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The impact of CYP2B6 polymorphisms on the interactions of efavirenz with lumefantrine: implications for paediatric antimalarial therapy

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ABSTRACT

Lumefantrine is a widely used antimalarial in children in sub-Saharan Africa and is predominantly metabolised by CYP3A4. The concomitant use of lumefantrine with the antiretroviral efavirenz, which is metabolised by CYP2B6 and is an inducer of CYP3A4, increases the risk of lumefantrine failure and can result in an increased recrudescence rate in HIV-infected children. This is further confounded by CYP2B6 being highly polymorphic resulting in a 2-3 fold higher efavirenz plasma concentration in polymorphic subjects, which enhances the potential for an efavirenz-lumefantrine drug-drug interaction (DDI). This study developed a population-based PBPK model capable of predicting the impact of efavirenz-mediated DDIs on lumefantrine pharmacokinetics in African paediatric population groups, which also considered the polymorphic nature of CYP2B6. The validated model demonstrated a significant difference in lumefantrine target day 7 concentrations (C_{d7}) in the presence and absence of efavirenz and confirmed the capability of efavirenz to initiate this DDI. This was more apparent in the *6/*6 compared to *1/*1 population group and resulted in a significantly lower ($P < 0.001$) lumefantrine C_{d7} . A prospective change in dosing schedule from 3-days to 7-days resulted in a greater number of *6/*6 subjects (28-57%) attaining the target C_{d7} across age bands (0.25-13 years), with the greatest increase evident in the 1-4 year old group (3-day: 1%; 7-day: 28%).

KEYWORDS

Physiologically-based pharmacokinetics; malaria; HIV; paediatrics; Africa.

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1. INTRODUCTION

Malaria represents a considerable healthcare burden, with the World Health Organisation (WHO) attributing an estimated 212 million malaria cases and 429 000 malaria-related deaths in 2015. Out of those malaria cases and deaths, 92% are from African regions and predominantly occur in children aged under 5 years (World Health Organisation, 2016).

Lumefantrine, often combined with artemether, is one of the most widely used antimalarials in sub-Saharan Africa, and many countries adopted it as first line therapy for uncomplicated *falciparum malaria*, including children with HIV co-infection (Fleteau et al., 2011). Typical treatment regimens for lumefantrine in children include a 3 day six-dose regimen which is stratified based on body weight: 5-15 kg 1 tablet per dose; 15-25 kg 2 tablets per dose; 25-35 kg 3 tablets per dose and >35 kg 4 tablets per dose, with each tablet providing 120 mg lumefantrine (World Health Organisation, 2016a).

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and is predominantly metabolised by CYP2B6 (Ogburn et al., 2010) and is an inducer of CYP3A4 (Hariparsad et al., 2004; Shou et al., 2008). It is a common first-line treatment in paediatrics or pregnancy population groups (2013) (Dybul et al., 2002). Lumefantrine is predominantly metabolised by CYP3A4 and, although lumefantrine therapy has a wide therapeutic window (Bharti et al., 2016), patients who are exposed to CYP3A4 inducers, such as efavirenz, may demonstrate reduced lumefantrine exposure which can lead to increased recrudescence rates and therapeutic failure (Maganda et al., 2016a). However, this process is further confounded by the fact that CYP2B6 is highly polymorphic with at least 37 distinct star-alleles (Zanger and Klein, 2013) with *1/*1 carriers considered as wild-type. The most common variant alleles result in two amino acid changes, Q172H and K262R, and is termed *CYP2B6**6, and which has been reported to lead to a 65 % reduction in protein expression and 50 % reduction in mean enzyme activity in the homozygous state (Lang et al., 2001).

Although CYP2B6 contributes towards between 2-10% of total CYP content (Wang and Tompkins, 2008), the impact of the *6/*6 genotype can often result in a 2- or 3-fold higher efavirenz plasma concentration (Haas et al., 2004; Rodriguez-Novoa et al., 2005; Rotger et al., 2005; Tsuchiya et al., 2004). A consequence of this alteration in efavirenz plasma concentration would be a greater ability of efavirenz to induce CYP3A4 (Habtewold et al., 2013; Hariparsad et al., 2004; Mouly et al., 2002) and thereby enhance the potential for an efavirenz-lumefantrine DDI.

Importantly, the *6/*6 polymorphism is more frequent in African population groups than Caucasian population groups (King and Aberg, 2008; Klein et al., 2005), and this places a considerable risk-burden on this geographic population group. However, the impact of CYP2B6 polymorphisms in antiretroviral-antimalarial mediated DDIs in African paediatric populations is lacking, and warrants investigation as it may contribute to significantly increased the risk of recrudescence especially in highly endemic regions and potential resistance towards these antimalarial treatments (Achan et al., 2012; Barnes et al., 2008; Khoo et al., 2005; Organization, 2016). This is further confounded by the risk of placental transfer of HIV (Drake et al., 2014) and/or malaria (World Health Organisation, 2016b), and the lack of naturally acquired immunity towards children, often puts paediatric population groups at significant risk of succumbing to either infection or being exposed to complex DDIs (Doolan et al., 2009).

Due to the complexity and ethical issues of recruitment of paediatrics into complex DDI studies in HIV-infected malaria subjects, population-based physiologically-based pharmacokinetic (PBPK) modelling can be used to explore the potential risk of DDIs in adults (Feng and Varma, 2016; Johansson et al., 2016; Olafuyi et al., 2017a) and paediatric populations (Johnson et al., 2014; Olafuyi et al., 2017b; Salem et al., 2013a; Salem et al., 2013b). The benefit of this approach is both the ability to model population variability in physiology (Jamei et al., 2009a; Jamei et al., 2009b; Jamei et al., 2009c; Olafuyi et al., 2017a, b), but to also specifically develop a modelling approach that is tailored towards a specific geographical population group of interest rather than a standard healthy (Caucasian) adult male.

The objectives of the present study were 2-fold: (i) to predict efavirenz pharmacokinetics in African population groups (adults and paediatrics) and (ii) to assess the impact of efavirenz in the attenuation of lumefantrine pharmacokinetics through a CYP3A4 induction effect. In all cases, it was important also to address the impact of the *6/*6 CYP2B6 phenotype on efavirenz pharmacokinetic and the effect of this to alter lumefantrine pharmacokinetics following a DDI.

2. METHODS

Population based PBPK modelling was conducted using the virtual clinical trials simulator Simcyp (Simcyp Ltd., a Certara company, Sheffield, UK, Version 16). For all simulations, doses for both lumefantrine and efavirenz were employed according to the standard weight-based dose regimen (See supplementary materials table S1), unless stated otherwise. Further, for all lumefantrine simulations, dosing occurred under fed-conditions unless otherwise indicated.

2.1 Model development

A five-stage stepwise approach was implemented for model development, validation and model refinement (Figure 1) which is fully described below. Unless otherwise stated, efavirenz was dosed for 20 days prior to initiation of a DDI (and throughout the study). Lumefantrine was dosed at over 3 days at 0, 8, 24, 36, 48 and 60 hours.

2.1.1 Step 1: Adult simulations with efavirenz

The Simcyp library compound efavirenz was selected, having already been developed and pre-validated by Simcyp (Ke et al., 2016). The metabolism of efavirenz was modelled using the application of allele-specific intrinsic clearance (CL_{int}) for *1/*1 and *6/*6 genotypes, as described by Xu et al (Xu et al., 2013). Subsequently, when simulating either entirely *1/*1 (EM) or *6/*6 (PM) genotypes, the frequency of CYP2B6 genotype was set at 1 for either *1/*1 or *6/*6. For efavirenz, unless otherwise stated, doses were administered to steady state or beyond (at least 20 days) prior to the initiation of lumefantrine dosing.

Step 1 attempted to apply the compound file to model predictions in Healthy Volunteer (Caucasian), South African and Ugandan population groups, which were generally the focus of clinical studies identified.

Clinical studies selected included: (i) A single 600 mg oral dose to healthy adult volunteers with results genotyped for *1/*1 and *6/*6 (Xu et al., 2013) and (ii) a 600 mg once daily multi-dose study over 32 weeks in Ugandan adults (Mukonzo et al., 2014).

The Ugandan population group was developed from reported age-weight relationships for Ugandan males and females (Hayes et al., 2015), and are detailed in the supplementary materials. A similar approach was reported and applied in PBPK modelling by our group (Olafuyi et al., 2017a). In the absence of literature reported abundance of CYP2B6 in Ugandan subjects, we fixed *1/*1 and *6/*6 genotype abundances to 6.9 and 2.4 pmol/mg protein, respectively, based upon adaptations found in a South African population group developed by Simcyp as part of the Critical Path to TB Drug Regimens (CPTR) (Critical Path to TB Drug Regimens, 2016) and which is available from population library repository of Simcyp. The South African population group includes appropriate age-weight-height distributions, CYP expression and blood biochemistry changes compared to standard (Caucasian) Healthy Volunteer population group (Gardner, 2016). All simulations replicated the study design reported by the validation clinical studies cited above.

2.1.2 Step 2: Adult simulations with lumefantrine-efavirenz drug-drug interactions

The validation of the lumefantrine-efavirenz DDI was conducted using two published clinical studies: (i) dosing of a single oral dose of 480 mg lumefantrine and 600 mg efavirenz to a Healthy Volunteer population group (Huang et al., 2012) where no genotyping was reported and (ii) dosing of 480 mg lumefantrine (6 doses, twice daily for 3 days) and 600 mg efavirenz (20 days prior to lumefantrine and then throughout the study duration) to a Tanzanian population group where genotyping of plasma concentration profiles were reported (Maganda et al., 2016b). As a result of similar age-weight relationships (Hayes et al., 2015), the South African population group was used as a surrogate for a Tanzanian population.

2.1.3 Step 3: Paediatric simulations with efavirenz

After successful validation and refinement of efavirenz compound in the adult population, this step focussed on the validation of efavirenz in paediatrics.

The studies used for validation of efavirenz pharmacokinetics in paediatrics included: (i) weight-based once daily 300 mg dose of oral efavirenz in HIV-infected Ugandan children and simulated using the Ugandan population group with dosing to steady state (Fillekes et al., 2011) (ii) weight-based once daily dose of oral efavirenz in HIV-infected children with weight-based stratification of plasma concentration profiles (Luo et al., 2016) simulated using the Ugandan population group; (iii) a single high dose (25 mg/kg) dosed once daily to 2-3 year old Ugandan children and simulated using the Ugandan population group (Pressiat et al., 2017) and (iv) a single oral 300 mg dose administered to 6-7 year old South African subjects and simulated using the South African population group (Viljoen et al., 2012) with genotyping of plasma concentration profiles.

2.1.4 Step 4: Paediatric simulations with lumefantrine-efavirenz drug-drug interactions

To validate the prediction of a lumefantrine-efavirenz based DDIs in paediatrics, we utilised the only study reporting the lumefantrine plasma concentration-time profile in the absence and presence of efavirenz (Parikh et al., 2016), although this study did not report genotyped pharmacokinetics. Trial simulations were performed using a standard 6-dose regimen of weight-based dosing of lumefantrine (see supplementary materials table S1) administered on day 20 (unless otherwise indicated), with weight-based dosing of efavirenz from day 1 to day 40 in Ugandan children and simulated using the Uganda population group. To account for the

impact of genotype on the pharmacokinetics of lumefantrine, our results were stratified for two extreme cases of an entire population of CYP2B6 *1/*1 or entirely CYP2B6 *6/*6, and this represents the ‘best’ and ‘worst’ clinical scenarios.

2.1.5 Step 5: Paediatric dose evaluation prediction

Having validated the lumefantrine (LUM) and efavirenz (EFV) DDIs in Ugandan paediatric patients, this step simulated the potential impact of dosage regimen alterations on target day-7 (C_{d7}) lumefantrine plasma concentrations. Simulations were run using the Ugandan population group and stratified across 4 age groups with weight-based dosing (see supplementary materials): 0.25-1 year-old (120mg LUM/300mg EFV), 1-4 year-old (120mg LUM/400mg EFV), 4-8 year-old (240mg LUM/500mg EFV) and 8-13 year-old (240mg LUM/600mg EFV). Further, each simulation included 100 subjects (10 trials with 10 subjects per trial) where the age-weight distribution matched the appropriate dose banding.

2.2 Predictive performance

In all simulations, a prediction to within 2-fold of the observed data was generally accepted as part of the ‘optimal’ predictive performances range despite there being no uniform standard of acceptance to determine this criterion (Edginton et al., 2006; Ginsberg et al., 2004; Parrott et al., 2011). This acceptance criterion was used in our C_{max} and AUC comparisons with the published clinical data reported. For the efavirenz DDI simulations, since the therapeutic efficacy of lumefantrine is determined by its C_{d7} of 280 ng/mL (Ezzet et al., 2000), a direct analysis of lumefantrine day-7 concentration was set as a cut-off value to determine the impact of a DDI in lumefantrine pharmacokinetics.

2.3 Data analysis

The observed data that was used for visual predictive checks when compared with the simulated profiles were extracted using the WebPlotDigitizer v.3.10 (<http://arohatgi.info/WebPlotDigitizer/>). All simulations of plasma concentration-time profiles were presented in 5th to 95th percentiles and either in mean or median unless otherwise specified.

For all adult simulations, age ranges and subject gender ratios were matched, where possible, to reported clinical studies. Where this information was not cited in clinical studies, a default age range of 20-50 years and gender ratio of 50% was selected.

For simulations employing weight-based dosing, unless otherwise stated, a 100-subject simulation was run in a 10x10 trial (10 subjects per trial with 10 trials) to ensure that reasonable inter-/intra individual variability is captured within the model simulations. However, as simulations are not possible with defined age and weight ranges, pooling and post-processing of output data were conducted to match individuals to the required age-weight boundary conditions for the study.

3. RESULTS

3.1 Step 1: Adult simulations with efavirenz

In order to predict the impact of efavirenz-mediated DDIs on lumefantrine pharmacokinetics, the capability of the model to predict efavirenz pharmacokinetics alone within a Healthy Volunteer population was first assessed. Using the efavirenz compound file within the Simcyp library and the Simcyp ‘Healthy Volunteer’ population group, the predicted population plasma concentration time profile for a single 600 mg oral dose of efavirenz were within the range of observed reported values for both *1/*1 (Supplementary materials Figure S1A) and *6/*6 population groups (Supplementary materials Figure S1B)

Furthermore, the model predicted t_{max} , C_{max} and AUC were within 2-fold of the reported parameters for each genotype (Table 1).

Table 1: Summary of predicted and observed pharmacokinetic parameters of efavirenz in adults with CYP2B6*1/*1 and CYP2B6*6/*6 genotypes in a Healthy Volunteer population

	Genotype	Pharmacokinetic parameters		
		t_{max} (h)	C_{max} (ng/ml)	AUC _{0-inf} (ng/mL.h)
Observed	*1/*1	2.3 ± 1.0	2300 ± 700	79800 ± 28400
Predicted	*1/*1	1.5 ± 0.02	2643 ± 53	59126 ± 1520
Observed	*6/*6	2.7 ± 1.5	2400 ± 200	101700 ± 7900
Predicted	*6/*6	1.5 ± 0.02	2727 ± 136	93857 ± 4693

Data represents mean ± SD. Simulations: n=20 with 50 % female and age range of 20-50 years.

Subsequently, to further validate model simulations, the ability to predict efavirenz plasma concentrations following multi-dosing was assessed in each genotype patient group using a Ugandan population group. In both *1/*1 (Supplementary materials Figure S2A) and *6/*6 (Supplementary materials Figure S2B) populations, the predicted concentrations were all within 2-fold of reported concentrations (Mukonzo et al., 2014) (Table 2) (Supplementary materials Figure S2B)

Table 2: Summary of predicted and observed efavirenz plasma concentrations in Ugandan adults with CYP2B6*1/*1 and CYP2B6*6/*6 genotypes.

Sampling day		*1/*1	*6/*6
		Concentration (ng/mL)	
Day 14	Observed	1752 ± 197	2462 ± 130
	Predicted	1597 ± 80	3921 ± 196
Day 56	Observed	1466 ± 134	4600 ± 262
	Predicted	1594 ± 80	4074 ± 204
Day 84	Observed	1329 ± 166	3991 ± 194
	Predicted	1284 ± 64	3989 ± 199
Day 112	Observed	1446 ± 283	3741 ± 225
	Predicted	1140 ± 57	4170 ± 209
Day 140	Observed	1420 ± 221	5404 ± 319
	Predicted	1595 ± 80	5698 ± 285
Day 168	Observed	1292 ± 278	5621 ± 284
	Predicted	1284 ± 64	5219 ± 261
Day 224	Observed	1353 ± 198	6861 ± 382
	Predicted	1595 ± 80	5698 ± 285

Data represents mean ± SD. Simulations: n=100 with 38.5 % female and age-range of 20-40 years.

3.2 Step 2: Adult simulations with lumefantrine-efavirenz drug-drug interactions

To further validate the proposed model, DDIs were simulated between lumefantrine and efavirenz using the Simcyp ‘Healthy Volunteer’ population group. The impact of the predicted DDI on lumefantrine pharmacokinetics was within the range of the observed data reported in the absence and presence of efavirenz (Huang et al., 2012) (Supplementary materials Figure S3), with C_{max} , t_{max} and AUC predictions within 2-fold of those reported (Huang et al., 2012) (Table 3). Furthermore, the predicted day 7 lumefantrine concentration (C_{d7}) in the presence

of efavirenz, 679 ± 361 ng/mL, was within 2-fold of that observed (544 ± 432 ng/mL) (Table 3).

Table 3: Summary of predicted and observed PK parameters of lumefantrine in the absence and presence of efavirenz in healthy adults.

		C_{max} ($\mu\text{g/mL}$)	AUC_{last} ($\mu\text{g/mL}\cdot\text{h}$)	t_{max} (h)	C_{d7} (ng/mL)
- EFV	Predicted	12.39 (8.12-19.2)	276 (320-711)	3.6 (2-5.9)	1162 ± 557
	Observed	11.6 (9.5-17.4)	418 (339-693)	2.0 (2-6)	1020 ± 478
+ EFV	Predicted	12.42 (7.14-18.7)	$236 \pm (201-617)$	4.2 (0.4-5.7)	679 ± 361
	Observed	12.1 (10.6-16.4)	$331 \pm (270-503)$	6.0 (0.5-6)	554 ± 432

Data represents geometric mean \pm 90 % CI or mean \pm SD (for C_{d7}). Simulations: n=12 with 16.6 % female and age-range of 24-53 years. EFV: efavirenz.

The model was then extended to assess its application within an African population group. A recent study reported lumefantrine C_{d7} in a Tanzanian population group for *1/*1 and *6/*6 population groups (Maganda et al., 2016b; Maganda et al., 2015). *In lieu* of the development of a Tanzanian population group, a recently developed Simcyp ‘South-African’ population group was used as a surrogate for a Tanzanian population group to predict lumefantrine C_{d7} in *1/*1 and *6/*6 population groups (Figure 2). In this simulation, we assumed some similarity between the population groups in terms of body weight demographics (Hayes et al., 2015).

Predictions of median lumefantrine C_{d7} in the absence of efavirenz, for both *1/*1 and *6/*6, were well predicted and within 2-fold of that reported (Maganda et al., 2016b) (Table 4). In the presence of efavirenz, the *1/*1 and *6/*6 predicted median C_{d7} were within 2-fold of that reported. In the absence of efavirenz, CYP2B6 genotypes had no significant impact on any pharmacokinetic parameters. However, in the presence of efavirenz, the C_{max} and C_{d7} for the *6/*6 population were significantly reduced ($P < 0.01$), 17500 to 9010 ng/mL and 901 to 201 ng/mL, when compared to the *1/*1 population group (Table 4).

Table 4: Summary of the simulated lumefantrine pharmacokinetics parameters in the absence and presence of efavirenz in South African adults with CYP2B6*1/*1 and CYP2B6*6/*6 genotypes.

Parameters	Lumefantrine alone		Lumefantrine plus Efavirenz	
	*1/*1 Median (Range)	*6/*6 Median (Range)	*1/*1 Median (Range)	*6/*6 Median (Range)
C _{max} (ng/mL)	17500 (8350-28500)	17500 (8350-28500)	12100 (5550-21500)	9010 (4200-16800)
AUC _{0-inf} (ng/mL.d)	60002 (20195-138896)	60002 (20195-138896)	38243 (12171-92638)	25578 (7685-67347)
t _{max} (h)	22.59 (22.54-22.64)	22.59 (22.54-22.64)	22.59 (22.10-22.60)	22.59 (22.05-22.64)
Predicted C _{d7} (ng/mL)	901 (13-4620)	901 (13-4620)	382 (1-1650)	201 (1-1280)
Observed C _{d7} (ng/mL)	1000 (686-1929)	893 (562-1732)	299 (253-384)	226 (173-278)

Data represents median (range). Simulations: n=59 with 52.3 % female and age-range of 21-65 years.

3.3 Step 3: Paediatric simulations with efavirenz

Having established the ability of the proposed model to predict adult lumefantine-efavirenz DDIs in genotyped African population groups, the model was expanded to assess the impact of such interactions in paediatric population groups.

Simulations were performed in a custom developed Ugandan paediatric population group (see supplementary materials) with validation of efavirenz pharmacokinetics based on WHO weight based dosing recommendation for children and compared to a report of efavirenz dosing in Ugandan paediatric malaria patients (Fillekes et al., 2011) where 41 children were dosed at 300 mg once daily. Based on this dosing approach, predicted efavirenz concentration profiles (Supplementary materials Figure S4) were within the range reported (Fillekes et al., 2011) with the predicted C_{max} for the population group, 3.59 mg/L (1.65-9.52 mg/L), within 2-fold of that reported 3.62 mg/L (2.86-4.38 mg/L) (Table 5).

Table 5: Summary of predicted and observed efavirenz pharmacokinetics parameters in HIV-infected Ugandan children.

	C_{max} (mg/L)	AUC _{0-last} (mg/L.h)	t_{max} (h)
Predicted	3.59 (1.65-9.52)	33.52 (11.24-94.43)	2.4 (2.3-2.6)
Observed	3.62 (2.86-4.38)	54.52 (44.53-64.51)	4.0 (3.2-4.8)

Data represents mean (range). Simulations: n=41 with 58.5 % female and age-range of 3-12 years.

To further validate the ability of the model to predict efavirenz plasma concentrations in paediatric population groups, predictions were stratified over weight ranges, where efavirenz was dosed based on body weight using the Ugandan population group, and compared with published population-based data (Luo et al., 2016) for matching weight ranges (Figure 3). In all weight groups, the predicted mean plasma concentration profiles were generally well predicted, and the 5th and 95th prediction percentiles spanned the range of observed population data points for each weight banding.

Further, following a high dose of efavirenz (25mg/kg), dosed once daily to 2-3 year old Ugandan children, simulations of the mean efavirenz plasma concentration profile (Figure 4)

were in good agreement with data published (Pressiat et al., 2017), with prediction of C_{12h} and AUC_{0-24} within 2-fold of that reported (Supplementary materials Table S3).

To further validate the ability of the model to predict efavirenz concentrations in CYP2B6 genotyped paediatric subjects, simulations were performed in a South African paediatric population group where efavirenz was dosed at 300 mg to 6-7 years old (Figure 5), and where genotyped plasma concentration had been previously published in a South African paediatric population group (Viljoen et al., 2012) for 2 hours around the C_{12h} . Model predictions for both *1/*1 and *6/*6 were generally in good agreement with that published, with a slight under prediction for *1/*1 subjects. Further, *6/*6 demonstrated a significantly higher C_{max} , 8.27 ng/mL (3.18-17.44 ng/mL), when compared to *1/*1, 4.26 ng/mL (1.45-7.98 ng/mL) ($P < 0.001$) (Supplementary materials Table S4).

3.4 Step 4: Paediatric simulations with lumefantrine-efavirenz drug-drug interactions

Having demonstrated the ability to predict efavirenz concentrations in paediatric subjects, we next attempted to predict lumefantrine-efavirenz based DDIs in African population groups. We utilised the Ugandan paediatric population group and reported pharmacokinetics in a Ugandan population group (Parikh et al., 2016), to predict lumefantrine concentrations in the presence and absence of efavirenz (Figure 6). This clinical study used for validation (Parikh et al., 2016) applied weight-based dosing across a wide age range (3.1-8.6 years) with dosing that spanned different weight bands, but did not present plasma concentration profiles genotyped for CYP2B6. Results presented in Figure 6 are therefore stratified for two extreme cases of an entire population of CYP2B6 *1/*1 or entirely CYP2B6 *6/*6, and this represent the 'best' and 'worst' clinical scenarios.

When considering the two extreme scenarios, model predictions of the final dose C_{max} were within 2-fold of that reported (Parikh et al., 2016), and spanned a range of total values (2060-11083 ng/mL) that were similar to the population range reported (2611-6673 ng/mL) (Table 6). In the absence of efavirenz, the predicted *1/*1 and *6/*6 lumefantrine profiles are largely overlapping with no significant differences in the last C_{max} between genotypes (Table 6). Further, model prediction of C_{d7} , (Figure 6), were within 2-fold of that reported (Parikh et al., 2016) (Table 6) in the absence of efavirenz.

In the presence of efavirenz, the median last-dose C_{\max} was significantly lower ($P < 0.01$) in the *6/*6 group (for both weight bands) (5-15 kg: 1532 ng/mL; 15-25kg: 1979 ng/ml) compared to the *1/*1 group (5-15 kg: 2494 ng/mL; 15-25kg: 2994 ng/ml) (Table 6). Similarly C_{d7} was significantly lower ($P < 0.01$) in the *6/*6 group (5-15 kg: 20 ng/mL; 15-25kg: 51 ng/ml) compared to the *1/*1 group (5-15 kg: 120 ng/mL; 15-25kg: 221 ng/ml) (Table 6).

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Table 6: Summary of simulated lumefantrine pharmacokinetic parameters in the absence and presence of efavirenz in children with CYP2B6 *1/*1 and CYP2B6 *6/*6 genotypes.

			Weight Band	C _{max} (ng/mL)	AUC _{0-inf} (ng/mL.d)	t _{max} (h)	C _{d7} (ng/mL)
Lumefantrine	<i>CYP2B6</i> *1/*1	Predicted	5-15 kg	3649 (2093-5857)	15669 (6194-40144)	22.5 (14.1-30.9)	555 (58-2027)
			15-25 kg	5007 (2060-10055)	19855 (5906-60356)	22.5 (14.1-30.9)	675 (27-3115)
	<i>CYP2B6</i> *6/*6	Predicted	5-15 kg	3649 (2093-5857)	15669 (6194-40144)	22.5 (14.1-30.9)	555 (58-2027)
			15-25 kg	5215 (2151-11083)	21083 (6056-64479)	22.5 (14.1-30.9)	672 (22-3105)
	<i>Non-genotyped</i>	Observed	-	4642 (2611-6673)	10547 (5822-15271)	23.3 (19.2-27.1)	340 (199-481)
	Lumefantrine + Efavirenz	<i>CYP2B6</i> *1/*1	Predicted	5-15 kg	2494 (702-5193)	7617 (1299-18962)	22.5 (14.1-30.9)
15-25 kg				2994 (893-5933)	10005 (1949-25938)	22.5 (14.1-30.9)	221 (1-1049)
<i>CYP2B6</i> *6/*6		Predicted	5-15 kg	1532 (383-3420)	3691 (596-9807)	22 (14-30)	20 (1-81)
			15-25 kg	1979 (505-4582)	5146 (912-13743)	22.5 (14.1-30.3)	51 (1-269)
<i>Non-genotyped</i>		Observed	-	3795 (2543-5047)	6053 (3840-8267)	23.3 (19.2-27.1)	111 (65-157)

Data represents median (range). Simulations: n=31 with 50 % female and age-range of 3.1-8.6 years.

3.5 Step 5: Paediatric dose evaluation prediction

Given the variability in CYP2B6 polymorphisms across different population groups, it was essential to assess the risks associated with antiretroviral agents, such as efavirenz, attenuating CYP-mediated drug metabolism of antimalarials, where variability in efavirenz plasma concentrations, as a result of poor metabolism, may alter antimalarial concentrations in a highly polymorphic CYP2B6 population groups. Confounding this is CYP2B6 ontogeny, where expression is known to be low at < 1 years (10-30% of adult mRNA/protein/activity) and stabilising at approximately 18 years of age (Croom et al., 2009; Pearce et al., 2015). Simulations were therefore conducted to predict the impact of efavirenz on lumefantrine plasma concentrations for subjects aged 0.25-13 years of age where weight-based dosing was used, with efavirenz dosed for at least 20 days prior to the initiation of lumefantrine to establish the stable induction of CYP3A4.

In the absence of an increase in the treatment duration, significant difference in the percentage of subjects possessing a C_{d7} above the 280 ng/mL threshold were apparent ($P < 0.001$) between $*1/*1$ and $*6/*6$ alleles for all dosing bands, with 1-11% of $*6/*6$ subjects attaining this threshold (see supplementary materials Table S5). With an increase in treatment duration of 5- or 7-days (Figure 7), an increase in the percentage of subjects attaining the target C_{d7} was evident (see supplementary materials Table S5) and most noticeable for the $*6/*6$ population group for the 7-day regimen where 28-57% of subjects attained the target concentrations across all dosing bands (Table 7).

Further, extension of the dosing interval from 5-days to 7-days did not alter the half-life within the same genotyped subjects ($*1/*1$ or $*6/*6$). However a significant decrease in the half-life ($P < 0.001$) was noted when comparing the same dosing regimen extension but across the different genotypes. Additionally, when comparing the half-life across increasing dosing-bands, the half-life increased but this was not significant ($P > 0.05$) (Table 7).

Table 7: Summary of predicted mean day 7 lumefantrine concentrations during a 5 and 7-day treatment schedule in children stratified for CYP2B6*1/*1 and CYP2B6*6/*6 genotypes (n=100).

Age-band (years)	Weight-band (kg)	Dosing		Mean C _{d7} (Range) (ng/mL)				Mean half-life (t _{1/2}) (SD) (h)			
		Lumefantrine (mg)	Efavirenz (mg)	CYP2B6*1/*1		CYP2B6*6/*6		CYP2B6*1/*1		CYP2B6*6/*6	
				5-day	7-day	5-day	7-day	5-day	7-day	5-day	7-day
0.25 - 1	5 - 6.9	120	300	1100 (1-5585)	2800 (45-10345)	306 (1-1497)	1030 (11-4308)	27.18 (8.36)	27.18 (8.36)	19.12 (6.98)	19.12 (6.98)
1 - 4	7 - 13.9	120	400	325 (1-1515)	1013 (17-3327)	65 (1-259)	305 (3-1072)	28.12 (10.68)	28.12 (10.68)	21.03 (7.91)	21.03 (7.91)
4 - 8	14 - 16.9	240	500	496 (1-1735)	1456 (45-4374)	100 (1-380)	438 (7-1568)	29.12 (11.11)	29.12 (11.11)	22.11 (8.51)	22.11 (8.51)
8 - 13	17 - 24.9	240	600	397 (3-1843)	1053 (56-3456)	100 (1-390)	359 (9-1315)	30.62 (12.28)	30.62 (12.28)	24.93 (9.78)	24.93 (9.78)

Half-life was calculated from the final dose. SD: standard deviation.

4. DISCUSSION

In 2013 it was estimated that 35 million people were living with HIV worldwide with sub-Saharan Africa accounting for 71% of the total global burden, predominantly centred around Southern and Eastern African countries (South Africa > Nigeria > Mozambique > Uganda > Tanzania > Zambia > Zimbabwe > Kenya > Malawi > Ethiopia) ((UNAIDS). 2014). Further at least 1 million pregnancies are complicated by the co-infection of malaria and HIV, resulting in a paediatric population group which may be subjected to complex pharmacotherapy (Gonzalez et al., 2012).

Although HIV infection can directly impact upon malaria through changes in parasitaemia (Omoti et al., 2013), as well as the severity of the disease and mortality rates during pregnancy (Desai et al., 2007), the use of weight-based dosing strategies and fixed-dosed combination system for both antiretroviral therapies and antimalarial therapies can hinder mitigation of DDIs.

A recent systematic review (Seden et al., 2017), assessed the literature reported DDIs between subjects co-administered with HIV and malaria pharmacotherapy. They identified efavirenz as being an important mediator of DDIs, particularly when co-administered with artemisinins (and lumefantrine), leading to reduced antimalarial plasma concentrations and potential recrudescence.

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI), being predominantly metabolised by CYP2B6 and an inducer of CYP3A4. These interactions with CYP isozymes can complicate both antiretroviral therapy and antimalarial therapy. Further confounding this, is the fact that CYP2B6 is highly polymorphic, with the most common variant alleles resulting in two amino acid changes, Q172H and K262R, and referred to as the *6/*6 polymorphism with wild-types as *1/*1. The *6/*6 polymorphism has been reported to lead to a 65 % reduction in protein expression and 50 % reduction in mean enzyme activity in the homozygous state (Lang et al., 2001). Further, although CYP2B6 contributes towards between 2-10 % of total CYP content (Wang and Tompkins, 2008), the *6/*6 genotype can result in a 2- or 3-fold higher efavirenz plasma concentrations (Haas et al., 2004; Rodriguez-Novoa et al., 2005; Rotger et al., 2005; Tsuchiya et al., 2004), with this genotype being more frequent in African population groups than Caucasian population groups (King and Aberg, 2008; Klein et al., 2005). A consequence of this alteration in efavirenz plasma concentration would be a greater

ability of efavirenz to induce CYP3A4 (Habtewold et al., 2013; Hariparsad et al., 2004; Mouly et al., 2002).

Pharmacokinetics assessment of drug therapy in paediatric population groups is often neglected due to ethical complications and the sparse plasma sample collections inherent in such population groups. However, PBPK represents a novel modelling strategy that has gained regulatory acceptance (Wagner et al., 2015) in its applications in the paediatric population and has been used previously to model malaria pharmacokinetic in special population groups such as paediatrics (Olafuyi et al., 2017b) and pregnancy populations (Olafuyi et al., 2017a).

Recently, we demonstrated the ability of rifampicin to alter antimalarial concentrations through a CYP3A4-induction process, and thereby reducing C_{d7} (Olafuyi et al., 2017b). In this present study, we addressed the impact of a similar induction process with the confounding complexity of potential CYP2B6 polymorphisms on the pharmacokinetics of lumefantrine in paediatric population groups. In this manuscript, we adopted a 5-stage modelling approach which spanned efavirenz and efavirenz-lumefantrine DDI model predictions in adults in African and Healthy Volunteer population groups (Steps 1 and 2), followed by efavirenz and efavirenz-lumefantrine DDI model predictions in African and Healthy Volunteers paediatric populations (Steps 3 and 4). This culminated in predictions of potential DDI risks in CYP2B6 genotyped paediatric population groups with an assessment of the impact of a revised dosing adjustments on C_{d7} (Step 5).

Although efavirenz is a compound that has been previously developed and validated by researchers associated with Simcyp (Ke et al., 2016), Step 1 attempted to predict efavirenz concentration profiles in a African population groups with altered CYP2B6 abundances (6.9 and 2.4 pmol/mg for *1/*1 and *6/*6 genotypes), in contrast to those of the default values set by Simcyp within the Healthy Volunteer population group (17 pmol/mg and 6 pmol/mg). This step integrated revised CYP2B6 abundances for EM and PM phenotypes, based on the incorporation of these abundances into a Simcyp developed South African population group, and *in-lieu* of any further published Ugandan CYP2B6 abundances (Critical Path to TB Drug Regimens, 2016; Gardner, 2016).

In adults, successful predictions of efavirenz concentrations were validated against 2 clinical in both single (Table 1) and multiple dosing (Table 2) regimens in Ugandan population groups, with model predictions within 2-fold of the reported C_{max} , AUC or single day point concentrations from clinical studies for each genotype. Following validation of genotype-

specific efavirenz pharmacokinetics, we next attempted to simulate the proposed lumefantrine-efavirenz DDI, whereby efavirenz would induce CYP3A4 expression, resulting in reduce lumefantrine plasma concentrations.

A Healthy Volunteer population was first utilised to demonstrate successful C_{d7} predictions, which were within 2-fold of that reported (Huang et al., 2012). This was subsequently extended to simulate the DDI in a South-African population group, as a surrogate for the use of a Tanzanian population group, where the clinical DDI was reported within each CYP2B6 genotype (Maganda et al., 2016b; Maganda et al., 2015). Based on recent age-weight relationships for malaria subjects in Africa, we assumed that the South African population group would demonstrate similar demographics to that of the Tanzanian population (Hayes et al., 2015), and this was further supported by the lack of reported CYP2B6 specific abundance data for EM or PM phenotypes within the Tanzanian population group.

As expected, in the absence of efavirenz, C_{d7} were well predicted for both genotypes (Table 4), confirming that in the absence of efavirenz, CYP2B6 *6/*6 has no significant effect on lumefantrine pharmacokinetics (Maganda et al., 2016b). In the presence of efavirenz, simulations with the *1/*1 and *6/*6 population groups resulted in predictions for median C_{d7} to within 2-fold of those reported, albeit with a broader range of values across the simulation study (Maganda et al., 2016b; Maganda et al., 2015), and demonstrated that lumefantrine pharmacokinetics are significantly altered following co-treatment with efavirenz.

Having established a working model for genotype-based DDI predictions in adults, we subsequently assessed the ability to predict efavirenz plasma concentrations within an African paediatric population group, using a custom developed Ugandan paediatric population group and validated against 3 clinical studies employing/reporting weight-based dosing strategies in non-genotyped (Fillekes et al., 2011; Luo et al., 2016; Pressiat et al., 2017) and genotyped subjects (Viljoen et al., 2012). The validation of efavirenz concentrations within an African paediatric population group was important as any changes in efavirenz plasma concentrations, for example as a result of the impact of CYP2B6 genotypes, would be the key function for driving a DDI with lumefantrine, the extent of which would, therefore, be sensitive to change in the availability of efavirenz. For both non-genotyped (Supplementary materials Figure S4, Figure 3-4) and genotype predictions (Figure 5), overall 5th and 95th percentiles of the mean/median predicted profiles were within the range reported in existing published literature and contributed to our efavirenz validation attempts. However, for the high dose administration

(25 mg/kg) (Figure 4), we were unable to capture the wide variability in the absorption phase reported (Pressiat et al., 2017) and this may have been a result of the influence of food on the absorption and bioavailability of efavirenz.

Having established a working model for efavirenz-mediated DDIs in paediatric predictions, we addressed the ability of this model to predict the lumefantrine-efavirenz DDI, and validation was attempted based upon a report of the interaction in Ugandan children across an age range of 3.1-8.6 years and weight range of 11.4-25.1 kg, using weight-based dosing for both efavirenz and lumefantrine (Parikh et al., 2016) (Figure 6). The reported study did not differentiate lumefantrine plasma concentration profiles by genotype, although our simulations were stratified for two extreme cases of an entire population of CYP2B6 *1/*1 or entirely CYP2B6 *6/*6, and this represent the 'best' and 'worst' clinical scenarios. Further, the wider C_{d7} range simulated in our studies for purely *1/*1 populations was outside of the range reported (Parikh et al., 2016), however we considered the 'best' and 'worst' clinical scenarios and a proportional 'mixed' genotype population (as potentially sampled by the reported study (Parikh et al., 2016)) would have corrected this disparity.

This study demonstrated a significant difference in median C_{d7} in the presence and absence of efavirenz and confirmed the capability of efavirenz to initiate this DDI. In our model simulations across all weight bands, we demonstrated a similar significant reduction in C_{d7} , which was more apparent and resulted in significantly lower ($P < 0.001$) lumefantrine in C_{d7} in the *6/*6 compared to *1/*1 population group (Table 6).

We previously demonstrated that an increase in lumefantrine treatment duration would significantly increase C_{d7} under rifampicin-mediated CYP3A4 induction, and this formed the basis of attempting to address the significantly lower number of *6/*6 subjects capable of attaining target C_{d7} (Olafuyi et al., 2017b). Orally administered lumefantrine is known to display saturated absorption kinetics (Ashley et al., 2007), and therefore increasing the dose of lumefantrine administered within each fixed-dose combination would not be appropriate. Therefore, having established the risk of DDI between efavirenz and lumefantrine (Step 4) the treatment duration was extended to a 5-day or 7-day regimen.

The change in dosing schedule to 7-day regime resulted in a greater number of *6/*6 subjects attaining the target C_{d7} , with 28-57% of subjects (Table 7) attaining this across the age bands studied (Figure 7). The greatest increase in those attained target C_{d7} was evident with 1-4 years old (3-day: 1%; 7-day: 28%) (Table 7), and this may be accounted for by the maturation of

CYP3A4 which would be the key driver for influencing lumefantrine plasma concentrations. CYP3A4 ontogeny is known to rapidly increase over this age range (Salem et al., 2014) to approach adult levels at approximately 6-years onwards.

Determination of the half-life of lumefantrine, typically difficult in paediatrics subjects, given the long-terminal half-life (2-6 days) (Djimde and Lefevre, 2009) and the often sparse nature of plasma collections. A recent study by Parikh et al (2016) in Ugandan children (3.1-8.6 years) reported the median half-life of lumefantrine, when dosing in the presence of efavirenz, as 23.7 hours. When comparing model predictions of the half-life across all age range simulations (0.25-13 years), our predictions are broadly in line with those of Parikh et al (2016). Further, the extension of the dosing interaction from 5-days to 7-days does not alter the half-life within the same genotype (Table 7), suggesting this extension would not alter the elimination clearance of lumefantrine. As expected, the *6/*6 subjects demonstrated a significant decrease in half-life, which would correspond to the increased circulating concentration of efavirenz, thereby handing the DDI and reducing the residency of lumefantrine within the subjects (Table 7). However, although CYP2B6 ontogeny may influence circulating efavirenz (Croom et al., 2009; Pearce et al., 2015), the impact of this DDI may be less apparent or masked by the rapid changes in CYP3A4 ontogeny across this range, which would directly influence circulating lumefantrine concentrations. Further clinical studies are encouraged to delineate the relative impact of CYP3A4 and CYP2B6 ontogeny on extent of this DDI.

Given the resource limitations and cost implications of dose extensions, the authors halted the study at a 7 day dosing regimen and believe further, extended dosing would likely not succeed clinically, due to medicine adherence concerns in resource limited countries (Yeung et al, 2005). Whilst a 5- or 7-day extension may not result in all subjects attaining the target C_{d7} , the proposed extension can be considered to be a pragmatic approach, given the complexity of treatment regimens in developing countries. Further, using a standard 3-day regimen Parikh et al (2016) demonstrated that the median lumefantrine C_{d7} for 3.1-8.6 year old, 111 ng/mL, was significantly below the target concentration in the presence of efavirenz. Therefore, although there may be some scope for further optimisation, the 5- or 7-day regimen would provide a greater level of subject's attainment appropriate C_{d7} compared to existing 3-day regimens. Despite the paucity in clinical data investigating altered dosing regimens for efavirenz-mediated DDI with lumefantrine, the relationship between the DDI, resultant reduction in lumefantrine concentration (primarily based around changes in day-7 concentration) and the

emergence of recurrent malaria has recently been highlighted in studies by Parikh et al (2016) and Maganda et al (2014). Finally, a number of groups are now advocating increasing the dosing duration to counteract this interaction (Hoglung et al, 2015) (Maganda et al, 2015).

5. CONCLUSION

Although the rates of malaria infections have decreased globally, persistent complications still exist in 'at-risk' population groups, particularly paediatrics and pregnant women (World Health Organisation, 2015, 2016b). This is further confounded in situations where genetic polymorphisms contribute towards cross-population variability in complex pharmacokinetics situation, such as antiretroviral mediated-DDIs, may increase the risk of parasite resistance and treatment recrudescence.

Exploration of these risks is difficult clinically, however population-based pharmacokinetic modelling provides a practical approach for simulating such complex interactions. This study focussed on predicting the risk of efavirenz-mediated DDIs on lumefantrine pharmacokinetics in African paediatric population groups with consideration of the polymorphic nature of CYP2B6. We demonstrated that an extension of the current artemether-lumefantrine treatment regimen from 3-days to 7-days would counteract the reduction in efavirenz metabolism common with the *6/*6 genotype and hence enhance the attainment of the target day-7 lumefantrine concentration in both *1/*1 and *6/*6 genotype groups, thereby reduce the risk of recrudescence.

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List Of Figures

Figure 1: Model development strategy

Figure 2: Simulated drug-drug interaction between lumefantrine and efavirenz in a South African population groups.

Lumefantrine was dosed for 6 doses of 480 mg, twice daily for three days commencing on day 20, in the absence (A) and presence (B) of efavirenz (600mg once daily) dosed for the entire study duration to 59 South African healthy adults. *1/*1 (EM) and *6/*6 (PM) genotype pharmacokinetic profiles are indicated by the black and red colour respectively. Median observed plasma day-7 lumefantrine concentrations (Maganda et al., 2016b) are represented by the black open circle (EM) and red closed square (PM) with error bars indicating standard deviations. Solid lines represent predicted median plasma concentration with dashed lines indicated 5th and 95th percentiles.

Figure 3: Simulated plasma concentration-time profile of body weight efavirenz dosing in Healthy Volunteers

A 10x10 trial was run with efavirenz dosed in a standard body-weight based single daily dose regimen for 30 days. Results are presented stratified based on body-weight bandings each possessing at least 80 virtual trial subjects. Solid lines represent mean predictions with dotted-lines representing 5th-95th percentile range for the final dose. Open circles represent data for observed study for each body weight banding (Luo et al., 2016).

Figure 4: Simulated plasma concentration-time profile of efavirenz dosed at 25 mg/kg to 2-3 years old Ugandan children

A dose of 25 mg/kg efavirenz was administered once daily to 2-3 years old Ugandan children (n=53) using the Ugandan Simcyp population group. Solid lines represent mean predictions with dotted-lines representing 5th-95th percentile range. Open circles represent data for observed study (Pressiat et al., 2017) originating from Burkina Faso and Cote d'Ivoire.

Figure 5: Simulated plasma concentration-time profile of efavirenz in South African children stratified by genotype

A dose of 300 mg was administered to 60 children (using the South African population group), aged between 6-7 years and possessing a weight of 20-24.9kg, for 25 days. Simulations were run for genotypes of all subjects being either *1/*1 or all *6/*6 genotypes. Results are presented for day 20 with solid lines representing median predictions with dotted-lines representing 5th-95th percentile range. Open circles represent data for observed study (Viljoen et al., 2012) from a South African population group.

Figure 6: Simulated median plasma concentration-time profile of lumefantrine administered to Ugandan children in the absence and presence of efavirenz.

Lumefantrine (LUM) and efavirenz (EFV) were dosed to Ugandan children aged 3-9 years of age using weight-based dosing strategies of (A) 5-15 kg (LUM: 120 mg per dose; EFV: 200 mg per dose) and (B) 15-25 kg (LUM: 240 mg per dose; EFV: 250-300 mg per dose) with simulated profiles for population groups of all CYP2B6 *1/*1 or all CYP2B6 *6/*6. All simulations included 40-50 subjects per dosing band. Open circles and error bars represent mean and standard deviation respectively (Parikh et al., 2016). Dotted-lines are represent the 5th-95th percentile range. Dashed horizontal and vertical lines originated from each axis represent day 7 concentration (280 ng/mL) and simulation day.

Figure 7: Simulated mean plasma concentration-time profile of lumefantrine administered in a 5-day or 7-day regimen to Ugandan children

Lumefantrine (LUM) and efavirenz (EFV) were dosed to Ugandan children aged 0.25-13 years of age using weight-based dosing strategies (0.25-1 year-old (120mg LUM/300mg EFV), 1-4 year-old (120mg LUM/400mg EFV), 4-8 year-old (240mg LUM/500mg EFV) and 8-13 year-old (240mg LUM/600mg EFV)), with population groups simulating all EFV extensive metabolisers (CYP2B6 *1/*1) or poor metabolisers (CYP2B6 *6/*6). All simulations included 40-50 subjects per dosing band. Solid lines represent mean plasma concentrations.

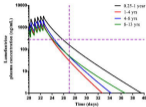
Lumefantrine
Efavirenz

0.25-12 year
olds
(CYP2B6 *6/*6)

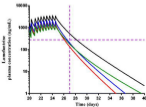
3 Days

5 Days

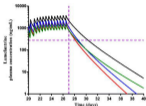
7 Days



Target day 7
concentration not
achieved



Target day 7
concentration
achieved



Target day 7
concentration
achieved

Graphics Abstract

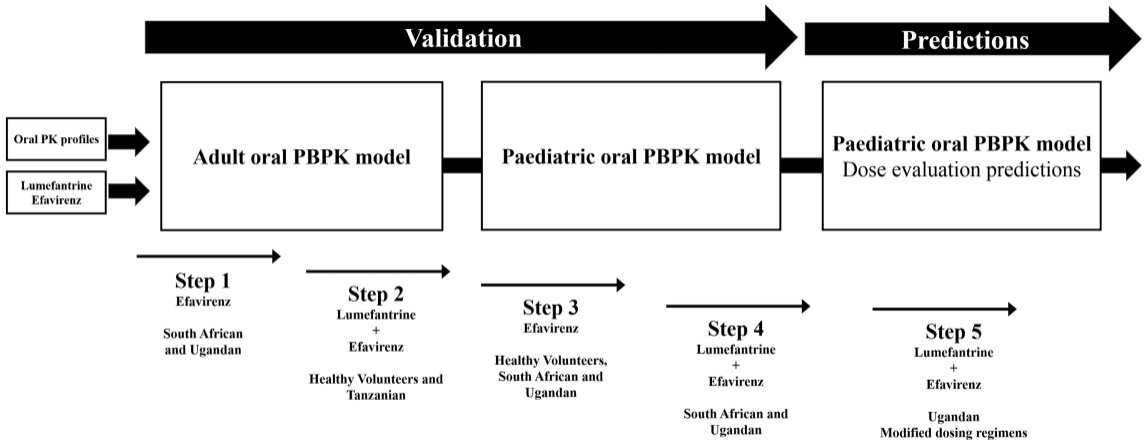


Figure 1

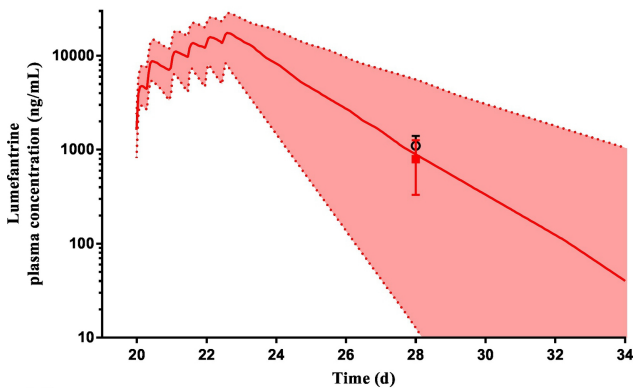
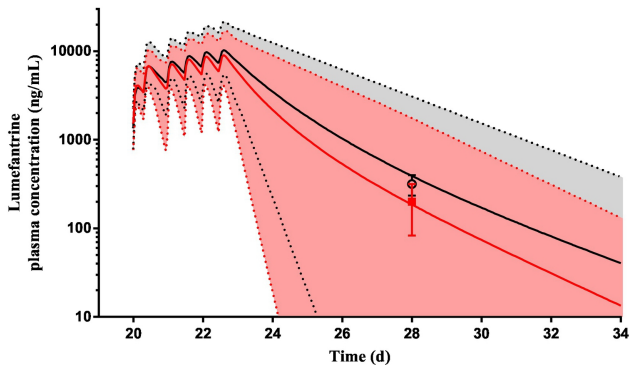
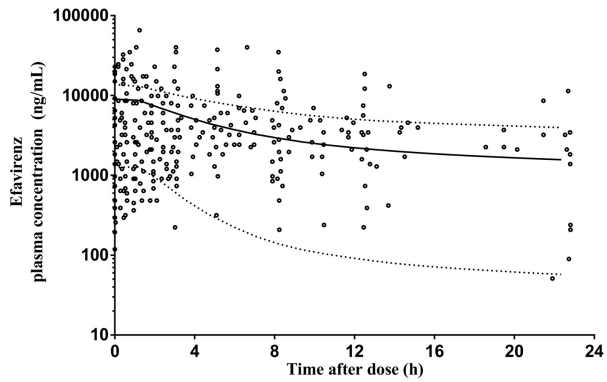
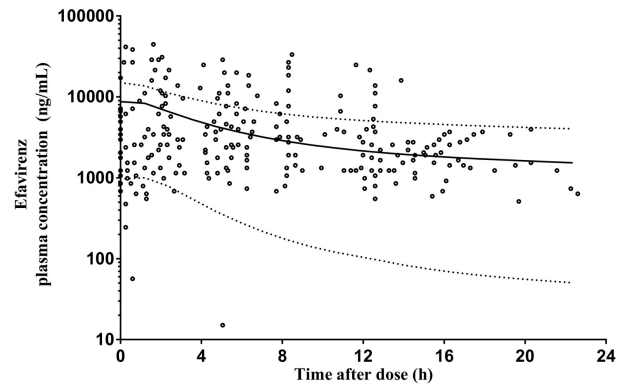
(A)**(B)**

Figure 2

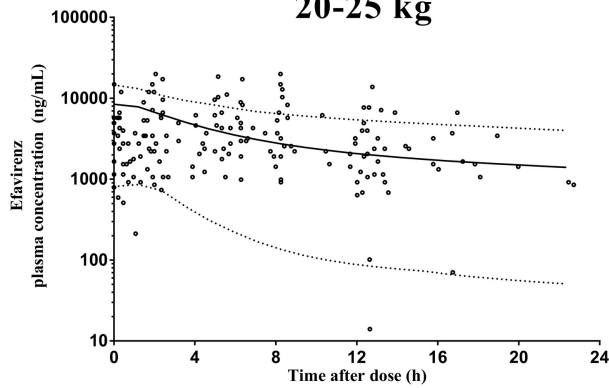
10-15 kg



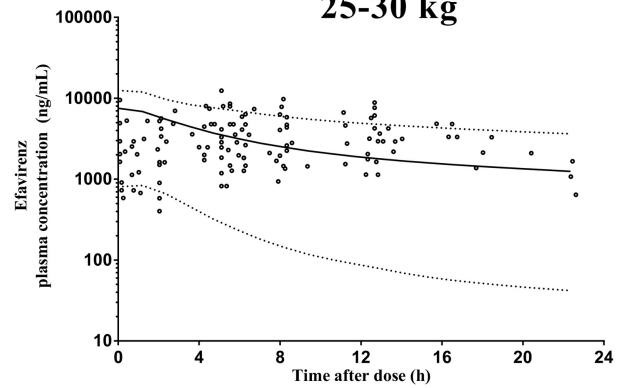
15-20 kg



20-25 kg



25-30 kg



30-35 kg

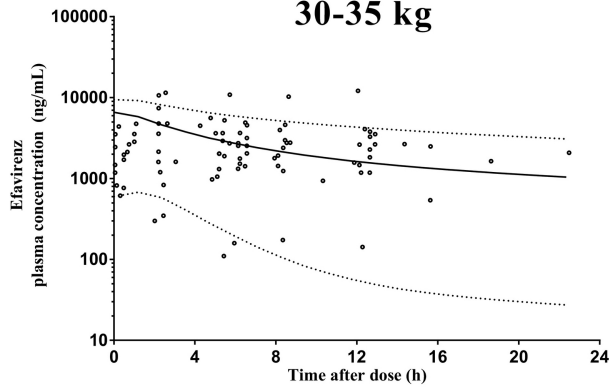


Figure 3

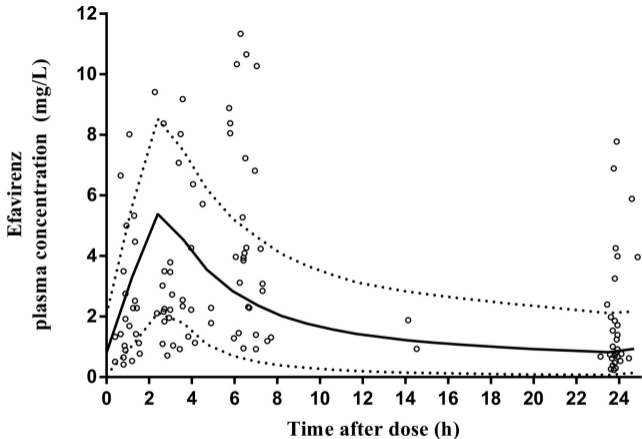
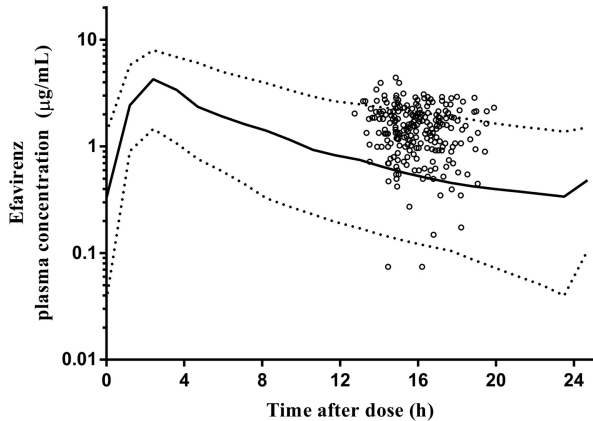


Figure 4

CYP2B6 *1/*1



CYP2B6 *6/*6

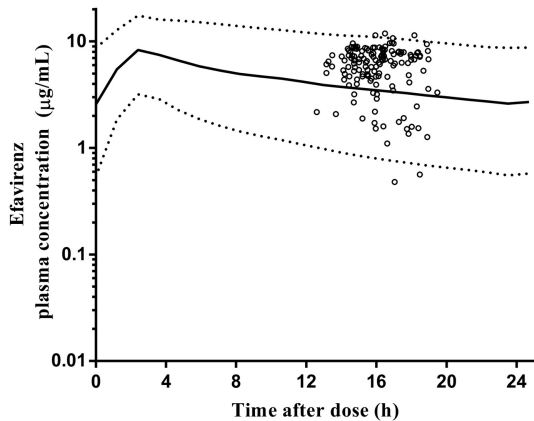
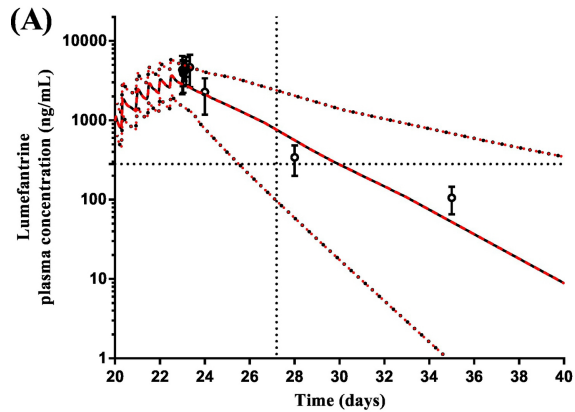


Figure 5

Lumefantrine



Lumefantrine + Efavirenz

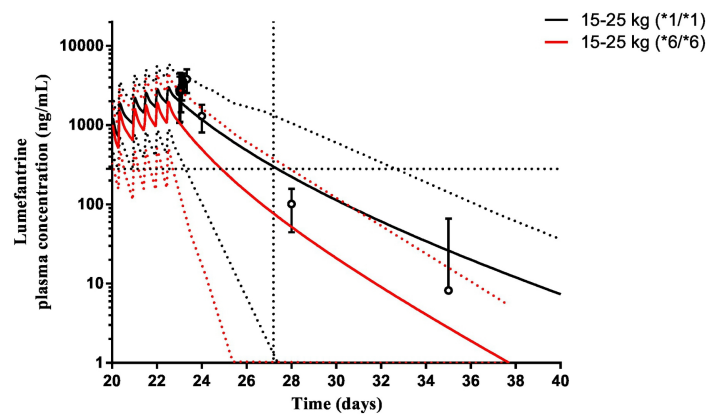
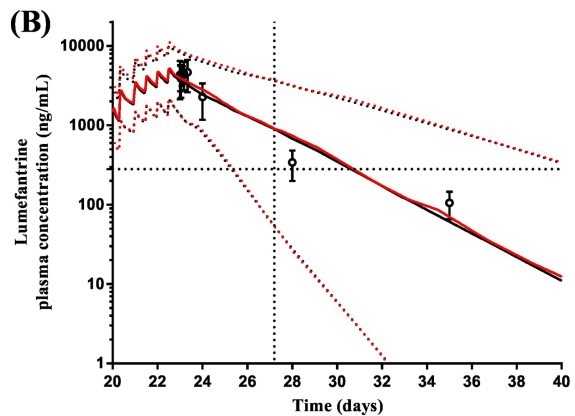
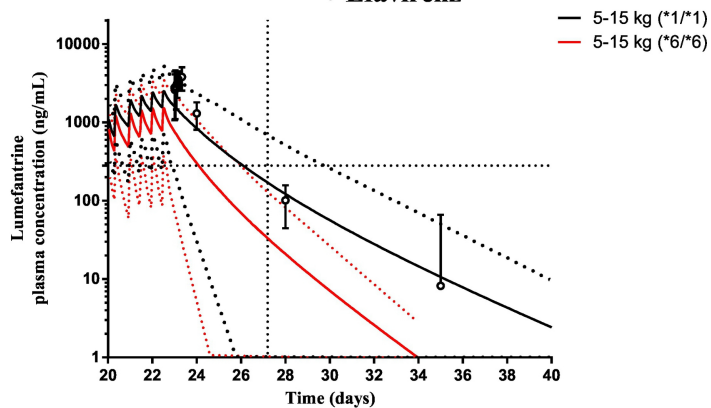
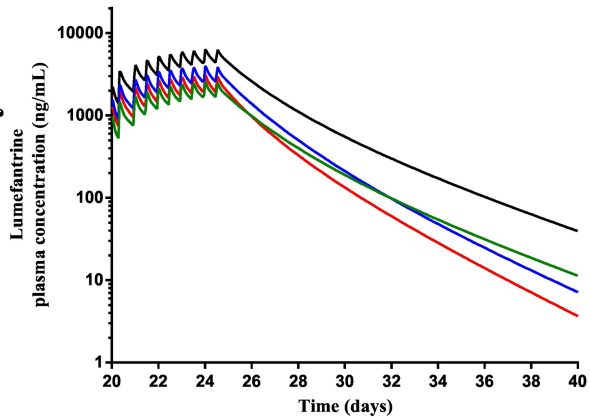


Figure 6

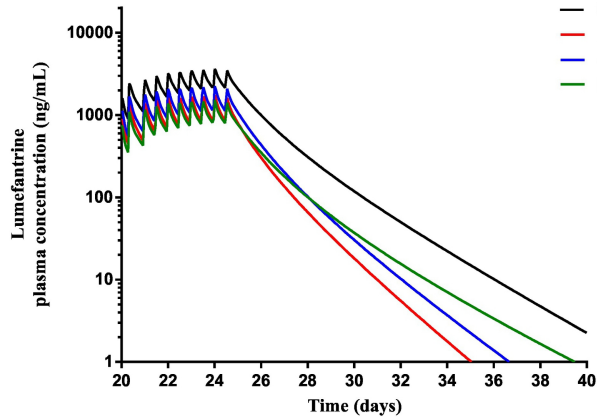
CYP2B6 *1/*1

5-day



CYP2B6 *6/*6

- 0.25-1 years
- 1-4 years
- 4-8 years
- 8-13 years



7-day

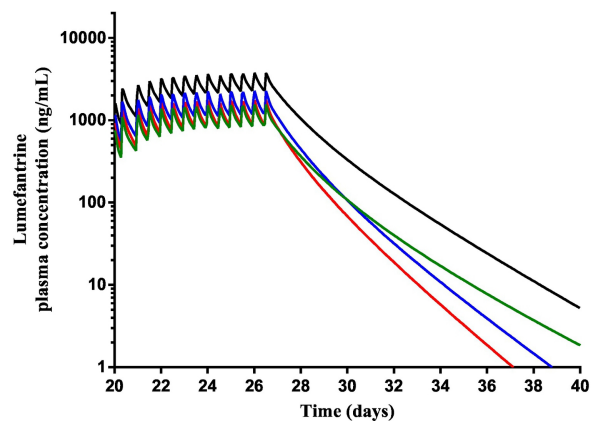
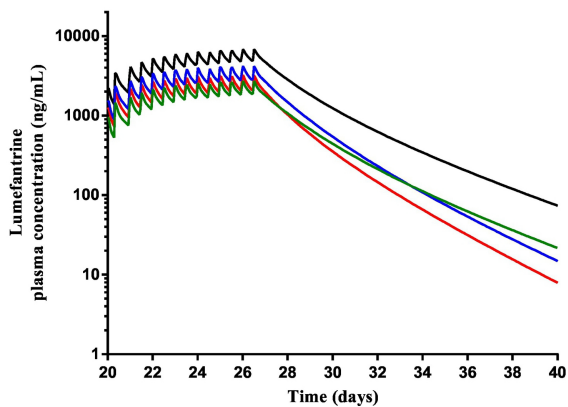


Figure 7