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Persistent and multiclonal malaria parasite dynamics despite extended artemether-lumefantrine treatment in Ugandan children

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Background

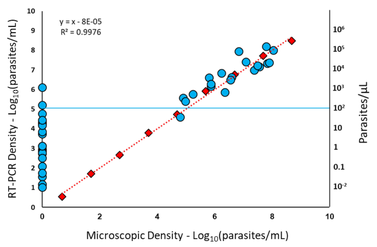
- Artemether-lumefantrine (AL) is the most widely used artemisinin-based combination therapy (ACT) in sub-Saharan Africa, but the emergence of partial artemisinin resistance in Africa threatens its useful therapeutic life.
- The use of standard diagnostics in longitudinal antimalarial studies constrains our understanding of the potential contribution of submicroscopic parasitemia to recurrence, transmission, and resistance.
- Extending the duration of AL treatment is a strategy to optimize antimalarial treatment in the face of emerging ACT resistance.
- We evaluated total parasite, asexual ring-stage, and multiclonal parasite dynamics in a randomized trial of both HIV-infected and HIV-uninfected Ugandan children for six weeks after treatment with a 3-day or extended 5-day regimen of AL.
- We combined this parasitological data with longitudinal measurement of ACT drug levels and clinical data to provide novel insights into post-treatment parasite dynamics and their association with drug exposure.

Methods

This study is part of EXALT, a prospective, randomized, open-label pharmacokinetic (PK)/pharmacodynamic (PD) study of 3-day versus 5-day AL for the treatment of malaria in HIV-uninfected and HIV-infected children (**Table 1**; ClinicalTrials.gov number NCT03453840).

To study longitudinal post-treatment parasite dynamics we used:

- Molecular markers **18S rRNA** and **SBP1 mRNA** (ring-stage marker)
- Amplicon deep sequencing of polymorphic loci (cpmp, cpp, and csp)
- Lumefantrine intensive and population PK data



Quantification of parasite density by 18S rRNA RT-PCR. The limit of detection (LOD) of 18S rRNA was 5-50 parasites/mL. The LOD of SBP1 was 100-1000 parasites/mL.

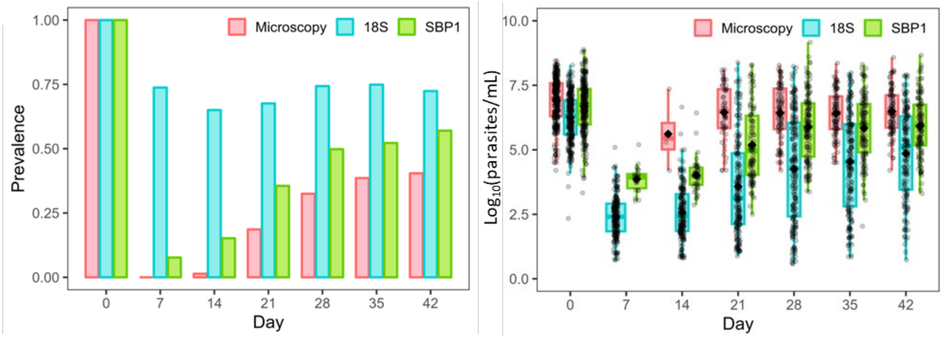
Table 1: Study Participant Characteristics

	Treatment group		
	3-Day AL (N = 153)	***5-Day AL (N = 150)***	***Total (N = 303)***
HIV status			
Uninfected	114 (74.5%)	113 (75.3%)	227 (74.9%)
Infected	39 (25.5%)	37 (24.7%)	76 (25.1%)
Sex			
Male	65 (42.5%)	75 (50.0%)	140 (46.2%)
Female	88 (57.5%)	75 (50.0%)	163 (53.8%)
Age, y			
Mean (SD)	7.2 (3.7)	7.4 (3.5)	7.3 (3.6)
Weight, kg			
Mean (SD)	22.3 (9.2)	21.9 (7.5)	22.1 (8.4)
Baseline microscopic parasite density (parasites/µL)			
Geometric mean (95% CI)	7801 (5628, 10813)	7802 (5557, 10955)	7802 (6174, 9859)
Baseline multiplicity of infection			
Median (IQR)	5.0 (3.3 – 7.0)	4.0 (2.7 – 7.0)	4.5 (3.0 – 7.0)
WHO outcome, day 28			
ACPR	76 (52.8%)	91 (62.8%)	167 (57.8%)
LCF	16 (11.1%)	11 (7.6%)	27 (9.3%)
LPF	52 (36.1%)	43 (29.7%)	95 (32.9%)
WHO outcome, day 42			
ACPR	38 (26.2%)	48 (32.9%)	86 (29.6%)
LCF	34 (23.4%)	33 (22.6%)	67 (23.0%)
LPF	73 (50.3%)	65 (44.5%)	138 (47.4%)
Recurrent parasitemia, day 28†			
No	84 (54.9%)	96 (64.0%)	180 (59.4%)
Yes	69 (45.1%)	54 (36.0%)	123 (40.6%)
Recurrent parasitemia, day 42†			
No	46 (30.1%)	52 (34.7%)	98 (32.3%)
Yes	107 (69.9%)	98 (65.3%)	205 (67.7%)

ACPR = Adequate clinical and parasitological response: absence of parasitemia irrespective of fever.
LCF = Late clinical failure: parasitemia beyond day 3 with evidence of fever (clinical malaria).
LPF = Late parasitological failure: parasitemia beyond day 6 without evidence of fever.
†Missing outcomes censored.

Results

Longitudinal prevalence and density of 18S rRNA and SBP1 mRNA

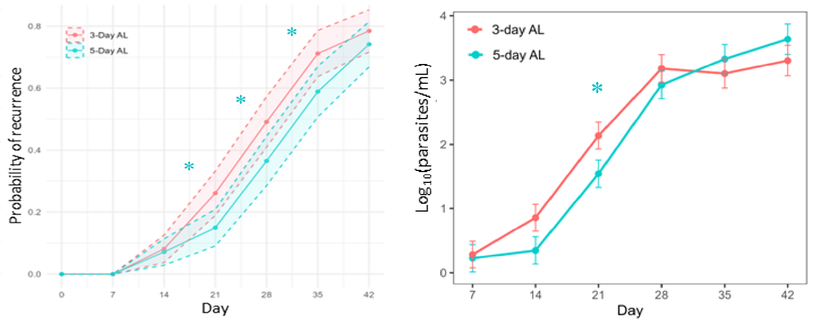


Parasite prevalence (left) and density (right) after AL treatment using 18S and SBP1 RT-PCR.

- Nearly **3/4** children had detectable 18S rRNA throughout 6 weeks of follow-up
- Nearly **1/6** children had detectable ring-stage parasites 14 days after treatment

This is despite efficacious AL therapy with 98.6% of children microscopy negative on day 3

Extended 5-day AL treatment reduces the rate and density of recurrent ring-stage parasitemia

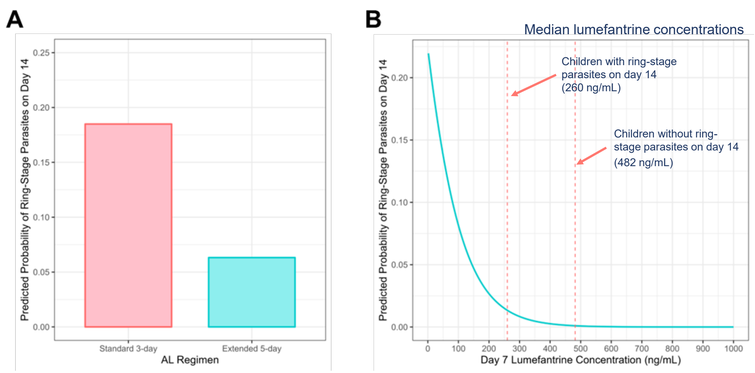


Ring-stage recurrence rate after treatment with standard 3-day or extended 5-day AL. Life table estimations with shaded regions representing 95% CI.

Ring-stage parasite densities after treatment with standard 3-day or extended 5-day AL. Least squares means estimated parasite densities with standard error bars.

Ring-stage recurrence was significantly lower during the 14-21, 21-28, and 28-35 day intervals in the extended 5-day regimen compared to the 3-day regimen.

Predicted probability of ring-stage parasites on day 14 based on treatment regimen or lumefantrine exposure

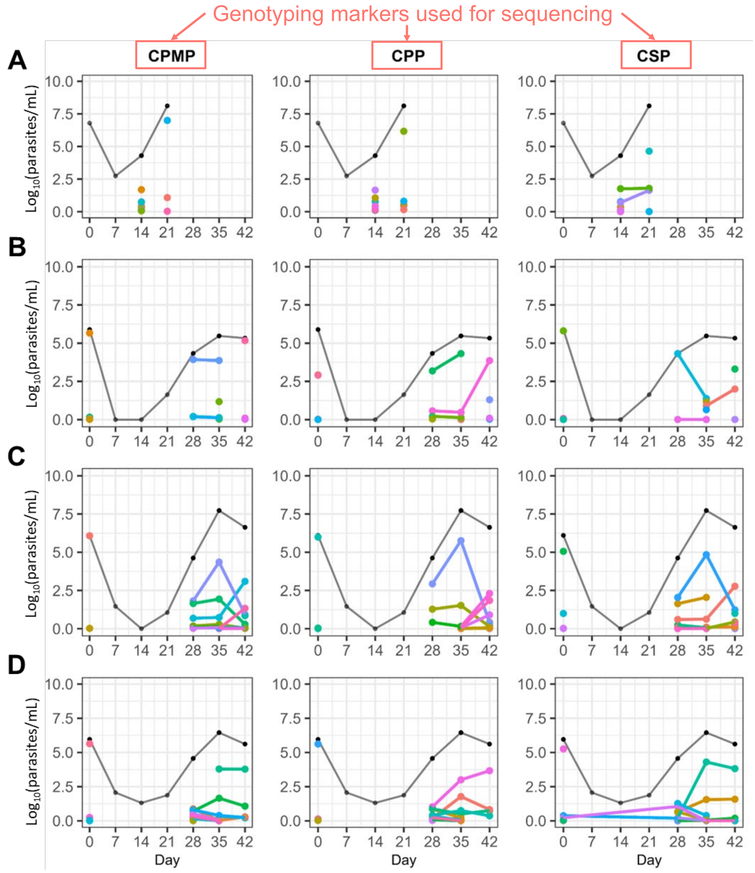


A. Probability of having ring-stage parasites on day 14 based on AL treatment regimen. **The probability of having ring-stage parasites on day 14 was 18.5% with the standard 3-day regimen, and 6.3% with the extended 5-day regimen.**

B. Probability of having ring-stage parasites on day 14 based on day 7 lumefantrine concentrations. **Higher lumefantrine concentrations were associated with a lower probability of ring-stage parasites on day 14.**

Results continued

Multiclonal infection dynamics using amplicon deep sequencing



The left, middle, and right columns represent *cpmp*, *cpp*, and *csp* clonal dynamics, respectively. The black line represents 18S parasite densities. **Each clone is a point represented by a color. Lines connecting two or more points represent identical genotypes (clones) over time.** The y-axis represents the absolute and relative abundance of each clone.

Overarching patterns characterizing longitudinal multiclonal dynamics in our high transmission setting

A. Continuous reinfection by new clones over time following treatment.

B. Continuous reinfection by new clones and the persistence of older clones leads to polyclonal infections of new and old clones over time.

C and **D**. Infections typically stratify into a dominant clone with multiple minority clones, but this can change week-to-week (**C**), or remain stable over time (**D**).

Conclusions

- Nearly 3 out of 4 of children are persistently positive for 18S throughout the 6-week post-treatment period, and the ring-stage specific marker SBP1 showed that up to 15% of children had ring-stage parasites on day 14 after AL in this high transmission setting
- **A 5-day extended AL regimen significantly reduced the recurrence of ring-stage parasites over follow-up, and was associated with a lower predicted probability of having ring-stage parasites on day 14.**
- Longitudinal amplicon sequencing revealed remarkably dynamic patterns of multiclonal infections that included new and persistent clones in both the early post-treatment and later time periods.
- Our molecular stage-specific and clonal parasite data, the largest such dataset published to date, challenges previously held notions of within-host post-treatment and reinfection dynamics, and should inform strategies to optimize regimens in the face of emerging artemisinin and partner drug partial resistance in Africa.

Acknowledgements

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