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Therapeutic Efficacy of Artemether–Lumefantrine for Uncomplicated Falciparum Malaria in Northern Zambia

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Abstract. Artemether–lumefantrine (AL) is a first-line agent for uncomplicated malaria caused by *Plasmodium falciparum*. The WHO recommends periodic therapeutic efficacy studies of antimalarial drugs for the detection of malaria parasite drug resistance and to inform national malaria treatment policies. We conducted a therapeutic efficacy study of AL in a high malaria transmission region of northern Zambia from December 2014 to July 2015. One hundred children of ages 6 to 59 months presenting to a rural health clinic with uncomplicated falciparum malaria were admitted for treatment with AL (standard 6-dose regimen) and followed weekly for 5 weeks. Parasite counts were taken every 6 hours during treatment to assess parasite clearance. Recurrent episodes during follow-up (n = 14) were genotyped to distinguish recrudescence from reinfection and to identify drug resistance single nucleotide polymorphisms (SNPs) and multidrug resistance protein 1 (*mdr1*) copy number variation. Day 7 lumefantrine concentrations were measured for correspondence with posttreatment reinfection. All children who completed the parasite clearance portion of the study (n = 94) were microscopy-negative by 72 hours. The median parasite elimination half-life was 2.7 hours (interquartile range: 2.1–3.3). Genotype-corrected therapeutic efficacy was 98.8% (95% CI: 97.6–100). Purported artemisinin and lumefantrine drug resistance SNPs in *atp6*, *3D7_1451200*, and *mdr1* were detected but did not correlate with parasite recurrence, nor did day 7 lumefantrine concentrations. In summary, AL was highly effective for the treatment of uncomplicated falciparum malaria in northern Zambia during the study period. The high incidence of recurrent parasitemia was consistent with reinfection due to high, perennial malaria transmission.

INTRODUCTION

Artemisinin-based combination therapies (ACTs) are a mainstay of malaria control. The WHO advises national malaria control programs to periodically conduct therapeutic efficacy studies of ACTs for uncomplicated *Plasmodium falciparum* malaria to inform case management and assist in the detection of drug resistance.[1] In recent years, stalling gains against malaria have highlighted the need to remain vigilant to emerging drug resistance as one of several threats to malaria control.[2]

The government of the Republic of Zambia was an early adopter of ACTs. In 2002, the Ministry of Health designated artemether–lumefantrine (AL) as first-line therapy for uncomplicated falciparum malaria. In conjunction with countrywide insecticide-treated bed net (ITN) distributions, indoor residual spraying (IRS) campaigns, reactive case detection, and mass drug administration in low-transmission areas, AL-based case management contributed to reducing malaria throughout Zambia.[3–6] Prior studies of AL carried out in Zambia in 2005 and 2013 showed sustained efficacy, as did a nearly contemporaneous study of AL in 2014–2015 for uncomplicated malaria in Zambian adults with HIV infection.[7–9] To the best of our knowledge, the present study is the most recent assessment of AL in Zambia.

Therapeutic efficacy studies of antimalarial drugs often incorporate some measure of parasite clearance. Blood smear positivity 72 hours after the first dose of ACT and a prolonged parasite clearance half-life, derived from the parasite clearance curve, are generally accepted to signal the presence of drug-resistant parasites.[10] Parasite clearance curves contain information about the pharmacodynamics of the artemisinin derivative, its companion drug, and their active metabolites. They also reflect host genetics and immunity, parasite biology, and parasitological diagnostic sensitivity and reliability. Historically, parasite clearance studies have been conducted mainly in Southeast Asia, with relatively few studies in sub-Saharan Africa.[11] The Worldwide Antimalarial Drug Resistance Network (WWARN) has supported these efforts by contributing analytic tools for parasite clearance, curating information, and publishing meta-analyses.[12–15] Through these efforts, resistance to first-line ACTs has been well documented in Southeast Asia.[16] The WWARN has measured parasite clearance half-lives twice as long in zones harboring resistant parasites compared with susceptible parasites.[14] To date, no evidence of widespread ACT resistance in sub-Saharan Africa has been produced. However, there have been reports of artemisinin or ACT-resistant sub-Saharan African strains of *P. falciparum* in returned travelers, echoing the historical experience with chloroquine and heightening concern that ACT resistance has to some extent already arrived or

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emerged there.[17,18] Genotypic markers with known associations to artemisinin-resistant Southeast Asian strains have been reported in eastern Africa, although only in rare instances with phenotypic correlate.[19,20]

Major challenges to conducting therapeutic efficacy studies of antimalarials in hyper- and holoendemic settings include the high proportion of multiclonal infections and high incidence of reinfection that occurs within the follow-up windows used to define treatment success or failure, typically a minimum of 4 weeks and up to 9 weeks. Conventional molecular methods used to disentangle coinfecting parasite clones and discern recrudescent parasites from new parasites are rendered imperfect at best and inaccurate at worst in high-transmission settings.[21] Updated or augmented methods are needed for these settings. Here, we coupled standard methods using length-polymorphic markers with amplicon deep sequencing of *apical membrane antigen-1* (*ama1*), an approach recently shown by Gruenberg et al.[22] to improve genotyping sensitivity and reliability.

High malaria transmission study settings provide an opportunity to examine the posttreatment chemoprotective effects of the long-acting partner drug, lumefantrine. Lumefantrine's terminal elimination half-life ranges from 3 to 6 days and, although shorter lived than piperaquine, mefloquine, and amodiaquine, it confers some posttreatment chemoprotection against repeat infection. Lumefantrine is notable among current antimalarials for its unpredictable pharmacokinetics on the basis of variable absorption between individuals and dose to dose.[23] Pharmacologic differences are likely amplified in infants and children in whom drug dosing is less finely tuned than in adults. Pediatric pharmacokinetics differ on account of organ development, enzyme expression, and allometrics, and lumefantrine exposure has been shown to vary significantly with a child's age and weight.[24] Dosing by weight group rather than on a continuum (e.g., a 5-kg child receives the same absolute dose as a 14-kg child) is an additional source of pharmacokinetic variability. Therapeutic efficacy studies in high-transmission settings therefore frequently incorporate a measure of drug exposure.

To evaluate for sustained effectiveness of AL in Zambia, and in response to anecdotal reports of treatment failure locally, we conducted a therapeutic efficacy study in a high-transmission area in the northern province of Luapula between December 2014 and July 2015.

METHODS

Study site. The study site was a rural health center in Nchelenge district, Luapula Province, where malaria transmission is perennial and *P. falciparum* parasite prevalence is > 50%.[25] The economy is predominantly fishing and farming based, with a median monthly household income of US$16 (unpublished data). *Anopheles funestus* and *Anopheles gambiae* sensu stricto are the primary malaria vectors, the former predominating throughout the year and peaking in the dry season (April–October).[26] Indoor residual spraying campaigns are conducted annually, targeted to the lakeside area, and ITN distributions have taken place periodically. Malaria accounts for 33% of pediatric hospital admissions and 38% of pediatric in-hospital deaths deaths at the district hospital.[27]

Study design. A modified WHO protocol for therapeutic efficacy studies of antimalarial drugs was followed.[1] Participants were admitted to the health center for directly observed treatment and serial parasite count measurements every 6 hours from 0 (pre-dose) to 48 hours, once each at 72 hours and 7 days, and then weekly for 5 weeks. Ethical approval was obtained from the Tropical Diseases Research Centre Ethics Review Committee in Ndola, Zambia. The study was approved by the Zambian Ministry of Health and registered retrospectively with the Pan African Clinical Trial Registry (PACTR201905783261453).

Treatment. Artemether–lumefantrine (pediatric dispersible, Coartem D®) was purchased from Novartis (Basel, Switzerland). Each tablet contained 20 mg of artemether and 120 mg of lumefantrine. Medication was administered with fatty food at 0, 8, 24, 36, 48, and 60 hours according to dosing guidelines (5 to < 15 kg: one tablet per occasion; 15 to < 25 kg: two tablets per occasion). Because of the high-transmission setting and in accordance with treatment guidelines, primaquine was not administered.

Inclusion and exclusion criteria. Inclusion criteria included age 6–59 months, *P. falciparum* monoinfection diagnosed by microscopy, and fever on examination (axillary temperature ≥ 37.5°C) or reported fever during the prior 24 hours. Exclusion criteria included the presence of signs or symptoms of severe malaria, and severe malnutrition (weight-for-age *Z* score < –3)[28]; presence of febrile illness other than malaria; underlying chronic disease (cardiac, renal, and hepatic); known infection with HIV; home medication with potential for interaction with AL; and history of hypersensitivity or adverse reaction to AL.

Primary and secondary outcomes. The primary endpoint was treatment outcome according to standard WHO definitions for early treatment failure, late treatment failure, late parasitological failure, and adequate clinical and parasitological response, adjusted by parasite genotype to distinguish treatment failure (recrudescence) from reinfection.[29,30]

Secondary outcomes were parasite clearance half-life (parasite clearance was modeled using the online WWARN estimator),[12] incidence of reinfection, gametocyte carriage, gametocyte clearance time among individuals with microscopic gametocytemia, hemoglobin recovery (day 0 versus 35), and drug resistance allele frequency in baseline versus recurrent parasites.

Microscopy. Asexual parasite densities were calculated by licensed and certified microscopists as the number of parasites per 200 leukocytes assuming 8,000 leukocytes/μL (or per 500 leukocytes if fewer than 10 parasites were seen). Blood smears were deemed negative if no parasites were identified against 1,000 leukocytes, providing a theoretical limit of detection of 16 parasites/μL. Gametocytemia was determined by the presence or absence of gametocytes on Giemsa-stained thick blood films.

Lumefantrine pharmacokinetics. Lumefantrine concentrations were determined from day 7 dried blood spots using liquid chromatography tandem mass spectrometry from 71 participants with available samples, as previously published.[31] In brief, analytes were extracted from dried blood spots on a filter paper using organic solvents and measured using instrument and chromatographic conditions adopted from a protocol for a plasma assay.[32] A deuterated internal standard was used to compensate for matrix effect, and the dynamic range was 100–5,000 ng/mL. Detectable concentrations below the lower limit of quantitation were assigned the measured value.[33,34]

Parasite genotyping. For participants with recurrent parasitemia, treatment outcome was assigned after parasite genotyping of *merozoite surface protein-1* (*msp1*), *msp2*, and *ama1*.[30] Nested PCR was used to amplify *msp1* and *msp2* loci as described by Snounou et al.[35] Amplicon deep sequencing of *ama1* used a modified nested PCR as described by Miller et al.[36] Paired-end sequencing was performed on Illumina Miseq platform (2 × 150 bp) at the Brown University Genomics Core. Forward and reverse reads were stitched, filtered for quality, and collapsed into unique *ama1* haplotypes within samples using SeekDeep's (version 2.6.4, seekdeep.brown.edu) default Illumina settings on samples with ≥ 250 total combined replicate reads.[37] Haplotypes were required to occur in both PCR replicates at a minimum frequency of 0.5% for inclusion. Drug resistance genotyping for single nucleotide polymorphisms (SNPs) at 45 known and candidate drug resistance loci was performed using molecular inversion probes as previously described.[38] MIPWrangler software (Hathaway, unpublished, https://github.com/bailey-lab/MIPWrangler) was used to stitch forward and reverse sequencing reads and to filter sequences for length, quality, and PCR error, discarding sequences with the proportion *Q*30 (quality score > 30) bases of less than 70%.[38] Filtered sequences were processed based on shared unique molecular identifiers to correct for PCR and sequencing errors. Gene copy numbers of *mdr1*, amplification of which is a marker of reduced lumefantrine susceptibility,[39] were estimated based on normalized depth of *mdr1* sequence coverage relative to other copy-invariant loci and calculated as a continuous variable, given the potential of mixed infections. Control strains all showed expected copy numbers.

Statistical analysis. Incidence of reinfection was determined by the number of cases divided by the total person-time for participants who completed any follow-up. The Kruskal–Wallis test was used to compare times-to-reinfection. For comparisons between those with and without reinfection, Student's *t*-test or Pearson's χ^2 test was used for crude comparisons of continuous or dichotomous variables, respectively. Multivariable linear and logistic regression models were used to examine associations between outcome variables (parasite clearance and gametocytemia) and covariates of interest, and Cox proportional hazard models were used to analyze incident reinfection. The proportional hazard assumption was tested using Schoenfeld residuals for each variable. Sensitivity analyses excluding two participants with high outlying day 7 lumefantrine blood concentrations, presumed due to surreptitious medication use outside of the study, were performed for all analyses and yielded similar results. These participants were excluded from secondary analyses of posttreatment reinfection but retained for all other analyses. Statistical analyses were performed in Stata (version 14.0, StataCorps LLC, College Station, TX).

RESULTS

Participant characteristics. One hundred children were enrolled between December 2014 and July 2015. Ninety-four children completed the parasite clearance portion of the study, and 81 children completed follow-up to 5 weeks (Figure 1). All children had a history of fever within 24 hours before presentation, and 20 children (21%) had fever on intake examination (Table 1). One child lost to follow-up after 28 days

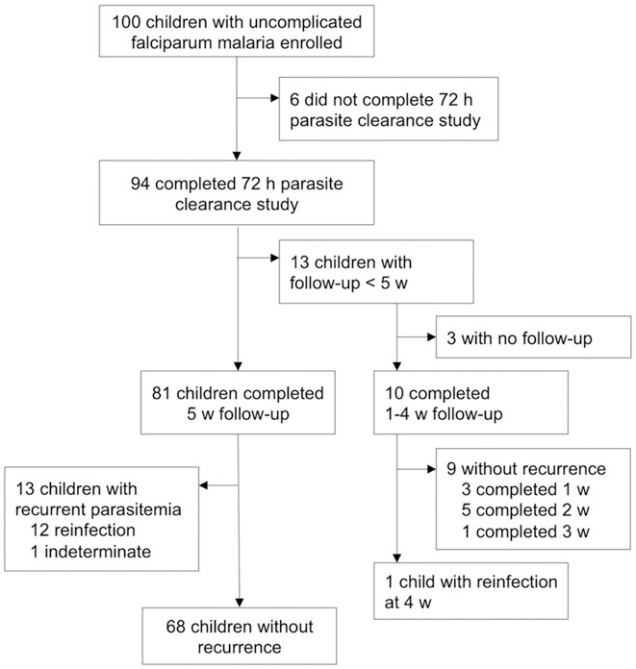

FIGURE 1. Study flow diagram.

experienced recurrent parasitemia at 21 days. Those lost to follow-up were similar to those who completed follow-up across all covariates (data not shown).

Therapeutic efficacy. Genotype-corrected adequate clinical and parasitological response among those who completed follow-up was 98.8% (Table 2). Thirteen children had asymptomatic reinfections (including the one child lost to follow-up after 28 days). There were no confirmed treatment failures, but one child with recurrent, asymptomatic parasitemia had an indeterminate genotype result. This child had low-level parasitemia (192 parasites/μL) at day 14 which was not treated, with all subsequent slide examinations negative. No serious adverse events occurred.

Parasite clearance. The median parasite clearance half-life was 2.7 hours (interquartile range [IQR]: 2.1–3.3). Thirty-five participants (37%) experienced an initial plateau or rise in parasite count (lag phase) during treatment followed by declining counts. Fever on presentation was associated with a subsequent rise in the parasite count at 6 hours (Figure 2B), including after adjustment for age, gender, and initial parasite density (adjusted odds ratio: 3.2, 95% CI: 1.1–9.5, $P = 0.04$). Parasite rebounding, defined as a second peak in the parasite concentration–time curve, was observed in 28 participants (Figure 3). Children with the lowest lumefantrine blood concentrations had parasite clearance times that were 7.9 hours slower on average than those of children with the highest concentrations, defined as the lower and upper quartiles of day 7 lumefantrine concentration (95% CI: 1.6–14.2, $P = 0.015$) (Figure 2D).

Gametocytemia. Twelve participants (13%) had microscopically detectable gametocytemia on presentation. Most (8 of 12) cleared by 30 hours. Three remained gametocytemic up to 14 days, and one remained gametocytemic at 7 days and was subsequently lost to follow-up. Children with gametocytemia had hemoglobin concentrations 1.4 g/dL lower on average than those without (8.1 ± 1.4 versus 9.5 ± 1.4, $P = 0.002$) and an

TABLE 1
Baseline characteristics of study participants who completed the parasite clearance portion of the study

Characteristic	Value (n = 94)
Age (months), median (interquartile range)	23 (16–42)
Female gender, no. (%)	47 (50)
Weight (kg), no. (%)	
< 15	70 (86)
15 to < 25	11 (14)
Undernutrition (Z score ≤ –2), no. (%)	20 (21)
Antimalarial use within the prior 2 weeks, no. (%)*	9 (10)
Bed net use, no. (%)*	78 (89)
Household with IRS during the prior 6 months, no. (%)*	79 (98)
Temperature > 37.5°C on presentation, no. (%)	20 (21)
Parasitemia (per μL), geo. mean ± SD (range)	13,000 ± 3,430 (1,120–460,200)
Gametocytemia, no. (%)	12 (13)
Hemoglobin (g/dL), mean ± SD	9.3 ± 1.5

IRS = indoor residual spraying.
* Data missing for these variables (antimalarial use within the prior 2 weeks: two participants with missing data, bed net use: three participants with missing data, household with IRS during the prior 6 months: 13 participants with missing data).

average weight-for-age Z score of –1.3 compared with –0.6 (P = 0.03). There were no significant associations of gametocytemia with age, gender, asexual parasite density, fever, or reported recent use of antimalarials.

Posttreatment reinfection. Thirteen posttreatment reinfections occurred during 399 weeks of total follow-up time (incidence rate: 1.6 reinfections per person-year, 95% CI: 1.0–3.1). Participant characteristics stratified by reinfection are shown in Table 3. None of the variables differed significantly in univariable analyses. Children with reinfection had day 7 lumefantrine concentrations similar to those without reinfection (median 106 ng/mL, IQR: 81–166).

Reinfections occurred at posttreatment day 14 (n = 1), day 21 (n = 3), day 28 (n = 7), and day 35 (n = 2). The number of reinfections at day 28 was significantly greater than that of other follow-up days (P = 0.01). Participants with reinfection showed a trend toward lower initial parasite burden and had significantly faster parasite clearance than those without reinfection including after adjustment for age, gender, and lumefantrine concentration (9.2 hours faster, 95% CI: 1.2–17.2, P = 0.025) (Figure 2C). There were no significant associations between the hazard of reinfection and age, gender, weight-normalized antimalarial dose, nutrition status, lumefantrine exposure, or presence of the 184F, N71, or 431K mutations.

TABLE 2
Clinical, parasitological, and laboratory endpoints

Endpoint	Value
Treatment efficacy, no. (%)*	
Recurrent parasitemia	14 (17)
Efficacy without genotype correction	68 (83)
Efficacy with genotype correction	81 (99)
Parasite clearance	
Parasite clearance half-life (hours), median (interquartile range)	2.7 (2.1–3.3)
Parasite clearance rate constant (per hour), mean ± SD	0.28 ± 0.10
Parasite count rise 6–24 hours posttreatment, no. (%)	35 (37)
Parasitemia at 72 hours, no. (%)	0 (0)
Gametocytemia, no. (%)	
Day 1	12 (12)
Day 2	4 (4)
Day 3	3 (3)
Day 7	2 (2)
Days 14, 21, 28, and 35	0 (0)
Hemoglobin at day 35 (g/dL), mean ± SD	11.1 ± 1.4

* Includes only those who completed follow-up (n = 82).

Hemoglobin recovery. Follow-up hemoglobin measurements (7 days and 35 days) and blood smear evaluations (weekly up to 5 weeks) showed good hemoglobin recovery without evidence of post-artemisinin hemolysis.

Drug resistance. The frequencies of drug resistance SNPs among participants with recurrent parasitemia (n = 14) are shown in Figure 4, stratified by baseline and recurrent parasites. Fifty-seven percent of samples harbored the 184F mutation in *mdr1*, associated with reduced susceptibility to lumefantrine when it co-occurs with N86, which was not detected,[40–42] and 39% had the N71 mutation in 3D7 1451200, a purported marker of artemisinin resistance.[43] There were higher frequencies of both mutations in recurrent compared with baseline parasites, although the differences were not statistically significant. Parasite genotypes from nine of the 14 participants had sufficient coverage to estimate *mdr1* copy number, none of which showed gene amplification (mean copy number 1.0, range: 0.6–1.2).

The 431K mutation adenosine triphosphate *synthase membrane subunit 6* (*atp6*), another candidate marker of artemisinin resistance, was seen in 27% of samples. No *kelch 13* or *chloroquine resistance transporter* (*crt*) drug-resistant genotypes were identified. Various parasite *dihydrofolate reductase* (*dhfr*) and *dihydropteroate synthase* (*dhps*) resistance SNPs were identified in 100% and 83% of those tested, respectively (Figure 4).

DISCUSSION

We conducted a therapeutic efficacy study of AL for uncomplicated falciparum malaria in Zambian children and measured a genotype-corrected efficacy > 98%, similar to contemporaneous therapeutic studies of AL in sub-Saharan Africa.[44–48] Reinfection was common, consistent with the high incidence of malaria in the study area. Genotyping revealed the presence of purported drug resistance polymorphisms, but we found no evidence of treatment failure or delayed parasite clearance to implicate phenotypic drug resistance; all participants were blood smear negative by 72 hours, and their parasite clearance rates were comparable to those observed elsewhere in Africa and parts of Southeast Asia where drug-susceptible parasites predominate.[14] Day 7 lumefantrine concentrations did not correlate with risk of subsequent reinfection.

Parasite clearance patterns (e.g., lagged parasite clearance, fever-associated late peaking, and parasite rebounding) were consistent with what is known about intra-treatment parasite kinetics and the stage-varying susceptibility of *P. falciparum* to antimalarial drugs.[49,50] Parasite life stages in which the plasmodia are most metabolically active are more vulnerable to artemisinins and likely the partner drug as well.[51] In line with this, human challenge experiments conducted by Khoury et al.[52] demonstrated how the timing of treatment in relation to the parasite life cycle influences the shape of the parasite clearance curve, and a recent report by Intharabut et al.[53] showed the same holds true in natural infections.

A lag phase was observed in a sizeable proportion of participants (37%). Previous studies reported a similar finding, and among currently available ACTs, AL appears to be most

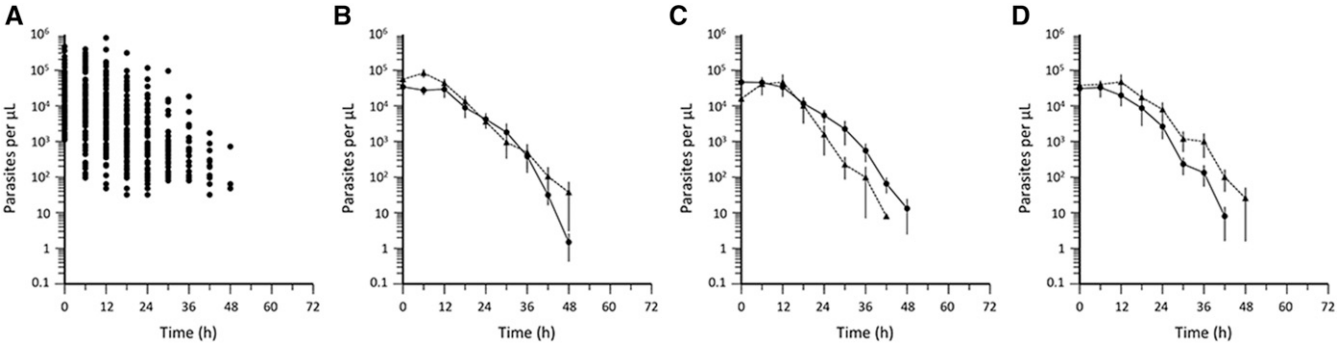

FIGURE 2. Parasite concentration–time profiles of children with uncomplicated malaria during treatment with artemether–lumefantrine. (**A**) Scatter plot of parasite count against time for all participants (nil values not shown). (**B**) Parasite clearance comparison in children with (dash) and without (solid) fever on presentation. (**C**) Parasite clearance in children who went on to have reinfection (dash) or no reinfection (solid). (**D**) Parasite clearance in participants with high (solid) or low (dash) lumefantrine exposure, defined as day 7 concentrations in the upper or lower quartile. Points and error bars are means and standard errors.

associated with lagged parasite clearance.[14] Fever on presentation was associated with a late peak in parasitemia (6 hours post-dose). This sequence—fever followed by a rise in peripheral blood parasites—could correspond to rupture of synchronized schizonts sequestered in the microvasculature that triggers a host pyrogenic response, and whose release of merozoites leads to a new generation of parasites observed hours later during the interval between the first and second doses, when drug concentrations are falling.

An interesting pattern was seen when parasite clearance curves were aligned according to the time of peak parasitemia (0, 6, or 12 hours). For the average participant with peak parasitemia at 0 or 6 hours post-dose, a second peak appeared 30 hours after the first (Figure 3). This second peak might correspond to a synchronized wave of newly invading merozoites originating from sequestered schizonts. We speculate that by this point in the treatment course, AL may have induced an increasingly synchronized infection through selection of drug-insensitive parasite stages, that is, parasites that are in a drug-susceptible part of their life cycle are preferentially killed with each dose, whereas parasites in less drug-sensitive stages preferentially remain. Treatment might thereby narrow the distribution of parasite life stages among surviving parasites. Rebounding (the appearance of a second peak) was not observed in the latest peaking infections (peak time of 12 hours post-dose), perhaps because of the additional suppression of parasites by the fourth AL dose, a further possible demonstration of the influence of dosing time relative to the parasite life cycle. Together, these patterns support a potentially useful role for compartmental models or other modeling approaches to represent antimalarial drug response in clinical trials.[49,54]

Gametocyte carriage decreased from 12% on presentation to 2% 1 week later, similar to prior studies.[55] *Plasmodium falciparum* gametocytes take 7–15 days from the onset of bloodstream infection to appear in the peripheral blood, so their presence on microscopy is partly a function of the duration of infection.[56] The association of gametocytemia with low hemoglobin and low weight likely reflects a longer duration of infection in those individuals than in those who had no detectable gametocytes; a meta-analysis of gametocytemia following ACT treatment identified similar associations.[57]

Whereas parasite clearance in the immediate posttreatment period is driven mainly by the fast-acting artemisinin derivative, patterns of reinfection in the follow-up period reflect properties of the long-acting partner drug. Measured day 7 concentrations were systematically lower than predicted

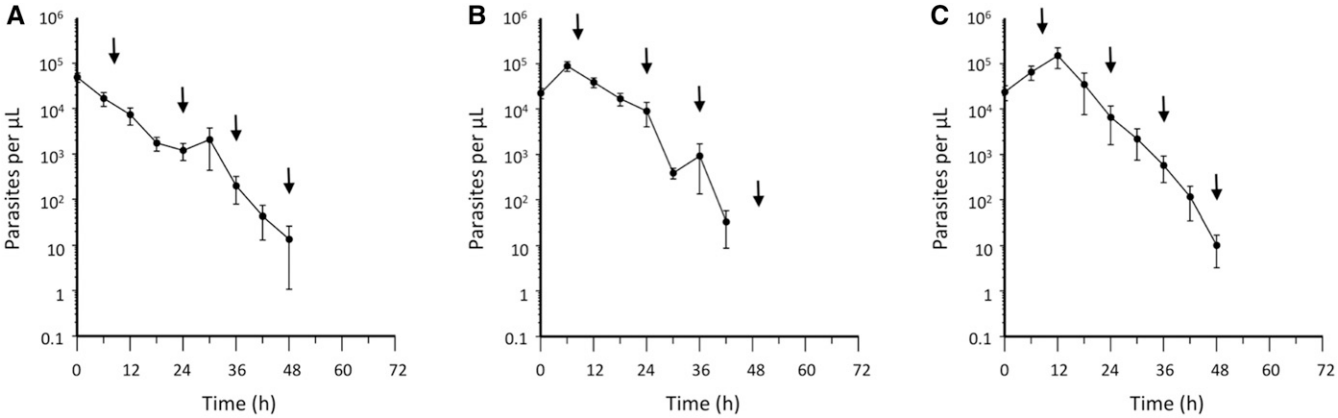

FIGURE 3. Parasite concentration–time profiles aligned by time of peak parasitemia (T_{peak}). (**A**) Parasite clearance of participants with T_{peak} of 0 hour ($n = 61$). (**B** and **C**) Parasite clearance of participants with T_{peak} of 6 hours ($n = 23$) and 12 hours ($n = 11$), respectively. All participants received an initial dose of artemether–lumefantrine at 0 hour and doses two through five at times indicated by the arrows. Curves (**B** and **C**) peak after initial treatment, and curves (**A** and **B**) rebound 30 hours after T_{peak}, a possible indication of recent schizont rupture and emancipation of a new generation of ring-stage trophozoites. Data are means and standard errors.

TABLE 3
Characteristics of participants included in the secondary analysis of post-treatment reinfection

Characteristic	No reinfection (n = 76)	Reinfection (n = 13)	P-value
Baseline characteristics			
Age (months), median (IQR)	23 (16–42)	24 (16–42)	0.92
Female gender, no. (%)	39 (51.3)	6 (46.2)	0.76
Weight (kg), no. (%)	–	–	0.22
< 15	69 (91)	10 (77)	–
15 to < 25	7 (9)	3 (23)	–
Undernutrition (Z score ≤ –2), no. (%)	18 (23.7)	1 (7.7)	0.21
Antimalarial use within the prior 2 weeks, no. (%)*	7 (9.9)	2 (15.4)	0.61
Bed net use, no. (%)*	63 (90.0)	12 (92.3)	0.75
Household with IRS within the prior 6 months, no. (%)*	62 (96.9)	12 (92.3)	0.52
Temperature > 37.5°C on presentation, no. (%)	18 (23.7)	3 (23.1)	0.99
Parasitemia at baseline (per μL), geo. mean (range)	15,230 (1,120–460,200)	8,610 (1,160–56,360)	0.19
Gametocytemia on presentation, no. (%)	9 (11.8)	3 (23.1)	0.13
Lumefantrine day 7 concentration (ng/mL), median (IQR)	106 (78–147)	121 (98–201)	0.30
Clinical and parasitological endpoints			
Time to negative blood smear (hours), mean ± SD	31.3 ± 11.4	26.3 ± 12.9	0.09
Parasite elimination rate constant (per hours), mean ± SD	0.28 ± 0.10	0.31 ± 0.09	0.24
Parasite elimination half-life (hours), median (IQR)	2.8 (2.1–3.4)	2.4 (1.4–4.7)	0.21
Hemoglobin at day 1 (g/dL), mean ± SD	9.3 ± 1.4	9.2 ± 1.8	0.77
Hemoglobin recovery (g/dL), mean ± SD	1.9 ± 1.4	1.6 ± 2.3	0.65

IQR = interquartile range; IRS = indoor residual spraying. P-values were calculated using Student's t-test or Pearson's chi-squared test for continuous or dichotomous variables, respectively.
* Data missing for these variables (antimalarial use within the prior 2 weeks: five participants with missing data, bed net use: six participants with missing data, household with IRS during the prior 6 months: 12 participants with missing data).

concentrations from population pharmacokinetic models (data not shown) and lower than previous reports,[24,58] believed due to the lower yield from dried blood spots relative to plasma matrices used in other studies.[59] In contrast to previously published studies,[24,60] day 7 lumefantrine concentrations were not associated with the prospective risk of reinfection. Interestingly, the greatest number of reinfections occurred between the third and the fourth weeks of follow-up, consistent with a pharmacology-driven, population-level "bunching" effect described by Watson et al.,[61] predicted by the long half-life of lumefantrine and the underlying rates of reinoculation and reinvasion by hepatic merozoites. The late timing of bunching (28 days after the start of treatment) suggests that the in vivo minimum inhibitory concentration of lumefantrine might be lower than the previously estimated threshold of 280 ng/mL,[60] or that the terminal elimination half-life of the drug was on average longer in our participants than in those previously studied, or a combination of both.

In addition to the extent and duration of the posttreatment chemoprotective effect of lumefantrine, risk of reinfection is driven by the uptake, or not, of vector control measures in combination with focal transmission intensity that varies geographically and seasonally. Yet, despite high penetration of ITNs (90%) and IRS (97%), reinfection was common. The high incidence of reinfection (1.6 per person-year), comparable to that seen in other studies of AL in similar settings during the early-to-mid-2010s,[9,44,62] underscores the structural impediments to malaria control in rural impoverished areas where inadequate housing and local ecologies that favor mosquito breeding and foraging contribute to intransigent malaria transmission, compounded further by the threat of insecticide resistance, outdoor-biting vector mosquitoes, and imported cases.[63,64]

The relationship between parasite clearance and subsequent risk of reinfection indirectly highlights the contribution of natural immunity to parasite clearance. Faster parasite clearance was associated with a greater chance of subsequent reinfection, a finding that could be explained by the effects of residing in a household or locale with high focal transmission. Those participants are both more likely to exhibit greater clinical immunity (premunition) cultivated by repeated exposures to *P. falciparum* over time and to have a higher prospective risk of reinfection by virtue of living in a highly malarious area. Greater premunition was manifested, we believe, as lower parasite counts and faster parasite clearance, which we observed in those who developed repeat infections during the follow-up window. This comports with our earlier findings in the same study site which showed focal heterogeneity of transmission throughout the area.[65]

Single nucleotide polymorphisms associated with artemisinin and lumefantrine resistance were detected, but occurred in similar frequencies in both baseline and recurrent parasites. There was no evidence of increased copy numbers of *mdr1*, and all copy numbers were < 1.5, which implies the absence of any major strains with multiple *mdr1* copies. We found genotypic evidence of chloroquine sensitivity, consistent with prior reports in Zambia and elsewhere in sub-Saharan Africa, where the reversion of *crt* to the wild type was seen after withdrawal of chloroquine.[66–69] A similar distribution of mutations in *dhps* and *dhfr* was described in nearby areas of the Democratic Republic of Congo from samples collected in 2013 and 2014,[38] conforming to a contiguous geography of drug resistance.

There were limitations to this study. The study was conducted over 5 years ago and as such may not reflect current drug resistance patterns or AL efficacy in northern Zambia. Loss to follow-up was high (19%) in this rural setting with a large itinerant farming and fishing population. We did not ascertain reasons for loss to follow-up, hence, treatment failures or other relevant outcomes may have been missed; for longitudinal analyses, these individuals were censored at the last study visit. All doses of AL were directly observed, limiting comparisons to previous studies where nonadherence might have influenced drug efficacy. Evaluation of *mdr1* copy number variation was limited by insufficient depth of coverage in five of the 14 participants with recurrent parasitemia; thus, we were unable to assess for the presence or absence of *mdr1* gene amplification in these participants' parasites. Artemisinin exposure likely tracks with lumefantrine to some degree because of weight-based dosing

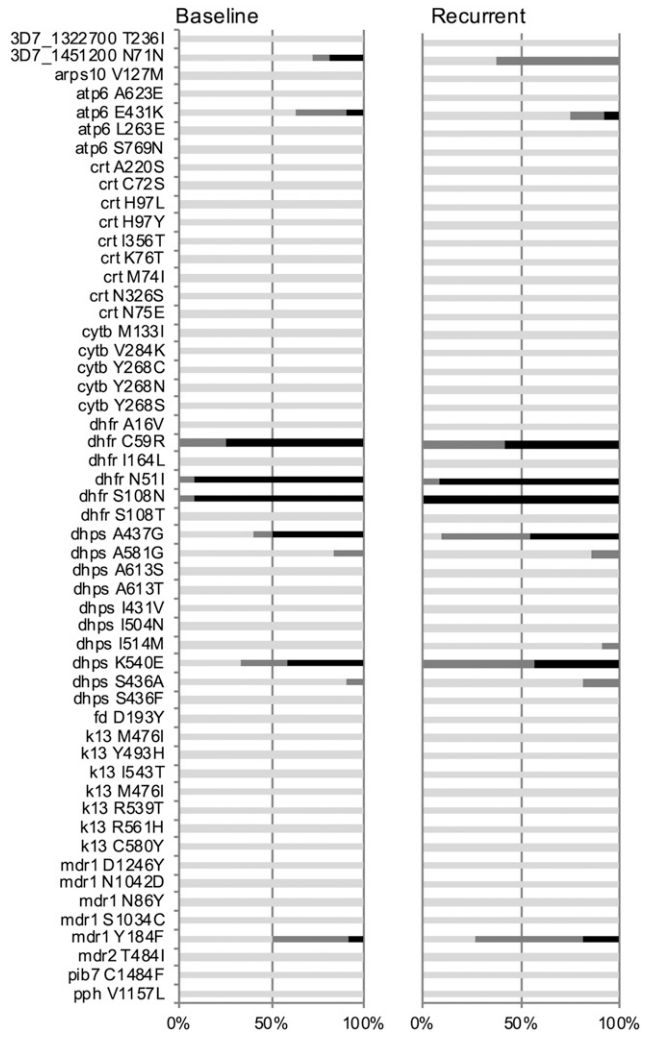

FIGURE 4. Prevalence of drug resistance single nucleotide polymorphisms in parasites among the 14 participants with recurrent parasitemia, comparing baseline with recurrent parasites. Bar graphs show wild-type (light gray), mixed wild-type and mutant (dark gray), and mutant (black) parasites. There were higher frequencies of N71, 431K, and 184F mutations in recurrent than in initial parasite infections, but the differences were not statistically significant. The N71 mutation in *3D7_1451200* and 431K mutation in *atp6* possibly reduce susceptibility to artemisinins.[43] The 184F mutation in *mdr1* is associated with reduced lumefantrine susceptibility but likely requires the N86 mutation.[40–42] There were no *chloroquine resistance transporter* (*crt*) or *cytochrome b* mutations found. There was a high prevalence of a *dihydrofolate reductase* (*dhfr*) and *dihydropteroate synthase* (*dhps*) drug resistance polymorphisms.

and factors that affect drug absorption; therefore, any observed associations with lumefantrine may instead (or additionally) reflect the effect of the artemisinin derivatives.

With a genotype-corrected treatment success of more than 98%, AL retained its efficacy against uncomplicated falciparum malaria in northern Zambia over the study period, December 2014–July 2015. Parasite kinetics during treatment appeared to correspond to perturbations in the parasite life cycle imposed by AL. These observations have implications for modeling and interpreting parasite clearance in the context of antimalarial drug efficacy and drug resistance surveillance—vital exercises in the malaria community's efforts to contend with diminishing gains and looming threats to malaria control. When chloroquine resistance first appeared in sub-Saharan African decades ago, the impact was catastrophic.[70] At present, an industrious antimalarial drug pipeline, watchful monitors such as WWARN, and ongoing containment efforts and clinical trials investigating alternative regimens for ACT-resistant *P. falciparum*[71] lend hope that history will not be repeated.

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