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1 **HIV-positive Nigerian adults harbour significantly higher serum lumefantrine levels than HIV-**
2 **negative individuals seven days after treatment for *Plasmodium falciparum* infection**

3
4 *Running title:* lumefantrine-nevirapine interaction in HIV patients

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26 ABSTRACT

27 Management of co-infection with malaria and HIV is a major challenge to public health in
28 developing countries and yet potential drug-drug interactions between antimalarial and antiviral
29 regimens have not been adequately investigated in people with both infections. Each of the
30 constituent components of artemether-lumefantrine, the first-line regimen for malaria treatment
31 in Nigeria, and nevirapine, a major component of highly active antiretroviral therapy, are drugs
32 metabolised by the cytochrome P450 3A4 isoenzyme system, which is also known to be induced
33 by nevirapine. We examined potential interactions between lumefantrine and nevirapine in 68
34 HIV-positive adults, all of whom were diagnosed with asymptomatic *Plasmodium falciparum*
35 infections by microscopy. *Post hoc* PCR analysis confirmed the presence of *P. falciparum* in only a
36 minority of participants. Day 7 capillary blood levels of lumefantrine were significantly higher in
37 HIV positive participants than in 99 HIV negative controls (P=0.0011). Associations between day 7
38 levels of lumefantrine and risk of persistent parasitaemia could not be evaluated due to
39 inadequate power. Further investigations of the impact of nevirapine on *in vivo* malaria treatment
40 outcomes in HIV-infected patients are thus needed.

41

42

43

44 **Introduction**

45 Malaria and HIV are two of the most important health problems facing developing countries and
46 are among the most common infections in sub-Saharan Africa. HIV co-infection is thought to
47 contribute to 3 million additional malaria cases, higher malaria parasite densities in
48 immunosuppressed children and a 5% greater mortality rate (1, 2). HIV also increases the risk of
49 *Plasmodium falciparum* infection progressing to clinical malaria in adults, especially in those with
50 advanced immunosuppression, by eroding the efficacy of acquired immunity (3). The choice of
51 antimalarial drug for the treatment of HIV patients therefore is of utmost importance considering
52 the dangers of comorbidity, but sufficient pharmacokinetic and parasitological evidence to make
53 this choice is currently lacking.

54

55 Combination therapies in current use for malaria in Africa comprise a derivative of the artemisinin
56 family of drugs combined with at least one non-artemisinin partner drug. The most widely used
57 such combination is artemether plus lumefantrine (co-artemether, AL). Artemether is metabolized
58 in the liver by the isoenzyme CYP3A4, to its active metabolite dihydroartemisinin (DHA) with peak
59 plasma concentration being reached around 2-3 hours after oral administration (4); elimination
60 half life is estimated at approximately 1 hour. There is thus only limited opportunity for DHA to
61 participate in drug-drug interactions. Lumefantrine is partially metabolised to desbutyl-
62 lumefantrine, predominantly through CYP3A4, reaching peak plasma levels approximately 10
63 hours after oral administration and is then cleared slowly, showing a terminal half life of 4-6 days
64 in *P. falciparum* malaria cases (5-9). Oral bioavailability of lumefantrine is variable and highly
65 dependent on administration with fatty foods (5, 9, 10).

66

67 The anti-retroviral drug nevirapine (NVP) is a non-nucleoside reverse-transcriptase inhibitor that is
68 well absorbed after oral administration with >90% bioavailability, generally achieved about 4 hours
69 after oral dosing and has a long half-life (11). NVP is extensively metabolised by the same CYP3A4
70 isoform as artemether and lumefantrine, and is also known to upregulate the isoenzyme (12, 13).
71 Thus NVP autoinduces its own metabolism, and potentially that of any other drugs metabolised
72 through this route. This raises the possibility of significant drug-drug interactions of NVP with
73 lumefantrine and other anti-malarials (1). Kredo and colleagues (6) initiated a pharmacokinetic
74 study in 18 South African volunteers that were HIV-infected and receiving NVP therapy, compared
75 to 18 naïve controls, each of whom took a full adult course of AL; none of the study subjects were

76 infected with *Plasmodium sp.* This study found differences between NVP recipients and controls in
77 several pharmacokinetic parameters for lumefantrine, the most important of which was a
78 significantly higher day 7 lumefantrine concentration in the NVP group. These authors concluded
79 that further studies of drug-drug interactions between NVP and lumefantrine were urgently
80 needed in malaria-infected subjects.

81

82 Artemether-lumefantrine (AL), which is currently the recommended therapy for malaria
83 treatment, was introduced in Nigeria in 2005 as the first line regimen for uncomplicated malaria.
84 Rivers State, in the Niger Delta area of southern Nigeria, has a high prevalence of HIV infection
85 (7.4% of the population) and is hyperendemic for malaria transmission. The study was designed to
86 address the lack of data regarding the pharmacokinetics of AL among HIV-positive subjects in this
87 setting, where asymptomatic parasite carriage is common. We hypothesised that following
88 treatment with AL for concomitant *P. falciparum* infections, day 7 blood concentrations of
89 lumefantrine in HIV-positive individuals on NVP therapy would differ from those in HIV-negative
90 individuals. Any such difference may also have a measurable impact on parasite clearance in
91 treated asymptomatic individuals, as day 7 lumefantrine concentration is known to be an
92 important determinant of antimalarial efficacy in individuals with symptomatic malaria (4).

93

94

95 **Materials and methods**

96 *Study area*

97 The study was carried out at the University of Port Harcourt Teaching Hospital and the Braithwaite
98 Memorial Specialist Hospital Port Harcourt, Nigeria, from September 2010 to May 2011. Port
99 Harcourt is the capital of Rivers State in the Niger Delta, rich in the nation's oil resources. The
100 region is dotted with oil and gas activities which attract an international workforce, and
101 commercial sex workers follow the camp (14). These socio-economic conditions contribute to a
102 high estimated population prevalence of HIV infection of 7.4% (15). Malaria is holoendemic in
103 Nigeria with transmission all the year round, but malaria cases are most common during the rainy
104 season from April to September, with peak of the rains and intense transmission between May
105 and July. Annual rainfall averages more than 3,550 millimeters in the region.

106

107 *Patients and samples*

108 This paper describes an exploratory pharmacokinetic study with a simple unmatched case-control
109 design, ancillary to a study designed to track molecular markers of drug resistance in HIV-infected
110 individuals, using active detection of *P. falciparum* infection followed by treatment with AL as the
111 regimen recommended by the University of Port Harcourt Teaching Hospital guidelines for
112 uncomplicated malaria in adults. The work was conducted from September 2010 to May 2011. The
113 main endpoints of the current analysis were day 7 peripheral blood lumefantrine levels, and
114 parasite carriage at day 3 and day 28 post-treatment. The primary endpoint for which the study
115 was designed and powered was carriage of parasite genetic markers of antimalarial resistance.
116 This analysis is ongoing and will be reported elsewhere.

117

118 Participants were recruited if they met the following eligibility criteria: age 16-65 years, willingness
119 to have HIV status confirmed from clinical records or by a point-of-care test, *P. falciparum* positive
120 by microscopic examination of a blood film and provision of a signed informed consent form. HIV-
121 positive patients were recruited from the HIV adult clinic of both hospitals. HIV-negative
122 participants were recruited from the hospital communities including staff and students. HIV-
123 negative patients were screened and confirmed virus-negative with the use of the HIV Determine
124 point-of-care test (Alere Medical Co. Ltd Matshuhidai-shi, Chiba, Japan). Each was then screened
125 for malaria by standard microscopy. Permission for the study was obtained from the Research

126 Ethics Committees of the University of Port Harcourt Teaching Hospital, the Braithwaite Memorial
127 Specialist Hospital and the London School of Hygiene and Tropical Medicine, London.

128

129 Enrolled patients were treated with AL ('Coartem', Novartis Pharma, Nigeria) according to
130 manufacturer's dosing regimen: 4 tablets twice daily for 3 days for persons with weight>35kg.

131 Patients were advised to eat before taking the tablets. Most of the patients took their first dose at
132 the site having been pre-informed to eat before coming. Patients were followed up till day 28. On
133 day 7, capillary blood samples were taken from a finger prick.

134

135 For drug measurements, 100µl of blood were measured using a pipette and dropped on a filter
136 paper (Glass microfibers paper, Fisherbrand FB59431) pre-treated with 0.75M tartaric acid (Fisher
137 Scientific). The papers were allowed to air-dry and then stored in individual pouches with a silica
138 desiccant to absorb moisture. The preserved papers were transferred to the London School of
139 Hygiene and Tropical Medicine. Filter paper adsorbed blood samples were analysed for
140 lumefantrine using liquid chromatography-mass spectrometry (LCMS; Thermo Finnigan LCQ
141 instrument) following a modified protocol based on previously published methods (16). Briefly
142 bloodspots were extracted in methanol / water (4:1; 350 µl), and the extracts were filtered
143 through a cotton wool plug. Each sample (20 µl) was separated on a Dionex Acclaim® 120 3µm C18
144 (4.6 x 150 mm, with 120 Å pore size, fitted with a guard column) eluting with mobile phase MeOH:
145 20 mM formate buffer, pH 2.7 (85:15) isocratically at a flow rate of 500µl/min. The column
146 temperature was maintained at 35°C. The ESI source was operated in positive mode with the
147 capillary temperature set to 350°C and sheath and auxiliary gas (nitrogen) flow rates of 60 and 20
148 arbitrary units respectively. Peak identity was confirmed by using blood spiked with lumefantrine
149 standards (0-30 µg/ml), adsorbed onto filter paper and extracted in the same manner as the
150 patient samples. Quantitation was performed using selective ion monitoring for the transitions
151 m/z 530 to 512. LLOD was determined to be 0.1 µg/ml, LLOQ 1.0 µg/ml and ULOQ 20.0 µg/ml.

152

153 *Plasmodium falciparum* DNA was prepared from dried spots (10–20 µl) on Whatman paper as
154 previously described (17) and codons 24 to 201 of the *pfmdr1* locus amplified by nested PCR (18).
155 Relative quantification of parasite DNA was performed by an established qPCR method as
156 previously described (19).

157

158 Data were entered into spreadsheets and analysed in STATA 11 (Stata Corp, Madison WI).
159 Continuous data were compared between groups using Wilcoxon's rank sum test, while
160 categorical comparisons in 2 x 2 format were performed using the χ^2 distribution.

161 **Results**

162 Out of 80 attendees at the two HIV clinics who agreed to have a malaria film read, 68 were
163 identified as positive for *P. falciparum* and returned for day 7 follow-up (85%). None of these
164 individuals reported concurrent symptoms suggestive of clinical malaria. 126 individuals agreed to
165 have a rapid HIV test performed, of which 99 were found to be negative for HIV-specific
166 antibodies, were identified as infected with *P. falciparum* and attended for day 7 follow-up (79%);
167 none of these individuals were symptomatic. These 167 participants were treated with a full adult
168 course of AL, and followed up on day 3, day 7 and day 28 for repeat blood sampling.

169

170 To confirm microscopic diagnosis of *P. falciparum* parasitaemia at enrolment, nested PCR
171 amplification of the amino-terminal fragment of the *pfmdr1* gene was carried out on DNA
172 extracted from the first blood sample taken from each participant. Nested PCR was also
173 performed on DNA extracted from all day 3 and day 28 filter paper blood samples. Unexpectedly,
174 a high proportion of enrollees (78.1%) were found to be aparasitaemic by nested PCR, suggesting
175 poor specificity of the original microscopic diagnosis (Table 1). There was a strong association
176 between PCR positivity at day 0 and day 3 (O.R. 5.56, 95% C.I. 1.76 - 17.32; P = 0.0004), suggesting
177 good reproducibility of parasite detection for the PCR method, in contrast to results obtained with
178 microscopy.

179

180 Using the PCR data as a more reliable test for parasite carriage, we found weak evidence that HIV
181 positive people were more likely to be parasitaemic at day 0 (OR 2.05, 95% C.I. 0.917 - 4.60; P =
182 0.054), which may reflect slightly higher parasite densities in this group, and thus a greater
183 likelihood of parasites being correctly identified by the screening microscopists. HIV-positive
184 subjects were not significantly more likely to be PCR positive for *P. falciparum* at day 3 and/or day
185 28 after AL treatment than were HIV negative individuals (OR 1.75, 95% C.I. 0.776 - 3.95; P =
186 0.141).

187

188 Both HIV status and lumefantrine concentration at day 7 were recorded for all 167 individuals. We
189 examined the distribution of lumefantrine concentration at day 7 in all study participants, and
190 found highly significant departure from normality ($z=7.581$, $P<0.0001$), which remained after
191 (natural) logarithmic transformation ($z=5.372$, $P<0.0001$). In an exploratory analysis following the
192 methods of Kredo *et al.*, (6), we removed as “outliers” 5 samples with extremely low measured
193 lumefantrine concentrations (0, 0, 0.01, 0.08, 0.08 μ M respectively, all in the HIV neg group) and

194 log-transformed. Departure from the normal distribution was then marginally non-significant
195 ($z=1.594$, $P = 0.054$). After consideration of these findings, we decided to take the conservative
196 approach of using only non-parametric tests for testing statistical significance of comparisons, and
197 retained all data in the analysis.

198

199 HIV status, and thus nevirapine use, was found to have a significant effect on the concentration of
200 lumefantrine 7 days after treatment (Wilcoxon ranksum test $z = -3.270$, $P=0.0011$), with a median
201 concentration in the HIV negative group of $2.75\mu\text{M}$ (IQR $1.03 - 4.31$), and in the HIV positive group
202 of $3.55\mu\text{M}$ (IQR $2.07 - 5.37$) (Figure 1). However, the 5 individuals with extremely low
203 lumefantrine readings (identified in the previous paragraph) were all in the HIV-negative group, so
204 to test for possible bias caused by this group, we performed the comparison with these 5
205 measurements removed. In this exploratory analysis, a significant association remained between
206 HIV status and lumefantrine concentration at 7 days post AL treatment ($z = -2.830$, $P=0.0046$).

207

208 As many of our subjects were shown to be parasite negative by PCR, we tested for any effect of
209 parasitaemia on lumefantrine concentrations at day 7. Overall, in all 166 evaluable individuals, PCR
210 positive parasitaemia at day 0 was not associated with any difference in day 7 lumefantrine
211 concentration in our sample set (37 positive vs 129 negative individuals). There was a weak
212 association between day 3 PCR-detected parasitaemia and higher lumefantrine concentration ($z =$
213 -2.305 , $P = 0.021$), suggesting that greater lumefantrine bioavailability among NVP recipients was
214 not improving AL treatment outcomes. This effect was not strong enough to confer a statistically
215 significant deficit in parasite clearance for HIV-positive individuals as a group; considering only
216 those participants with follow-up data from both day 3 and day 28 ($N = 140$) 33.9% of HIV-positive
217 individuals had PCR-detectable parasitaemia on either or both days 3 and 28, compared to 22.7%
218 of HIV negative individuals (O.R. 1.75; 95% C.I. 0.776 - 3.95; $P = 0.141$). Assessment of day 0
219 parasitaemia using qPCR was performed for 8 individuals (including 5 HIV+) who were
220 subsequently PCR-positive on day 3, and 15 who had cleared parasites by day 3 (including 9 HIV+).
221 This exploratory analysis did not provide any evidence that higher starting parasitaemia increased
222 the likelihood of an individual remaining PCR-positive for *P. falciparum* on day 3 (Wilcoxon ranksum
223 test: $z = -0.904$, $P = 0.37$).

224

225

226

227 **Discussion**

228 The co-formulated combination of artemether, a sesquiterpene lactone derived from the natural
229 compound artemisinin, with the aryl amino-alcohol lumefantrine, as a systemic racemic flourene
230 mixture, has become the most widely distributed and available ACT throughout Africa. As anti-
231 retroviral chemotherapies have also become more widely available for treatment of HIV patients
232 in health systems in Africa, detailed understanding of any interactions between these two
233 chemotherapies is urgently needed. In this study we show that HIV positive adults taking regular
234 NVP who were treated with AL for microscopically apparent *P. falciparum* infection, had
235 significantly higher day 7 plasma concentrations of lumefantrine compared to treated adults who
236 were HIV test-negative and not receiving NVP. However, we found no evidence that sub-
237 microscopic parasite persistence at day 3 after AL treatment was prevented in individuals with
238 higher day 7 plasma levels of lumefantrine, in fact HIV-positive individuals were slightly more likely
239 to have PCR-detectable parasitaemia on day 3 or day 28 than were HIV-negative participants,
240 although this was not significant.

241

242 Our findings are consistent with those of Kredo *et al.* (6) and confirm that drug-drug interactions
243 between AL and NVP are potentially important. However, NVP-stimulation of the CYP3A4
244 isoenzyme would be expected, *a priori*, to lower peripheral lumefantrine levels, due to an increase
245 in the amount of lumefantrine metabolised to desbutyl-lumefantrine, a potent derivative that is
246 normally found at a concentration between 0.5% and 5% of that of the parent compound at day 7
247 in the few studies available (8, 20). Food intake also alters lumefantrine metabolism; we were not
248 able to supervise the food intake of our participants while they were taking AL, but all were
249 informed of the need to accompany their medication with fatty food. The apparently increased
250 bioavailability of lumefantrine in NVP recipients produced no measurable parasitological benefit in
251 our patients; on the contrary, one third of HIV-positive (and thus NVP-receiving) participants were
252 found to have persisting PCR-detectable *P. falciparum* parasitaemia at day 3 and/or day 28,
253 compared to less than a quarter of the control group. This difference, which suggests perturbation
254 of the immune system in HIV infection remains a significant factor in these dual-treated patients,
255 was not statistically significant. The case-control design used here may be prone to selection bias,
256 and this could affect parasitological outcomes. However univariate analysis of post-treatment
257 parasitaemia versus age, weight, gender and educational attainment found no evidence of

258 confounding by any of these parameters (data not shown). The recent observation that co-
259 administration of NVP with AL leads to reduced maximal concentration of both artemether and
260 DHA (21) suggests an alternate explanation for reduced parasite clearance at day 3 in patients
261 receiving both regimens. Nevertheless, further studies of the parasitological impact of
262 antiretroviral-antimalarial drug-drug interactions in adequately powered studies are urgently
263 needed, not least because of the important role of the host immune system in clearing drug-
264 treated malaria parasites (3, 22). In our study, all HIV-infected participants were identified through
265 attendance at a weekly clinic in which all received NVP (except for a single patient on efavirenz;
266 when this patient was excluded from the analysis the relationship between NVP use and
267 lumefantrine concentration at day 7 remained significant). Compliance with antiretroviral
268 treatment was not evaluated directly. Future studies with HIV patients not receiving NVP may
269 permit discrimination between drug-drug interactions, and the impact of retroviral disease *per se*
270 on lumefantrine bioavailability.

271

272 A major weakness of our study was the poor quality of enrolment microscopic diagnosis, such that
273 the majority of participants had in fact failed a major inclusion criterion. This had two main
274 impacts. Firstly, the study was greatly under-powered to evaluate any parasitological outcomes, as
275 so many participants were actually uninfected (with *P. falciparum*). Secondly, we were not able to
276 analyse parasite densities with any confidence, and thus were left with the binary variable of PCR
277 positivity as the remaining reliable measure of malaria infection. Further, by this method we
278 cannot rule out the possibility that some of our positive PCR reactions on post-treatment blood
279 samples were detecting gametocytes of *P. falciparum* only. These sexual stage parasite forms are
280 infective to *Anopheles sp.* mosquitoes, but do not contribute to clinical malaria symptoms and
281 cannot divide. Gametocytes are well known to survive in a minority of AL-treated patients after
282 clearance of the actively dividing asexual parasite stages (23, 24). Nevertheless, we have recently
283 described persistence of asexual parasites in asymptomatic Ghanaian school children treated with
284 ACT, suggesting that sub-clinical parasitaemia may be more difficult to clear than previously
285 thought (25).

286

287 In conclusion, this is the second study to find evidence that NVP-recipient HIV patients harbour a
288 significantly higher peripheral blood concentration of lumefantrine than do HIV-negative controls,
289 7 days after receiving a full treatment course of AL. Our findings corroborate the findings of Kredo
290 *et al.* (6) in a larger group of AL-treated individuals, some of whom were infected with *P.*

291 *falciparum*. Insufficient parasitological data were available to determine whether this difference in
292 lumefantrine concentration provides any parasitological benefit to NVP-treated HIV patients with
293 malaria infections.

294

295

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300 **References**

- 301 1. **Khoo S, Back D, Winstanley P.** 2005. The potential for interactions between antimalarial and
 302 antiretroviral drugs. *AIDS*. **19**: 995-1005.
- 303 2. **Korenromp EL, Williams BG, de Vlas SJ, Gouws E, Gilks CF, Ghys PD, Nahlen BL.** 2005. Malaria
 304 attributable to the HIV-1 epidemic, sub-Saharan Africa. *Emerg Infect Dis*. **11**: 1410-1419.
- 305 3. **Whitworth J, Morgan D, Quigley M, Smith A, Mayanja B, Eotu H, Omoding N, Okongo M, Malamba S,**
 306 **Ojwiya A.** 2000. Effect of HIV-1 and increasing immunosuppression on malaria parasitaemia and clinical
 307 episodes in adults in rural Uganda: a cohort study. *Lancet* **356**: 1051-1056.
- 308 4. **Ezzet F, Mull R, Karbwang J.** 1998. Population pharmacokinetics and therapeutic response of CGP 56697
 309 (artemether + benflumetol) in malaria patients. *Br J Clin Pharmacol*. **46**: 553-561.
- 310 5. **Djimé A, Lefèvre G.** 2009. Understanding the pharmacokinetics of Coartem. *Malar J*. **8 Suppl 1**: S4.
- 311 6. **Kredo T, Mauff K, Van der Walt JS, Wiesner L, Maartens G, Cohen K, Smith P, Barnes KI.** 2011.
 312 Interaction between artemether-lumefantrine and nevirapine-based antiretroviral therapy in HIV-1-
 313 infected patients. *Antimicrob Agents Chemother*. **55**: 5616-5623.
- 314 7. **Lefèvre G, Bindschedler M, Ezzet F, Schaeffer N, Meyer I, Thomsen MS.** 2000. Pharmacokinetic
 315 interaction trial between co-artemether and mefloquine. *Eur J Pharm Sci*. **10**: 141-151.
- 316 8. **Salman S, Page-Sharp M, Griffin S, Kose K, Siba PM, Ilett KF, Mueller I, Davis TM.** 2011. Population
 317 pharmacokinetics of artemether, lumefantrine, and their respective metabolites in Papua New Guinean
 318 children with uncomplicated malaria. *Antimicrob Agents Chemother*. **55**: 5306-13.
- 319 9. **van Vugt M, Ezzet F, Phaipun L, Nosten F, White NJ.** 1998. The relationship between capillary and
 320 venous concentrations of the antimalarial drug lumefantrine (benflumetol). *Trans R Soc Trop Med Hyg*.
 321 **92**: 564-565.
- 322 10. **Ezzet F, van Vugt M, Nosten F, Looareesuwan S, White NJ.** 2000. Pharmacokinetics and
 323 pharmacodynamics of lumefantrine (benflumetol) in acute falciparum malaria. *Antimicrob Agents*
 324 *Chemother*. **44**: 697-704.
- 325 11. **Gerber JG.** 2000. Using pharmacokinetics to optimize antiretroviral drug-drug interactions in the
 326 treatment of human immunodeficiency virus infection. *Clin Infect Dis*. **Suppl 2**: S123-129.
- 327 12. **Back DJ, Khoo SH.** 2003. The role of clinical pharmacology in optimizing antiretroviral therapy. *Br J Clin*
 328 *Pharmacol* **55**: 473-476.
- 329 13. **Havir D, Cheeseman SH, McLaughlin M, Murphy R, Erice A, Spector SA, Greenough TC, Sullivan JL, Hall**
 330 **D, Myers M, Lamson M, Richman DD.** 1995. High-dose nevirapine: safety, pharmacokinetics, and
 331 antiviral effect in patients with human immunodeficiency virus infection. *J Infect Dis*. **171**: 537-545.
- 332 14. **Nwauche CA, Akani CI.** 2006. An assessment of high risk sexual behavior and HIV transmission-among
 333 migrant oil workers in the Niger Delta area of Nigeria. *Niger J Clin Pract* **9**: 48-51.
- 334 15. **Federal Ministry of Health, Nigeria.** 2008. Technical report (2008). *National HIV sero-prevalence*
 335 *sentinel survey among pregnant women attending antenatal clinics in Nigeria*. FMOH, Abuja.

- 336 16. **Blessborn D, Römsing S, Annerberg, A, Sundquist, D, Björkman A, Lindegardh N, Bergqvist Y.** 2007.
 337 Development and validation of an automated solid-phase extraction and liquid chromatographic
 338 method for determination of lumefantrine in capillary blood on sampling paper. *J Pharmaceut Biomed.*
 339 **45:** 282-287.
- 340 17. **Dlamini SV, Beshir K, Sutherland CJ.** 2010. Markers of anti-malarial drug resistance in *Plasmodium*
 341 *falciparum* isolates from Swaziland: identification of pfmdr1-86F in natural parasite isolates *Malaria J* **9:**
 342 68.
- 343 18. **Humphreys GA, Merinopoulos I, Ahmed J, Whitty CJM, Mutabingwa TK, Sutherland CJ & Hallett RL.**
 344 2007. Amodiaquine and artemether-lumefantrine select distinct alleles of the *Plasmodium falciparum*
 345 *pfmdr1* gene in Tanzanian children treated for uncomplicated malaria *Antimicrob. Agents Chemother.* **51:**
 346 991-997.
- 347 19. **Beshir K, Hallett RL, Eziefula AC, Bailey R, Watson J, Wright SG, Chiodini PL, Polley SD, Sutherland CJ.**
 348 2010. Measuring the efficacy of anti-malarial drugs *in vivo*: quantitative PCR measurement of parasite
 349 clearance. *Malaria J.* **9:** 312.
- 350 20. **McGready R, Stepniewska K, Lindegardh N, Ashley EA, La Y, Singhasivanon P, White NJ, Nosten F.**
 351 2006. The pharmacokinetics of artemether and lumefantrine in pregnant women with uncomplicated
 352 falciparum malaria. *Eur J Clin Pharmacol.* **62:** 1021-1031.
- 353 21. **Byakika-Kibwika P, Lamorde M, Mayito J, Nabukeera L, Namakula R, Mayanja-Kizza H, Katabira E,**
 354 **Ntale M, Pakker N, Ryan M, Hanpithakpong W, Tarning J, Lindegardh N, de Vries PJ, Khoo S, Back D,**
 355 **Merry C.** Significant pharmacokinetic interactions between artemether/lumefantrine and efavirenz or
 356 nevirapine in HIV-infected Ugandan adults. *J Antimicrob Chemother.* 2012 Sep;67(9): 2213-21.
- 357 22. **Djimde´ AA, Guindo AB, Kayentao K, Diourte Y, Niare-Doumbo S, Coulibaly D, Kone AK, Cissoko Y,**
 358 **Tekete M, Fofana B, Dicko A, Diallo DA, Wellems TE, Kwiatkowski D, Plowe CV.** 2003. Clearance of
 359 drug-resistant parasites as a model for protective immunity in *Plasmodium falciparum* malaria. *Am J*
 360 *Trop Med Hyg* **69:** 558–63.
- 361 23. **Bousema JT, Schneider P, Gouagna LC, Drakeley CJ, Tostmann A, Houben R, Githure JI, Ord R,**
 362 **Sutherland CJ, Omar SA, Sauerwein RW.** 2006. Moderate effect of artemisinin-based combination
 363 therapy on transmission of *Plasmodium falciparum*. *J. Infect. Dis.* **193:** 1151-1159.
- 364 24. **Sutherland CJ, Ord R, Dunyo S, Jawara M, Drakeley CJ, Alexander N, Coleman R, Pinder M, Walraven**
 365 **G, Targett GAT.** 2005. Reduction of malaria transmission to *Anopheles* mosquitoes with a six-dose
 366 regimen of Co-artemether. *PLoS Med* **2:** e92.
- 367 25. **Dinko B, Oguike MC, Larbi JB, Bousema JT, Sutherland CJ.** 2013. Persistent detection of *Plasmodium*
 368 *falciparum*, *P. malariae*, *P. ovale curtisi* and *P. ovale wallikeri* after ACT treatment of asymptomatic
 369 Ghanaian school-children. *Int J Parasitol DDR* **3:** 45-50.
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373 TABLES

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375 TABLE 1. **Parasite carriage by microscopy and PCR in 68 HIV positive and 99 HIV negative**
 376 **individuals.**

377 All 167 individuals were reported as positive for *P. falciparum* parasites on
 378 microscopic examination of thick blood films.

379

Numbers PCR positive for <i>P. falciparum</i>	Day 0	Day 3	Day 28
HIV pos N = 68*	17 (29.9%)	8	12
HIV neg N = 99	20 (17.2%)	12	12

380 *67 of these individuals were receiving daily nevirapine anti-retroviral therapy; one received
 381 efavirenz; all HIV positive patients also received the nucleoside reverse-transcriptase inhibitors
 382 lamivudine and zidovudine.

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387 Figure Legends

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389 **Figure 1. Day 7 lumefantrine concentration in AL-treated participants.**

390 Mid-line of each box-plot is the median, with the edges of the box representing the
391 inter-quartile interval. Whiskers delineate the 5th and 95th percentile. Lumefantrine
392 concentration was below the normal limits of detection in five individuals, all in the
393 HIV negative group (see text).

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Day 7 lumefantrine concentration, by HIV status

