

1 **Development of a paediatric physiologically based pharmacokinetic model to assess the**  
2 **impact of drug-drug interactions in tuberculosis co-infected malaria subjects: a case**  
3 **study with artemether-lumefantrine and the CYP3A4-inducer rifampicin**

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24 **ABSTRACT**

25 The fixed dosed combination of artemether and lumefantrine (AL) is widely used for the  
26 treatment of malaria in adults and children in sub-Saharan Africa, with lumefantrine day 7  
27 concentrations being widely used as a marker for clinical efficacy. Both are substrates for  
28 CYP3A4 and susceptible to drug-drug interactions (DDIs); indeed, knowledge of the impact  
29 of these factors is currently sparse in paediatric population groups. Confounding malaria  
30 treatment is the co-infection of patients with tuberculosis. The concomitant treatment of AL  
31 with tuberculosis chemotherapy, which includes the CYP3A4 inducer rifampicin, increases the  
32 risk of parasite recrudescence and malaria treatment failure. This study developed a  
33 population-based PBPK model for AL in adults capable of predicting the pharmacokinetics of  
34 AL under non-DDI and DDI conditions, as well as predicting AL pharmacokinetics in  
35 paediatrics of 2-12 years of age. The validated model was utilised to assess the concomitant  
36 treatment of rifampicin and lumefantrine under standard body-weight based treatment  
37 regimens for 2-5 year olds, and demonstrated that no subjects attained the target day 7  
38 concentration ( $C_{d7}$ ) of 280 ng/mL, highlighting the importance of this DDI and the potential  
39 risk of malaria-TB based DDIs. An adapted 7-day treatment regimen was simulated and  
40 resulted in 63 % and 74.5 % of subjects attaining the target  $C_{d7}$  for 1-tablet and 2-tablet  
41 regimens respectively.

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60 **KEYWORDS**

61 Physiologically-based pharmacokinetics; malaria; tuberculosis; paediatrics;  
62 pharmacokinetics.

## 63 1. INTRODUCTION

64 Malaria is a deadly parasitic disease spread by female *anopheles* mosquitoes infected with  
65 *Plasmodium falciparum* [1, 2]. The World Health Organisation's (WHO) target is to eliminate  
66 malaria in 35 countries by 2030 and this has led to several measures being taken over the past  
67 few decades directed towards malaria prevention and treatment in order to reduce its prevalence  
68 and mortality rates [2]. At the turn of the millennium, the global estimate of malaria cases  
69 averaged 262 million which, by 2015, had fallen to 214 million, reflecting a decrease of 18 %  
70 [3]. Furthermore, 88 % of these malaria cases were reported in the sub-Saharan African region.  
71 Alarmingly however, within the paediatric population group 70% of the total malaria related  
72 deaths were attributed to children under five years of age [2].

73 In 2006, artemisinin or artemisinin derivatives were recommended by the WHO for the first  
74 line treatment of malaria in endemic areas. During every 48 hour *P. falciparum* replication  
75 period, artemether and its active metabolite dihydroartemisinin (DHA) decreases parasite load  
76 by approximately 10,000 fold [4, 5]. Artemether's oral absorption and onset of action are both  
77 rapid, with an approximate  $t_{max}$  following oral administration of two hours [6, 7].  
78 Furthermore, oral absorption is improved following a fat-rich meal [8], with bioavailability  
79 increasing by 2-fold compared to a fasted-state in healthy volunteers [9]. Hepatic metabolism  
80 of artemether is rapid and predominantly mediated by CYP3A4, as well as CYP2B6 [5, 10,  
81 11]. Lumefantrine is a racemic fluorine mixture possessing a chemical structure related to the  
82 arylaminoalcohol group of antimalarials such as quinine, halofantrine and mefloquine [12].  
83 Lumefantrine is well orally absorbed but, as with artemether, demonstrates absorption  
84 pharmacokinetics which are highly variable in malaria patients [9]. As with artemether, the  
85 administration of food increases the bioavailability by 16-fold when compared to the fasted  
86 state in healthy volunteers [9]. CYP3A4 is primarily responsible for the metabolism of  
87 lumefantrine. As a result of low hepatic intrinsic clearance and negligible renal excretion,  
88 lumefantrine possess a prolonged half-life [8] of up to six days in healthy volunteers [13] [14].  
89 Artemether is recommend for dosing in conjunction with lumefantrine (AL) as a fixed dose  
90 combination (FDC) of 20mg/120mg respectively, in six doses usually over three days  
91 (commonly at 0, 8, 24, 26, 48 and 60 hours). Typical treatment regimens for children include  
92 a similar 3 day six-dose regimen stratified based on body weight: 5-15 kg 1 tablet per dose; 15-  
93 25 kg 2 tablets per dose; 25-35 kg 3 tablets per dose and >35 kg 4 tablets per dose [15], with  
94 the latter dose primarily being the default adult dose.

95 Contrary to adults who possess naturally acquired immunity, children often do not, this  
96 puts them at risk of succumbing to the infection [16] and this is further complicated by possible  
97 trans-placental transmission in pregnant women leading to congenital malaria [2]. Whilst  
98 malaria is endemic to many areas of sub-Saharan Africa, other communicable diseases such as  
99 tuberculosis are also commonplace, and particularly impacts upon paediatric population  
100 groups. In 2015, there were an estimated 10 million new TB cases worldwide of which 10 %  
101 were children [17]. Worryingly, the mainstay treatments for tuberculosis, namely a FDC of  
102 rifampicin (10-20 mg/kg), isoniazid (10-15 mg/kg), pyrazinamide (30-40 mg/kg) and  
103 ethambutol (15-25 mg/kg), can directly affect CYP3A4 activity through primarily rifampicin  
104 being a strong inducer [18, 19] or isoniazid being a moderate inhibitor [19, 20]. Thus, drug-  
105 drug interactions are commonplace in patients who are likely to present with both malaria and  
106 tuberculosis making dosing strategies in paediatrics complex. Although data is sparse and the  
107 connection between malaria and tuberculosis co-infection has not been widely investigated (in  
108 contrast to HIV and tuberculosis coinfection), one study in Angola reported that the presence  
109 of malaria in patients admitted for tuberculosis as 37.5 % [21]. Furthermore, the risk of  
110 rifampicin-mediated induction in CYP3A4 expression/activity would have the potential to  
111 significantly increase the clearance of AL, as has been demonstrated in adult populations [22]  
112 and has further been contraindicated when used with strong inducers such as rifampicin [23].

113 However, the magnitude of this induction effect on AL pharmacokinetics has not been  
114 investigated. DDIs between antimalarials and other drugs in paediatrics are not well studied  
115 and this may impact on the clinical efficacy, and safety of antimalarial drug therapy. *In-lieu*  
116 of complex clinical studies, physiologically-based pharmacokinetic (PBPK) modelling has  
117 been used to explore the potential risk of DDIs in adults [24, 25] and paediatric populations  
118 [26-28].

119 The objective of the current study was to demonstrate the application of PBPK modelling to  
120 the prediction of DDI risks in malaria-tuberculosis co-infection paediatric population groups.  
121 Specifically, the potential for a DDI between the CYP3A4 inducer rifampicin and AL will be  
122 explored over 2-5 year old population groups.

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## 125 2. METHODS

126 All population based PBPK modelling was conducted using the virtual clinical trials simulator  
127 Simcyp® (Simcyp® Ltd, a Certara company, Sheffield, UK, Version 16) using either the pre-  
128 validated in-built ‘Healthy Volunteer’ or ‘Paediatric’ population groups. The latter population  
129 group accounts for age-related changes in systems-parameters such as organ volumes, organ  
130 perfusion and ontogeny of drug metabolising enzymes[29] [30] [31] and allows for the  
131 prediction of drug behaviour in paediatric population groups . In the case of both models,  
132 population variability is accounted for by the inclusion of a variability metric (% coefficient  
133 variability) having been established from public health data bases such as the US National  
134 health and Nutrition Examination Survey (<https://www.cdc.gov/nchs/nhanes/>).

### 135 2.1 Study design

136 A four stage strategy was employed for model development and validation (Figure 1).

137 Step 1: this step focussed on the development of Simcyp® compound files and validation of  
138 simulations with published clinical studies. For artemether and lumefantrine, these included a  
139 study conducted in 120 adult subjects who were orally dosed the branded combination  
140 Coartem® [69], and studies conducted in 16 subjects who were orally dosed the branded  
141 combination Riamet® [32]. For lumefantrine an additional study included a 6-dose study  
142 conducted in 17 subjects [33].

143 Step 2: this step focussed on the validation of the adult DDI predictions. CYP3A4 inhibition  
144 and induction mechanisms were simulated using ketoconazole and rifampicin respectively.  
145 Clinical studies demonstrating such a DDI were obtained from Lefèvre *et al* who studied AL  
146 with ketoconazole [32] (single dose of 80/480 mg of AL and 5 day treatment with  
147 ketoconazole) and Lamorde *et al* [34], who studied AL DDI with rifampicin where rifampicin  
148 was dosed at 10 mg/kg for the duration of the study with AL dosed as six 80/480 mg doses (12  
149 hourly) on days 8, 9 and 10.

150 Step 3: this step focussed on the validation of artemether and lumefantrine model predictions  
151 in paediatrics. In these studies, weight bandings were simulated based on dosing strategies  
152 for AL if the clinical study did not use a weight normalised dosing method. Dosing boundaries  
153 were set at 1 tablet for 5-14.9 kg, 2 tablets for 15-24.9 kg and 3 tablets for 25-34.9 kg and trials  
154 were run to ensure, where possible, an equal proportion of subjects were included into each  
155 distribution banding based on the total number of subjects recruited within each reported trial.

156 Simulated profiles were body weight stratified and analysed accordingly. Clinical studies used  
157 are detailed the results section 3.3 and 3.4.

158 Step 4: this step focussed on simulations to predict the impact of rifampicin-mediated DDIs on  
159 artemether and lumefantrine pharmacokinetics in children of 2-5 years of age over a weight  
160 boundary of 5-14.9 kg or 15-24.9 kg. In these simulations, trials of 100 subjects were simulated  
161 and analysed with appropriate weight-based dosing (see above) and under treatment of  
162 rifampicin with AL.

163 For all validation steps, unless otherwise stated, all observed data sets were obtained from  
164 ‘supervised’ administration groups in reported clinical studies and simulated under ‘fed’  
165 conditions. Furthermore, unless otherwise stated all simulations included subjects of  $\geq 5$  years

## 166 **2.2 Artemether-lumefantrine model development**

167 The physicochemical and pharmacokinetic parameters required to describe the  
168 pharmacokinetic properties of artemether, lumefantrine and isoniazid are detailed in table 1.  
169 For artemether, literature- reported isozyme specific hepatic intrinsic clearances were utilised  
170 for the description of drug metabolism (Table 1). For lumefantrine, the isozyme specific  
171 hepatic intrinsic clearance ( $CL_{int}$ ) was back-calculated using the Simcyp® retrograde calculator  
172 from the oral clearance and assuming CYP3A4 was the predominant isozyme for lumefantrine  
173 metabolism[6]. This approach is essential in order to mechanistically model DDIs.

174 Where necessary, the human jejunal effective permeability ( $P_{eff}$ ) and  $K_p$  scalar were further  
175 optimised for AL using a parameter estimate method within Simcyp® to yield optimal  
176 estimates for the absorption ( $P_{eff}$ ) and tissue distribution/ $V_{ss}$  prediction ( $K_p$  scalar).  
177 Furthermore, for artemether, where necessary, the *in-vitro* metabolic clearance was optimised  
178 through the parameter estimation of the Inter System Extrapolation Factor (ISEF) (Table 1).

179 Rifampicin and ketoconazole compounds were used in simulations without modification from  
180 the library of pre-validated drug molecules within the Simcyp® simulator, using a 1<sup>st</sup>-order  
181 absorption model and assuming dosing in solution form. Where Simcyp® ADAM (Advanced  
182 Dissolution Absorption Model) was used, an immediate release formulation with an applied  
183 diffusion layer model was utilised for modelling with literature-reported solubility parameters  
184 included. Where simulations were performed in paediatrics, all APIs were assumed to be dosed  
185 in solution form, mimicking the dispersible/crushed application of AL in paediatric subjects  
186 [15].

187 **2.2.1 Artemether-lumefantrine DDI model development**

188 The successful development and validation of AL compounds within Simcyp® was followed  
189 by assessing the ability to predict DDIs in adults and paediatrics. All adult DDI simulations  
190 were, where possible, run identically to the reported clinical study with which the validation  
191 was conducted against, and primarily included matching age ranges, male-to-female ratios and  
192 identical dose/dosing intervals. In order to validate the capability of the model to predict a  
193 broad range of DDIs, the prevalidated Simcyp® in-built compounds ketoconazole and  
194 rifampicin were directly utilised in simulations as candidates to simulate CYP3A4 inhibition  
195 DDIs (ketoconazole) and CYP3A4 induction DDIs (rifampicin).

196 A previously validated isoniazid compound file [35] was used for all rifampicin DDI  
197 simulations to account for the impact of isoniazid mediated CYP3A4-inhibition associated with  
198 TB chemotherapy. All simulations included both rifampicin (as the primary perpetrator) and  
199 isoniazid (as the secondary perpetrators), however results are presented for the key interactions  
200 between AL and rifampicin only, and reflects the clinical net effect of CYP3A4 induction with  
201 the clinical use of the combination of rifampicin and isoniazid in DDI-focussed studies [36-  
202 38].

203 For paediatric DDI simulations (Step 4), a 100 subject simulation was run in a 10x10 trial (10  
204 subjects per trial with 10 trials) to ensure that reasonable inter-/intra individual variability is  
205 captured within the model simulations. However, as simulations are not possible with defined  
206 age and weight ranges, pooling and post-processing of output data was conducted to match  
207 individuals to the required age-weight boundary conditions for the study.

208



209 **Table 1. Input parameter values and predicted PBPK values for use in the simulation of**  
 210 **artemether, lumefantrine and isoniazid.**

Parameters	Artemether	Lumefantrine	Isoniazid <sup>d</sup>
<b>Compound type</b>	Monoprotic base	Diprotic base	Monoprotic base
<b>Molecular weight (g/mol)</b>	298.4 <sup>[39]</sup>	528.94 <sup>[39]</sup>	137.1
<b>Log P</b>	3.53 <sup>[40]</sup>	8.70 <sup>[41]</sup>	-0.7
<b>fu</b>	0.05 <sup>[42]</sup>	0.003 <sup>[42]</sup>	0.95
<b>pKa 1</b>	3.9 <sup>[39]</sup>	14.1 <sup>[39]</sup>	1.82
<b>pKa 2</b>	-	9.80 <sup>[39]</sup>	-
<b>B/P</b>	0.55 <sup>a</sup>	0.80 <sup>[43]</sup>	0.825
<b>Vss (L/kg)</b>	1.77 <sup>b</sup>	0.70 <sup>b</sup>	0.63 <sup>a</sup>
<b>P<sub>eff</sub> (10<sup>-4</sup> cm/s)</b>	3.67 <sup>a</sup>	0.97 <sup>a</sup>	10.23 <sup>a</sup>
<b>Kp scalar</b>	0.21 <sup>a</sup>	0.10 <sup>a</sup>	-
<b>Solubility (mg/mL)</b>	0.012 <sup>[44]</sup>	0.002 <sup>[45]</sup>	-
<b>CL<sub>po</sub> (L/min)</b>	-	0.25 <sup>[7]</sup>	12
<b>CL<sub>int3A4</sub> (μL/min/pmol)</b>	1.47 <sup>[11]</sup>	2.61 <sup>a,c</sup>	-
<b>CL<sub>int2B6</sub> (μL/min/pmol)</b>	9.31 <sup>[11]</sup>	-	-
<b>ISEF CYP 3A4</b>	2.424 <sup>a</sup>	-	-
<b>ISEF CYP 2B6</b>	1.697 <sup>a</sup>	-	-
<b>Ki (μM)</b>	-	-	36 <sup>[20]</sup>
<b>K<sub>inact</sub> (min<sup>-1</sup>)</b>	-	-	0.08 <sup>[20]</sup>
<b>K<sub>app</sub> (μM)</b>	-	-	228 <sup>[20]</sup>
<b>Absorption model</b>	ADAM	ADAM	1 <sup>st</sup> order
<b>Distribution model</b>	Full	Full	Minimal

211 <sup>a</sup> Parameter estimated; <sup>b</sup> Simcyp® mechanistic prediction; <sup>c</sup> Simcyp® retrograde calculation  
 212 from population estimates of CL<sub>po</sub> followed by parameter estimation (final optimised value:  
 213 0.85 μL/min/pmol for CYP3A4); <sup>d</sup> Unless otherwise detailed data was obtained from Gaohua  
 214 *et al* (2015) [46]. MW: Molecular weight; P<sub>eff</sub>: human effective permeability; B/P: blood-to-  
 215 plasma ratio; CL<sub>int</sub>: *in vitro* intrinsic clearance; Vss: Steady state volume of distribution; ISEF:  
 216 Intersystem extrapolation factor for scaling CYP *in-vitro* kinetic data; Ki: concentration of  
 217 inhibitor supporting half-maximal inhibition; K<sub>inact</sub>: inactivation rate of the enzyme; K<sub>app</sub>:  
 218 concentration of mechanism based inhibitor associated with half-maximal inactivation rate.

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### 224 **2.3 Predictive performance**

225 Whilst no agreed criterion has been suggested for an ‘optimal’ predictive performance range,  
226 it is generally considered that a prediction to within 2-fold of the observed data is acceptable  
227 [47]. Given the wide inter-subject variability in artemether pharmacokinetics, we selected this  
228 2-fold range (0.5-2.0) as our criterion for comparing  $C_{\max}$  and AUC parameters between model  
229 predictions and those clinically reported. Where a DDI was simulated, the model performance  
230 was primarily dictated by a comparison of the AUC ratio (ratio of AUC in the absence and  
231 presence of the perpetrator agent) ( $AUC_r$ ), with a prediction of  $AUC_r$  to within 2-fold of the  
232 reported  $AUC_r$  being considered as acceptable, with an  $AUC_r$  greater than 1.25 being indicative  
233 of an inhibition reaction whereas an  $AUC_r$  less than 0.8 indicating an induction reaction whilst  
234 an AUC ratio of between 0.8 – 1.25 indicating no interaction.

### 235 **2.4 Data analysis**

236 Unless otherwise stated, all simulations of plasma concentration-time profiles were presented  
237 as arithmetic mean and 5-95<sup>th</sup> percentiles. In circumstances where reported concentration-time  
238 profiles did not provide corresponding tabulated summary data, the observed data points were  
239 retrieved using the WebPlotDigitizer v3.10 [48] and superimposed onto simulated profiles for  
240 visual predictive checks.

## 241 **3. RESULTS**

### 242 **3.1 Step 1: Predictive performance for artemether-lumefantrine models for adults**

243 Following optimisation of parameter estimates (Table 1) the predicted population plasma  
244 concentration profile for both artemether and lumefantrine were within the observed trial  
245 means for plasma concentration profiles. The model predicted  $C_{\max}$  values were within 2-fold  
246 of the reported  $C_{\max}$  for each clinical study for both artemether ( $139.1 \pm 116.2$  ng/mL; table 2;  
247 figure 2A) and lumefantrine (single dose:  $6.31 \pm 3.72$   $\mu$ g/mL; six dose:  $9.56$   $\mu$ g/mL; range:  
248  $5.67$ - $16.78$   $\mu$ g/mL; table 2; figure 2B and 2C). The 24 h, 48 h, 72 h and day 7 lumefantrine  
249 concentrations were also predicted to within 2-fold of those reported by Ashley  
250 *et al* [33].

251 Similarly, the model predicted  $AUC_{\text{last}}$  for artemether ( $521.2 \pm 254.1$  ng/mL.h) (Table 2) and  
252 lumefantrine (single dose:  $251.4 \pm 1.45$   $\mu$ g/mL; six dose  $AUC_{0-\infty}$ :  $387.4$   $\mu$ g/mL.h (98-1157  
253  $\mu$ g/mL.h) (Table 2) were within 2-fold of the reported  $AUC_{\text{last}}$ .

254 **Table 2: Summary of predicted and observed pharmacokinetic parameters of artemether and lumefantrine in healthy adults**  
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					256
		Prediction	Lefevre et al 2013 <sup>[49]</sup>	Lefevre et al 2002 <sup>[32]</sup>	Ashley et al 2007 <sup>[33]</sup>
<b>Artemether</b>	<b>Dose (mg)</b>	80	80	80	258
	<b>Population size (n)</b>	100	58	16	259
	<b>C<sub>max</sub> (ng/ml)</b>	139.1 ± 116.2	113 ± 69.5	104 ± 40	260
	<b>AUC<sub>last</sub> (ng/ml.h)</b>	521.2 ± 254.1	408 ± 209	302 ± 135	261
<b>Lumefantrine</b>	<b>Dose (mg)</b>	480	480	480	262
	<b>Population size (n)</b>	100	58	16	263
	<b>C<sub>max</sub> (µg/ml)</b>	6.31 ± 3.72	8.92 ± 3.18	7.91 ± 3.49	264
	<b>AUC<sub>last</sub> (µg/ml.h)</b>	251.4 ± 112.3	236 ± 93	195 ± 119	265
<b>Lumefantrine<sup>a</sup></b>	<b>Dose (mg)</b>	6 dose regimen			6 dose regimen <sup>265</sup>
	<b>Population size (n)</b>	100			17 <sup>266</sup>
	<b>C<sub>max</sub> (µg/ml)</b>	9.56 (5.67-16.78)			6.89 (3.69-13.19) <sup>267</sup>
	<b>C<sub>24h</sub> (pre-dose)</b>	3.39 (1.98-9.28)			2.53 (0.68-9.8) <sup>268</sup>
	<b>C<sub>48h</sub> (pre-dose)</b>	5.81 (1.48-13.14)			3.84 (1.91-6.80) <sup>269</sup>
	<b>C<sub>72h</sub> (pre-dose)</b>	5.84 (1.12-12.75)			3.91 (2.15-9.64) <sup>270</sup>
	<b>C<sub>d7</sub></b>	0.32 (0.11-0.78)			0.35 (0.20-0.87) <sup>271</sup>
	<b>AUC<sub>0-∞</sub> (µg/ml.h)</b>	387.4 (98-1157)			432 (308-991) <sup>272</sup>

271 Data represent mean ± SD or mean (range).

272 <sup>a</sup> Concentrations measured at 24, 48 and 72 hour immediately pre-dose are labelled by the subscript time (hour) nominals, with all concentrations  
 273 units express as µg/ml. C<sub>d7</sub> indicates the 7<sup>th</sup> day concentration.

274 **3.2 Step 2: Simulation of the AL DDIs following exposure to ketoconazole and**  
275 **rifampicin**

276 The artemether and lumefantrine compound files were further assessed for the ability to  
277 recapitulate the literature reported extent of DDIs on plasma concentration profiles in adults.

278 Predictions for inhibition-based DDIs with artemether and ketoconazole resulted in predicted  
279 plasma-concentration profiles for the simulated population within the observed range reported  
280 by Lefevre *et al* 2002 [13] (Figure 3A). The predicted  $C_{\max}$  ratio was  $2.49 \pm 0.51$  compared  
281 with a reported ratio of 2.24 and predicted  $AUC_r$  was  $2.96 \pm 0.80$  compared to a reported ratio  
282 of 2.51 (Table 3).

283 Predictions for inhibition-based DDIs with lumefantrine and ketoconazole, resulted in plasma-  
284 concentration profiles for the simulated population within the observed range reported by  
285 Lefevre *et al* 2002 [13] (Figure 3B). The predicted  $C_{\max}$  ratio was  $1.16 \pm 0.89$  compared with  
286 a reported ratio of 1.26 and predicted  $AUC_r$  was  $2.10 \pm 0.54$  compared to a reported ratio of  
287 1.65 (Table 3).

288 **Table 3: Summary of predicted and observed pharmacokinetic parameters of artemether and lumefantrine in the absence and presence**  
 289 **of ketoconazole in healthy adults**

		<b>-Ketoconazole</b>		<b>+Ketoconazole</b>		<b>Ratio</b>	
		<b>C<sub>max</sub></b> <b>(ng/mL)</b>	<b>AUC<sup>a</sup></b> <b>(ng/mL.h)</b>	<b>C<sub>max</sub></b> <b>(ng/mL)</b>	<b>AUC<sup>a</sup></b> <b>(ng/mL.h)</b>	<b>C<sub>max</sub></b>	<b>AUC</b>
<b>Artemether</b>	<b>Predicted</b>	71.2 ± 62.7	316.2 ± 96.05	171.39 ± 115.21	911.24 ± 324.60	2.49 ± 0.51	2.96 ± 0.80
	<b>Observed</b>	104 ± 40	302 ± 135	225 ± 77	718 ± 279	2.24	2.51
<b>Lumefantrine</b>	<b>Predicted</b>	5476 ± 2168	118211 ± 57079	6305 ± 2432	235041 ± 97260	1.16 ± 0.89	2.10 ± 0.5
	<b>Observed</b>	7910 ± 3490	195000 ± 119000	10100 ± 4740	312000 ± 181000	1.26	1.65

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292 <sup>a</sup> Artemether: AUC<sub>(0-∞)</sub>; lumefantrine: AUC<sub>(0-last)</sub>

293 Data represent mean ± SD.

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295 For induction based DDI studies, only one clinical study was identified with rifampicin  
296 mediated DDIs reporting the impact on both artemether and lumefantrine in the same subjects  
297 [22]. However, due to the small clinical study size (6 subjects) and narrow age and weight  
298 range used in the study, we simulated a virtual clinical trial of 10 trials consisting of 10 subject  
299 per trial within the weight and age boundaries reported by Lamorde *et al* [22]. As there was  
300 no direct way to specify an age boundary, the trials containing at least 6 subjects within the  
301 correct weight boundaries were selected for study and subsequent analysis.

302 Predictions for induction-based DDIs with artemether and rifampicin were validated against a  
303 single study reporting a single time point artemether concentration at 12-hours ( $C_{12h}$ ) post final  
304 dose [22] in six subjects in the absence and presence to subjects taking a FDC for tuberculosis  
305 which included rifampicin [22]. Predicted  $C_{12h}$  was  $3.56 \pm 3.13$  ng/mL which reduced to  $0.77$   
306  $\pm 1.14$  ng/mL in the presence of rifampicin, and was within 2-fold of the reported  $C_{12h}$  of  $0.5$   
307  $\pm 1$  ng/mL (Figure 4A).

308 Predictions for induction-based DDIs with lumefantrine and rifampicin were validated against  
309 a single study reporting a single time point lumefantrine concentration on the 8<sup>th</sup> day after  
310 initiating lumefantrine dosing ( $C_{d8}$ ) (7.3 days' post first dose). Using this approach, the  
311 predicted  $C_{d8}$ ,  $59.83 \pm 24.86$  ng/mL, was within 2-fold of the observed reported  $C_{d8}$  of  $107.75$   
312  $\pm 19.58$  ng/mL [22] (Figure 4B).

### 313 **3.3 Step 3: Predictive performance for artemether in children**

314 The majority of clinical studies assessing AL pharmacokinetics in children often focus on the  
315 longer-half life drug lumefantrine. Existing arthemeter clinical studies are sparse and include  
316 either sampling around the expected  $C_{max}$  (1-2 hours) [50, 51] or limited large population based  
317 sampling approaches [10], with dosing based on the body weight stratification.

318 The model predicted mean artemether plasma concentration for the lower doses ( $221.25 \mu\text{g/mL}$   
319  $\pm 104.51 \mu\text{g/mL}$ ) and higher doses ( $293.51 \pm 98.62 \mu\text{g/mL}$ ) were within the 2-fold of the  
320 literature reported plasma concentrations for both lower ( $150 \pm 206 \mu\text{g/mL}$ ) and higher doses  
321 ( $196 \pm 204 \mu\text{g/mL}$ ) (Figure 5A) [50].

322 Similarly when using a single lower dose and stratifying further for weight into  $5 < 10\text{kg}$  and  
323  $10 \text{ to } < 15 \text{ kg}$ , the reported concentrations for the lower and higher weight banding,  $295 \pm 214$   
324  $\mu\text{g/mL}$  and  $137 \pm 111 \mu\text{g/mL}$ , were within the 5<sup>th</sup> and 95<sup>th</sup> percentiles of the mean prediction  
325 profiles (Figure 5B), with a predicted mean concentration (mean of 1 and 2 hour time points)

326 of  $225.59 \pm 187.27 \mu\text{g/mL}$  for the lower weight boundary and  $238.84 \pm 187.12 \mu\text{g/mL}$  for the  
327 higher weight boundary [51] (Figure 5B).

328 To confirm a successful model prediction of the distribution and elimination phases of  
329 artemether pharmacokinetics, Figure 5C illustrates model predicted concentration-time profiles  
330 for artemether dosing at the lowest (5-14 kg) and highest (25-34 kg) doses, where observed  
331 sampling points were obtained from a population study reported by Hietala *et al* (2010) [10] at  
332 2, 4, 8, 16, 24, 36, 48 and 60 hours. The predicted profile for each dosing band fell within the  
333 range reported by Hietala *et al* [10]. However, due to the well documented variability in the  
334 absorption phase of artemether, the predicted concentrations during the absorption phases (0-4  
335 hours) were slightly over-predicted.

### 336 **3.4 Step 3: Predictive performance for lumefantrine in children**

337 Lumefantrine is often studied, in preference to artemether, in clinical trials due its longer half-  
338 life [13] [14], and a range of clinical studies are available to support PBPK-based model  
339 development where 7-day post-dosing concentration ( $\sim 280 \text{ ng/mL}$  [7]) is used as a marker of  
340 successful ‘target’ concentration to obtain parasite clearance.

341 To validate the lumefantrine compound we first assessed the predictive performance against  
342 two studies reporting mean plasma concentration through the study duration period. Based on  
343 a study by Borrmann *et al* (2010) [8] where mean  $\pm$  SD plasma concentration data was available  
344 for 30, 54, 66, 84 and 168 hours post first dose, the  $CL_{\text{int},3A4}$  was optimised to 0.71 and Kp  
345 scaler optimised to 0.05 ( $V_{\text{ss}}$ : 0.53 L/kg). Using this revised lumefantrine compound file, we  
346 are able to capture the 4 time-points reported by Borrmann *et al* over the 3 doses stratification  
347 used (Figure 6A).

348 This optimised compound file was then applied to all subsequent simulations, and was  
349 confirmed with a second study reported by Piola *et al* [52] where 5-14 year olds were simulated  
350 with appropriate weight-based dosing, and where observed mean  $\pm$  SD plasma concentration  
351 data was available for day 3 and day 7 (Figure 6B). Day 3 predicted concentration was  $7958$   
352  $\pm 2381 \text{ ng/mL}$  and  $8246 \pm 5478 \text{ ng/mL}$  for the 5-15 kg and 15-25 kg doses, and day 7 predicted  
353 concentrations of  $658.5 \pm 289 \text{ ng/mL}$  and  $718.9 \pm 554 \text{ ng/mL}$  for the 5-15 kg and 15-25 kg  
354 doses. The observed day 3 ( $7050 \pm 3560 \text{ ng/mL}$ ) and day 7 ( $376 \pm 217 \text{ ng/mL}$ ) mean plasma  
355 concentration were within 2-fold of the predicted mean concentrations, in addition to being  
356 within the 5<sup>th</sup> and 95<sup>th</sup> percentiles of the mean lumefantrine predicted plasma concentration for  
357 the two weight-based doses (Figure 6B).

358 This optimised compound file was further utilised to assess the predictive performance for  
359 median day 3 and day 7 (predominantly) concentrations (Table 5), and was able to capture  
360 day 3 and day 7 concentrations to within 2-fold of those reported in clinical studies.



361 **Table 4: Summary of simulated and observed median day 3 or day 7 lumefantrine concentrations in children**  
 362

Study	Notes	Observed		Simulated	
		Median Concentration [Range] (ng/mL)			
		Day 3	Day 7	Day 3	Day 7 <sup>a</sup>
Mayxay <i>et al</i> (2004) <sup>[53]</sup>	n=77; 95% CI reported	-	520 [390–650]	-	1 dose: 374.12 [0.1-2341] 2 doses: 392.32 [0.1-4719] 3 doses: 411.12 [0.3-4853]
Schramm <i>et al</i> (2013) <sup>[54]</sup>	n=139; IQR reported; ACRP results	-	356 [211-547]	-	1 dose <sup>b</sup> : 368.43 [37-885]
Ngasala <i>et al</i> (2011) <sup>[55]</sup>	n=177; Range reported	-	205 [0-1887]	-	1 dose: 392.15 [0.12-6785] 2 doses: 408.29 [0.13-7511]
Borrmann <i>et al</i> (2011) <sup>[56]</sup>	n=15; Range reported from 2005-2006 study	-	536 [178-3270]	-	369.89 <sup>c</sup> [0.1-5028]
Checchi <i>et al</i> (2006) <sup>[57]</sup>	n=70; Range reported in supervised group in under 5 years	7050 [1876-14985]	367 [0.12-768]	4877 [1678-25285]	1 dose: 389.752 [0.1-7544] 2 doses: 347.93 [0.3-8641]

363 <sup>a</sup> Simulated day 7 median concentrations were predicted following dosing based on body-weight stratification as a result of the lack of clear age-  
 364 weight dosing strategies detailed in the observed studies.

365 <sup>b</sup> Observed study demographics required single dose of AL based on weight

366 <sup>c</sup> Dosed as 12mg/kg

367

368 **3.5 Step 4: Simulating the impact of rifampicin-mediated CYP3A4 induction on**  
 369 **artemether and lumefantrine pharmacokinetics in children**

370 The presence of tuberculosis is thought to occur in at least 37.5 % of subjects infected with  
 371 malaria [21], and given the potential for TB treatments to attenuate CYP-mediated drug  
 372 metabolism (rifampicin being a CYP3A4 inducer and isoniazid a CYP3A4 inhibitor), the  
 373 potential risk in paediatrics patients is important to assess considering the ontogeny CYP3A4  
 374 expression during the first 5 years of life [26-28]. Simulations to predict the potential impact  
 375 of TB treatment on subjects established on anti-malarial treatment was assessed to quantify the  
 376 change in AL plasma concentrations in the absence and presence of dosing with rifampicin  
 377 (and isoniazid) for subjects of 2-5 years of age with weight-based dosing (1 tablet: 5-14.9 kg  
 378 and 2 tablets 15-24.5 kg) where rifampicin (and isoniazid) was dosed daily for 7 days prior to  
 379 the initiation of AL.

380 **3.5.1 Artemether**

381 A DDI initiated with a combination of rifampicin and isoniazid significantly reduces the  $C_{max}$   
 382 for both one and two table regimens by approximately 80 %, with a calculated  $C_{max}$  ratio of  
 383 0.21 (Table 5) (Figure 7). Similarly a significant reduction in the AUC following the DDI  
 384 resulting an  $AUC_r$  of 0.22 (Table 5) (Figure 7). No differences in the overall impact of the  
 385 DDI between the two dosing groups was reported suggesting the magnitude of the DDI is  
 386 similar across the 2-5 years' age range.

387 **Table 5: Summary of predicted artemether pharmacokinetics in the absence and**  
 388 **presence of a DDI in children aged 2-5 year.**

	No Rifampicin		Rifampicin		Ratio	
	$C_{max}$ (ng/mL)	AUC (ng/mL.h)	$C_{max}$ (ng/mL)	AUC (ng/mL.h)	$C_{max}$	AUC
One	89.12 ± 78.93	563.60 ± 316.64	18.47 ± 31.18	121.53 ± 143.43	0.21	0.22
Two	210.95 ± 179.81	1127.21 ± 633.27	39.12 ± 136.37	243.06 ± 290.1	0.18	0.21

390

391  $C_{max}$  data is from the final dose; AUC calculated from final dose to end of study period.

392

393 **3.5.2 Lumefantrine**

394 In the absence of a DDI (i.e. malaria only patients), the predicted mean day 7 concentration  
 395 was above the minimum therapeutic target of 280 ng/mL (Figure 8) for both the single tablet  
 396 per dose (5-14.9 kg) and two tablets per dose (15-24.9 kg) strategies, 300.49 ng/mL (range:  
 397 0.1-4442 ng/mL) and 614.37 ng/mL (range: 0.14-6485 ng/mL) respectively (Table 6).

398 However, in TB co-infected patients, the predicted day 7 concentration fell significantly below  
399 the therapeutic target of 280 ng/mL (Table 6) for both the single and two tablet regimens, with  
400 a resultant  $AUC_r$  of 0.41 and  $AUC_r$  0.40 respectively (Figure 8) and no subjects presenting with  
401 a simulated day 7 concentration of  $> 280$  ng/mL (Table 6). The potential risk for failure of  
402 AMT is therefore of significant concern in TB co-infected paediatric patients, particularly those  
403 falling into the lower body-weight stratification who would typically be younger in age and  
404 therefore more prone to treatment failure.

405 Given that orally administrated AL often shows absorption saturation kinetics, to overcome the  
406 risk of significant treatment failure increasing the dose of AMT administrated in each FDC  
407 would not be appropriate. We assessed the impact of increasing the duration of treatment from  
408 3 days to 5 or 7 days on the potential impact on day 7 lumefantrine concentrations (Figure 9).

409 Increasing the duration of treatment to 5 days had a minimal impact on day 7 mean  
410 concentrations, with a modest increase for the single tablet to 63.63 ng/mL leading to a 11.1 %  
411 ( $n=5/46$ ) increase in the subjects with day 7 target  $> 280$  ng (Table 6) (Figure 9A). Similarly,  
412 for the two tablet treatment an increase in the mean day 7 concentration was simulated 76.93  
413 ng/mL which resulted in an overall increase in subjects with a target concentration  $> 280$  ng of  
414 11.3 % ( $n=6/53$ ) (Table 6) (Figure 9B).

415 However, for a 7-day treatment 63 % (one tablet) and 74.5 % (two tablets) of subjects  
416 demonstrated day 7 concentration in excess of 280 ng/mL (Table 6) (Figure 9A and B: lower  
417 panels).

418

419 **Table 6: Summary of predicted mean day 7 lumefantrine concentrations during a 3, 5 and 7-day treatment schedule in children**

420

Dosing	Mean C <sub>d7</sub> (Range) (ng/mL)			Lumefantrine ≥ 280ng/mL <sup>a</sup>		
	Regimen			Regimen		
	3 day	5 day	7 day	3 day	5 day	7 day
<b>1 tablet/NI</b>	300.49 (0.1-4442)	1451.01 (15.2-8367)	7509.77 (79.67-12438.06)	47.8 (n=22)	86.7 (n=39)	95.6 (n=44)
<b>1 tablet/I</b>	18.12 (0.01-88.91)	63.63 (0.01-578.12)	329.71 (0.12-4385.12)	0	11.1 (n=5)	63 (n=29)
<b>2 tablets/NI</b>	614.37 (0.14-6485)	1516.07 (14.9-9656)	9748.96 (28.55-14375.5)	46.6 (n=21)	60.3 (n=32)	85 (n=40)
<b>2 tablets/I</b>	42.69 (0.01-154.3)	76.93 (0.02-1087.99)	704.25 (0.08-7895.21)	0	11.3 (n=6)	74.5 (n=35)

421

422 <sup>a</sup> Percentage (number) of subjects with C<sub>d7</sub> ≥ 280ng/mL.

423 3 days: 1 tablet (n=53), 2 tablets (n=45); 5 days: 1 tablet (n=46), 2 tablets (n=53); 7 days: 1 tablet (n=46), 2 tablets (n=47).

424 NI: no interaction; I: interaction. C<sub>d7</sub>: mean day 7 concentration.

425

426

427

#### 428 **4. DISCUSSION**

429 The study of pharmacokinetics in paediatric population groups is often neglected for many  
430 therapeutic agents because of complexities in ethical/legal and recruitment strategies coupled  
431 with the requirement for limited sample collection and often diverse population based data  
432 analysis.

433 Although allometric scaling remains a useful tool for first predictions of primary  
434 pharmacokinetics parameters such as  $V_{ss}$  or clearance [58, 59] it can often fail for example in  
435 the prediction of clearance [60-63]; when assessing dosing-optimisation strategies in  
436 paediatrics [30]; in situations where body weight may be significantly variable based on  
437 geographical locations [64]. Further allometry often does not address the impact of maturation  
438 at early ages of childhood and can often over-predict clearance during the maturation of  
439 metabolic elimination pathways [65]. However PBPK modelling can often be used to support  
440 population modelling approaches with deviations in covariate models can be build and based  
441 upon the mechanistic knowledge for the population to study allowing the rational extrapolation  
442 of a drug pharmacokinetics across age groups. In light of these facts, PBPK is now gaining  
443 regulatory acceptance [66-70] as one approach to assess pharmacokinetics in paediatric patients  
444 [71] and complex scenarios such DDI [72, 73].

445 Although standard regimens for malaria treatment have shown positive treatment benefits with  
446 a reduction in mortality rates [2], in many developing countries with a high burden of  
447 communicable disease such as HIV/AIDS and tuberculosis, the risk potential of DDIs with co-  
448 infected malaria patients is high [21]. Such DDI issues are more apparent in children where  
449 the recruitment and inclusion of children onto antimalarial clinical trials is limited.  
450 Pragmatically assessing the risk of a DDIs in paediatrics is difficult due to CYP-ontogeny  
451 observed in key drug metabolic pathways associated the AMT metabolism, mainly CYP3A4,  
452 during the first 5 years of life [26-28], where maturation of CYP3A4 expression will lead to  
453 both altered plasma concentrations of CYP3A4-substrates (such as AL) whilst also dynamically  
454 altering the magnitude of any CYP3A4-induction process.

455 Furthermore, rifampicin is a known potent CYP3A4 inducer, and therefore has the potential to  
456 lead to AMT treatment failure if the AMT metabolic pathway favours CYP3A4-mediated  
457 transformation.

458 The ultimate goal of this study was to address the potential risk associated with DDIs related  
459 to tuberculosis therapy in children between 2-5 years of age, which accommodate the lowest

460 dosing range (age based) for use of both AMT and rifampicin. Our modelling strategy included  
461 a 4-step approach commencing in prediction AL pharmacokinetics in adult population groups  
462 to develop and optimise compound files (Step 1 and 2) before scaling to paediatrics and  
463 conducting validation with published non-DDI clinical studies (Step 3) before finally making  
464 predictions for potential DDI risks in co-infected malaria-tuberculosis children (Step 4).

465 In adults, successful AL compound development (Step 1) was achieved through comparison to  
466 3 key clinical studies quantifying both artemether and lumefantrine in each study and all  
467 predictions were within 2-fold of the reported  $C_{max}$  and AUC from clinical studies (Table 2).  
468 The large variability in the absorption phase of artemether and lumefantrine (Figure 2) was  
469 evident in the observed clinical data and the slight model over prediction may be a result of  
470 the lower limit of detection for artemether in the studies reported by Lefevre et al [49] [32]  
471 compared to that reported by Bindschedler *et al* 2002[74].

472 Following successful compound development, the ability of each compound file to mechanistic  
473 predict a DDI was then assessed through the use of two inbuilt Simcyp® inhibitors, namely  
474 ketoconazole (CYP3A4 inhibitor) and rifampicin (CYP3A4 inducer) (Step 2). For CYP3A4  
475 inhibition, the model was able to recapitulate the extent of DDIs with reported plasma  
476 concentration within the predicted 5<sup>th</sup>-95<sup>th</sup> percentiles for the simulation for artemether and  
477 lumefantrine (Figure 3 and Table 3).

478 For the induction based interactions of CYP3A4 with AL, very few reports have characterised  
479 rifampicin mediated DDIs and we utilised a study reported AL concentration within the same  
480 subjects [22]. Under these circumstances, the model predicted 12-hour post final dose  
481 concentration (artemether) and day 8 concentration (lumefantrine) was similar (within 2-fold)  
482 to that reported by Lamorde *et al* [22]. Steps 1 and 2 demonstrate the ability of the development  
483 AL model compounds to capitulate pharmacokinetic parameters reported from a range of non-  
484 DDI and DDI studies, confirming successful model development.

485 To consider the potential impact of DDI on AL pharmacokinetic in 2-5 year olds, it was  
486 important to demonstrate the capability of the developed model to predict AL pharmacokinetics  
487 in children. To this end step 3 focussed on validation of artemether and lumefantrine in  
488 children. Artemether model predictions in children were able to capture the difference in  
489 weight based dosing strategies on the outcome pharmacokinetic profiles, both in 'single' point  
490 concentrations centred around the  $C_{max}$  (Figure 5A and B) and population based sampling over  
491 a dosing period (Figure 5C). Lumefantrine model predictions required an optimisation step and

492 following this optimisation procedure, observed time-point data for 30, 54, 66, 84 and 168  
493 hours [8] and model predictions and day 3 and day 7 points [52] were within 2-fold of the  
494 simulated profiles and within the 5<sup>th</sup> and 95<sup>th</sup> percentiles of the mean predicted profiles (Figure  
495 6). Lumefantrine model predictions were finally further validated using median concentration  
496 data at day 3 or day 7 (Table 4), which were found to be well predicted and within 2-fold of  
497 the reported concentrations. The approach described in Step 3 resulted in appropriate model  
498 predictions based on existing published literature detailing either single-time point or multiple-  
499 time point concentration data of AL in children.

500 Having established a working model for AL pharmacokinetics in adults and children, along  
501 with a working model for quantifying AL DDIs in adults, we addressed the major focus of this  
502 study, the prediction of potential AL based DDIs in children between the ages of 2-5 years of  
503 age. As expected the impact of rifampicin on the pharmacokinetics of artemether was  
504 significant, reducing both the final dose  $C_{max}$  for both one and two tablet regimens by  
505 approximately 80 % ( $C_{max}$  ratio: 0.18-0.21) (Table 5) along with an  $AUC_r$  of 0.21-0.22 for both  
506 dosing regimens.

507 To infer a clinical consequence of this is difficult, given the shorter half-life of artemether  
508 compared to lumefantrine. AL is a very efficacious therapy in uncomplicated malaria patients  
509 with the recommend 6-dose treatment show efficacy of 97.6% on day 28 and 96.0% on day 42  
510 [75], however the efficacy of treatment reduces with patients receiving lower doses (an 8%  
511 decrease in patients for every 1 mg/kg decrease in dose received). However, the overall  
512 determinant of artemether–lumefantrine clinical efficacy is the area under the curve of  
513 lumefantrine [6], with day 7 concentration (~280 ng/mL) being the primary marker for  
514 successful therapy under dosing with 3-day dosing regimen.

515 The DDI has a detrimental effect on lumefantrine  $C_{d7}$ , significantly reducing this below the  
516 target concentration for both one and two dose treatment (Figure 8). Although data on such  
517 interactions in paediatric is lacking, Lamorde *et al* [22] have demonstrated a similar effect in  
518 adults with a significant decrease (3-10 fold) in lumefantrine concentrations during TB  
519 treatment [22].

520 Artemether and lumefantrine have been reported to show saturation in the absorption  
521 pharmacokinetics and it would be expected that dose increases would have a limited impact on  
522 resultant pharmacokinetics lumefantrine [33] [76]. Therefore, to overcome the DDI-based  
523 decrease in  $C_{d7}$ , an increase in the dose administered would not be viable for increasing  $C_{d7}$ .

524 We then simulated the impact of a change in dosing frequency would influence the plasma  
525 concentration of AL, and whether an increase in  $C_{d7}$  would be evident.

526 Whilst a 3-day treatment is viable for patient compliance, day 7 concentration in malaria-TB  
527 co-infected children are significantly lower than this target concentration. An increase in  
528 dosing frequency was investigated to assess the impact on the predicted target concentration.  
529 Whilst a 5-day course resulted in some modest increase in the percentage of subjects with a  $C_{d7}$   
530  $> 280\text{ng/mL}$  (~11% increase), this increase was far greater for a 7-day treatment regimen with  
531 ~63-75% of subjects demonstrated  $C_{d7} > 280\text{ng/mL}$  across both dosing bandings (Table 6). A  
532 recent population pharmacokinetic study by Hogleung *et al* (2015) [77] assessed the potential  
533 for DDI with malaria-HIV co-infected adult patients. In prospective simulations they  
534 demonstrated a similar beneficial effect of an increase in dosing frequency to counteract the  
535 induction effect of antiretroviral on malaria (AL) treatment regimens.

536 Whilst the impact of this will require prospective clinical analysis, it is suggested that an  
537 increase in the dosing frequency for children who are co-infected with malaria and TB and  
538 subjected to TB chemotherapy, including rifampicin, may benefit from an increase in treatment  
539 duration to 7 days to full ensure parasite clearance. Our results have demonstrated that children  
540 aged 2-5 years of age are susceptible to significant DDI when being co-treated with TB  
541 chemotherapy, which directly impacts upon the potential for AL therapy failure.

542 Challenges remain however, the impact of non-adherence to designated treatment regimens  
543 would render the impact of the induction effect as variable and unpredictable [78]. However,  
544 given the erratic absorption of lumefantrine (and artemether) [79], the extension of a dosing  
545 regimen from 3 to 5 days would not alter the peak concentrations significantly (Figure 9) and  
546 would be within this erratic absorption range absorption range (Figure 6).

547 Furthermore, it should be noted that simulations were performed in healthy subjects in our  
548 simulations, and therefore we have assumed that any physiological changes associated with  
549 malaria are negligible and does not impact upon the extent of the DDI in our simulation trials.

550 Malaria patients are susceptible to reduced albumin and  $\alpha 1$ -acidic glycoprotein, which can  
551 directly impact upon the extent of plasma protein binding and therefore exposure of AL to  
552 metabolic extraction with reports demonstrating a decrease of  $\geq 30\%$  of serum albumin, ( $\leq 35$   
553 g/L) [80-82]. For highly protein bound drugs, such as lumefantrine, any change subsequent  
554 changes in the extent of protein binding (e.g. reduce binding due to reduced serum protein) will



555 inevitably increase the unbound drug fraction and potentially enhance both drug tissue  
556 distribution along with metabolic clearance.

557 The potential impact of such a change was assessed in 2-5 year olds (1 tablet per dose over the  
558 7 day optimised regimen) (Figure 10) and demonstrated that a modest increase in  $f_{u,plasma}$  from  
559 0.003 to 0.005, results in all subjects possessing a  $C_{d7}$  of just below the target  $< 280$  ng/mL  
560 subjects (when considering the range of simulated values). Furthermore a 10-fold increase in  
561  $f_{u,plasma}$  (0.003 to 0.03) yields  $C_{d7}$  which would be irreconcilable by dosing adjustments.

562 In adults, it has been noted that changes in body weight (malnutrition) and potentially changes  
563 which can impact upon absorption, distribution, metabolism and excretion. Nevertheless, our  
564 dosing range for the age selection (5-15kg and 15-25kg) is broad enough to simulate the impact  
565 on potential underweight children who are within the simulated age range (2-5 years).

566 Interesting, a clinical trial is on-going [83] to assess the impact of an increased treatment  
567 frequency to 5 days for AL, the outcomes of which may support the requirement for an increase  
568 in dosing frequency for patients subjected to induction-based DDIs.

## 569 **5. CONCLUSION**

570 The WHO have highlighted the increased risks of mortality children face with malaria infection  
571 [2, 3] and coupled with the innate complications of co-infection with tuberculosis, children are  
572 at significant risk of potential drug-drug interactions in many areas of sub-Sahara Africa which  
573 may inadvertently impact upon parasite clearance. Whilst clinical studies exploring this risk  
574 of DDI in co-infected paediatric population groups are sparse, mechanistic population-based  
575 PBPK modelling provides a potential approach to assess this risk-potential. The  
576 pharmacokinetics of artemether and lumefantrine has been simulated for two-body weights in  
577 children ages 2-5 years old, who would be a greater risk of mortality associated with both  
578 malaria and tuberculosis. We demonstrated that an extension of the current recommend dosing  
579 range for AL, from 3 to 7 days, would counteract the potential rifampicin-mediated induction  
580 on lumefantrine (and artemether) metabolic clearance and yields a significantly greater  
581 proportion of subjects attaining a target lumefantrine concentration thereby preventing  
582 recrudescence and potential mortality.

583

584

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588

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810 ClinicalTrials.gov.

811

812

813 **List of Figures**

814

815 **Figure 1**

816 **Model development strategy.**

817

818 **Figure 2**

819 **The simulated plasma concentration-time profile of artemether and lumefantrine.**

820 Simulation of (A) artemether and (B and C) lumefantrine plasma concentration-time profile  
821 following a single oral dose of 80mg (artemether) (A), a single oral dose of 480 mg  
822 (lumefantrine) (B) and a six-dose three-day regimen (lumefantrine) (C) [29]. For all  
823 simulations a standard population size of 100 individuals was used. Solid line represents  
824 population mean prediction with dashed lines representing the 5<sup>th</sup> and 95<sup>th</sup> percentiles of  
825 prediction. Mean observed plasma concentrations represented by the solid circles [28] and  
826 diamonds [42].

827

828 **Figure 3**

829 **The simulated plasma concentration-time profile of artemether and lumefantrine in the**  
830 **absence and presence of ketoconazole**

831 (A) Artemether was dosed as a single 80 mg oral dose in the absence and presence of  
832 ketoconazole, dosed as a single 400 mg oral dose over a 24-hour period under fed-conditions.  
833 Open circles represent observed mean data points [13]. (B) Lumefantrine was dosed as a single  
834 480 mg oral dose in the absence and presence of ketoconazole, dosed as a single 400 mg oral  
835 dose over a 24-hour period under fed-conditions. Open circles represent observed mean data  
836 points [13]. Solid line represents population mean prediction with shaded regions representing  
837 the 5<sup>th</sup> and 95<sup>th</sup> percentiles of prediction (grey: no interaction; red: interaction).

838

839 **Figure 4**

840 **The simulated plasma concentration-time profile of artemether and lumefantrine in the**  
841 **absence and presence of rifampicin.**

842 (A) Artemether was dosed as 6 doses (80 mg per dose) over 3 days (on days 8-10) of a 14-day  
843 trial with rifampicin dosed at 10 mg/kg once daily during the duration of the trial. Isoniazid  
844 was also dosed at 10 mg/kg and used as a secondary perpetrator in light of its inclusion in anti-  
845 Tb therapy. Open circle represents observed mean 12-hour post final dose concentration  $\pm$  SD  
846 [22]. Solid line represents population mean prediction with shaded regions representing the 5<sup>th</sup>  
847 and 95<sup>th</sup> percentiles of prediction (grey: no interaction; red: interaction). (B) Lumefantrine was  
848 dosed as 6 doses (480 mg per dose) over 3 days (on days 8-10) of a 14-day trial with rifampicin  
849 dosed at a dose of 10mg/kg once daily and isoniazid (secondary perpetrator) administered at a  
850 dose of 5mg/kg during the duration of the trial. Open circles represent observed mean day 8



851 concentration (7.3 hours after final dose)  $\pm$  SD [22]. Solid line represents population mean  
852 prediction with shaded regions representing the 5<sup>th</sup> and 95<sup>th</sup> percentiles of prediction (grey: no  
853 interaction; red: interaction). Dashed line represents minimum effective parasite clearance  
854 plasma concentration for lumefantrine (280 ng/mL).

855

## 856 **Figure 5**

### 857 **The simulated plasma concentration-time profile of artemether in paediatrics.**

858 Six doses of artemether were administered at 0, 8, 24, 36, 48 and 60 hours based on patient  
859 weight (20 mg: 5-15 kg or 40 mg: 15-25kg). Shaded regions between 1-2 hours indicates  
860 observed sampling times (1-2 hours). (A) Red circle and black square are observed data from  
861 subjects receiving the lower dose and higher doses respectively [45] with red and black solid  
862 lines indicating mean profiles with 5<sup>th</sup> and 95<sup>th</sup> percentiles illustrates by dashed coloured lines.  
863 (B) Circle and triangle symbols are observed data from subjects receiving the lower dose but  
864 stratified for body weight [44] with red solid line indicating mean profile for the lower weight  
865 range and black solid line indicating mean profile for the higher dose range. Dashed lines  
866 indicate 5<sup>th</sup> and 95<sup>th</sup> percentiles. (C) Black line represents simulated lower doses (5-14 kg) and  
867 red line represents simulated highest dose (25-34 kg). Observed data points are represented by  
868 solid red circles [10] with red and black solid lines indicating mean profiles with 5<sup>th</sup> and 95<sup>th</sup>  
869 percentiles illustrated by dashed coloured lines.

870 Shaded regions representing the 5<sup>th</sup> and 95<sup>th</sup> percentiles range of the prediction

## 871 **Figure 6**

### 872 **The simulated plasma concentration-time profile of lumefantrine in children.**

873 (A) Blue, green and black solid lines indicate 1 (5-14.9 kg), 2 (15-24.9 kg) or 3 (25-34.9 kg)  
874 tablet dosing regimens respectively. Upper and lower dashed lines represent the 95<sup>th</sup> percentile  
875 for the 360 mg (3 tablet) dose and 5<sup>th</sup> percentile for the 120 mg (1 tablet) dose, respectively.  
876 Red circles represent mean population observed concentrations reported in Borrmann *et al*  
877 (2010) [8]. (B) Black and green solid lines indicate increasing doses of lumefantrine (1 tablet:  
878 5-14.9 kg); 2 tablets 15-24.9 kg). Upper and lower dashed lines represent the 95<sup>th</sup> percentile  
879 for the 240 mg dose and 5<sup>th</sup> percentile for the 120 mg dose, respectively. Red circles represented  
880 mean population observed concentration reported in reported by Piola *et al* (2005) [46].

881

## 882 **Figure 7**

### 883 **The simulated mean plasma concentration-time profile of artemether in paediatrics in 884 the absence and presence of a DDI.**

885 Artemether plasma concentrations following dosing with 1 tablet (5-14.9kg) or 2 tablets (15-  
886 24.5kg) to children (2-5 years). Solid lines represent clinical trials with artemether alone.  
887 Dashed lines represented artemether dosing with rifampicin (10mg/kg). One tablet doses are  
888 indicated in black and two tablet doses in blue. Isoniazid was also dosed at 10 mg/kg and used  
889 as a secondary perpetrator in light of its inclusion in anti-Tb therapy

890

891 **Figure 8**

892 **The simulated mean plasma concentration-time profile of lumefantrine in paediatrics in**  
893 **the absence and presence of a DDI for a standard 3 day regimen.**

894 Lumefantrine plasma concentrations following dosing with 1 tablet (5-14.9 kg) or 2 tablets (15-  
895 24.5 kg) to children (2-5 years). Solid lines represent clinical trials with lumefantrine alone.  
896 Dashed lines represented lumefantrine dosing with rifampicin (10 mg/kg). Isoniazid was also  
897 dosed at 10 mg/kg and used as a secondary perpetrator in light of its inclusion in anti-Tb  
898 therapy.

899

900 **Figure 9**

901 **The simulated mean plasma concentration-time profile of lumefantrine in paediatrics in**  
902 **the presence of a DDI for an adapted 5 and 7-day regimen.**

903 Lumefantrine plasma concentrations following dosing with (A) 1 tablet (5-14.9 kg) or (B) 2  
904 tablets (15-24.5 kg) to children (2-5 years) in the presence of rifampicin (10 mg/kg) when dosed  
905 for 5 days (upper panels) or 7 days (lower panels). Solid lines represent mean and dashed line  
906 represents upper and lower ranges of predicted concentrations with shaded regions representing  
907 the range of predictions concentrations. Isoniazid was also dosed at 10 mg/kg and used as a  
908 secondary perpetrator in light of its inclusion in anti-Tb therapy

909

910

911 **Figure 10**

912 **The impact of alterations in lumefantrine plasma unbound fraction on simulated  $C_{d7}$  in**  
913 **paediatrics in the presence of a rifampicin-mediated DDI for a 7-day regimen (one**  
914 **table/dose)**

915 Day 7 lumefantrine plasma concentrations ( $C_{d7}$ ) were simulated for 56 subjects within a weight  
916 range of 5-15 kg (1 tablet/dose) in the presence of rifampicin (10mg/kg) following a treatment  
917 regimen described in section 3.5.2. Solid line represents 280 ng/mL lumefantrine ‘target’  
918 concentration. Dashed lines represented simulated range (upper and lower) and  $C_{d7}$  target  
919 concentration when  $f_{u,plasma} = 0.003$ . Dotted lines represented simulated range (upper and  
920 lower) concentrations when  $f_{u,plasma} = 0.005$ . Isoniazid was also dosed at 10 mg/kg and used  
921 as a secondary perpetrator in light of its inclusion in anti-Tb therapy