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3 **Efficacy of a novel sublingual spray formulation**

4 **of artemether in African children with falciparum malaria.**

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21

22 **Running title:** Efficacy of sublingual artemether in children

23

24 **ABSTRACT**

25

26 The efficacy of sublingual artemether (ArTiMist) was investigated in two studies. In Study 1,

27 31 children were randomised to sublingual artemether (n = 16) or i.v. quinine (n = 15). In

28 Study 2, 151 children were randomised to sublingual artemether (n = 77) or i.v. quinine (n =

29 74). For both studies, patients weighed between 5 and 15 kg and had either severe or

30 complicated malaria based on the WHO criteria, or uncomplicated malaria but were unable to

31 tolerate oral medication as a result of nausea, vomiting or diarrhoea. Patients received either 3

32 mg/kg sublingual artemether or a loading dose of 20 mg/kg i.v. quinine followed by 10

33 mg/kg 8 hourly i.v. thereafter. The primary endpoint was parasitological success, defined as a

34 reduction in parasite count of ≥ 90 % of baseline at 24 h after the first dose. Other endpoints

35 based on parasite clearance and clinical response were evaluated. In Study 1, there were

36 93.3 % (14/15) and 66.7 % (10/15) parasitological successes for the sublingual artemether

37 and quinine treatments respectively. In Study 2, 94.3 % (66/70) ArTiMist treated patients and

38 39.4 % (28/71) patient treated with quinine had parasitological success (p < 0.0001).

39 Indicators of parasite clearance (PCT, PCT_{50}; PCT_{90}, PRR_{24}) were significantly superior for

40 children treated with sublingual artemether than those treated with i.v. quinine. There were no

41 differences between treatments for the clinical endpoints such as fever clearance time. Local

42 tolerability of sublingual artemether was good. Sublingual artemether leads to rapid parasite

43 clearance and clinical recovery.

44

45

46 **INTRODUCTION**

47

48 Malaria remains a major health challenge in developing countries especially in sub Saharan

49 Africa (SSA). Approximately 207 million cases of malaria were reported world wide, of

50 which 80 % were in sub Saharan Africa. Overall, 90 % of the reported 627 000 deaths, of

51 which 77 % were in children under five were in SSA (1). The overwhelming majority (98 %)

52 of malaria cases in the African region are due to *Plasmodium falciparum* (1).

53

54 In highly endemic countries, 20 % - 46 % of child deaths can be attributed to malaria or

55 febrile illness (2).

56

57 In moderate to high transmission settings, such as is found in most endemic SSA countries,

58 young children are disproportionately affected by malaria (3). A child presents with an

59 average 1.6 - 5.4 episodes of febrile malaria per year, with about 5 % of malaria episodes

60 becoming severe disease (4). Less than one third attend clinics and many receive malaria

61 treatment outside of the health care system. The majority of treatments are initiated on a

62 presumptive diagnosis with a high false positive rate, resulting in challenges in accurate

63 monitoring of the malaria burden in SSA (5).

64

65 Despite advances in the treatment of malaria in children, '*the majority of deaths from severe*

66 *malaria in childhood are caused by the delayed administration of effective antimalarial*

67 *treatment. There is a relentless deterioration in the clinical condition of a young child with*

68 *malaria who fails to get effective treatment, with death ensuing in a matter of hours or days.*

69 *Any successful attempt to reduce mortality from malaria will have to explore novel*

70 *possibilities for minimizing such delays'* (6).

71

72 There is a clear need for a formulation of an artemisinin derivative that can be easily

73 administered and adequately absorbed in a child who may be unconscious or uncooperative,

74 or in whom nausea and vomiting preclude oral dosing. Artemether can be used as initial

75 intramuscular (i.m.) monotherapy for severe malaria in children (7). It is also a recommended

76 first-line oral therapy in combination with the longer half-life partner drug lumefantrine for

77 uncomplicated *P.falciparum* (8) and *P. vivax* (9) infections in pediatric age-groups.

78

79 A new formulation of artemether in neutral oil, ArTiMist (Suda Ltd, Perth, Australia) has

80 been developed that has the potential to minimize delay in administration of an effective anti

81 malarial agent in children with complicated or severe malaria. ArTiMist is administered as a

82 metered sublingual spray that is more rapidly and extensively absorbed than artemether given

83 in tablet form in healthy adult volunteers (10).

84

85 In the present study, we assessed the safety, tolerability and clinical efficacy of ArTiMist and

86 intravenous (i.v.) quinine for severe and complicated *P.falciparum* malaria in African

87 children and in infected children who were unable to take oral therapy.

88

89

90 **SUBJECTS AND METHODS**

91

92 **Study site, approvals, and patients**

93 The present study was conducted in two parts. Study 1 was an open label randomized

94 comparative trial of ArTiMist and i.v. quinine conducted in Rwanda (Rwinkwavu district

95 hospital) between 01 December 2009 and 19 January 2010. Study 2 was a Phase III,

96   randomized, open label, multi-center superiority trial of ArTiMist versus i.v. quinine

97   conducted between 16 November 2010 and 07 September 2012 at three different sites;

98   Rwanda (Rwinkwavu district hospital, Eastern Province, Rwanda), Ghana (Navrongo Health

99   Research Centre, Upper East Region, Ghana) and Burkina Faso (Centre National de

100   Recherche et de Formation sur le Paludisme (CNRFP), Ouagadougou, Burkina Faso). Details

101   of the pharmacokinetic procedures and results have been published elsewhere (10), (11). The

102   present paper provides details of the trial procedures and efficacy and safety outcomes.

103

104   Children weighing between 5 and 15 kg were eligible for the studies if i) they had falciparum

105   malaria confirmed by either blood film microscopy showing a *P. falciparum* density ≥

106   500/µL whole blood (including those positive for other plasmodial species), ii) they had

107   severe or complicated malaria based on the WHO criteria (12) or uncomplicated malaria but

108   were unable to tolerate oral medication as a result of nausea, vomiting or diarrhoea, iii) had

109   not received any antimalarial therapy within the 7 days prior to first study drug

110   administration, iv) did not have evidence of significant co-morbidity including other

111   infections, v) had no contraindication or allergy, or history of intolerance, to either artemether

112   or quinine, and vi) their parents or attendant relatives/guardians gave witnessed informed

113   consent and, where possible, the child assented to participation.

114

115   Study 1 was approved by the University Teaching Hospital Kigali Research Ethics

116   Committee [EC/CHUK/002/09] and Study 2 the University Teaching Hospital Kigali

117   Research Ethics Committee [EC/CHUK/015/10], the Navrongo Health Research Centre

118   Institute Review Board [NHRCIRB107], and Centre National de Recherche et de Formation

119   sur le Paludisme Comite Institutionnel de Bioethique [AEP-002/02/2011/CIB-CNRFP]. Both

120   studies were registered on Clintrials.gov (NCT01047436 and NCT01258049 for Study 1 and

121 Study 2 respectively). In both studies, allocation bias was avoided by randomization, using a

122 computer-generated schedule, and ensuring that the Investigator remained blinded until after

123 the randomly allocated medication was dispensed from the pharmacy.

124

125

126 **Patients**

127 In Study 1, 31 eligible children were randomized to either receive ArTiMist (n = 16) or i.v.

128 quinine (n =15). There were no screening failures. In Study 2, of the 180 children that were

129 screened for study entry, 151 eligible children were randomized to receive ArTiMist (n = 77)

130 or i.v. quinine (n = 74).

131

132 For both studies, ArTiMist (Essential Nutrition Ltd., Brough, England) was administered at a

133 dose of 3.0 mg/kg at 0, 8, 24, 36, 48 and 60 h, or until initiation of oral antimalarial therapy.

134 I.v. quinine (Martindale Pharmaceuticals) was administered as a 20 mg/kg infusion over 4

135 hours at 0 h, followed by 10 mg/kg (infusion over 4 hours) 8 hourly thereafter for at least 24

136 hours and until resumption of oral therapy. The exact timing of each dose was recorded.

137

138 On resumption of oral therapy, patients could, at the discretion of the local investigator,

139 receive further doses of oral quinine (Teva UK Limited) or further doses of ArTiMist to

140 complete seven days of treatment or be converted to another suitable treatment (typically

141 artemisinin combination therapy (ACT)) in accordance with the respective national treatment

142 guidelines.

143

144 **Clinical Procedures**

145 Patients were initially treated as hospital inpatients, and could be discharged from Day 4

146 onwards at investigator discretion. Patients returned for outpatient visits on Days 7, 14, 21

147 and 28. Vital signs (temperature, pulse rate, blood pressure, hydration status and respiratory

148 rate), and physical examination (including neurological examination, level of consciousness,

149 and ability to eat, drink and mobilize normally for age) were evaluated regularly.

150

151 Determination of parasite counts, physical examinations and vital signs were as follows: Day

152 1: predose, 3 h, 6 h, 12 h, and 18 h post dose; Days 2 and 3: predose, 6 h, and 12 h post dose;

153 Day 4 and discharge day: prior to discharge or prior to converting to quinine or oral therapy;

154 every second day thereafter if prolonged hospitalization; each of the outpatient visits on Days

155 7, 14, 21 and 28.

156

157 Dipstix urinalysis was performed prestudy and on Days 1, 2, 3, 4, 7, 14, 21, and 28 when

158 urine was available or clinically indicated. Adverse events were regularly elicited and

159 concomitant medications recorded. Clinical laboratory assessments (biochemistry and

160 haematology) were evaluated at baseline (screening or Day 0), Day 4 and Day 21.

161

162 **Parasite counts**

163 In Study 1, three independent microscopists blinded to treatment read the blood smears at the

164 study site. For Study 2, Phoenix Pharma Central Services (S) Pte Ltd in Singapore, which is

165 accredited by the Ministry of Health in Singapore and the College of American Pathologists

166 was the central laboratory for the evaluation of parasite counts. The laboratory remained

167 blinded to treatment at all times. Two trained microbiologists read all slides independently,

168 with results averaged. A third independent microbiologist read discordant results and the

169 closest two parasite counts were averaged. Thick blood films were stained with Giemsa.

170

171 For both studies, the number of asexual parasites/µl of blood was determined by dividing the

172 number of asexual parasites by the number of white blood cells (WBC) counted (500) and

173 then multiplying by an assumed WBC density of 6000 – 8000/µl.

174

175 **Assessments of efficacy**

176 In Study 1, the primary efficacy parameters were; parasitological success, defined as a

177 reduction in parasite count of $\geq$ 90 % of baseline at 24 h after the first dose; time for parasite

178 count to fall by 90 % (PCT_{90}) and time for parasite count to fall by 50 % (PCT_{50}). In Study 2,

179 the primary efficacy parameter was parasitological success, defined as a reduction in parasite

180 count of $\geq$ 90 % of baseline at 24 h.

181

182 Secondary endpoints included parameters of parasite clearance; parasite clearance time (PCT)

183 [the time in hours from the initiation of therapy until the first of two successive parasite-

184 negative smears were obtained]; percentage reduction in parasitaemia from baseline at 12 h

185 (PRR_{12}) and 24 h (PRR_{24}); PCT_{90} and PCT_{50}.

186

187 Early treatment failure was defined as parasitaemia on Day 2 > baseline irrespective of

188 axillary temperature; parasitaemia on Day 3 with axillary temperature $\geq$ to 37.5°C;

189 parasitaemia on Day 3 $\geq$ 25 percent of count at baseline; or the requirement for rescue

190 antimicrobial treatment. Late parasitological failure was defined as parasitaemia on any day

191 from Day 7 to Day 28 and tympanic temperature $\geq$ 38.0°C.

192

193 Clinical endpoints included fever clearance time (FCT) [time in hours from the initiation of

194 therapy until the disappearance of fever (tympanic temperature < 38.0°C) for at least 24 h],

195 the time taken for patients to return to normal *per os* status; time to full consciousness in

196 patients admitted with impaired level of consciousness; number of patients with neurological

197 sequelae and number of deaths. In Study 2, late clinical failure was defined as signs of severe

198 malaria on any day between Day 4 and Day 28 in the presence of parasitaemia, without

199 previously meeting any of the criteria of early treatment failure; or presence of parasitaemia

200 and tympanic temperature $\geq 38.0°C$ (or history of fever), on any day between Day 4 and Day

201 28, without previously meeting any of the criteria of early treatment failure.

202

203 Complete cure was defined as the complete resolution of clinical signs and symptoms,

204 malaria related laboratory abnormalities, and elimination of asexual parasites by Day 7, with

205 no recurrence up to Day 28 (± 2 days), and the 48 h parasite count to be < 25% of baseline

206 with no clinical deterioration.

207

208 Adverse events and concomitant medications were recorded throughout both studies. For

209 patients allocated to the ArTiMist treatment, the Investigator evaluated local tolerability.

210

211 **Statistical analysis**

212 Statistical programming and analyses were performed using SAS® version 9.2 (SAS Institute,

213 Cary, NC). For Study 1, the sample size was not formally calculated. For Study 2, sample

214 size was calculated using the primary efficacy endpoint data from Study 1, where the parasite

215 success rate for quinine was 67.7 %. On the assumption of a parasite success rate of 70 % for

216 quinine in Study 2, and to demonstrate that ArTiMist was superior to quinine by at least 20 %

217 the parasite success rate for ArTiMist should be at least 90 %. Assuming a power of 80 %,

218 an alpha of 0.05 (two-sided) and equal allocation to the ArTiMist and quinine treatment arms,

219 the number of evaluable patients (n) required on each treatment was 59.

220

221 Efficacy analysis was based on a modified intent to treat (MITT) population (full analysis set

222 (FAS) in Study 1), which included all randomized patients receiving at least one dose of

223 study medication with evaluable parasite counts at 12 h (Study 1) and 24 h (Study 1 and

224 Study 2). A second efficacy population, which for Study 1 was the modified FAS (MFAS),

225 was defined as all patients in the FAS receiving all 6 doses of ArTiMist or equivalent of

226 quinine, and had parasite density count after the final dose. For Study 2, the per protocol (PP)

227 population included patients in the MITT population receiving at least 80 % of doses at

228 discharge from hospital, evaluable data up to and including Day 28 and no major protocol

229 violations. Efficacy analyses were conducted and presented for both populations. The safety

230 analysis population included all randomized patients receiving at least one dose of study

231 medication was used for all safety analyses. The intention to treat population (ITT) included

232 the set of enrolled patients who were assigned to a treatment group (randomized), regardless

233 of whether or not they took any study drug was used for summarizing demographic and

234 baseline data.

235

236 For parasitological success, each patient was defined as to whether they had success or not,

237 by treatment. Parasitological success was appropriately summarized using descriptive

238 statistics by treatment. The difference between treatments and its 95 % confidence interval

239 was determined using a logistic model with site as a prognostic factor.

240

241 The secondary endpoints and safety data were appropriately summarized using descriptive

242 statistics by treatment, overall and by site.

243

244 For both studies, the statistical analysis was pre specified in a statistical analysis plan, which

245 for Study 2 was finalized prior to parasite count data being received from the central

246 laboratory. In Study 2, data was analyzed overall and by site. There were no changes to the

247 planned analysis for either study.

248

249 Data are presented as means ± SD and differences between treatments are presented as Δ

250 (95% confidence interval; p value) unless otherwise indicated.

251

252

253 **RESULTS**

254

255 **Patient characteristics**

256 In Study 1, one patient (ArTiMist) was withdrawn after the second dose due to a protocol

257 violation (incorrect drug storage) and was replaced. Data for this patient was included in all

258 analysis populations, apart from the PP population.

259

260 Patient disposition for Study 2 is provided in Figure 1.The baseline characteristics of

261 participants in each study are summarized in Table 1, and are well balanced between

262 treatments for both studies. For Study 2, treatment allocation by site is provided in Table 3.

263

264 One patient allocated to ArTiMist treatment had mixed infection with *P.ovale*.

265

266 **Efficacy analysis – parasite response**

267

268 The efficacy endpoint parameters are presented in Table 2 for the main analysis population for

269 both studies.

270

11

271 Primary efficacy endpoint:

272 In Study 1, there were 93.3 % (14/15) and 66.7 % (10/15) parasitological successes for

273 ArTiMist and quinine treatments respectively, with no difference between the FAS and

274 MFAS. The difference between treatments was not statistically significant.

275

276 In Study 2, ArTiMist demonstrated superiority over i.v. quinine in both efficacy populations.

277 For the MITT population 94.3 % (66/70) ArTiMist and 39.4 % (28/71) quinine treated

278 patients had parasitological success, which was statistically significant ($p < 0.005$).

279

280 For the PP population, 95.6 % (65/68) ArTiMist and 40.6 % (28/69) quinine treated patients

281 had parasitological success ($p < 0.005$).

282

283 Table 3 provides further details regarding parasite success rate at each site for both studies.

284 By site, the parasite response differed and the factor 'Site' was a statistically significant

285 factor in the logistic model for both populations ($p = 0.034$ for MITT; $p = 0.008$ for PP). At

286 each study site, the difference between treatments was statistically significant.

287

288 The study site in Rwanda was the same for Study 1 and Study 2. The difference between

289 parasite success rates between the studies of 2.4 (-12.1 to 16.9) % and 19.1 (-13.8 to 52.0) %

290 for ArTiMist and quinine treatments respectively was not statistically significant.

291

292 Secondary parasitological efficacy endpoints:

293 As there was no difference in outcome for any of the secondary endpoints in either study

294 between the MITT/FAS and the PP/MFAS populations, only details of the MITT/FAS

295 populations are described below.

296

297 In Study 1, the PCT_{90} was 17.6 ±7.3 h and 19.8 ±13.6 h and PCT_{50} was 12.0 ±6.5 h and 10.8

298 ±7.4 h for ArTiMist and quinine treatments respectively; the differences between the survival

299 curves were not statistically significant. There was no difference in the PRR_{12}, PRR_{24} or PCT

300 between treatments.

301

302 For Study 2, 53 % (37/70) patients treated with ArTiMist cleared their parasites within 24 h.

303 By comparison, 4 % (3/71) of the quinine treated patients cleared their parasites within 24 h

304 (Figure 2). No ArTiMist treated patient was parasitaemic at 72 h, whereas 13 % (9/71) of the

305 quinine treated patients remained parasitaemic at 72 h. As indicated in Table 2, the secondary

306 efficacy parameters relating to parasite clearance; PCT, PRR_{24}, PCT_{50}, and PCT_{90}

307 demonstrated a statistically significant difference overall between the treatments ($p < 0.005$)

308 for both populations. Although the parasite counts had almost halved by 12 h (PRR_{12}) for

309 ArTiMist treated patients, it increased for quinine treated patients (p=0.064).

310

311 In Study 1, there were no early treatment failures. In Study 2, there were no early treatment

312 failures for the ArTiMist treated patients, for the quinine group, there were 14.1 % (10/71)

313 early treatment failures. Eight were due to parasite counts on Day 2 greater than the baseline

314 parasite count and 2 due to the Day 3 parasite count being greater than or equal to 25 % of the

315 baseline parasite count. One patient developed cerebral malaria, severe anemia and

316 convulsions following 24 h of i.v. quinine treatment. Quinine study treatment was

317 discontinued and rescue treatment (i.v. artesunate) was administered, following which the

318 patient recovered.

319

320 In Study 2, there were 17.1 % (12/70) and 19.7 % 14/71) patients with late parasitological

321 failures in the ArTiMist and quinine treatment groups respectively.

322

323 For both studies, after the initial treatment with either ArTiMist or i.v. quinine, patients were

324 given a full course of oral therapy in accordance with the applicable treatment guidelines in

325 effect in the country at the time. For Study 2, 73.5 % (111/151) patients received a full course

326 of artemether-lumefantrine, 8.5 % (13/151) continued with oral quinine for at least 7 days and

327 17.9 % (27/151) continued with ArTiMist for at least 7 days.

328

329 **Secondary clinical efficacy endpoints**

330 In both Study 1 and Study 2, there was no difference between treatments for FCT, or time to

331 normal *per os* status.

332

333 Time to return to full level of consciousness (LOC) (for patients with decreased LOC prior to

334 treatment) was evaluated in Study 2 only. All 17 (ArTiMist) and 21 (quinine) patients with

335 reduced level of consciousness before dosing were at the Burkina Faso study site. Time to

336 return to full consciousness was 20.8 ±9.6 h and 23.0 ±16.3 h for the ArTiMist and quinine

337 treatments respectively, which was not statistically significant.

338

339 4.3 % (3/70) patients treated with ArTiMist and 1.4 % (1/71) patients treated with quinine

340 had late clinical failures which was not statistically significant.

341

342 **Cure rates**

343 Cure rates were evaluated for Study 2 only. Overall, the complete cure rate was similar for

344 ArTiMist and quinine treated patients for both the MITT and PP populations.

345

14

346   One patient with a mixed infection cleared parasites of both plasmodium species (*falciparum*

347   and *ovale*) within 24 h of starting ArTiMist treatment and had a complete cure at Day 28.

348

349   **Safety evaluation and adverse events**

350   There were no deaths in either of the studies. One patient (quinine) had a serious adverse

351   event (SAE) in Study 1; malaria recrudescence/reinfection at the Day 28 visit requiring

352   inpatient treatment with i.v. quinine. Of the 14 SAE's reported in Study 2, 71.4 % (10/71)

353   were in quinine treated patients, the remaining 28.6 % (4/70) in ArTiMist patients. There

354   were 9 reports (nine patients) of anemia, all of which were unrelated to study medication that

355   was either life threatening or prolonged hospitalization. All required blood transfusion,

356   following which all events resolved. 77.8 % (7/9) were in quinine treated patients, the

357   remaining 22.2 % (2/9) in ArTiMist treated patients. There was one report of

358   bronchopneumonia (1 patient) and one report of sepsis (1 patient) in patients allocated to

359   ArTiMist treatment that either required, or prolonged inpatient hospitalization. Both are

360   common comorbidities in children with severe malaria and were considered unrelated to

361   ArTiMist treatment.

362

363   One patient allocated to quinine treatment failed to respond to i.v. quinine therapy and

364   developed cerebral malaria. Quinine treatment was stopped and the patient received i.v.

365   artesunate as rescue therapy, following which the patient recovered. The investigator

366   considered the SAE not related to the study medication.

367

368   Local tolerability was assessed by regular physical examination of the mouth and

369   documentation of local adverse events. There were no adverse events reported or

370   physical/oral examination observations noted that related to local tolerability in any of the

371   patients treated with ArTiMist.

372

373   In Study 1, there were no reported AE's that were considered by the investigator to be

374   treatment related. In Study 2, 6.5 % (5/70) patients treated with ArTiMist experienced six

375   related treatment emergent adverse events (TEAE's); 2cases of diarrhoea, 2 cases of

376   vomiting, 1 case of parotitis, and 1 case of cough.

377

378   8.1 % (6/71) quinine treated patients had six related TEAEs, which included 1 case of

379   anemia, 2 cases of abdominal pain, 1 case of headache, 1 case of diarrhoea, and 1 case of

380   vomiting.

381

382   In both studies a number of patients had abnormalities in clinical status, vital signs and

383   laboratory parameters at baseline for both treatment groups (Table 1). In Study 2, there were

384   no differences between the treatment groups for the mean values (overall and by site) for

385   body weight changes, systolic blood pressure, diastolic blood pressure, heart rate, respiratory

386   rate, and temperature during the study. Following treatment with ArTiMist, these

387   abnormalities resolved as expected, and consistent with the patients in the comparator

388   treatment arm. No new abnormalities emerged that were associated to ArTiMist treatment.

389

390

391   **DISCUSSION**

392   Death from malaria reflects delay in administration of effective antimalarial treatment,

393   immediate treatment with pre-referral rectal artesunate substantially reduces the risk of death

394   or permanent disability in severely ill young children where there is a delay in access to

395   treatment (15).

396

397   The present data show that ArTiMist given at a doses of 3.0 mg/kg twice daily for three days

398   to young African children who have severe malaria or who are unable to tolerate

399   conventional oral therapy leads to rapid clearance of parasites and clinical recovery.

400

401   Pharmacokinetic analysis shows that ArTiMist is promptly and adequately absorbed,

402   regardless of indices of severity including conscious level and even in patients with a history

403   of vomiting at presentation (11). Simulations demonstrated that sublingual artemether

404   bioavailability (artemether and DHA) was at least equivalent to that after conventional

405   artemether-lumefantrine oral therapy in older Melanesian children with uncomplicated

406   malaria (11).

407

408   I.v. quinine was chosen as the active comparator for both studies. This was the standard of

409   care for children with severe or complicated falciparum malaria, or uncomplicated falciparum

410   malaria with gastrointestinal complications during Study 1 and at the start of Study 2 (13).

411

412   During the course of Study 2, the treatment recommendations for severe childhood malaria

413   were updated (14), following the publication of a large randomised controlled trial, enrolling

414   5425 children < 15 years of age across Africa, showing a significant mortality reduction by

415   22.5 % in the artesunate group when compared to the quinine group (16). I.v. or i.m.

416   artesunate was recommended as the treatment of choice in children with severe malaria,

417   although i.v. quinine remained an acceptable alternative if artesunate was not available (14).

418

419   The concerned Ethics Committees, Regulatory Authorities and investigators were notified

420   and considered that i.v. quinine remained an acceptable comparator treatment in this study as

421   i) i.v. quinine continued to be an acceptable treatment in the updated WHO treatment

422   guidelines; ii) i.v. artesunate was not on the national treatment guidelines for any of the

423   concerned countries at the time; and iii) GMP quality i.v. artesunate was not available to

424   complete the trial.

425

426   The dose of 3 mg/kg ArTiMist was based on a previous pharmacokinetic study in healthy

427   adults (10) with extrapolation to children. The dosing regimen was the same as the twice

428   daily 3 day course of artemether-lumefantrine treatment.

429

430   In our studies, baseline *P.falciparum* parasite count was required to be ≥ 500/μl. Although

431   some studies of severe malaria required a higher parasite count for study entry (18) (19), this

432   level was considered appropriate as patients unable to tolerate oral medication could also be

433   included. The concern of including patients with low baseline counts would be that a patient

434   has incidental parasitaemia whilst being ill due to a different condition. Clinically however,

435   as it is often difficult to differentiate the symptoms from malaria and other co-infections or

436   conditions, patients would require treatment for malaria, in which case ArTiMist may be of

437   potential benefit for their initial treatment.

438

439   The primary endpoint used for both studies was parasitological success, which has been used

440   previously to evaluate early treatment for severe malaria following rectal artemisinins (27).

441   Although the PCT is the most commonly used primary endpoint in the reported literature, it is

442   a function of the pre-treatment parasite count (28) that could potentially be biased over the 24

443   h period, detracting from the study objectives. Once parenteral antimalarials had been started,

444   it was required to administer them for at least 24 hours before converting patients to suitable

445   oral treatment irrespective of the patient's ability to tolerate oral medication earlier (13).

446 Therefore it was essential to have the primary endpoint evaluated at 24 h, as the quinine

447 treated patients could then be changed to oral therapy, potentially confounding PCT

448 thereafter. PCT, PCT_{50}, PCT_{90}, PRR_{12} and PRR_{24} were evaluated as secondary endpoints for

449 parasitological clearance, with FCT, time to normal per *os* and time to return to full

450 consciousness as secondary endpoints for clinical response in both studies to allow wider

451 comparability to other reported studies.

452

453 **Comparisons with other published data**

454

455 In both our studies, children had less severe malaria than reported in some other studies of

456 children with severe malaria; notably, there were no deaths or patients with neurological

457 sequelae in either of our studies. In the AQUAMAT study for example, mortality was 10.9 %

458 for the quinine treatment (control). At baseline, 35 % of patients in the quinine treatment arm

459 had coma (16), whereas 20 % and 28.1 % of children in the quinine treatment had reduced

460 level of consciousness in our Study 1 and Study 2 respectively.

461

462 ArTiMist has been developed to minimize the delay in initial administration of an effective

463 anti malarial in children with severe malaria. In this regard, the most important consideration

464 is rapid clearance of parasites with corresponding improvement of clinical signs, or delay in

465 disease progression while the patient is transported to a suitable facility for further treatment.

466

467 As clearance of parasites is a surrogate endpoint, it cannot be assumed that greater parasite

468 clearance results in an improved clinical outcome and lower mortality. However, both the

469 AQUAMAT (16) and SEAQUAMAT (17) trials demonstrated that more rapid parasite

470 clearance with artesunate resulted in significantly lower mortality. Without direct evidence,

471 the same cannot be inferred for other artemisinin derivatives such as artemether.

472

473 Comparing our results to those in the literature for both the ArTiMist and quinine treatments,

474 with respect to parasite clearance and clinical response contributes to the validity of our

475 studies and the weight of evidence of the proposed intervention.

476

477 The parameters for PCT is consistent with those reported in the literature for both the

478 ArTiMist (artemether) and quinine treatments arms. For ArTiMist treated patients, mean

479 (35.7±42.0 h and 30.3±13.2 h) and median (24 (18-182) h and 24.0 (12-72) h) PCT for Study

480 1 and Study 2 respectively are in accord with reported studies ranging from 16.0±9.2 h to

481 54.2±33.6 h (18), (19), (20), (21), and 32 (24-52) h to 48 (36-60) h (22), (23), (24), (25), (26).

482

483 Similarly, corresponding parameters for quinine treated children (51.2±79 h and 68.3±98.0

484 h); (30 (12-331) h and; 47.8 (3-526) h) were in accord with reported PCT for the quinine

485 control groups, ranging from 22.4±11.5 h to 55.0±24.3 h (20), (21), (26), and 40 (32-48) h to

486 60 (48-72) h (22), (23), (25).

487

488 Gomes *et al* (27) pooled individual patient data from 1167 patients in 15 clinical trials of

489 rectal artemisinin derivative therapy (artesunate, artemisinin and artemether) in order to

490 compare the rapidity of clearance of *P. falciparum* parasitaemia. The reported parasite

491 reduction ratio at 12 h (PRR_{12}) ranged from 32.7 % to 73.5 %. By comparison, for Study 1

492 and Study 2, PRR_{12} for ArTiMist was 45.1±85.1 % and 47.6±70.3 % respectively. Similarly

493 for the PRR_{24}, reported parameters ranged from 83.1 % to 96.7 % (27), the respective

494 parameters for Study 1 and Study 2 were 97.7±6.0 % and 98.2±6.1 %. The reported values

20

495   for the i.v. quinine arm for PRR_{24} ranged between 63.7 % and 68.7 % (27) in comparison to

496   89.1±16.1 % and 44.5±114.3 % for Study 1 and Study 2 respectively.

497

498   In a Cochrane review conducted by Sinclair *et al*, eight trials enrolling 1664 adults and 5765

499   children comparing i.v. i.m. or rectal artesunate with i.v. or i.m. quinine for treating adults

500   and children with severe malaria who are unable to take medication by mouth were included

501   (29). In this analysis it was reported that artesunate appeared superior to quinine at reducing

502   the PCT_{50} by -8.14 h (-11.55 to -4.73) [292 participants, three trials], the PCT_{90} by -18.50 h (-

503   24.13 to -12.87); [61 patients; one trial], and PCT by -9.77 h (-18.11 to -1.44) [419 patients;

504   four trials]; (29). For Study 2, the corresponding parameters were -9.2 (-11.71 to – 6.61) h; -

505   12.1 (-17.38 to – 8.44) h; and -39.0 (-62.2 to -15.7) h. There was no difference in coma

506   recovery time or FCT (29).

507

508   In a similar review by McIntosh *et al*, eleven studies compared artemether (1069 patients)

509   with quinine (1073 patients) (30). The median PCT from the three largest studies was 32 to

510   72 h with artemether compared with 40 to 90 h for quinine (30).

511

512   Five studies showed no difference in FCT, three showed that artemether was faster, and one

513   showed that quinine was faster than artemether (30). In both our studies, there was no

514   difference in FCT.

515

516   Only one study reported fully on time to drink, eat, sit, stand and walk. As in our studies,

517   there was no significant difference was shown between the treatment groups. Another study

518   reported no significant difference in time to walk (30).

519

21

520 Cure rates were secondary endpoints of lesser importance in this study, as they are not a

521 reflection of the evaluation of the treatment intervention under investigation. There was no

522 difference in cure rates between the ArTiMist and quinine treated patients,

523

524 ArTiMist treatment was well tolerated. Most of the clinical abnormalities reported were due

525 to malaria and not study treatment; no local adverse events were reported.

526

527 Given WHO recommendations regarding the need for artemisinin drugs to be administered in

528 combination with a longer half-life partner (such as lumefantrine) except in situations in

529 which initial oral therapy cannot be given safely and reliably (13), ArTiMist is likely to have

530 application as one- or two-dose pre-referral treatment as sick children are transferred for

531 parenteral artemisinin therapy or oral ACT. Given potential pharmacokinetic (32), cultural

532 (15) and efficacy (33) concerns regarding artesunate suppositories in this situation, ArTiMist

533 appears a valuable alternative in this situation.

534

544

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654 **Table 1. Demographics and baseline characteristics of African children in Study 1 and**

655 **Study 2 who were treated for malaria using sublingual artemether (ArTiMist) or i.v.**

656 **quinine. Data presented as means ±SD, medians (range), or number (%).**

	Study 1		Study 2	
	ArTiMist	**Quinine**	**ArTiMist**	**Quinine**
Number of patients	16	15	77/70[a]	74/71[a]
Age (Years)	3.0±1.5	3.6 ±2.5	2.8 ±1.3	2.5 ±1.2
Sex, number (%) male	9 (56.3)	7 (46.7)	37 (48.1)	35 (47.3)
Disease Definition				
Severe or Complicated Malaria (n; %)	10 (62.5)	12 (80.0)	49 (63.6)	51 (68.9)
Uncomplicated Malaria with GI Complications (n; %)	6 (37.5)	3 (20.00	28 (36.4)	23 (31.1)
≥Mixed with ≥ 500 P *falciparum*/µL (n, %)			1 (1.3)	0 (0)
Vital Signs				
Weight [kg]	11.2 ±2.5	11.4 ±3.4	11.7 ±2.4	11.2 ±2.5
Respiratory Rate [b/min]	31.9 ±9.1	32.8 ±10.7	36.7 ±11.0	35.9 ±11.3
Pulse rate [bpm]	142.4 ±24.7	144.3 ±15.1	146.5 ±20.2	147.5 ±26.3
Tympanic Temperature [°C]	38.2 ±1.3	37.8 ±0.8	38.6 ±1.1	38.6 ±1.0
Systolic Blood Pressure [mmHg]	87.5 ±10.0	84.3 ±6.5	99.3 ±12.3	99.4 ±11.6
Diastolic Blood Pressure [mmHg]	50.5 ±10.0	50.7 ±7.3	58.7 ±10.8	58.5 ±11.4
Blantyre Coma Scale < 5 (n; %)	3 (18.7)	3 (20.0)	17 (22.1)	21 (28.4)
Parasite Count /µl) (Rwanda)	63430	30989.7	69752	65537
Mean±SD	± 173551	± 33841	±94146	±63357
Median (range)	19660 (1480 -712307)	21800 (1120-109440)	28958 (933-374248)	51061 (1067-216601)
Parasite Count (/µl) (Ghana)			151014.	190723
Mean±SD			±124850	±243425
Median			138021 (8067-515556)	128686.0 (1225-843746)
Parasite Count (/µl) (Burkina Faso)			123905	125557
Mean±SD			±137880	±151246
Median			62431 (581-494500)	48415 (6679 –551067)

657

658

659   [a] patient numbers are for safety analysis population, apart from parasite counts, which are for MITT population.

660 **Table 2. Parasitological and clinical endpoints for African children who were treated for**

661 **malaria using sublingual artemether (ArTiMist) or i.v. quinine for Study 1 (MFAS**

662 **population) and Study 2 (MITT population) by study and treatment group presented as**

663 **means ± SD, medians (range), or number (%).**

Endpoint	Study 1			Study 2		
	ArTiMist	Quinine	Δ(95% CI)[a]; Hazard ratio (95% CI)[b]	ArTiMist	Quinine	Δ(95% CI; p)[a]; Hazard ratio (95% CI; p)[b]
Number of patients	15	15		70	71	
Parasitological Success n (%)	14 (93.3)	10 (66.7)	26.6 (-0.3 to 53.7)	66 (94.3)	28 (39.4)	54.9 (42.25-67.45; p<0.005)
PCT (h) mean±SD	35.7±42.0	51.2±79.0	N/A	30.3±13.2	68.3±98.0	39.0 (-62.2 to -15.72; p<0/005)
PCT (h) median (range)	24 (12-182)	30 (12-331)	N/A	24.0 (12-72)	47.8 (3-526)	N/A
PCT_{90} (h) mean±SD	17.6±7.3	19.8±13.6	1.05 (0.5 – 3.0)	15.0±5.8	27.93±18.0	-12.9 (-17.4 to -8.4; p<0.005)
PCT_{90} (h) median (range)	18 (6-36)	18 (3-60)	N/A	15.8 (3-32)	26.4 (3-156)	0.2 (0.1-0.3; p<0.005))
PCT_{50} (h) mean±SD	12.0±6.5	10.8±7.4	0.9 (0.3-2.2)	9.4±5.7	18.6±9.2	-9.2 (-11.7 - -6.6; p<0.005))
PCT_{50} (h) median (range)	12 (3-24)	12 (3-24)	N/A	10.5 (2-22)	20.1(1-42)	0.2 (0.1-0.3; p<0.005)
PRR_{12} (%) mean±SD	45.1±85.1	58.5±53.1	-13.5 (-66.0 – 39.1)	47.6±70.3	-132.2±765.9	174.1 (-10.4 to -358.6; p=0.064)
PRR_{12} (%) median (range)	79.6 (-220-100)	75.9 (-58-100)	N/A	72.3 (-287-100)	-10.3 (-635-100)	N/A
PRR_{24} % mean±SD	97.7±6.0	89.1±16.1	8.7 (-0.4 – 17.7)	98.2±6.1	44.5±114.3	54.0 (27.1 – 81.0; p<0.005)
PRR_{24} % median (range)	100 (77-100)	96.7 (53-100)	N/A	100.0 (69-100)	80.6(-664-100)	N/A
Time to normal *per os* (h) mean ±SD	17.7±11.3	20.7±9.4	N/A	22.1±12.9	25.3±16.3	-3.2 (-8.2 - 1.8;p=0.20)

30

Time to normal *per os* (h) median (range)	17.2 (0-48)	18.0 (6-36)	N/A	23.8 (2-50)	23.9 (1-64)	0.9 (0.7-1.3)
FCT (h) mean±SD	89.9±72.8	86.1±25.8	N/A	42.6±34.5	41.6±22.7	1.0 (-10.2 to 12.1; p=0.86)
FCT (h) median (range)	75.9 (30-331)	91.7 (30-126)	N/A	38.1 (3-229)	42.6 (3-96)	0.9 (0.6-1.3)
Time to return to full consciousness (h)[d] mean±SD	9.0±6.5	11.0±6.2	N/A	20.8±9.6	25.3±16.3	-3.2 (-8.2 - 1.8; p=0.62)
Time to return to full consciousness (h)[c] median (range)	6 (3-18)	12 (3-18)	N/A	23.8 (3-36)	17.8 (3-60)	0.8 (0.4-1.6)
Early treatment failures	0 (0.0)	0 (0.0)	N/A	0 (0.0)	10 (14.1)	N/A
Late clinical failure		N/A		3 (4.3)	1 (1.4)	2.6 (0.3 to 26.6; p=0.41)
Late parasitological failure		N/A		12 (17.1)	14 (19.7)	1.0 (0.4 - 2.6; p=0.95)

664

665　[a] Differences between groups (Δ) and their 95% confidence intervals (CI) are shown.

666　[b] Hazard ratio (95% CI) and difference in survival curves compared by Log rank (Study 1) and Cox regressions analysis

667　(Study 2).

668　[c] For patients whose level of consciousness was reduced prior to dosing.

669　N/A not applicable as was not assessed or calculated.

670

671 **Table 3. Primary efficacy endpoint of African children in Study 1 and Study 2 who were**

672 **treated for malaria using sublingual artemether (ArTiMist) or i.v. quinine by site**

673 **presented as number (%).**

Study	Site		Number of patients	Parasitological Success n (%)[d]	Parasitological Non Success n (%)[d]	Δ (95% CI)%; p value
Study 1[a]	Rwanda[c]	ArTiMist	15	14 (93.3)	1 (6.7)	26.7 (-0.3-53.7); p = 0.17
		Quinine	15	10 (66.7)	5 (33.3)	
Study 2[b]	Rwanda[c]	ArTiMist	23	22 (95.7)	1 (4.3)	48.1 (21.8-74.4); p < 0.05
		Quinine	21	10 (47.6)	11 (52.4)	
	Ghana	ArTiMist	23	23 (100.0)	0 (0.0)	88.0 (59.8-116); p < 0.05
		Quinine	25	3 (12.0)	22 (88.0)	
	Burkina Faso	ArTiMist	24	21 (87.5)	3 (12.5)	27.5 (2.8 – 52.2); p < 0.05
		Quinine	25	15 (60.0)	10 (40.0)	

674

675 [a] Modified full analysis set population (MFAS).

676 [b] Modified intent to treat population (MITT).

677 [c] Study site was the same in Study 1 and Study 2.

678 [d] Parasitological success was defined as a reduction in parasite count of $\geq$ 90% of

679 baseline at 24 h after the first dose.

680

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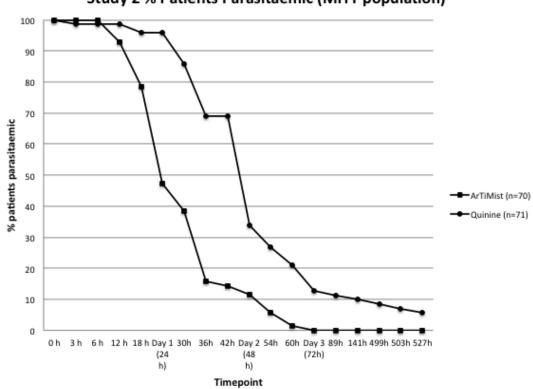
681 **FIGURE CAPTIONS**

682 **Figure 1.** Flow chart of patient disposition for the treatment of African children with malaria
683 using sublingual artemether (ArTiMist) or i.v. quinine in Study 2.

684

685 **Figure 2.** Percent of patients still parasitaemic over time in African children who were
686 treated for malaria using sublingual artemether (ArTiMist) or i.v. quinine in Study 2.

N = 180
Patients screened*

N = 29
Screen Failures

Reasons:
Did not meet criteria for severe malaria = 15
Low/Negative parasitemia = 8
ICF withdrawn = 3
Death = 1
Co - infection = 1
Co - infection associated low parasitemia < 500/μL = 1

N = 151
Subjects
Randomised

N = 10 for MITT, N = 14 for PP
Excluded from Efficacy Analysis

Reasons (MITT and PP):
No baseline parasite count
(Art =1, Quin = 1)
Baseline parasite count < 500 μl
(Art = 4, Quin =2)
No baseline parasite at 24 hours
(Art = 2)

Reasons (PP only):
Early withdrawal (missed visits)
(Art = 2)
Compliance < 80% while inpatient
(Quin = 1)
Received non-study quinine
(Quin = 1)

N = 141
Efficacy Analysis
(MITT)

N = 137
Efficacy Analysis
(PP)

N = 151
ITT and Safety
Analysis Population

N = 70
Art

N = 71
Quin

N = 68
Art

N = 69
Quin

N = 77
Art

N = 74
Quin

N = 75
Completed

N = 2
Withdrawn

Reasons:
Lost to
follow-up (2)

N = 72
Completed

N = 2
Withdrawn

Reasons:
Other (2)

Study 2 % Patients Parasitaemic (MITT population)

Legend: ArTiMist (n=70); Quinine (n=71)

X-axis (Timepoint): 0 h, 3 h, 6 h, 12 h, 18 h, Day 1 (24 h), 30h, 36h, 42h, Day 2 (48 h), 54h, 60h, Day 3 (72h), 89h, 141h, 499h, 503h, 527h

Y-axis: % patients parasitaemic