44, 1.86)	0.788

Abbreviations: AL, artemether-lumefantrine; CI, confidence interval; DBL, desbutyl-lumefantrine; LF, lumefantrine; OR, odds ratio.
All covariates were tested in multivariate models. None were statistically significant.

Chapter 4: Conclusions and Perspectives

The findings presented in this dissertation contribute to filling a gap in knowledge of antimalarial pharmacokinetics (PK) and pharmacodynamics (PD) in pediatric populations. This research brings us closer to determining the optimal dosing of antimalarial medications in children, the most relevant population for malaria, including those who are co-infected with HIV and taking antiretroviral therapies (ART). As discussed in Chapter 1, young children and HIV-infected children on ART are at a high risk of being underdosed for malaria treatment with artemether-lumefantrine (AL). In an effort to increase AL exposure and mitigate the risk of underdosing, an extended treatment duration was tested. The “extended duration artemether-lumefantrine treatment for malaria in children (EXALT)” is the first study to directly evaluate an extended dosing regimen in both an HIV-uninfected and HIV-infected pediatric population.

In Chapter 2, we focused on testing an extended AL treatment (5 days) for uncomplicated malaria against the standard of care treatment (3 days of AL) in young, malaria-infected, Ugandan children. The literature shows that young, small children are often underdosed for malaria treatment when weight-based dosing is utilized. As discussed in Chapter 2, groups using PK/PD modeling have suggested an extended duration treatment with AL may remedy the lower AL exposure seen in young, smaller children. We implemented a study to test the suggestions in the literature and determine if extending treatment with AL from 3 days to 5 days would result in higher AL exposure and reduced recurrence of parasitemia. Based on the results presented in Chapter 2, the following conclusions can be drawn: (i) 5 days of AL treatment significantly increases overall exposure of both the artemisinins and long acting LF; specifically day 7 concentrations of LF (a key surrogate for clinical outcomes) are higher compared to 3 days of AL, (ii) higher LF day 7 concentrations significantly reduce the risk of recurrent

parasitemia, (iii) children in the lowest weight band for weight-based AL dosing who are treated with 3 days of AL are at high risk of reduced LF exposure, which can largely be mitigated with two additional days of AL treatment. Given this specific finding for our lowest weight children, further research is needed to explore the PK/PD impacts of low weight for age (i.e. "malnourishment") for children receiving AL and how extended AL dosing may alter clinical outcomes in this patient population. Overall, 5 days of AL is a safe and effective treatment for uncomplicated malaria in young children and could be considered as a treatment option for small children that fall in the lower weight bins of AL weight-based dosing.

For Chapter 3, we pivoted to explore the use of extended AL dosing in HIV-infected children on efavirenz (EFV)-based ART. HIV-infected children on ART are at high risk of drug-drug interactions that can lead to drug toxicity or loss of efficacy. With this work, we attempted to compensate for a known drug-drug interaction between AL and EFV that results in reduced AL exposure. Our study revealed that 5 days of AL dosing successfully compensates for the EFV-driven CYP3A4 induction effect and returns LF exposure to levels seen in HIV-uninfected children treated with the standard 3 days of AL. We were able to enroll our target number of subjects in the intensive PK sampling arm to confirm this compensatory PK effect. However, for relating PK exposure to clinical outcomes, we found that recruiting HIV-infected participants on EFV-based ART near our study site was difficult. Due to the associated costs with running a longer duration clinical trial, we terminated our recruitment early before we attained the desired sample size for the sparse PK sampling arm. This limited our ability to detect meaningful differences in clinical outcomes between the 3-day AL and 5-day AL HIV-infected groups and rendered it unable to show that extended AL treatment results in better clinical outcomes. Despite these results, based on our previous research, we still believe that AL exposure does impact clinical outcomes and that 5 days of AL could be a viable malaria treatment regimen for HIV-infected children on EFV-based ART. However, more research is needed, with larger

sample sizes, to truly assess the impact of 5-day AL dosing on clinical outcomes in HIV-infected pediatric populations.

In addition to our work in children, studies have begun to emerge evaluating extended AL treatment duration in pregnant women and adults. Thus, the next step for assessing the utility of an extended AL dosing regimen will come from testing extended AL treatment in areas with emerging artemisinin resistance. As artemisinin-based combination therapies are the gold standard treatment options for uncomplicated malaria, it is crucial that we explore methods to preserve the efficacy of these essential medications. By extending the duration of AL dosing, additional parasites emerging from the liver will be subjected to antimalarial treatment, with the expectation that the two extra days of AL exposure will clear out any lingering parasites and reduce the risk of parasites being exposed to sub-therapeutic levels of AL.

While young (small) children and HIV-infected children on EFV-based ART would likely benefit from the implementation of a 5-day AL regimen, in addition to the possible use of an extended regimen to slow artemisinin resistance, there are some drawbacks to utilizing extended duration AL dosing. As patients generally feel better and are clinically improving after 2 or 3 days of ACT treatment, adherence may be an issue if a 5-day AL regimen is implemented. Additionally, though we have shown that extended duration AL treatment is safe in HIV-uninfected children and HIV-infected children on EFV-based ART, caution may be needed in children taking concomitant CYP3A4 inhibitors, like lopinavir/ritonavir, where LF concentrations are already high after only 3 days of AL dosing. As LF has the potential to increase the QTc interval, additional studies would be needed to examine the use (and need) of extended duration AL in this population.

In summary, the work presented in this dissertation contributes to knowledge of AL PK/PD in pediatric and HIV-infected populations and brings us closer to fully optimizing AL dosing in these populations. These findings may impact future malaria treatment guidelines as further research is published on extended AL treatment duration

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