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The Impact of Extended Treatment With Artemether-lumefantrine on Antimalarial Exposure and Reinfection Risks in Ugandan Children With Uncomplicated Malaria: A Randomized Controlled Trial

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Background. Artemether-lumefantrine (AL) is the most widely used artemisinin-based combination therapy 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/pharmacodynamic study of extended duration AL 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, and lumefantrine over 42-day clinical follow-up. Primary outcomes were (1) comparative pharmacokinetic parameters between regimens and (2) recurrent parasitemia analyzed as intention-to-treat.

Results. A total of 177 children aged 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 ($P < .001$). A predefined day 7 lumefantrine threshold of 280 ng/mL was strongly predictive of recurrence risk at 28 and 42 days ($P < .001$). Kaplan–Meier estimated 28-day (51% vs 40%) and 42-day risk (75% vs 68%) did not significantly differ between 3- 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 did not lead to significant reductions in recurrent parasitemia risk in our high-transmission setting. However, day 7 levels were strongly predictive of recurrent parasitemia risk, and those in the lowest weight-band were at higher risk of underdosing with the standard 3-day regimen.

Clinical Trial Registration. ClinicalTrials.gov number NCT03453840.

Keywords. malaria; antimalarial; pharmacokinetics; pharmacodynamics; randomized trial.

Plasmodium falciparum malaria remains one of the most devastating infectious diseases, with gains stalling since 2015. Malaria continues to cause roughly 242 million clinical cases and 627 000 deaths in 2020, >90% of which are in Sub-Saharan Africa (SSA) [1]. Children younger than age 5 years, for whom antimalarial dosing guidelines are not fully optimized, account for the majority of cases and 80% of all deaths in the region [1]. Treatment currently relies on artemisinin-based combination therapies (ACTs), all dosed over 3 days, and most effectively targets the initial 48-hour blood stage lifecycles. Short-acting artemisinins rapidly reduce parasite burden, whereas 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 [2, 3]. 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 [4–7].

Of the 6 World Health Organization-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

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by various cytochrome p450 (CYP) enzymes into dihydroartemisinin (DHA), its active metabolite [9]. DHA then undergoes glucuronidation before it is excreted [10]. Lumefantrine, the long-acting partner drug, has a slow absorption phase and is metabolized primarily by CYP3A4 with a terminal half-life of ~3 to 4 days [11, 12].

With this pharmacokinetic (PK) mismatch in mind, children in high-transmission settings can experience 5 or more episodes per year, indicating the need for optimized regimens that consider both treatment of the current infection and posttreatment prophylaxis against new or recurrent infections [13, 14]. Consideration of these dynamics can reduce the risk of true treatment failure (recrudescence), extend the period of posttreatment prophylaxis (in high-transmission settings), and mitigate the selection of resistance [15].

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 PK and pharmacodynamics (PD) [16–20]. For AL, our group and others have documented low lumefantrine exposure and worse outcomes following currently recommended doses in young children [17, 18, 21–23]. Day 7 lumefantrine concentration thresholds from 175 to 280 ng/mL have been commonly linked to outcomes [11, 21, 24]. Improving lumefantrine exposure, and thereby improving outcomes, requires lengthening the treatment duration (vs increasing mg/kg per dose) because absorption is dose-limited [2, 17, 21, 23, 25–28]. 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 [28–31].

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.

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 children without human immunodeficiency virus (HIV) in holoendemic high-transmission Busia, Uganda. Children ages 6 months to 18 years were recruited at our clinic, which is 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 reenrolled/rerandomized for up to 4 episodes (Supplemental text; Supplementary Figure 1*A*). The study was open-label, with weight-based AL dosing (Supplemental text; Coartem Dispersible 20 mg/120 mg, Novartis, Switzerland). Clinic doses were observed and administered with milk, and milk was provided for nonobserved evening doses. Primary outcomes were (1) comparative plasma PK parameters for artemether, DHA, and lumefantrine between regimens in the intensive PK subcohort 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 at all participating institutions (ClinicalTrials.gov number NCT03453840).

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). Active and passive 42-day follow-up was conducted (Supplementary Figure 1*B*). Participants were encouraged to return to clinic on any nonstudy days for any concerns. The National Institute of Allergy and Infectious Disease Division of AIDS criteria (version 2.1) were used to assess safety and tolerability, including adverse events. Electrocardiograms were performed in a subset of participants (Supplementary Figure 1*B*). Episodes were considered recrudescent (true failures) only if strain genotypes matched at all successfully genotyped loci. Recurrences occurring after 14 days were retreated with AL, as per standard of care.

Pharmacokinetic Sampling and Analysis

PK sampling schedule and analytic details for the intensive and sparse cohorts are in the Supplemental text and Supplementary Figure 1*B*. Analyte concentrations were determined using liquid chromatography tandem mass spectrometry. For intensive PK studies, PK parameters for each subject around the final dose were determined using noncompartmental analysis and followed a linear up-log down trapezoidal rule in conjunction with first-order input (Phoenix WinNonlin 64). For sparse PK data, lumefantrine concentrations at day 7, day 14, and day 21 were combined with the terminal concentration data from the intensive PK cohort to compare exposure between the 3- and 5-day AL groups. Capillary and venous samples collected concurrently at 2- and 8-hours after the final dose were used to compute capillary-venous correlations of artemether and DHA concentrations which permitted merging of measurements for analysis (Supplemental text).

Statistical Analysis

Analysis included the intention-to-treat (ITT) population (all those who were enrolled and randomized) and the per protocol population (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- and 5-day

groups and terminal concentrations for the combined intensive and population 3- and 5-day groups, after accounting for correlation within rerandomized subjects. For the primary clinical outcome, the cumulative risk of recurrent parasitemia at days 28 and 42 was assessed using Kaplan–Meier curves with differences between arms 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 per dose, lumefantrine PK exposure, and crossover status (ie, enrolled in the 3-day arm and reenrolled with a separate episode in the 5-day arm; Supplemental text). Supremum test was performed to assess proportional hazards assumption. P value $< .05$ indicated failure of the assumption.

RESULTS

Study Profile

Children were randomized into 3- or 5-day regimens, first into the intensive cohort, and then into the sparse cohort once sample sizes or maximum enrollment was reached (Supplementary Figure 1*A*). Intensive and sparse PK cohorts were combined for all analyses, except for intensive PK parameters. Enrolment and follow-up took place between 21 February 2018 and 29 August 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 (Figure 1). For the sparse PK cohort, 276 episodes were screened, 125 episodes met eligibility criteria, and 119 episodes were included in the final analysis (Figure 1). For all randomized children, the median age (interquartile range) was 5.8 years (4.1–8.0 years) and median (interquartile range) weight was 18.4 kg (15.3–22.9) (Table 1).

PK of Artemether and DHA in the 3- Versus 5-day Study Arms

Capillary and venous measurements of both artemether and DHA were found to have a 1:1 linear relationship, and for lumefantrine, a 1:1 correlation was previously found [32]. PK parameters for 3- and 5-day episodes (n = 50 each) with complete intensive PK sampling are summarized in Table 2 and

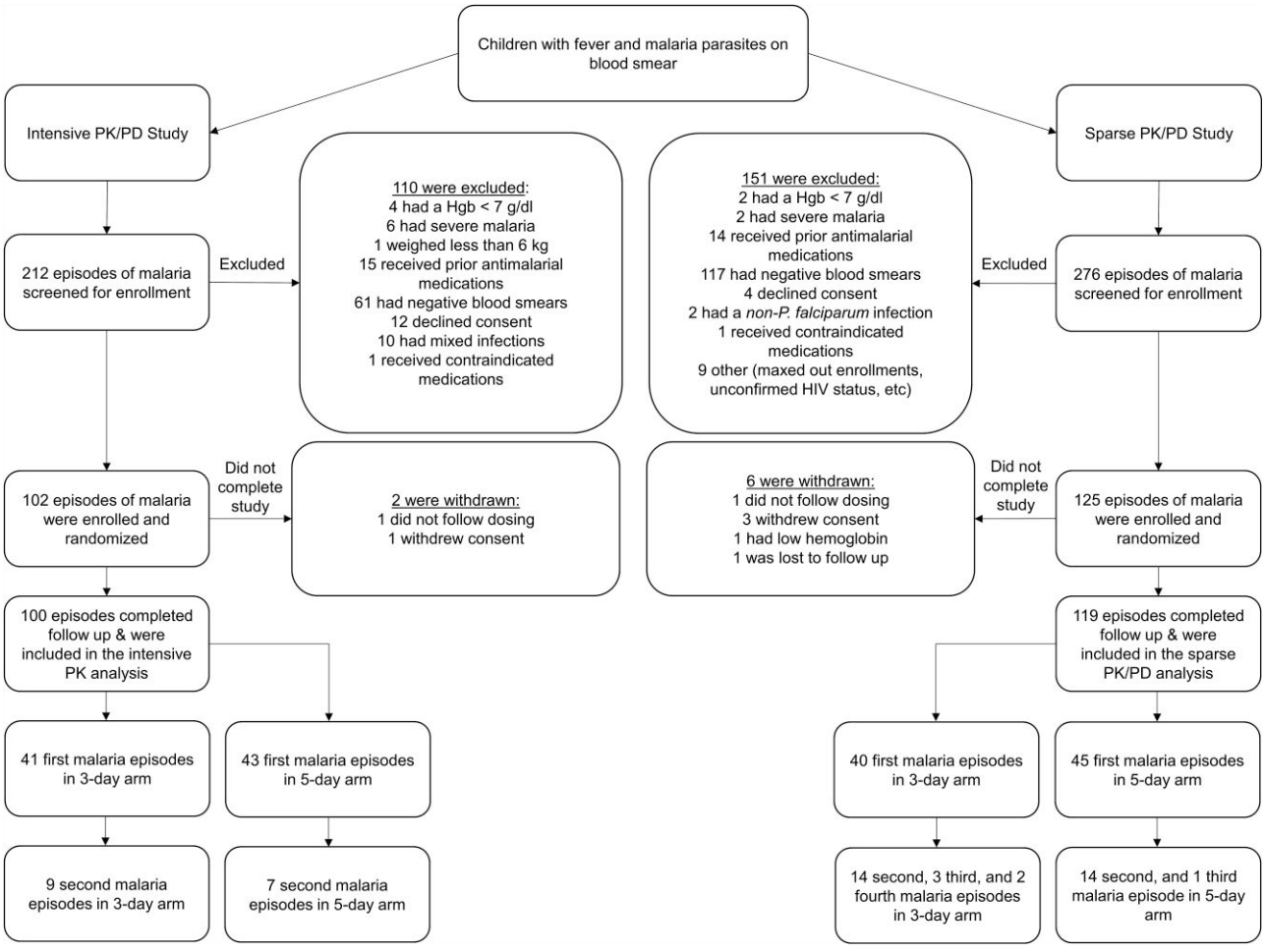

Figure 1. Trial profile. Study screening and enrollment flowchart for the intensive and sparse PK sampling arms showing intention to treat (ITT) and per protocol cohorts (CONSORT diagram).

Table 1. Demographics of Study Participants in the ITT Cohort

	AL Dosing Regimen Group			
	3-Day AL (N = 114 Episodes/87 Subjects)	5-Day AL (N = 113 Episodes/90 Subjects)	Total (N = 227)	*P* Value
Malaria episodes, per enrolled child				
1	87 (76.3%)	90 (79.6%)	177 (78.0%)	.69
2	22 (19.3%)	21 (18.6%)	43 (18.9%)	
3	3 (2.6%)	2 (1.8%)	5 (2.2%)	
4	2 (1.8%)		2 (0.9%)	
PK study arm				
Intensive	51 (44.7%)	51 (45.1%)	102 (44.9%)	.95
Sparse	63 (55.3%)	62 (54.9%)	125 (55.1%)	
Age, y				
Median (IQR)	5.3 (4.1–7.9)	5.9 (4.1–8.0)	5.8 (4.1–8.0)	.24
Sex				
Female	64 (56.1%)	60 (53.1%)	124 (54.6%)	.65
Height, cm				
Median (IQR)	105.0 (93.0–118.0)	108.0 (96.0–124.0)	107.0 (95.0–122.0)	.26
Weight, kg				
Median (IQR)	17.3 (15.1–23.0)	19.1 (15.4–22.6)	18.3 (15.3–22.9)	.26
Parasite density at diagnosis				
Geometric mean (95% CI)	8552 (6112–12 821)	10 293 (7065–14 995)	9542 (7342–12 403)	.50
Gametocytes detected by microscopy on the day of diagnosis				
Yes	34 (29.8%)	30 (26.5%)	64 (28.2%)	.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)	.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)	.37

Abbreviations: AL, artemether-lumefantrine; CI, confidence interval; IQR, interquartile range.

Figure 2*A* and *B*. For children undergoing intensive PK, cumulative artemether and DHA exposure (cumulative area under the curve [AUC_{cum}]; after the third to 8 hours after the final dose) showed a 1.70- and 1.82-fold increase in artemether and DHA exposure in the 5- versus 3-day group ($P = .001$ and <.0001, respectively). To investigate artemisinins' exposure changes with repeated dosing, postdose 2-hour concentrations were compared over the course of dosing (Table 3, Figure 3). Artemether concentrations were 68% and 65% lower following the last versus the third dose in the 3- and 5-day regimens, respectively ($P < .0001$ for both); DHA concentrations were 43% and 29% lower following the last versus third dose in the 3- and 5-day regimens, respectively ($P \leq .0039$ for all comparisons; Table 3, Figure 3).

PK of Lumefantrine in 3- Versus 5-day Study Arms

PK parameters for the 3- and 5-day (n = 50 each) intensive episodes are in Table 4 and Figure 2*C*. An estimate of cumulative lumefantrine exposure (AUC_{cum}; after third dose to day 21) showed a 1.82-fold increase in lumefantrine exposure in the 5- versus 3-day group ($P = .0001$) (Table 4 and Figure 2*D*). Combining data from the intensive and sparse PK cohorts, those receiving the 5- versus 3-day regimen exhibited markedly higher median lumefantrine concentrations on days 7, 14, and 21 (2.25-, 1.52-, and 1.37-fold, respectively; $P \leq .0001$ for all comparisons; Table 4).

Treatment Outcomes at 28- 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 28-day cumulative recurrence risk was 51% versus 40% in 3-day versus 5-day AL, respectively ($P = .091$), and at day 42 was 75% versus 68% in 3- versus 5-day AL, respectively ($P = .10$; Figure 4*A*). At 42 days, 24% and 23% were symptomatic reinfections in the 3- and 5-day regimens, respectively (Supplementary Table 1; WHO outcomes). Overall, 7.1% (n = 10/140) of recurrences were recrudescent, and equally proportioned between regimens (Supplemental text).

Multivariate Cox regression was performed to evaluate the risk of recurrent parasitemia after adjusting for the previous covariates, as well as whether a participant was reenrolled and participated in both arms (Table 5). No violation of proportionality was evident. The adjusted analysis risk differences

Table 2. Artemisinin Pharmacokinetics Following a 6-dose or 10-dose Regimen of AL in Children Undergoing Intensive PK Sampling

Pharmacokinetic Parameter	3-Day AL n = 50	5-Day AL n = 50	*P* Value 5-Day AL/3-Day AL
Artemether			
C_{max}, ng/mL, GM (95% CI)	32.5 (25.4–41.5)	27.3 (20.5–36.3)	.34
T_{max}, h, median (IQR)	1.10 (0.98, 2.03)	1.08 (0.97, 2.02)	.74
$AUC_{0–8h}$, h·ng/mL, GM (95% CI)	95.8 (77.5–118)	78.6 (61.3–101)	.25
C_{8hr}, ng/mL, GM (95% CI)	3.6 (2.58–6.33)	2.72 (1.51–5.33)	.07
AUC_{cum}, h·ng/mL, GM (95% CI)[a]	792 (645–974)	1344 (1090–1656)	.001
Dihydroartemisinin			
C_{max}, ng/mL, GM (95% CI)	89.0 (77.4–102)	87.9 (75.8–02)	.83
T_{max}, h, median (IQR)	2.00 (1.00, 2.03)	2.00 (1.08, 2.08)	.68
$AUC_{0–8h}$, h·ng/mL, GM (95% CI)	241 (216–269)	229 (202–261)	.52
C_{8h}, ng/mL, GM (95% CI)	4.09 (2.73–6.32)	3.36 (2.48–6.11)	.23
AUC_{cum}, h·ng/mL, GM (95% CI)[b]	1670 (1467–1901)	3038 (2629–3510)	<.0001

Per protocol cohort.

Abbreviations: AL, artemether-lumefantrine; $AUC_{0–8h}$, area under the concentration-time curve after last dose; AUC_{cum}, area under the concentration-time curve after third dose to day 21; C_{8h}, concentration 8 hours after last dose; CI, confidence interval; C_{max}, maximal concentration after last dose; IQR, interquartile range; T_{max}, time to maximal concentration after last dose; GM, geometric mean; GMR, geometric mean ratio.

[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.

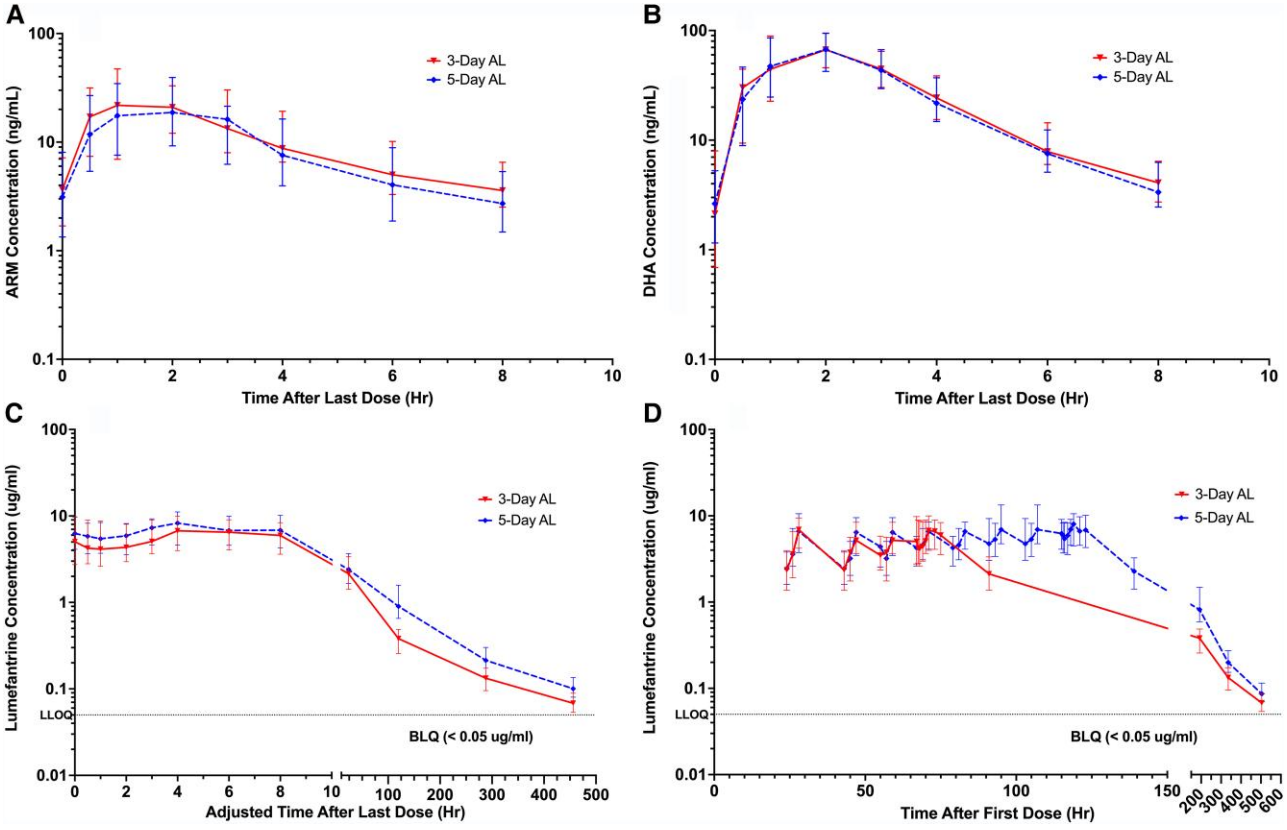

Figure 2. (*A*) Plasma concentration-time profiles of artemether, (*B*) dihydroartemisinin (DHA), and (*C*) lumefantrine in children treated with 3 days of AL and children treated with 5 days of AL, and (*D*) estimated cumulative AUC (AUC from the third dose to day 21; AUC_{cum}). Data are represented as median, and values below the limit of quantitation (BLQ) are shown. Note that lumefantrine concentrations are shown in μg/mL.

Table 3. Comparing Artemether and DHA After Third Dose Exposure to After Last Dose Exposure in Children Receiving 3- or 5-day AL in the Intensive PK Sampling Study Arm

	After 3rd Dose of AL GM; 95% CI	After Last Dose of AL (Dose 6 or 10) GM; 95% CI	After Last/After 3rd Dose GMR (*P* Value)
Artemether, C_{2h}, ng/mL			
3-day AL regimen[a]	60.1 (47.3–76.4)	19.4 (15.3–24.7)	0.32 (<.0001)
5-day AL regimen[b]	51.5 (39.9–66.5)	18.0 (13.6–23.8)	0.35 (<.0001)
DHA, C_{2h}, ng/mL			
3-day AL regimen[c]	105 (83.6–132)	59.2 (49.4–71.0)	0.57 (<.0001)
5-day AL regimen[d]	89.3 (72.5–110)	63.8 (53.8–75.5)	0.71 (.0039)

Wilcoxon signed-rank test used for all; GM (95% CI). Per protocol cohort.

Abbreviations: AL, artemether-lumefantrine; C2hr, concentration 2 hours after dose; CI, confidence interval; DHA, dihydroartemisinin; GM, geometric mean; GMR, geometric mean ratio.

[a]3-day AL: after 3rd dose, n = 49; after last dose AL, n = 50.

[b]5-day AL: after 3rd dose, n = 49; after last dose AL, n = 50.

[c]3-day AL: after 3rd dose, n = 50; after last dose AL, n = 50.

[d]5-day AL: after 3rd dose, n = 49; after last dose AL, n = 50.

between 3- and 5-day regimens were not significantly different at 28 or 42 days.

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 previous covariates found that at 28 days, children in the 5- versus 3-day regimen had a hazard ratio (HR) of 0.47 (95% confidence interval [CI]: .25–.88; $P = .019$), though differences were not significant at 42 days (HR, 0.74; $P = .23$) (Table 5). Lumefantrine AUC_{0-21d} (AUC after the last dose to 21 days) was significantly associated with malaria risk at both 28 and 42 days (HR, 0.54, $P = .028$ and HR, 0.61, $P = .038$; 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 (Figure 4*B* and *C*) [11, 26]. Approximately 3.7 times as many children were found to have a day 7 level ≤280 ng/mL in the 3- versus 5-day regimen (Table 4). 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 5).

In the 3-day arm, height and weight were significantly higher in those achieving targeted day 7 levels (Supplementary Table 2). We investigated whether certain dosing weight bins were associated with higher frequencies of falling below the protective lumefantrine threshold. For those in the 5- to 14-kg weight bin (1 AL tablet), 64.0% (n = 16/25) in the 3-day versus 18.8% (n = 3/16) in 5-day regimen fell below the day 7 threshold.

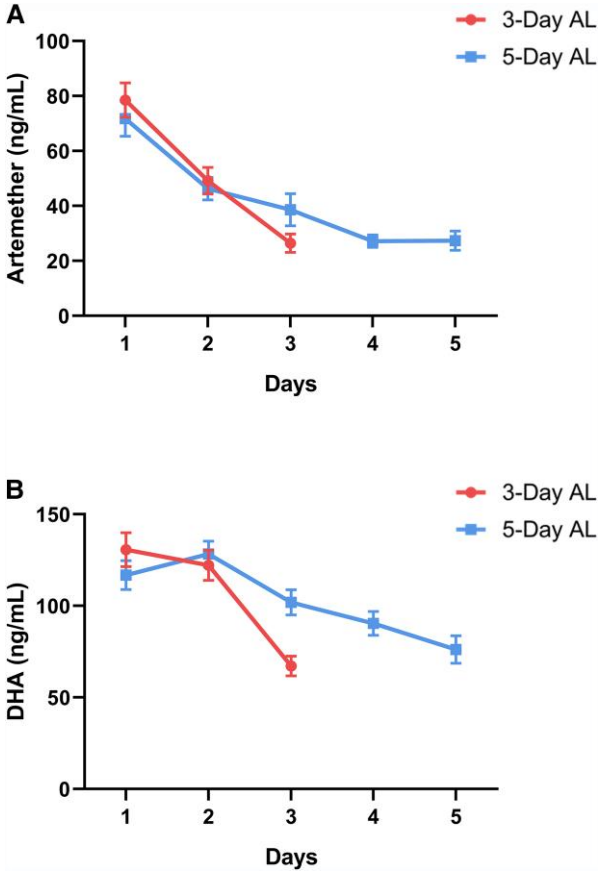

Figure 3. (*A*) Artemether and (*B*) DHA concentrations 2 hours following each morning dose in 3-day and 5-day regimens.

Safety and Tolerability of Artemether-lumefantrine

Artemether-lumefantrine was well tolerated. Two serious adverse effects occurred in the 3-day arm and were characterized as hypoglycemia on day 26 and grade 4 anemia on day 28, both of unclear etiology (Supplemental text). Graphs of chemistry and hematology values are presented in Supplementary Figure 3. Electrocardiograms conducted in subset of 101 children showed no QTc prolongation greater than 450 ms at any time point (Supplemental text). Detailed electrocardiogram results will be reported separately.

DISCUSSION

We conducted EXALT, the first study 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 in children. 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

Table 4. Lumefantrine Pharmacokinetics Following a 6-dose Regimen or 10-dose Regimen of Artemether-lumefantrine

Pharmacokinetic Parameter	3-day AL Median (IQR)	5-day AL Median (IQR)	Ratio (*P* Value)
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 (.39)
T_{max}, h	4.00 (0.00, 6.00)	4.00 (1.00, 6.00)	1.00 (.69)
$T_{1/2}$, h[a]	120 (91.4, 158)	97.4 (81.6, 119.1)	0.81 (.007)
$AUC_{0–21d}$, h·µg/mL, GM (95% CI)	259 (222, 302)	318 (274, 370)	1.22 (.12)
AUC_{cum}, h·µg/mL, GM (95% CI)[b]	468 (410, 534)	852 (746, 974)	1.82 (<.0001)
Intensive + sparse sampling PK episodes	n = 109	n = 110	
C_{7d}, ng/mL[c]	363 (188, 478)	816 (524, 1290)	2.25 (<.0001)
Day 7 > 280 ng/mL	69 (63.3%)	99 (90.8%)	<.001
C_{14d}, ng/mL[d]	122 (86.7, 171)	186 (122, 269.5)	1.52 (<.0001)
C_{21d}, ng/mL[e]	65.0 (BLQ, 85.2)	89.1 (63.5, 116)	1.37 (<.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}, $T_{1/2}$, and $AUC_{0–21d}$data all refer to after last dose values.

Abbreviations: AL, artemether-lumefantrine; AUC, area under the concentration-time curve; AUC_{cum}, area under the concentration-time curve from after third 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]Because of 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 with 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.

attain a previously defined day 7 protective lumefantrine threshold concentration, with 4-fold more children falling below this level in the 3- versus 5-day regimen.

In our high-intensity transmission setting, true failure remained rare, with 93% of 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. This is likely explained by posttreatment 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 (Figure 4C).

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 arm. A potential explanation may relate to levels of

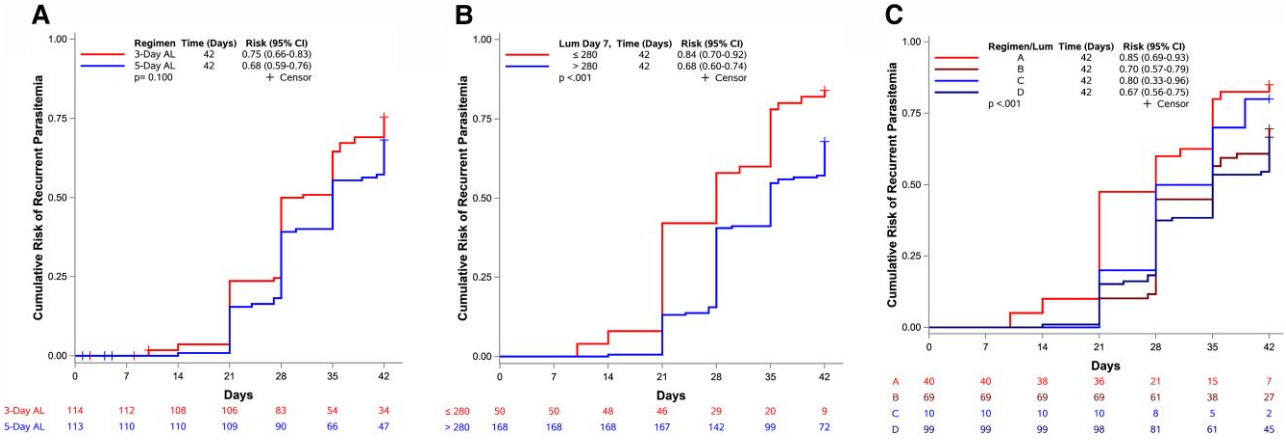

Figure 4. Kaplan–Maier estimate of time to microscopically determined recurrent parasitemia over 42-day follow-up for ITT cohort in (*A*) children randomized to the 3-day versus 5-day regimen, (*B*) children attaining a day 7 lumefantrine levels >280 ng/mL and ≤280 ng/L, and (*C*) children stratified by treatment regimen duration and day 7 lumefantrine level >280 ng/mL and ≤280 ng/L. (*A*) 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).

Table 5. Multivariate Cox Regression Analysis of PK Exposure and 28- 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)	.820	0.92 (0.66–1.27)	.61
Lumefantrine at day 7, >280 vs ≤280	0.54 (0.32–0.91)	.021	0.58 (0.38–0.88)	.010
Intensive PK participants only	n = 98		n = 98	
AL 5 day vs 3 day	0.47 (0.25–0.88)	.019	0.74 (0.46–1.21)	.230
Lumefantrine $AUC_{0–21d}$	0.54 (0.31–0.93)	.028	0.61 (0.38–0.97)	.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.

Abbreviations: AL, artemether-lumefantrine; AUC, area under the concentration-time curve; CI, confidence interval; HR, hazard ratio; ITT, intention to treat; PK, pharmacokinetics.

adherence, as intensive participants spent more time in the clinic, had 1 additional directly observed dose, and their regimen was extended by an additional 12 hours compared with children in the sparse cohort (Supplementary Figure 4). Indeed, for those in the 5-day regimen, day 14 and 21 lumefantrine levels were higher in the intensive versus sparse cohort ($P = .0018$ and $P = .0004$, respectively). It is also notable that parasite densities of recurrent episodes trended toward being lower in the 5- versus 3-day regimens, perhaps demonstrating a quantitative impact of the additional AL doses on parasite clearance (Supplementary Figure 2; Supplementary Table 3).

Our trial builds on a handful of other studies that confirm the safety and efficacy of extended duration of AL in different settings [25, 26, 33]. The first was a trial in Myanmar in 2013 to 2015, involving adults and children treated with 3 versus 5 days of AL, all of whom received a dose of primaquine. Both regimens were safe and effective [33]. A similar Tanzanian study involved adults and children treated with 3 versus 6 days of AL plus primaquine [25]. The extended regimen was safe and effective but did not meet superiority specifications. Finally, researchers compared 3-day versus 5-day AL in n = 48 pregnant and n = 48 nonpregnant Congolese women; again, regimens were safe and effective, and the extended regimen attained exposure comparable to nonpregnant adults [26].

An important additional finding our study was that those children in the lowest weight-band of 3-day AL dosing were 3.4 times as likely to fall below 280 ng/mL than those receiving the 5-day regimen. Previous work by our group showed that children younger than age 2 years were at risk for low AL exposure, which we hypothesized was due to lower bioavailability [23]. A population PK/PD meta-analysis also demonstrated that day 7 lumefantrine concentrations in children weighing <15 kg and 15 to 25 kg were 24.2% and 13.4% lower compared with levels in nonpregnant adults [21]. Perhaps the first study to demonstrate the potential impact of extending the duration of AL was in Thailand, where 6 doses were administered either over 3 or 5 days, with the 5-day dosing interval improving PK exposure and cure rates [28, 34]. 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 children be revisited.

Optimizing the dosing of the *artemisinin* component is also critical, particularly considering the recent emergence of artemisinin resistance in SSA [2, 3]. Five days of AL exposes the parasite to the artemisinin component for an additional 48-hour trophozoite cycle where artemisinins are most active [28]. 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 [29, 31]. We also observed a notable decrease in artemisinin PK 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 [35–40]. The clinical impact of declining artemisinin exposure with each dose is unclear. However, any impacts are more likely to be seen in the 3-day regimen because the extended regimen significantly enhanced artemisinin exposure.

Our study is subject to a few limitations. Evening doses were not observed. In addition, although active sampling occurred on up to 13 visits, alongside passive follow-up available daily, we are unable to comment on parasitemia occurring on nonvisit days or submicroscopic parasitemia. In addition, we are unable to comment on regimen effectiveness if deployed outside of a controlled study where adherence may be more problematic, a potential drawback of extending regimen duration. Our study was also unable to address the cost effectiveness of 4 additional doses of AL, challenges with modifying AL dosing packages, and the challenge of educating policy makers and healthcare service providers on the potential role of a longer regimen.

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 dosing days. 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 because of 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 efficacy 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.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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