

1 Pharmacokinetics and safety profile of artesunate-amodiaquine co-administered with  
2 antiretroviral therapy in malaria uninfected HIV-positive Malawian adults.

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26 **ABSTRACT**

27

28 There are limited data on the pharmacokinetic and safety profiles of artesunate-amodiaquine in  
29 human immunodeficiency virus infected (HIV+) individuals receiving antiretroviral therapy. In a  
30 two-step intensive sampling pharmacokinetic trial, we compared area under the concentration-  
31 time curve from 0 to 28 days ( $AUC_{0-28 \text{ days}}$ ) of an active metabolite of amodiaquine,  
32 desethylamodiaquine, and treatment-emergent adverse events between antiretroviral therapy-  
33 naive HIV+ adults and those taking nevirapine and ritonavir-boosted lopinavir-based  
34 antiretroviral therapy. In step 1, malaria uninfected adults ( $n=6/\text{arm}$ ) received half the standard  
35 adult treatment regimen of artesunate-amodiaquine. In step 2, another cohort ( $n=25/\text{arm}$ )  
36 received the full regimen. In step 1, there were no safety signals and significant differences in  
37 desethylamodiaquine  $AUC_{0-28 \text{ days}}$  among participants in the ritonavir-boosted lopinavir,  
38 nevirapine and antiretroviral therapy-naive arms. In step 2, compared with the antiretroviral  
39 therapy-naive arm, participants in the ritonavir-boosted lopinavir arm had 51% lower  
40 desethylamodiaquine  $AUC_{0-28 \text{ days}}$ , (geometric mean [95% CI]; 23,822 [17,458-32506] vs 48,617  
41 [40,787-57,950] ng.hr/mL,  $p < 0.001$ ). No significant differences in  $AUC_{0-28 \text{ days}}$  were observed  
42 between nevirapine and antiretroviral therapy-naïve arms. Treatment-emergent transaminitis  
43 was higher in the nevirapine (20% [5/25]) than the antiretroviral therapy naïve (0.0% [0/25]) arm  
44 (risk difference 20% [95% CI:4.3-35.7]  $p=0.018$ ). Ritonavir-boosted lopinavir antiretroviral  
45 regimen was associated with reduced desethylamodiaquine exposure which may compromise  
46 artesunate-amodiaquine's efficacy. Co-administration of nevirapine and artesunate-  
47 amodiaquine may be associated with hepatotoxicity.

48

49 **Key words:** Amodiaquine; Antiretroviral therapy; Malaria

50

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51 **INTRODUCTION**

52

53 Human immunodeficiency virus (HIV) and *Plasmodium falciparum* (*Pf*) malaria infections are  
54 endemic in most regions in sub-Saharan Africa (SSA) and co-infections occur frequently. HIV  
55 infection increases susceptibility (1–3) and severity of *Pf* malaria (4–6), and reduces the efficacy  
56 of antimalarial drugs (7). The World Health Organisation (WHO) recommends initiation of triple  
57 antiretroviral therapy (ART) in HIV-positive (HIV+) individuals regardless of CD4 cell count (8).  
58 The recommended ART in SSA contain non-nucleoside reverse transcriptase inhibitors  
59 (NNRTIs), such as efavirenz (EFV) and nevirapine (NVP), or protease inhibitors (PIs) such as  
60 ritonavir-boosted lopinavir (LPV/r). The WHO also recommends artesunate-amodiaquine (AS-  
61 AQ), as one of the first-line treatment for uncomplicated malaria (9).

62

63 HIV-malaria co-infected individuals require concurrent treatment with ACTs and ART, potentially  
64 resulting in pharmacokinetic interactions (10). Drug information sheets for ACTs caution against  
65 concurrent use of ACTs and ART because NNRTIs or PIs and ACTs are metabolized by  
66 cytochrome-P (CYP) 450 liver enzymes (particularly, CYP3A4). NNRTIs such as nevirapine and  
67 efavirenz usually induce various CYP450 enzymes but are also substrates for CYP450 isoforms  
68 (CYP3A4) (11, 12). AQ is rapidly metabolised, mainly by CYP2C8 but also CYP3A4, to its  
69 metabolite, desethylamodiaquine (DESAQ), which is responsible for almost all the antimalarial  
70 effect (13, 14). This metabolite has a longer half-life and is eliminated slowly compared to AQ  
71 (13–19). Thus, co-administration of NNRTI-based ART with AS-AQ could reduce AQ and  
72 DESAQ blood concentrations resulting in lower efficacy of AQ. Conversely, HIV protease  
73 inhibitors, particularly ritonavir, are potent inhibitors of the CYP3A4 isoform. Co-administration of  
74 protease inhibitor-based ART with AS-AQ could lead to elevated AQ and lower DESAQ  
75 concentrations, potentially resulting in toxicities or reduced AS-AQ efficacy (20).

76

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77 To characterize the interactions between AS-AQ and ART, we compared the pharmacokinetic  
78 parameters ( $AUC_{0-28 \text{ days}}$ ,  $C_{\max}$ ,  $t_{\max}$  and  $t_{1/2}$ ) of the longer acting partner drug of AS-AQ,  
79 amodiaquine and of its metabolite-DESAQ, and incidence of adverse events in HIV+ adults  
80 taking AS-AQ plus NVP-ART or LPV/r-ART and those taking AS-AQ only in a parallel design  
81 (two-step) study.

82

## 83 **RESULTS**

### 84 **Characteristics of study participants**

85 In step 1, 18 participants were successfully enrolled and followed up for 28 days, including 1  
86 subject who replaced a participant who was withdrawn following a protocol violation. In step 2,  
87 75 were enrolled and successfully followed up to 28 days, including 2 who replaced those who  
88 were lost to follow-up.

89

90 Supplementary Table 1 shows baseline characteristics of participants who completed follow-up  
91 in steps 1 and 2. In both step 1 and step 2, the majority of participants in all study arms, except  
92 the step 1 ART naïve arm, were females. Participants in the LPV/r arm had a tendency towards  
93 higher alkaline phosphatase levels at baseline than those in the ART-naïve arm. In step 2,  
94 participants in the LPV/r arm had a higher median age than those in the other study arms. The  
95 median duration on ART was longer in the LPV/r than the NVP arm. All the participants in step 1  
96 and the majority (80%) in step 2 were on cotrimoxazole prophylaxis.

97

### 98 **Pharmacokinetics of AQ and DESAQ and interactions with ART in step 1**

99 PK data were available for 17 of the 18 participants who completed follow-up in step 1. The  
100 excluded participant had unquantifiable drug or metabolite concentrations at nearly all follow up  
101 time points. AQ concentrations were well below the HPLC assay limit of quantification

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102 (LLQ=25ng/mL). Therefore, no formal comparisons of AQ PK parameters were performed  
103 across the study arms.

104

105 As shown in Table 1a, the geometric mean [95% CI] of DESAQ  $C_{max}$  was 60% lower in the  
106 LPV/r-ART arm (42 [34-51] ng/mL) compared to the ART-naïve arm (106 [63-179] ng/mL,  
107  $p=0.006$ ), while no significant difference in DESAQ  $AUC_{0-28\text{ days}}$  was observed between the  
108 LPV/r-ART (4,128 [1,946- 8,758] ng.hr/mL) and the ART-naïve (7,920 [5034-12459] ng.hr/mL,  
109  $p=0.10$ ) arms. The  $C_{max}$  for DESAQ were similar between participants in the NVP and ART-  
110 naïve arms. Similarly, no differences in mean  $AUC_{0-28\text{ days}}$  were observed between the NVP-ART  
111 and ART-naïve arms. As shown in the concentration-time plot in Figure 1, DESAQ  
112 concentration-time profile was notably lower in the LPV/r-ART arm compared to the ART-naïve  
113 and NVP-ART arms. There were no significant differences in half-life and  $T_{max}$  of DESAQ  
114 between the NVP and ART naïve arms as well as between the LPV/r and ART naïve arms.

115

#### 116 **Safety assessment in step 1**

117 After AS-AQ administration, one participant in the NVP arm developed headache and chills,  
118 which resolved without any treatment and were judged as not related to the study drug. As  
119 shown in Table 2a, treatment-emergent grade 3 or 4 neutropenia was observed in the NVP-ART  
120 arm (50% [3/6]), LPV/r-ART arm (33% [2/6]) and ART-naïve arm (17% [1/6]). One participant in  
121 the AS-AQ plus NVP arm had a car accident which was not thought to be related to the study  
122 drug.

123

#### 124 **Pharmacokinetics of DESAQ and interactions with ART in step 2**

125 In step 2, PK data were available for 74 of the 75 participants who completed follow-up. The  
126 excluded participant had unquantifiable drug or metabolite concentrations at nearly all follow up

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127 time points. Similar to our observation in step 1, AQ concentrations in step 2 were well below  
128 the HPLC assay limit of quantification (LLQ=25ng/mL).

129

130 Table 1b shows that the geometric mean [95% CI] of DESAQ  $C_{max}$  was 45% lower in the LPV/r-  
131 ART arm (248 [199, 310] ng/mL) compared to the ART-naïve arm (448 [374, 534] ng/mL,  
132  $p<0.001$ ), while DESAQ  $AUC_{0-28\text{ days}}$  was 51% lower in the LPV/r-ART arm (23,822 [17,458-  
133 32,506] ng.hr/mL) compared to the ART-naïve arm (48,617 [40,787-57,950] ng.hr/mL,  $p<0.001$ )

134 In contrast, there were no significant differences in  $AUC_{0-28\text{ days}}$  and  $C_{max}$  between the NVP-

135 treated and the ART naïve arms. Also, there were no significant differences in DESAQ  $T_{max}$

136 among the ART-naïve, LPV/r-ART and NVP-ART study arms. DESAQ half-life and clearance

137 were significantly shorter and faster, respectively, in the LPV/r-ART arm compared with the

138 ART-naïve arm.

139

140 Figure 1 shows the concentration-time plot for DESAQ in the study arms. Similar to the findings

141 in steps 1, DESAQ concentration-time profile in step 2 was notably lower in the LPV/r-ART arm

142 when compared with the ARV-naïve arm.

143

#### 144 **Day 7 plasma DESAQ levels by ART arm in step 2**

145 Compared with the geometric mean concentration [95% CI] of DESAQ at day 7 in the ART-

146 naïve arm (94 [73, 120] ng/ml), the concentration was 52% lower in the LPV/r arm (45 [29, 73]

147 ng/ml,  $p=0.011$ ) and was 28% lower in the NVP arm (68 [57, 80] ng/ml,  $p=0.092$ ). However,

148 there were no significant differences in the proportion of participants with Day 7 DESAQ levels

149 below 75ng/ml (a threshold associated with 100% parasitological cure rate (19)) between the

150 LPV/r arm (67%, [14/21]) and the ART-naïve arm (43%, [9/21],  $p=0.215$ ), and between the ART

151 naïve arm and the NVP arm (56%, [14/25],  $p=0.554$ )

152

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153 **Safety assessment in step 2**

154 Overall, gastrointestinal symptoms (such as vomiting or diarrhoea) or neurological symptoms  
155 (such as headache) were not reported following intake of AS-AQ in the different study arms.  
156 However, as shown in Table 2b, there was a statistically non-significant trend towards higher  
157 incidence of grade 3 or 4 treatment emergent neutropenia in the NVP arm (28.0% [7/25]  
158 compared to ART-naïve arm (16.0% [4/25] ,  $p=0.496$ ). The incidence of grade 3 or 4 post-  
159 dosing neutropenia was lower in the LPV/r arm (0.0% [0/25],  $p=0.110$ ). The incidence of  
160 treatment-emergent grade 3 or 4 transaminitis (concurrent ALT and AST elevation) was higher  
161 in the NVP arm (20% [5/25]) than the ART naïve arm (0.0% [0/25], risk difference 20% [95% CI:  
162 4.3, 35.7]  $p=0.018$ ). Similar to the ART-naïve arm, there were no cases of treatment-emergent  
163 grade 3 or 4 transaminitis in the LPV/r arm. Two cases of QTc prolongation (change in QTc  
164  $>60$ ms from baseline to  $C_{max}$ ) were detected in both the LPV/r-ART arm (8.0%,  $n=25$ ) and the  
165 NVP-ART arm (8.0%,  $n=25$ ) arms but none were detected in the ART-naïve arm (0.0%,  $n=25$ ).  
166 No significant differences were found between any of the ART arms and the ART-naïve arm  
167 ( $p=0.490$ ). These cases resolved spontaneously within two weeks of occurrence.

168

169 **DISCUSSION**

170 In this study, we found that median DESAQ AUC and  $C_{max}$  were significantly lower in the  
171 LPV/r- arm when compared to the ART-naïve arm but no differences were observed in these PK  
172 parameters between the NVP and ART-naïve arms. While AS-AQ appeared to be generally  
173 tolerated in all study arms, treatment-emergent transaminitis was more common in the NVP-arm  
174 than in the ART-naïve arm.

175

176 Our findings of insignificant differences in PK parameters of DESAQ between the ART naïve  
177 and NVP group are in contrast with those from a previous Nigerian open-label parallel-arm PK

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178 study which found a lower DESAQ AUC in HIV-infected adults on NVP-based ART than in ART  
179 naïve participants (21). These differences could be due to several reasons including genetic  
180 differences in CYP450 iso-enzymes of the study populations. Additional studies would be  
181 needed to explain the reasons for this discrepancy.

182

183 Although highly expressed in the liver, CYP family enzymes, especially CYP3A4 and CYP2C8,  
184 are expressed in the small intestinal epithelium and play an active role in the metabolism of  
185 drugs (22–24). Findings of significantly reduced DESAQ  $C_{max}$  in the LPV/r arm at full standard  
186 dose in step 2 may partly be due to reduced CYP2C8-mediated gut or liver metabolism of AQ to  
187 DESAQ. This is plausible as CYP2C8 is the main hepatic P450 isoform that clears AQ and  
188 catalyses the formation of DESAQ (13)(25). Consequently, inhibition of CYP2C8 by its known  
189 potent inhibitors, LPV and ritonavir (10), are likely to account for the observed reduction in  $C_{max}$ .  
190 Alternatively, the reduced DESAQ AUC in the LPV/r could be as a result of rapid clearance of  
191 DESAQ in the LPV/r arm compared to the ART naïve arm. However, this increased clearance is  
192 inconsistent with the known inhibitory effects of LPV/r on CYP2C8 (25). DESAQ is eliminated  
193 through extrahepatic CYP1A1 and CYP1B1 (25, 26), any potential impact that LPV/r may have  
194 on clearance of DESAQ by CYP1A1 and CYP1B1 needs to be further evaluated.

195

196 Since DESAQ is responsible for nearly all the antimalarial effect of AQ (13, 14), it is likely that  
197 lower DESAQ exposure (reduced  $C_{max}$  and AUC at full standard dose) in those taking LPV/r  
198 may result in lower treatment efficacy or prophylactic effect. Indeed, previous studies which  
199 administered amodiaquine base at a dosage of 10 mg/kg/day found that lower day 7 DESAQ  
200 concentrations were associated with an increased risk of treatment failure (14)(19). In the study  
201 by Strepniewska (19), patients with Day-7 DESAQ concentrations above 75 ng/mL achieved  
202 100% parasitological cure rate while 60% (n=5) of the participants who had Day-7 DESAQ  
203 concentrations of below 75 ng/mL had PCR confirmed recrudescence parasitaemia. The daily

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204 and total amodiaquine dose received by participants in step 2 (9.5 mg/kg/day and 28.5 mg/kg,  
205 respectively) falls within the middle of WHO's therapeutic dose range of 7.5 to 15 mg/kg/day for  
206 amodiaquine (27)(28)(14). The higher frequency of participants below the 75 ng/mL level in the  
207 LPV/r arm suggests that, in this population, the current dosage of AS-AQ may likely result in  
208 treatment failure or recurrent malaria infections.

209

210 Our finding of a higher incidence of neutropenia in the NVP-ART arm than the ART naïve arm is  
211 consistent with results from a previous Ugandan study which found an increased risk of  
212 neutropenia in children receiving AQ-AS and ART (20). Although blood levels of AQ and AS  
213 were not measured in the Ugandan study, the observed cases of neutropenia could have been  
214 due to high AQ or DESAQ levels. NVP has been associated with granulocytopenia as a marker  
215 of hypersensitivity (29). Any potential synergistic role of AQ and NVP in causing neutropenia or  
216 other haematological abnormalities requires further understanding. Additionally, administration  
217 of AS-AQ in our study was associated with transient liver function abnormalities, especially in  
218 people taking NVP-based ART. This finding is similar to significant increases in liver  
219 transaminase levels observed in a previous study when AS-AQ was co-administered with an  
220 NNRTI (efavirenz) (30). NVP is independently associated with hepatotoxicity (31, 32), so is AQ  
221 (33, 34). Thus, combining these drugs may have an additive hepatotoxic effect. The observed  
222 cases of transaminitis in the NVP arm could have been due to an increase in NVP  
223 concentrations following co-administration with AQ or a result of a synergistic effect of NVP and  
224 AQ as previously experienced among individuals taking an NNRTI (efavirenz) and AQ (30).  
225 Since we did not measure NVP concentrations, we were unable to ascertain the  
226 pharmacokinetic changes in steady state concentrations of NVP after administration of AQ and  
227 the impact this may have on incidence of transaminitis. Despite the fact that haematological and  
228 hepatic abnormalities found in our study were not clinically significant and did not persist beyond  
229 two weeks, our findings suggest that caution should be exercised when co-administering AS-AQ

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230 and NVP or the need for careful monitoring of liver function and haematological changes in  
231 malaria-infected HIV+ patients taking AS-AQ, particularly those taking AS-AQ plus NVP.

232

233 The present study was not adequately powered to detect adverse events such as cardiac  
234 toxicity. In our study, AQ levels were below the HPLC assay limit of quantification possibly due  
235 to lack of sensitivity of the assay in detecting very low plasma drug concentrations. Although this  
236 study was not aimed at examining dose proportionality between the two steps, the inability to  
237 observe this and to detect significant differences in PK parameters across arms and between  
238 steps may have been due to a very small sample size in step 1 relative to step 2 and the use of  
239 the parallel-arm design, which is more prone to effects of inter-individual anthropometric and  
240 genetic variations than a cross-over design. Genetic polymorphisms in CYP 450 iso-enzymes  
241 may have contributed to wide interquartile ranges of DESAQ PK parameters observed within  
242 each study arm. However, our study sample size is unlikely to have missed large (>2-fold)  
243 clinically important differences in AUC across the study arms. Future studies should explore  
244 dose linearity when AS-AQ is administered with antiretroviral drugs, assess the effect of genetic  
245 polymorphisms on the pharmacokinetics of DESAQ, quantify any changes in plasma ART levels  
246 when co-administered with antimalarial drugs and explore any potential impact of artesunate on  
247 the metabolism of amodiaquine when co-administered with antiretroviral drugs.

248

249 In conclusion, this study found significant PK interactions between LPV/r and AS-AQ, and  
250 signals of transaminitis and neutropenic effects among those taking NVP and AS-AQ. The  
251 clinical therapeutic implications of these findings in malaria-infected individuals on ART need  
252 further evaluation.

253

254

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255 **MATERIALS AND METHODS**

256 *Study Design*

257 We conducted an open-label, parallel arm, pharmacokinetic (PK) trial at Queen Elizabeth  
258 Central Hospital, Malawi, from August 2010 to March 2013. The study was implemented in two  
259 steps;

260

261 1. In step 1 (N=18) [PACTR2010030001871293], we administered half adult oral doses  
262 of AS-AQ (1 tablet of Coarsucam™, Sanofi-Aventis containing AS/AQ 100mg/270mg) at  
263 0, 24 and 48 hours, to HIV+ malaria-negative individuals in the following arms: (i) those  
264 on NVP-d4T-3TC, (ii) those on AZT-3TC-TDF-LPV/r and (iii) antiretroviral naive  
265 individuals which served as a control arm. step 1 served as a safety evaluation step,  
266 checking for unexpected clinical toxicities or interactions.

267

268 2. In step 2 (N=75) of the study [PACTR2010030001971409], after review of step 1  
269 safety data by an independent Data Safety Monitoring Board (DSMB), full treatment  
270 doses of AS-AQ (2 tablets of Coarsucam™, Sanofi-Aventis, each containing AS/AQ  
271 100mg/270mg) were administered to additional HIV+ individuals in the same arms and  
272 at the same time intervals as in step 1.

273

274 All doses of AS-AQ were administered with water only as recommended by Sanofi-Aventis.

275

276 *Study Population*

277 The target population for both steps were HIV+ male and non-pregnant female adults aged  $\geq 18$   
278 years residing in Blantyre or neighbouring districts of Thyolo and Chiradzulu. Individuals were  
279 eligible if they had been on NVP-ART or LPV/r-ART for  $\geq 6$  months and had CD4 cell count  $\geq$

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280 250 cells/mm<sup>3</sup>. At the beginning of the study, HIV+ antiretroviral naive individuals were eligible  
281 for recruitment into the study if they had a CD4 cell count  $\geq$  250/mm<sup>3</sup> but this cut-off point was  
282 increased to  $\geq$ 350/mm<sup>3</sup> when the new WHO criteria for ART initiation was implemented in  
283 Malawi in July 2011 (35). Other inclusion criteria were body weight  $\geq$ 40kgs, willingness to be  
284 admitted in the hospital for 3 days, to remain within the study sites and be contacted by phone  
285 or at home during the course of the study.

286

287 We excluded subjects who met any of the following criteria:

- 288 i. Body Mass Index  $\leq$ 18.5kg/m<sup>2</sup>  
289 ii. Haemoglobin concentration  $<$ 8.5 g/dL  
290 iii. Reported use of any antimalarial drugs within the preceding 4 weeks  
291 iv. Reported hypersensitivity to any of the ACTs  
292 v. Receipt of other drugs which are known inhibitors or inducers of P450 enzymes or P-  
293 glycoprotein (except cotrimoxazole prophylaxis)  
294 vi. History of regular intake of alcohol ( $>$ twice/week), tobacco ( $>$ 3 times/week) or use of  
295 illicit drugs  
296 vii. History or evidence of pre-existing liver, kidney or heart disease, including conductive  
297 abnormalities on electrocardiographs (*QTc interval* $>$ 450ms in men,  $>$ 470ms in females)  
298 viii. Clinical and/or laboratory evidence of *Pf* malaria, hepatitis B, pneumonia, tuberculosis,  
299 bacteraemia or laboratory evidence of potentially life threatening disorders  
300 ix. Karnofsky score of  $<$ 80%

301

302 *Sample size:*

303 The sample size in step 1 was 6 for each of the three arms. This approach was based on  
304 standard practice in early PK studies of antimalarial drugs which aim to safeguard the safety of  
305 study subjects and minimize the number of subjects who may be potentially exposed to harmful

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306 drug levels. The sample size for step 2 was 25 per arm which gave at least 90% power to detect  
307 a two-fold increase in the DESAQ AUC in any of the AS-AQ plus ART arms, assuming a mean  
308 DESAQ AUC of 154 ng/ml/hr (standard deviation of 150 ng/ml/hr (2)) in the AS-AQ control arm,  
309 at the 5% significance level.

310

### 311 *Ethics*

312 The study conformed to the principles of the International Conference on Harmonization on  
313 Good Clinical Practice and was approved by the College of Medicine Research Ethics  
314 Committee (COMREC) in Malawi. Written informed consent, to participate in the study, was  
315 sought from potential participants.

316

### 317 *Screening and enrolment*

318 Research nurses and clinicians sought written informed consent from individuals to perform  
319 screening procedures including physical medical and anthropometric assessment,  
320 electrocardiographs (ECGs) and blood tests to detect blood-borne infections, haematological,  
321 renal or hepatic abnormalities. Based on the results from screening procedures which were  
322 available within 7 days, potential study participants were informed about their eligibility to  
323 participate in the study. Consenting study participants were re-assessed by research nurses or  
324 clinicians to determine whether they still met all eligibility criteria, through repeat history taking  
325 and physical examination. Eligible participants were admitted in hospital and an indwelling  
326 cannula was inserted into a vein before their scheduled dose of ART and the first dose of the  
327 ACT. Approximately 1 hour before the scheduled time of ART and ACT dosing, blood samples  
328 were collected for haematological, renal and liver function tests and also random glucose test.

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330 *Blood sample collection and follow-up procedures*

331 While participants were hospitalized, blood samples for PK assays were collected in heparin  
332 vacutainer tubes, pre-treatment and at the following post-treatment times: 0, 0.25, 0.5, 1, 1.5, 2,  
333 3, 4, 5, 6, 8, 12, 24, 36, 48, 60 and 72hours. After discharge from hospital, blood samples were  
334 taken at 4, 5, 6, 7, 14, 21 and 28 days. Immediately after collection, samples were spun in a  
335 refrigerated centrifuge and the separated plasma was temporarily frozen in liquid nitrogen  
336 before being transferred to a -80°C freezer until PK analyses.

337

338 Participants were monitored for 28 days after administration of the first study dose to detect  
339 clinical adverse events. Blood samples to detect haematological, renal and liver function  
340 abnormalities were collected at 12, 48 and 72 hours and days 7, 14, 21 and 28. Participants  
341 were monitored for treatment emergent adverse events (AEs), defined as any clinical or  
342 subclinical abnormality which was absent before dosing with AS-AQ but emerged post dosing,  
343 or a clinical or subclinical abnormality which was present before dosing with AS-AQ but  
344 worsened post-dosing. Severity of AEs was graded using the DAIDS criteria (36). In addition,  
345 12-lead ECGs were performed pre-dosing, 2 hours after the first dose and 2 hours after the last  
346 dose in step 2 to assess Fridericia-corrected(37) QT interval.

347

348 *Pharmacokinetic assays*

349 Plasma samples were analysed for AQ and DESAQ levels using a validated HPLC-UV assay  
350 adopted and transferred to Malawi-Liverpool Wellcome Trust Clinical Research Programme in  
351 Blantyre, Malawi from Liverpool School of Tropical Medicine. The PK laboratory in Blantyre  
352 participated in WWARN's External Quality Assurance programme(38). Briefly, AQ/DESAQ and  
353 the internal standard (Quinidine) were recovered from plasma using liquid extraction (diethyl/tert-  
354 butyl ether). The supernatant was evaporated to dryness in a vacuum concentrator at 25 °C. The

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355 residue was re-dissolved in 200  $\mu$ l of the reconstitution mobile phase: Water–Acetonitrile–  
356 Triethylamine (85:15:1, v/v/v; pH 3) and 75  $\mu$ L was injected into the chromatograph (Agilent  
357 1100). The optimum detection wavelength for each drug was 345 nm. The lower limit of  
358 quantification (LLOQ) of the HPLC-UV assay was 25 ng/mL for the drugs AQ/DESAQ. Extracted  
359 plasma PK samples were run in batches. Each batch run included a blank plasma extract, two  
360 sets of 8-concentration-level calibration standards, and quality controls (QC) at three  
361 concentration levels: low, medium and high (0.025, 1500 and 3000 ng/mL for AQ/DESAQ). For  
362 batch assay to pass the measured concentrations, at least 67% of the QC samples had to be  
363 within +/-20% of their nominal value and at least one QC had to be acceptable at the LLOQ. In  
364 addition, 75% of each calibration curve's concentrations had to lie within +/-20% and +/-15% of  
365 the nominal concentration at the LLOQ or all other concentrations, respectively. The mean  
366 interassay precision for low, medium and high QCs was 15%, 9% and 6% respectively.

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#### 368 *Data analyses*

369 Plasma concentrations of AQ/DESAQ were analysed using non-compartmental  
370 pharmacokinetic analysis (NCA), employing the trapezoidal rule with cubic splines. Observed  
371 AQ/DESAQ concentrations below LLOQ were treated as missing data except for the pre-dose  
372 concentration which was imputed to 0 if below LLOQ. For each study participant, the following  
373 PK parameters were computed:  $AUC_{0-28 \text{ days}}$ , maximum concentration [ $C_{max}$ ], time to maximum  
374 concentration [ $t_{max}$ ] and terminal elimination half-life [ $t_{1/2}$ ]. We used STATA 15.0 for the NCA  
375 and to compare PK parameters. The Two-sample Wilcoxon rank-sum (Mann-Whitney-U test)  
376 was used to test any significant differences in PK parameters between each ACT/ART arm and  
377 the control arm ( $\alpha=0.05$ ). Geometric means and their 95% confidence intervals have been  
378 reported. Fisher's exact test was used to compare proportions of participants across the study  
379 groups with day 7 concentrations that were above a value known to predict treatment response

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380 by day 28, and of safety parameters across the different ACT/ART groups in comparison to the

381 ART naïve group. Data summaries and graphics were all performed in STATA 15.0.

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414

415 **CONFLICT OF INTEREST**

416 The authors do not have any association that might pose a conflict of interest (e.g.  
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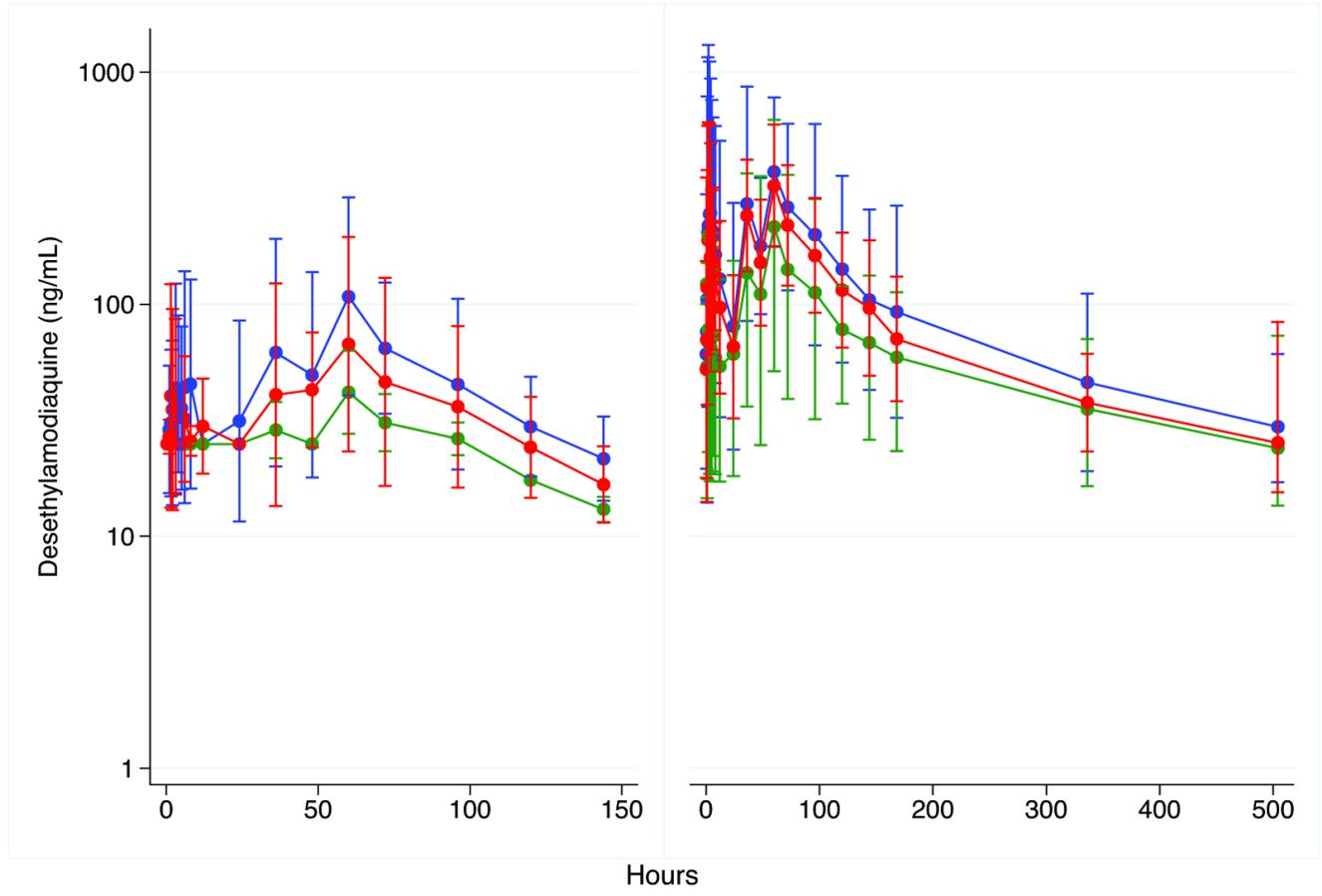
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562 **LEGEND**

563 **Figure 1:** Desethylamodiaquine concentration-time profile (semi-log scale) in step 1 (**left; n=17**)  
564 and step 2 (**right; n=74**) following oral administration of half and full standard artesunate-  
565 amodiaquine adult treatment courses, respectively, among HIV infected ART naïve (blue), those  
566 on nevirapine- (red) and ritonavir-boosted lopinavir-based (green) antiretroviral therapy. Below  
567 limit of quantification concentrations are not included (resulting in observation time up to 144  
568 hours in step 1 and 504 hours in step 2). Data are presented as mean (95% confidence interval)  
569

Desethylamodiaquine concentration–time profile in step 1 and 2



**Table 1a: Desethylamodiaquine pharmacokinetic parameters for participants in step 1**

	Study groups			Geometric Mean Ratio (p-value)	
	ART naïve n=5*	NVP n=6	LPV/r n=6	NVP/ART naïve	LPV/r/ART naïve
AUC <sub>0-28 days</sub> , hr.ng/mL	7,920 (5,034-12,459)	6,091 (3,096-11,983)	4,128 (1,946-8,758)	0.77 (0.465)	0.52 (0.100)
C <sub>max</sub> (ng/mL)	106 (63-179)	75 (54-105)	42 (34-51)	0.71 (0.273)	0.40 (0.006)
T <sub>max</sub> (hr)	60 (36-60)	60 (3-60)	60 (36-60)	(0.562) <sup>a</sup>	(0.484) <sup>a</sup>
t <sub>1/2</sub> (hr)	59 (9-381)	88 (23-331)	75 (16-334)	1.49 (0.715)	1.27 (0.715)

PK parameters are presented as geometric mean (95% confidence interval) except for T<sub>max</sub>, which is reported as median (range). P-value for the ratio is calculated using

Wilcoxon rank sum test in Stata 15.0

ART=antiretroviral therapy; NVP=Nevirapine-based ART; C<sub>max</sub>=maximal concentration, T<sub>max</sub>=time to reach maximal concentration, t<sub>1/2</sub>=drug elimination half-life.

AUC<sub>0-28 days</sub>=area under concentration-time curve from 0 hours

to 28 days

\* One participant did not have quantifiable DESAQ concentrations at nearly all follow up time points and was excluded from analysis

a: p-value only, calculated using Wilcoxon rank sum test

**Table 1b: Desethylamodiaquine pharmacokinetic parameters for participants in step 2**

	Study groups			Geometric Mean Ratio (p-value)	
	ART naïve n=25	NVP n=25	LPV/r n=24*	NVP/ART naïve	LPV/r/ART naïve
AUC <sub>0-28 days</sub> , hr.ng/mL	48,617 (40787- 57,950)	43016 (38,300-48,313)	23,822 (17,458-32,506)	0.88 (0.308)	0.49 (0.0005)
C <sub>max</sub> (ng/mL)	448 (374-534)	360 (322-403)	248 (199-310)	0.80 (0.067)	0.55 (0.0003)
T <sub>max</sub> (hr)	60 (1.5-96)	60 (3-60)	60 (2-72)	(0.887) <sup>a</sup>	(0.248) <sup>a</sup>
t <sub>1/2</sub> (hr)	166 (121-227)	234 (201-272)	90 (58-140)	1.41 (0.037)	0.54 (0.023)

PK parameters are presented as geometric mean (95% confidence interval) except T<sub>max</sub>, which is reported as median (range). P-value for the ratio is calculated using Wilcoxon rank

sum test in Stata 15.0;  $\alpha=0.05$

ART=antiretroviral therapy; NVP=Nevirapine-based ART; LPV/r=ritonavir-boosted Lopinavir-based ART; C<sub>max</sub>=maximal concentration, T<sub>max</sub>=time to reach maximal concentration,

t<sub>1/2</sub>=drug elimination half -life.

AUC<sub>0-28 days</sub> =area under concentration-time curve from 0 hours to 28 days

\* One participant did not have quantifiable DESAQ concentrations at nearly all follow up time points and was excluded from analysis

a: p-value only compared using Wilcoxon rank sum test

**Table 2a:** Summary of DAIDS Grade 3 or 4 Treatment-emergent adverse events in Step 1

DAIDS (Grade 3 or 4) Treatment-emergent abnormalities	AS-AQ (Without ART) N=6	AS-AQ +NVP N=6	AS-AQ +LPV/r N=6
	n (%)	n (%)	n (%)
<b>Haematological events</b>			
Anaemia	0 (0)	1 (17)	0 (0)
Leucopenia	0 (0)	0 (0)	0 (0)
Lymphopenia	0 (0)	0 (0)	0 (0)
Neutropenia	1 (17)	3 (50)	2 (33)
Thrombocytopenia	0 (0)	0 (0)	1 (17)
<b>Biochemical events</b>			
Elevated ALT and AST	0 (0)	0 (0)	0 (0)
Raised Creatinine	0 (0)	0 (0)	0 (0)
<b>Cardiac events</b>			
QTc prolongation	NA	NA	NA

NA: ECG assessment not conducted in step 1

**Table 2b:** Treatment-emergent DAIDS Grade 3/4 abnormalities in Step 2

DAIDS (Grade 3 or 4) Treatment-emergent abnormalities	AS-AQ (Without ART) N=25	AS-AQ +NVP N=25	AS-AQ +LPV/r N=25
	n (%)	n (%)	n (%)
<b>Haematological events</b>			
Anaemia	1 (4)	0 (0)	0 (0)
Leucopenia	0 (0)	0 (0)	0 (0)
Lymphopenia	1 (4)	1 (4)	0 (0)
Neutropenia	4 (16)	7 (28)	0 (0)
Thrombocytopenia	0 (0)	2 (8)	0 (0)
<b>Biochemical events</b>			
Elevated ALT and AST	0 (0)	5 (20)	0 (0)
Raised Creatinine	0 (0)	0 (0)	0 (0)
<b>Cardiac events</b>			
QTc prolongation	0 (0)	2 (8)	2 (8)