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A physiologically based pharmacokinetic model to characterise the association between CYP2B6 polymorphisms and efavirenz pharmacokinetics in pregnancy

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2017

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A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL TO CHARACTERISE THE ASSOCIATION BETWEEN *CYP2B6* POLYMORPHISMS AND EFAVIRENZ PHARMACOKINETICS IN PREGNANCY

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of MSc Med Pharmaceutical Affairs

Johannesburg, 2017

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Dear Miss Julsing

Master of Science in Medicine: Change of title of research

I am pleased to inform you that the following change in the title of your Research Report for the degree of **Master of Science in Medicine** has been approved:

From:

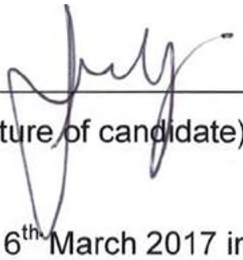
To: **A physiologically based pharmacokinetic model to characterise the association CYP2B6 polymorphisms and efavirenz pharmacokinetics in pregnancy**

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

DECLARATION

I Andrea Alison Julsing declare that this Research Report is my own, unaided work. It is being submitted in partial fulfilment of the requirements for the degree of Master of Science (Pharmaceutical Affairs), at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



(Signature of candidate)

on the 6th March 2017 in Johannesburg

DEDICATION

Foremost, I would like to express my sincere gratitude to my research supervisors, Dr. Manoranjenni Chetty and Ass. Prof. M Paul Danckwerts, for their continued support of my research. I attribute the level of my research to Dr. Manoranjenni Chetty who has driven me to pursue an upper echelon of research and who has given up her personal time to share her knowledge and assist me in navigating the Simcyp simulator. Ass. Prof. M Paul Danckwerts has been instrumental in encouraging and supporting me throughout this period. The suggestions and input provided by Ass. Prof. M Paul Danckwerts, through the evaluation, of each revision, of my research report, have been invaluable. To both, I am eternally grateful.

Particular acknowledgement and thanks go to my husband, Dr. Mark Keyter, who has been a constant source of support and encouragement. I am truly thankful for having you in my life. To my parents, who have been exemplary examples; achieving academically, through dedication and hard work, you have been my motivation for higher education and have instilled in me, a great aspiration to achieve. Thank you for your unrelenting enthusiasm and love.

A Physiologically Based Pharmacokinetic Model to Characterise the Association between *CYP2B6* Polymorphisms and Efavirenz Pharmacokinetics In Pregnancy

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Running head: *CYP2B6* polymorphism and EFVPK in pregnancy.

Keywords: Pregnancy, efavirenz PK, PBPK model, *CYP2B6* polymorphism

Word Count: 3 800

ABSTRACT

There is often limited information available to establish clear dosage requirements in pregnant women, due to ethical concerns about clinical trials on this patient population. Some clinical evidence suggests that the physiological and biological changes which occur in the maternal body impact the pharmacokinetics (PKs) of antiretroviral drugs. This study aimed to develop a physiologically based pharmacokinetic (PBPK) model to characterise the effect of *CYP2B6* metaboliser status on efavirenz (EFV) exposure through each trimester of pregnancy. Such models would be useful in predicting more appropriate dosing during pregnancy.

A PBPK model that accounted for the association between EFV PKs and *CYP2B6* polymorphism in a non-pregnant Caucasian population was developed using the Simcyp Population-Based Simulator (version 15; Simcyp, Sheffield, UK). Following verification of the model with clinical data, the model was applied to a virtual pregnant population in the third trimester. Clinical data, available in the public domain, was used to verify this model before it was used to predict EFV exposure in the first and second trimesters of pregnancy. The effect of *CYP2B6* phenotypic status on EFV exposure, during the first (T_1), second (T_2) and third trimester (T_3) of pregnancy, were compared to determine whether trough plasma concentrations of EFV were within the acceptable therapeutic range.

The EFV PBPK model that accounted for *CYP2B6* phenotypic status recovered the clinical data for extensive metabolisers (EM), intermediate metabolisers (IM) and poor metabolisers (PM), taking single and multiple doses of EFV, adequately. EFV PK changes associated with *CYP2B6* metaboliser status was also adequately recovered for the third trimester (T_3) of pregnancy, where median trough concentrations (C_{min}) were predicted at 0.99 mg l^{-1}, 1.21 mg l^{-1} and 2.54 mg l^{-1}, for EM, IM and PM, respectively, which were within 2-fold of the observed values. Predictions showed that during T_3, 50 % EM had EFV trough concentrations (C_{min}) below the accepted effective concentration of 1.0 mg l^{-1}, suggesting that these patients may require an increased dose. During T_1 and T_2, 45 % of EM experienced sub-therapeutic EFV plasma concentrations. The percentage of IM who

had C_{min} values below the therapeutic range were 27.14 % in T_1, 30.71 % in T_2 and 41.43 % in T_3. Predictions of PM during T_1 did not demonstrate any sub-therapeutic EFV plasma concentrations and only 3.33 % of PM experienced sub-therapeutic C_{min} during T_2 and T_3.

The PBPK models effectively identified differences in plasma concentrations due to *CYP2B6* metaboliser status, during the different trimesters of pregnancy. Such models can be used to facilitate the development of drug dosing regimens in pregnant populations. Furthermore the capability of the predictive model can be extended to evaluate untested drug dosages, drug-drug interactions and metabolic interactions associated with polymorphisms.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The physiologically altered maternal body has a large impact on the disposition and pharmacokinetics of antiretroviral drugs.
- Efavirenz is an important component of first-line antiretroviral regimens and is predominantly metabolised by the isoform *CYP2B6*.
- *CYP2B6* metaboliser status has been shown to affect the magnitude of efavirenz autoinduction and efavirenz exposure significantly.

WHAT THIS STUDY ADDS

- The pregnancy PBPK (p-PBPK) model in this study was used to characterise the association between *CYP2B6* polymorphisms and efavirenz pharmacokinetics during the first, second and third trimester of pregnancy.
- The model was used to identify the probability of pregnant women having sub-therapeutic plasma concentrations based on their *CYP2B6* metaboliser status.

ACKNOWLEDGEMENTS

I would like to acknowledge and thank the Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand and Ass. Prof. M Paul Danckwerts, for purchasing the Simcyp software and laptop, needed to perform the simulations, in order to pursue my studies; I truly appreciate it.

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LIST OF ABBREVIATIONS

AIDS:	Acquired immune deficiency syndrome
ART:	Antiretroviral treatment
ARV:	Antiretroviral
AUC:	Area under the concentration-time curve
CL/F:	Apparent oral clearance
CL_{int}:	Hepatic intrinsic clearance
C_{max}:	Maximum blood plasma concentration
C_{min}:	Minimum blood plasma concentration
CV:	Coefficient of variation
CYP:	Cytochrome P450
EFV:	Efavirenz
EM:	Extensive *CYP2B6* metaboliser
GFR:	Glomerular filtration rate
HIV:	Human immunodeficiency virus
IM:	Intermediate *CYP2B6* metaboliser
IQR:	Inter-quartile range
IVIVE:	*In vitro-in vivo* extrapolation
NNRTI:	Non-nucleoside reverse transcriptase inhibitor
NRTI:	Nucleoside reverse transcriptase inhibitors
RNA:	Ribonucleic acid
SNPs	Single nucleotide polymorphisms
TB:	Tuberculosis
PBPK:	Physiologically-based pharmacokinetic
PK:	Pharmacokinetic
PM:	Poor *CYP2B6* metaboliser
p-PBPK:	Pregnant-PBPK
T_1:	First trimester of pregnancy
T_2:	Second trimester of pregnancy
T_3:	Third trimester of pregnancy

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Figure 3: Simulated mean (green line) and observed [4] (coloured circles) EFV plasma concentrations, of extensive (A), intermediate (B) and poor (C) *CYP2B6* metabolisers, at steady-state. The grey lines represent the 95th and 5th percentiles of the predicted EFV concentrations.

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INTRODUCTION

Acquired immune deficiency syndrome (AIDS) is the most frequent cause of death in pregnant women in many Southern African countries [1] and antiretroviral treatment (ART) of maternal human immunodeficiency virus (HIV) infection is widely used. Changes in the body, as a result of pregnancy can influence the absorption, distribution, metabolism and elimination of drugs [2]. The physiologically altered maternal body has a large impact on the disposition and PKs of antiretroviral (ARV) drugs [3]. Significant inter-individual variability in the PKs of ARVs in pregnant women, due to single nucleotide polymorphisms (SNPs) in drug disposition genes, has been observed clinically [4]. Due to the clinical and ethical concerns associated with the inclusion of pregnant women in clinical trials, there is often limited or no information available to establish clear dosage requirements for pregnant women [5]. Suboptimal drug exposure can result in HIV ribonucleic acid (RNA) rebound, the presence of a resistant virus strain, or an increased risk of HIV-1 transmission to the infant [6]. Increased drug exposure can produce unwarranted maternal adverse effects and/or foetal toxicity [6].

First-line ART regimens include the use of a non-nucleoside reverse transcriptase inhibitor (NNRTI) and two nucleoside reverse transcriptase inhibitors (NRTI) [7]. Efavirenz (EFV) is a common component of first-line ART regimens [4], as it is considered to have extensive efficacy and safety documentation, in contrast to other NNRTIs [8]. EFV is predominantly metabolised by the isoform *CYP2B6* [4] and *CYP2B6* metaboliser status has been shown to affect EFV exposure significantly [4, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18].

Several studies have investigated EFV exposure, associated with *CYP2B6* polymorphism in subjects who were not pregnant. Xu *et al.* [9] and Haas *et al.* [10] studied the effect of *CYP2B6* polymorphism, following administration of a single dose of EFV in healthy populations. Both studies demonstrated an increase in EFV area under the concentration-time curve (AUC) and a decrease in apparent oral clearance (CL/F) in individuals carrying the *CYP2B6* 516T→T phenotype (poor *CYP2B6*

metabolisers (PM)) when compared to individuals carrying the *CYP2B6* 516G→G phenotype (extensive *CYP2B6* metabolisers (EM)) [9, 10].

A study of Zimbabwean patients showed that the *CYP2B6* 516G→T (intermediate *CYP2B6* metaboliser (IM)) and PM were associated with a decrease in EFV CL/F by 22% and 57 % respectively [11]. PM occur with high frequency in African populations and dose reductions have been recommended as very high EFV plasma concentrations may result in increased adverse effects, including serious central nervous system adverse reactions, and subsequent treatment discontinuation [12, 13]. Siccardi *et al.* [12] investigated the effects of EFV dose reduction (from 600 mg to 400 mg and 200 mg) on PKs in a cohort of 500 virtual subjects with different *CYP2B6* phenotypes. This study suggested that dose reductions would be favourable for both IM and PM [12]. Cabrera *et* al. [14] described a decrease in EFV CL/F of 50 % and 75 %, in a Caucasian population, in IM and PM, respectively [14].

Kwara *et al.* [15] considered the effect of *CYP2B6* genetic variation on the steady-state PKs of EFV in a Ghanaian, HIV/tuberculosis (TB) co-infected population. The study demonstrated that EFV AUC was significantly higher, and CL/F was significantly lower, in PM when compared to EM [15]. In a study of 273 HIV-infected patients, receiving 600 mg EFV daily, Dickinson *et al.* [16] reported low EFV median trough concentrations (C_{min}) in EM and described the associated risk of potential virological failure [16].

Studies on possible changes in EFV exposure, during pregnancy, are sparse. Cressey *et al.* [17] studied EFV exposure in a HIV-infected, pregnant population, during the third trimester (T_3) [17]. The study demonstrated that EFV CL/F was increased during pregnancy; however, *CYP2B6* polymorphisms were not explored in the study [17].

A study was conducted by Olagunju *et al.* [4] in 25 pregnant (first two trimesters (T_1 and T_2): n = 7; third trimester (T_3): n = 18) and 19 different postpartum, HIV-positive

women. Results obtained by Olagunju *et al.* [4] demonstrated no significant differences in EFV PKs between the first two trimesters and T_3 [4]. As such, all 25 pregnant women were included in the analysis and the results showed comparisons between pooled EFV PKs in pregnancy vs. post-partum [4]. CL/F in pregnant patients was 42.6 % higher than in postpartum patients [4]. The increase in CL/F was attributed to enhanced *CYP2B6* activity associated with the increased 17β-estradiaol plasma concentration, during pregnancy [4]. Pregnancy-induced changes in EFV PKs, were observed in EM and PM [4], whereby CL/F increased by 100 % and 45.6 % respectively [4]. EFV PKs were not affected by pregnancy-induced changes in IM [4].

Dooley *et al.* [18] studied a prospective cohort of 97 pregnant, HIV-infected women, during T_3. The findings of the study suggested that the main determinant of EFV hepatic intrinsic clearance (CL_{int}) was *CYP2B6* phenotype [18]. Women with extensive *CYP2B6* metaboliser status demonstrated rapid CL/F of EFV and were at increased risk of attaining EFV concentrations which fell below the therapeutic threshold, adopted in 2001, of 1.0 mg l^{-1} [18, 19].
The few studies in pregnant women suggest that EFV exposure during pregnancy may change considerably and is likely to be influenced by the *CYP2B6* metaboliser status of the individual.

Physiologically-based pharmacokinetic (PBPK) models can facilitate the development of drug dosing regimens for populations which are considered to be high-risk during clinical studies, such as pregnant populations [20]. PBPK models use a bottom-up approach whereby *in vivo* data are predicted through the *in vitro-in vivo* extrapolation (IVIVE) of known data [20]. PBPK modelling has the advantage of incorporating both physiological and biochemical parameters that are important for pharmacokinetic processes and drug-specific parameters (e.g. physico-chemical and drug disposition characteristics), into a quantitative predictive model, that can be used to predict the pharmacokinetics of drugs and develop suitable drug dosing regimens [21]. Anatomical, physiological and metabolic changes, occurring in the maternal body, can be incorporated into pregnant-PBPK (p-PBPK) models [22]. Thus, the p-PBPK model can be used to predict the impact of physiological changes

during pregnancy, such as enzyme induction or inhibition [22] on the PKs of drugs. As such, p-PBPK modelling can potentially be used to predict PK changes and corresponding dose adjustments in pregnancy to ensure adequate drug efficacy and avoid undesirable toxicity [23]. A PBPK pregnancy model is available in the Simcyp simulator. As discussed by De Sousa Mendes *et al* [22], "the model accounts for maternal changes such as increasing body weight, fat mass, cardiac output, plasma volume, glomerular filtration rate (GFR), renal plasma flow and decreasing haematocrit, albumin and α1-acid glycoprotein" and has been described by Gaohua *et al.* and Ke *et al.* [21, 24]. Changes in the activity of *CYP3A4*, *CYP2D6* and *CYP1A2* are also incorporated in the current model [24].

This study aimed to develop a model to describe the impact of *CYP2B6* metaboliser status on the PKs of a standard 600mg dose of EFV in pregnant women. Such a model will be useful to determine whether dosage adjustments are required during pregnancy, based on *CYP2B6* metaboliser status. In future studies, the p-PBPK model will be useful to predict interactions with EFV that may require dosage adjustments. This is of particular relevance for EFV since it is usually administered in combination with other drugs.

METHODS

Computer Software

All simulations in this study were performed using the Simcyp Population-Based Simulator (version 15; Simcyp, Sheffield, UK) [8].

Non-pregnant Caucasian Population Model

The Caucasian population in the Simcyp simulator was used to represent the non-pregnant individuals. However, the model does not account for differences in metabolism due to *CYP2B6* phenotypic differences.*CYP2B6* protein expression in liver tissue was revised to account for phenotypic differences, based on data from Lang and co-workers [25], as shown in Table 1.

Table 1

CYP2B6 input parameters.

CYP2B6 phenotype	CYP2B6 liver abundance[12,25]	CV%[12, 25]
EM	24 pmol/mg of protein	68 %
IM	17 pmol/mg of protein	80 %
PM	8 pmol/mg of protein	97 %

EM = extensive *CYP2B6* metabolisers; IM = intermediate *CYP2B6* metabolisers; PM = poor *CYP2B6* metabolisers, CV = coefficient of variation.

Pregnancy population model

The Pregnancy model available in the Simcyp simulator was used for simulations in this study. The general structure of the p-PBPK model, the associated assumptions made and verification were described in detail previously by Gaohua *et al.* [24]. The model accounts for systemic gestational age-dependent parameter changes in the physiology and biochemistry that occurs during the progression of pregnancy [26]. Since the model did not account for *CYP2B6* metaboliser status or pregnancy-induced hepatic *CYP2B6* induction, the *CYP2B6* abundance was increased 1.1-fold, 1.4-fold and 1.9-fold, during T_1, T_2 and T_3, respectively, as described by Ke *et al.* [21, 27].

Verification and application of an EFV PBPK model to predict the impact of *CYP2B6* metaboliser status on PKs.

The EFV PBPK model described by Ke *et al.* [28] was used in this study. The model accounts for metabolism, predominantly by *CYP2B6*, contribution by *CYP3A4*, *CYP2A6* and *CYP1A2*, and *CYP2B6* and *CYP3A4* autoinduction [28]. The EFV model was verified in Caucasian healthy volunteers by Ke *et al.* [28] but no differentiation, in the PKs of the drug, due to *CYP2B6* metaboliser status, was accounted for. In this study, the EFV model was evaluated and verified for the prediction of PK differences associated with *CYP2B6* metaboliser status in non-pregnant Caucasian subjects and thereafter verified in a pregnant population.

The general strategy used to evaluate the effect of *CYP2B6* phenotypic status on EFV PKs during pregnancy is represented in the workflow diagram in Figure 1.

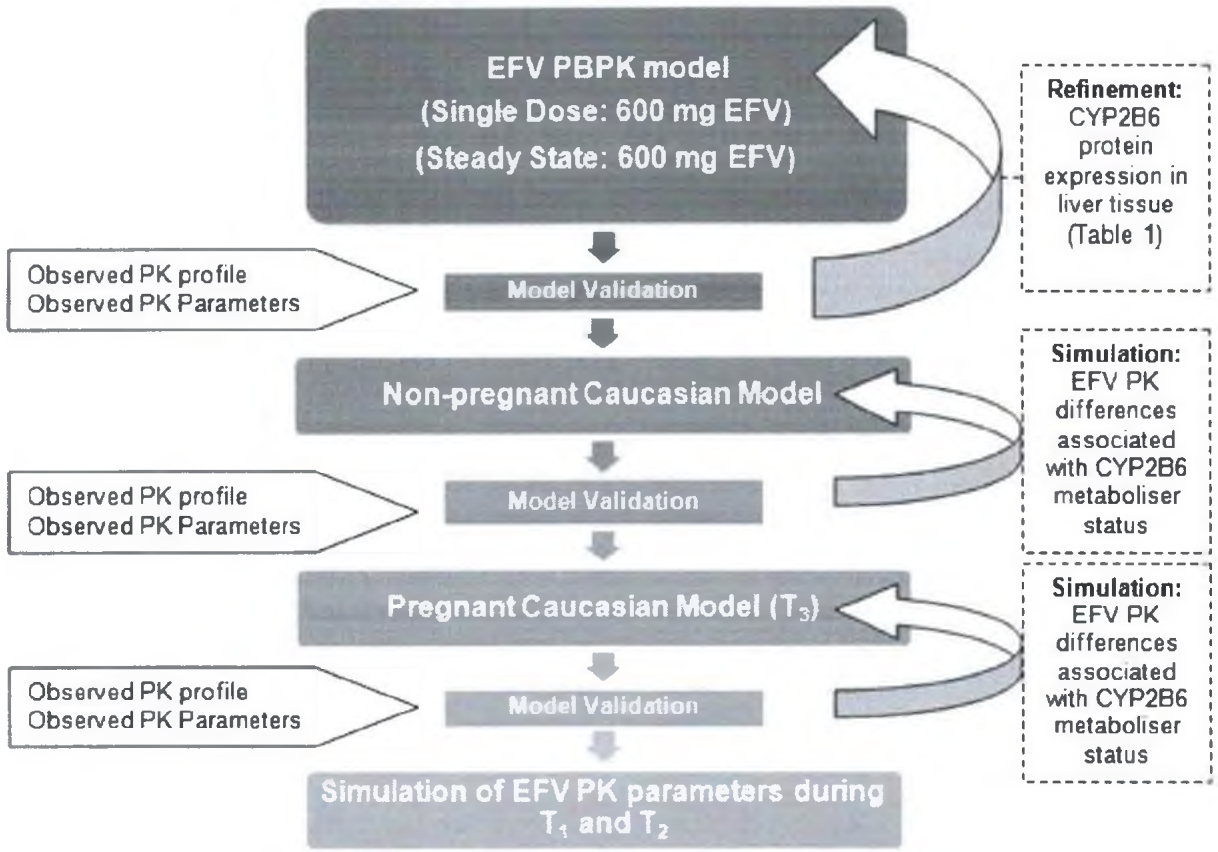


Figure 1

Schematic representation of the workflow of PBPK model development.

To verify and evaluate the PBPK models, predicted concentration-time profiles for EFV in the three *CYP2B6* phenotypes were compared to those observed in clinical studies. Visual predictive tests were performed on the concentration time profiles. In addition, the predicted median exposure parameters (AUC, C_{max} and CL/F) were compared with the observed values from the clinical studies. The 2-fold prediction error, used for model validation in this study, is commonly used in the justification of predictions in IVIVE studies [29]. Abduljalil *et al.* [29] described that, for drugs with high variability, a 2.5-fold metric system may be applied [29]. The 2-fold system would be appropriate for drugs with medium variability and a tighter boundary of 1.5-fold is suggested for drugs with low variable PK parameters [29]. The 2-fold criteria boundaries have been applied in this study.

Where tabulated concentration-time data was not available in the literature, data were extracted from the observed figures available, using the GetData graph digitiser, version 2.2. PBPK models were found to be acceptable when inspection of the median concentration-time profiles showed that majority of the clinically observed data points were within the 95% and 5% percentiles and the PK parameter estimations were predicted within 2-fold of the observed data.

The trial design of the simulations was based on the clinical studies that were used for comparison and verification. To verify the ability of the model to recover the phenotypic differences in EFV PKs in non-pregnant individuals, observed single-dose EFV PK data was obtained from Xu *et al.* [9] and Haas *et al.* [10]and PK data of EFV exposure at steady-state was obtained from Olagunju *et al.*(HIV-infected patients; pregnant: n = 25; post-partum: n = 19), 100 % female) [4], Kwara *et al.*(26 HIV/TB co-infected patients, 31 % female) [15] and Dickinson *et al.*(273 HIV-infected patients, 32 % female) [16].

The verified PBPK model was extrapolated to the pregnant population and was used to predict the PK profiles of EFV, for each phenotype. The trial designs were based upon studies conducted by Dooley *et al.* [18] and Olagunju *et al.* [4].The study by Dooley and co-workers [18] evaluated concentration-time profiles for 600 mg EFV given once daily in women in the third trimester of pregnancy in South African subjects. Olagunju and co-workers [4] studied concentration-time profiles for 600 mg EFV given once daily in women in pregnancy (first two trimesters (T_1 and T_2): n = 7; third trimester (T_3): n = 18), in Nigerian subjects. The median AUC, CL/F, C_{max} and C_{min} of EFV (600 mg) were predicted, for each phenotype, for T_3, and verified using the clinical data.

The PBPK model was then used to predict the impact of *CYP2B6* metaboliser status on the PKs of EFV in T_1 and T_2, in the absence of clinical data. 10 trials of 10 individuals were predicted for each phenotype to account for variability. For comparison with T_3, median EFV PK parameters and concentration-time profiles were predicted for T_1 and T_2 so as to characterise the association between *CYP2B6*

polymorphisms and EFV PKs throughout pregnancy. Predicted C_{min} concentrations during each trimester of pregnancy, for each *CYP2B6* phenotype, were then compared to determine whether dose adjustments may be required.

RESULTS

Model evaluation for the non-pregnant population

Single-dose EFV PKs

The observed and predicted values for the PK parameters (CL/F, $AUC_{0-\infty}$, and C_{max}), following a single dose of EFV (600 mg), are summarised in Table 2 and are all within a 2-fold range of observed values. 77.8 % of the results observed for PK parameters, in the single-dose study of EFV exposure, were within a 1.25-fold range of the reported data.

Table 2

Clinical and predicted PK parameters for EFV after single-dose oral administration (600 mg), in extensive, intermediate and poor *CYP2B6* metabolisers.

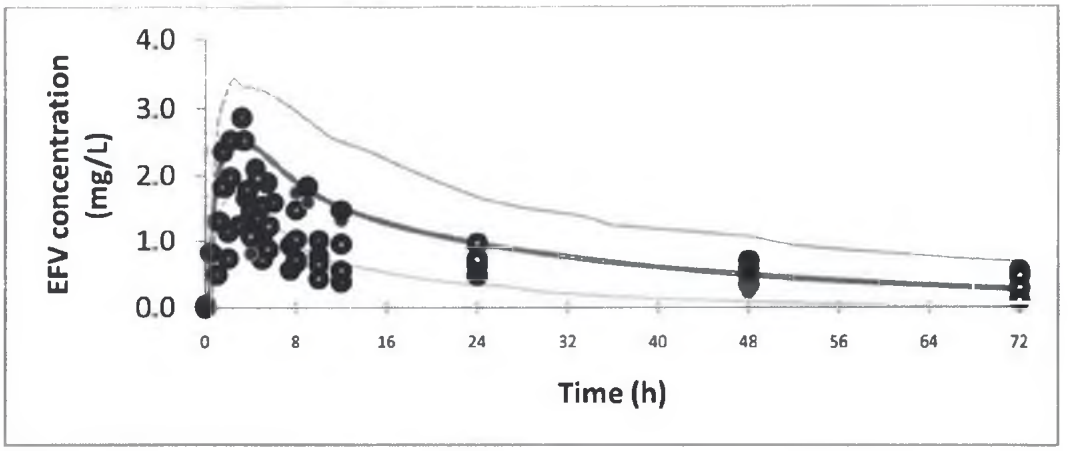
EXTENSIVE *CYP2B6* METABOLISERS			
	CL/F ($l\ h^{-1}$)	**AUC ($mg\ h\ l^{-1}$)**	**C_{max} ($mg\ l^{-1}$)**
Xu *et al.* [9] (*n* = 8) (mean values)	**8.5 (± 3.4)**	**79.8 (± 28.4)**	**2.3 (± 0.7)**
Simulated (*n* = 8)	11.16	63.73	2.59
Predicted/Observed	1.31	0.80	1.13
Haas *et al.* [10] (*n* = 10) (median values)	**7.57 (4.89 – 12.53)**	**68.00 (47.00 – 102.00)**	**1.64 (1.47 – 1.91)**
Simulated (*n* = 10)	8.02	74.78	2.65
Predicted/Observed	1.06	1.10	1.62
INTERMEDIATE *CYP2B6* METABOLISERS			
	CL/F ($l\ h^{-1}$)	**AUC ($mg\ h\ l^{-1}$)**	**C_{max} ($mg\ l^{-1}$)**
Xu *et al.* [9] (*n* = 9) (mean values)	**8.3 (± 2.8)**	**81.6 (± 33.7)**	**1.7 (± 0.5)**
Simulated (*n* = 9)	9.35	77.05	2.74
Predicted/Observed	1.13	0.94	1.61
Haas *et al.* [10] (*n* = 17) (median values)	**7.14 (5.47 – 8.38)**	**77.00 (63.00 – 99.00)**	**1.88 (1.38 – 2.40)**
Simulated (*n* = 17)	6.80	88.28	2.65
Predicted/Observed	0.95	1.15	1.41
POOR *CYP2B6* METABOLISERS			
	CL/F ($l\ h^{-1}$)	**AUC ($mg\ h\ l^{-1}$)**	**C_{max} ($mg\ l^{-1}$)**

Xu *et al.* [9] (*n* = 3) (mean values)	**5.9 (± 0.5)**	**101.7 (± 7.9)**	**2.4 (± 0.2)**
Simulated (*n* = 3)	6.85	93.96	2.85
Predicted/Observed	1.16	0.92	1.19
Haas *et al.* [10] (*n* = 7) (median values)	**4.09 (3.90 – 4.55)**	**123.00 (102.00 – 128.00)**	**2.34 (1.78 – 2.52)**
Simulated (*n* = 7)	4.20	142.93	2.89
Predicted/Observed	1.03	1.16	1.24

PK = pharmacokinetic; EFV = efavirenz; CL/F = oral clearance; AUC = area under the plasma concentration time curve; C_{max} = maximum plasma concentration; SD = standard deviation; IQR = interquartile range.

As shown in Figure 2, the overall PK profile showed satisfactory agreement with the clinical observations [9], although there appeared to be a trend towards over-prediction in the IM and PM groups. The high variability between the subjects could account for this. Predicted PK parameters demonstrated higher CL/F and lower AUC in EM when compared to IM and PM in accordance with observed PK data obtained from Xu *et al.* [9] and Haas *et al.* [10].

(A)



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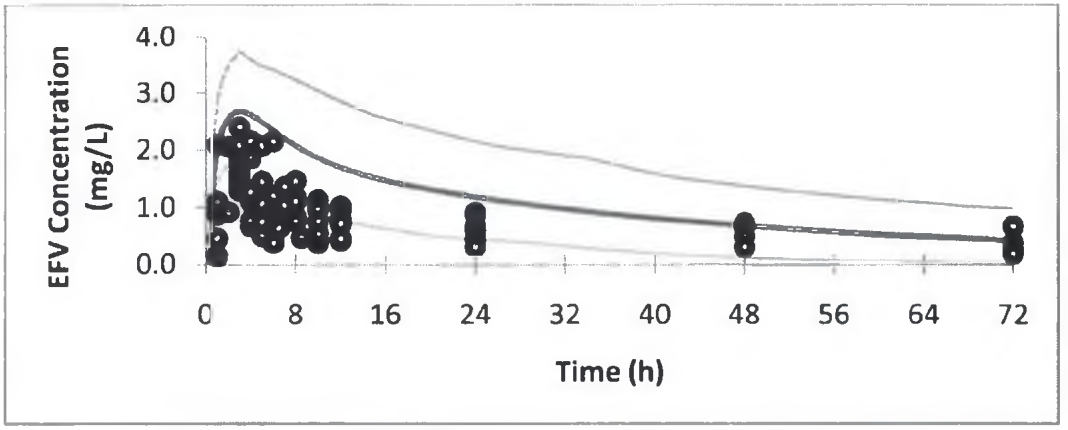


(C)

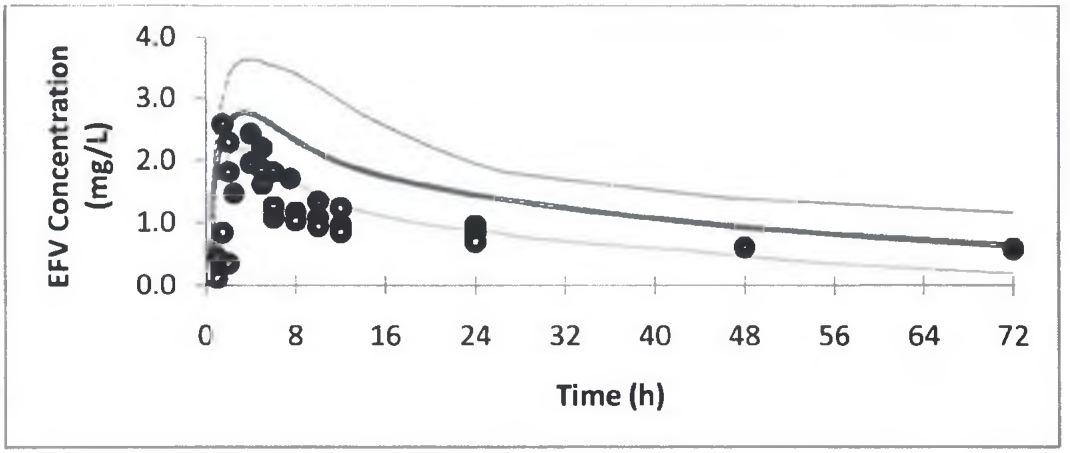


Figure 2

Simulated mean (green line) and observed [9] EFV plasma concentrations, of extensive (A), intermediate (B) and poor (C) *CYP2B6* metabolisers, after single-dosing (600 mg). The grey lines represent the 95th and 5th percentiles of the predicted EFV concentrations.

Multiple-dose EFV PKs

Predictions of multiple-dose EFV PK parameters were compared with clinical data obtained from Olagunju *et al.* [4], Kwara *et al.* [15] and Dickinson *et al.* [16]. Results provided in Table 3 demonstrate that 75 % of the results predicted for PK parameters (CL/F, AUC, C_{max} and C_{min}), were within a 1.25-fold range of the reported data and 96 % of the results were within a 2-fold range (Table 3).

Table 3

Median (IQR) clinical and predicted EFV PK parameters, at steady-state (600 mg), in extensive, intermediate and poor *CYP2B6* metabolisers.

EXTENSIVE *CYP2B6* METABOLIZERS				
	CL/F ($l\ h^{-1}$)	**AUC_{0-24} ($mg\ h\ l^{-1}$)**	**C_{max} ($mg\ l^{-1}$)**	**C_{min} ($mg\ l^{-1}$)**
Olagunju *et al.* [4] (*n = 6*)	**11.60 (9.37 – 18.40)**	**52.40 (32.60 – 64.00)**	**3.19 (2.70 – 3.80)**	**1.54 (0.87 – 2.31)**
Simulation (*n* = 6)	13.21	45.41	3.31	1.02 (0.56 – 1.55)
Predicted/Observed	1.14	0.87	1.04	0.66
Kwara *et al.* [11] (*n = 7*)	**24.60 (19.44 – 51.84)**	**24.40 (11.80 – 30.90)**	**1.60 (1.20 – 2.00)**	**0.60 (0.20 – 0.90)**
Simulation (*n* = 7)	14.75	40.67	2.94	0.89 (0.57 – 1.27)
Predicted/Observed	0.6	1.67	1.84	1.48
Dickinson *et al.* [16] (*n* = 107)	**12.40 (5.11 – 69.70)**	**49.20 (8.61 – 117.00)**	**2.80 (1.53 – 5.79)**	**1.28 (0.0025 – 3.94)**
Simulation (*n* = 107)	15.42	38.92	2.92	0.82 (0.54 – 1.26)
Predicted/Observed	1.24	0.8	1.04	0.64
INTERMEDIATE *CYP2B6* METABOLIZERS				
	CL/F ($l\ h^{-1}$)	**AUC_{0-24} ($mg\ h\ l^{-1}$)**	**C_{max} ($mg\ l^{-1}$)**	**C_{min} ($mg\ l^{-1}$)**

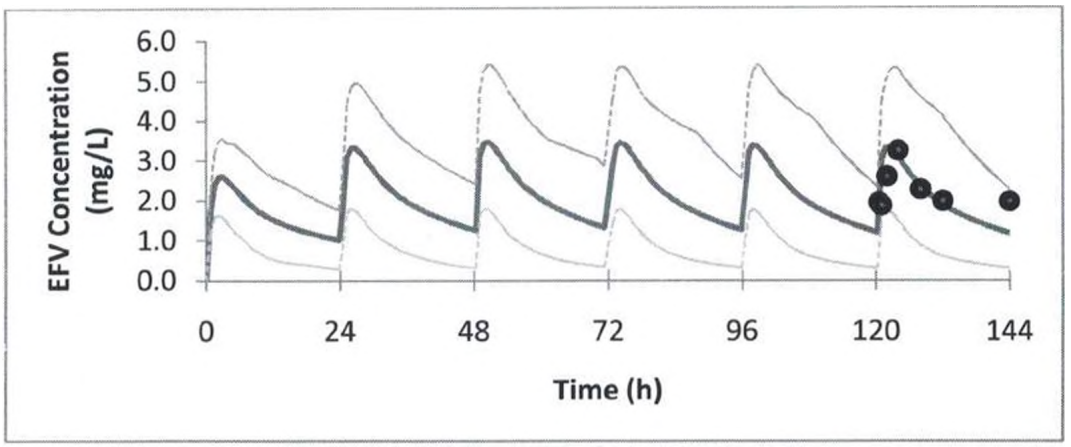
Olagunju *et al.* [4] (*n* = 7)	**11.90 (4.71 – 20.67)**	**50.70 (29.00 – 128.00)**	**4.85 (2.05 – 6.78)**	**1.52 (0.76 – 4.86)**
Simulation (*n* = 7)	9.90	60.58	4.06	1.55 (0.87 – 2.32)
Predicted/Observed	0.83	1.19	0.84	1.02
Kwara *et al.* [11] (*n* = 12)	**28.86 (18.42 – 38.82)**	**24.80 (16.60 – 40.30)**	**1.80 (1.20 – 2.10)**	**0.50 (0.30 – 1.20)**
Simulation (*n* = 12)	11.93	50.30	3.40	1.19 (0.70 – 1.79)
Predicted/Observed	0.41	2.03	1.89	2.38
Dickinson *et al.* [16] (*n* = 127)	**8.93 (2.98 – 65.40)**	**67.60 (12.40 – 202.00)**	**3.71 (1.69 – 9.03)**	**1.94 (0.079 – 7.55)**
Simulation (*n* = 127)	11.78	50.94	3.42	1.23 (0.80 – 1.82)
Predicted/Observed	1.32	0.75	0.92	0.63
POOR *CYP2B6* METABOLIZERS				
	CL/F (l h^{-1})	**AUC_{0-24} (mg h l^{-1})**	**C_{max} (mg l^{-1})**	**C_{min} (mg l^{-1})**
Olagunju *et al.* [4] (*n* = 6)	**4.69 (3.39 – 5.35)**	**129.00 (112.00 – 177.00)**	**6.94 (6.37 – 9.76)**	**5.13 (3.83 – 6.74)**
Simulation (*n* = 6)	6.43	93.35	5.47	2.75 (1.58 – 3.76)
Predicted/Observed	1.37	0.72	0.79	0.54
Kwara *et al.* [11] (*n* = 7)	**8.04 (4.62 – 10.14)**	**74.70 (59.10 – 133.70)**	**4.30 (2.90 – 7.00)**	**2.80 (2.10 – 4.70)**
Simulation (*n* = 7)	7.24	82.93	4.74	2.44 (1.54 – 3.22)
Predicted/Observed	0.90	1.11	1.10	0.87
Dickinson *et al.* [16] (*n* = 39)	**3.55 (1.69 – 58.20)**	**171.00 (19.20 – 359.00)**	**7.80 (1.75 – 15.60)**	**6.24 (0.24 – 14.10)**

Simulation (n = 39)	7.32	81.96	4.90	2.17 (1.72 – 3.46)
Predicted/Observed	2.06	0.48	0.63	0.35

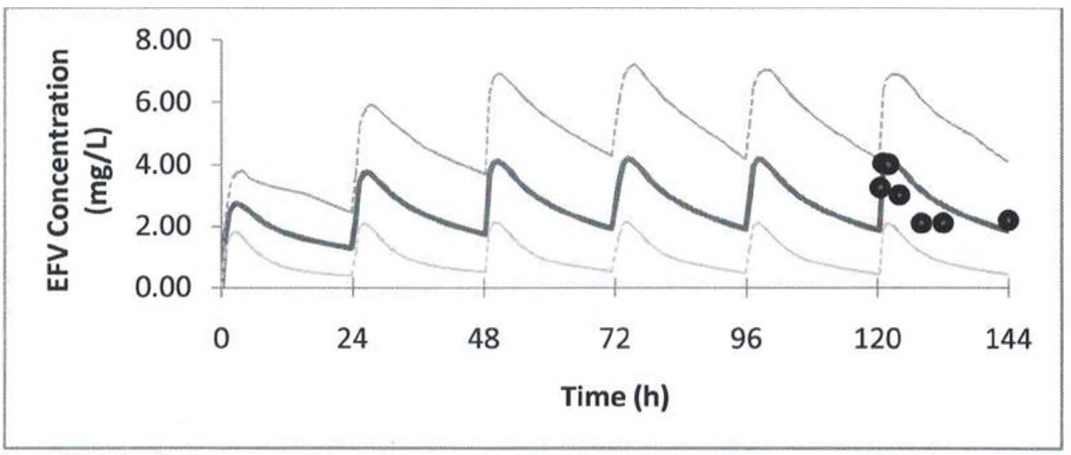
IQR = interquartile range; EFV = efavirenz; PK = pharmacokinetic; CL/F = oral clearance; AUC = area under the plasma concentration time curve; C_{max} = maximum plasma concentration; C_{min} = EFV trough concentration.

Figure 3 shows predicted vs. observed [4] concentration-time courses for EFV (600 mg) exposure at steady-state. The EFV plasma concentration values, reported by Olagunju *et al.* [4], were within the 5th and 95th percentile of the simulation, suggesting good recovery of the clinical data.

(A)



(B)



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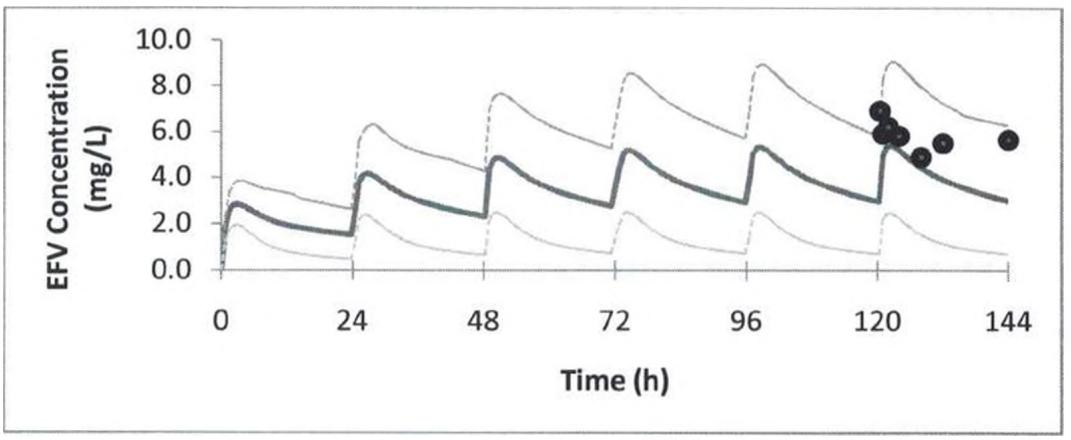


Figure 3

Simulated mean (green line) and observed [4] (coloured circles) EFV plasma concentrations, of extensive (A), intermediate (B) and poor (C) *CYP2B6* metabolisers, at steady-state. The grey lines represent the 95th and 5th percentiles of the predicted EFV concentrations.

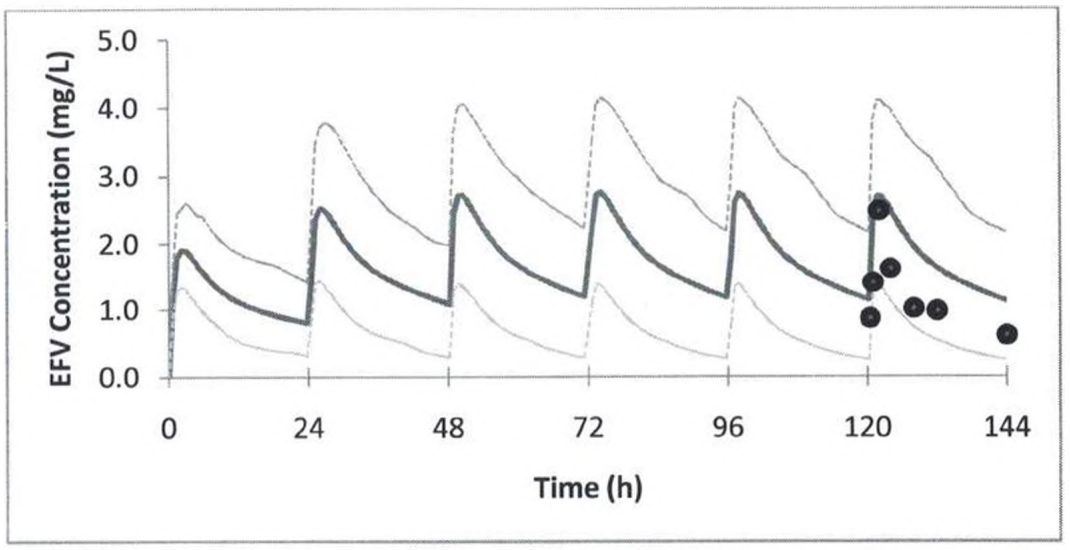
While the majority of the predicted parameters were within the 2-fold range, slight over-predictions were observed in the predicted median AUC (50.30 mg h l^{-1}) and C_{min} (1.19 mg l^{-1}), for IM, when compared with the observed median values of AUC (24.80 mg h l^{-1}) and C_{min} (0.50 mg l^{-1}) obtained from Kwara *et al.* [15]. The predicted median CL/F of 7.32 l h^{-1}, for poor *CYP2B6* metabolisers, was also slightly over-predicted when compared to the observed CL/F of 3.55 l h^{-1} described by Dickinson *et al.* [16].

Based on the trial design described by Olagunju *et al.* [4], the predicted EFV PK parameters demonstrated 105.44 % higher CL/F, 51.36 % lower AUC, 39.49 % lower C_{max} and 62.91 % lower C_{min} in EM when compared to PM.

EFV PKs in pregnancy (T_3)

The overall PK profiles, for EM, IM and PM, during T_3 showed good agreement with the clinical observations, for acceptable predictability, within the 5th and 95^{th}percentiles [4] (Figure 4). 83.33 % of the predicted PK parameters (CL/F, AUC, C_{max} and C_{min}), during T_3 were within a 1.25-fold range of the reported data, obtained from Olagunju *et al.* [4] and 100 % of the predictions were within 2-fold of the clinical data [4].

(A)


EFV Concentration (mg/L)
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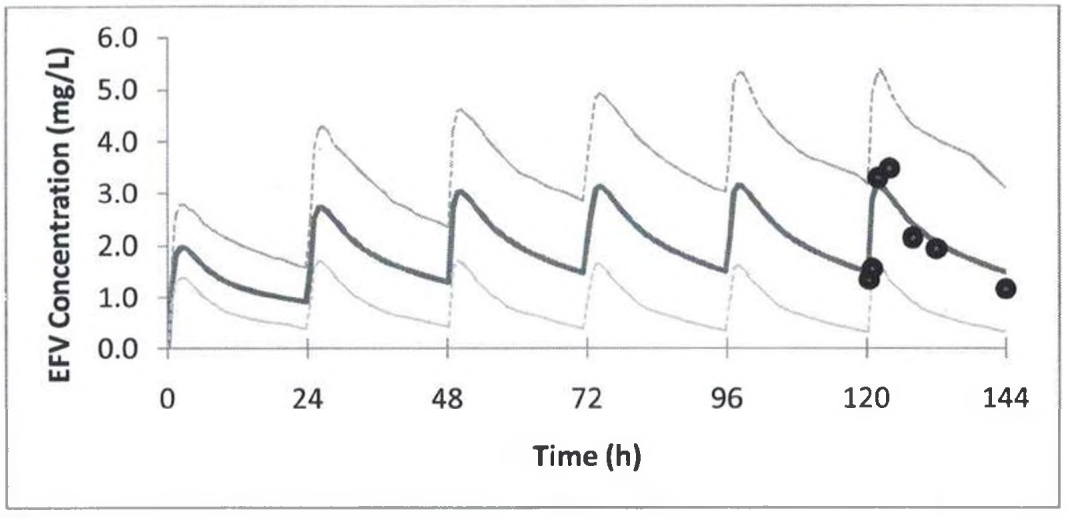
3.0
2.0
1.0
0.0
0
24
48
72
96
120
144
Time (h)

(B)


EFV Concentration (mg/L)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
24
48
72
96
120
144
Time (h)

(C)

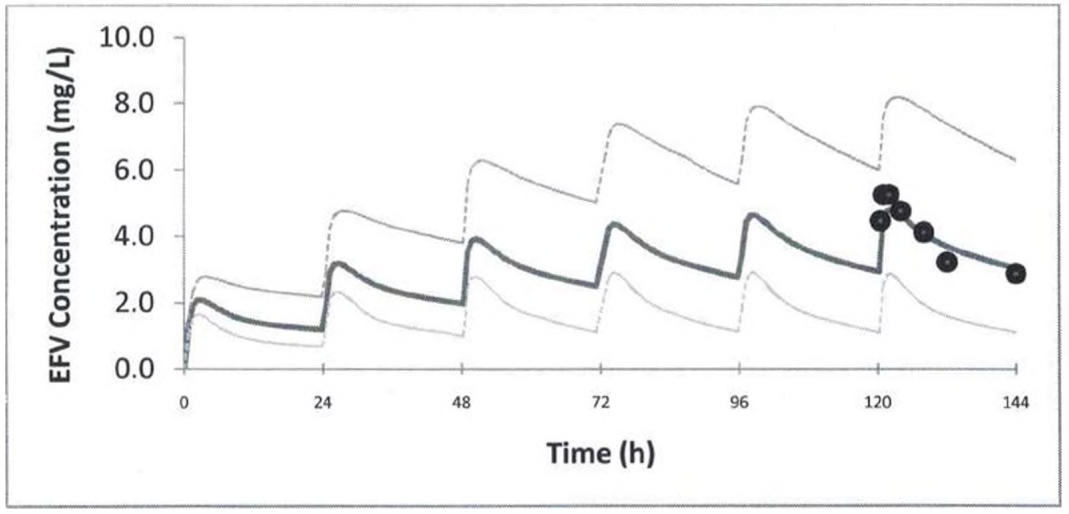

EFV Concentration (mg/L)
10.0
8.0
6.0
4.0
2.0
0.0
0
24
48
72
96
120
144
Time (h)

Figure 4

Simulated mean (green line) and observed [4] (coloured circles) EFV plasma concentrations, of extensive (A), intermediate (B) and poor (C) *CYP2B6* metabolisers, at steady-state, during third trimester (T_3: 37 weeks) of pregnancy. The grey lines represent the 95th and 5th percentiles of the predicted EFV concentrations.

As shown in table 4, the predicted median CL/F and AUC for each *CYP2B6* phenotype, during T_3 were well predicted and within 1.25-fold and1.5-fold, respectively, of the observed data [4]. The predicted C_{max} values, for EM, IM and PM, during T_3 were very well predicted [4]. C_{min} was predicted at 0.99 mg l^{-1}, 1.21 mg l^{-1} and 2.54 mg l^{-1}, for EM, IM and PM, respectively, which was within 2-fold of the observed C_{min} reported by Olagunju *et al.* [4].

Table 4

Median (IQR) clinical and predicted EFV PK parameters, at steady-state (600 mg), during pregnancy (T_3), in extensive, intermediate and poor *CYP2B6* metabolisers.

EXTENSIVE *CYP2B6* METABOLIZERS				
	CL/F ($l\ h^{-1}$)	**AUC ($mg\ h\ l^{-1}$)**	**C_{max} ($mg\ l^{-1}$)**	**C_{min} ($mg\ l^{-1}$)**
Olagunju *et al.* [4] (*n* = 8)	**23.2 (18.4 – 27.7)**	**25.9 (21.7 – 32.6)**	**2.64 (1.26 – 3.49)**	**0.59 (0.43 – 0.92)**
Simulation (*n* = 8)	15.86	37.85	2.56	0.99 (0.60 – 1.53)
Predicted/Observed	0.68	1.46	0.97	1.68
Dooley *et al.* [18] (*n* = 18)	-	-	-	**0.79 (0.67 – 1.02)**
Simulation (*n* = 18)	18.55	32.35	2.33	0.72 (0.47 – 1.21)
Predicted/Observed	-	-	-	0.91
INTERMEDIATE *CYP2B6* METABOLIZERS				
	CL/F ($l\ h^{-1}$)	**AUC ($mg\ h\ l^{-1}$)**	**C_{max} ($mg\ l^{-1}$)**	**C_{min} ($mg\ l^{-1}$)**
Olagunju *et al.* [4] (*n* = 14)	**13.7 (2.96 – 23.3)**	**43.90 (25.7 – 203.00)**	**3.66 (2.49 – 14.4)**	**1.12 (0.57 – 5.19)**
Simulation (*n* = 14)	13.66	43.93	2.89	1.21 (0.81 – 2.03)
Predicted/Observed	1.00	1.00	0.79	1.08
Dooley *et al.* [18] (*n* = 43)	-	-	-	**1.38 (1.09 – 1.81)**
Simulation (*n* = 43)	14.50	41.38	2.75	1.06 (0.65 – 1.72)

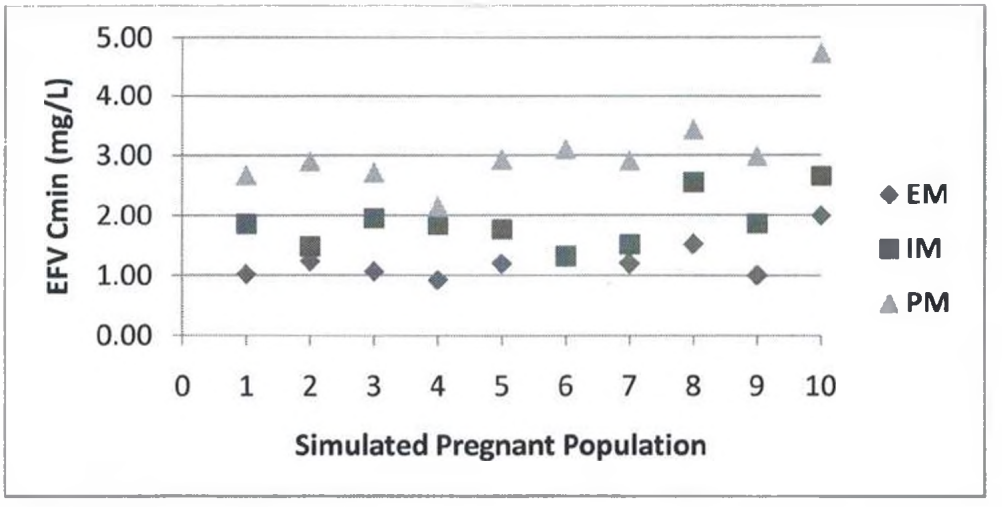
Predicted/Observed	-	-	-	0.77
POOR *CYP2B6* METABOLIZERS				
	CL/F (l h^{-1})	**AUC (mg h l^{-1})**	**C_{max} (mg l^{-1})**	**C_{min} (mg l^{-1})**
Olagunju *et al.* [4] (*n* = 3)	**6.83 (5.22 – 8.15)**	**87.9 (73.7 – 115.0)**	**5.77 (5.32 – 5.95)**	**2.89 (2.66 – 4.03)**
Simulation (*n* = 3)	7.72	77.68	4.28	2.54 (1.81 – 3.88)
Predicted/Observed	1.13	0.88	0.74	0.88
Dooley *et al.* [18] (*n* = 10)	**-**	**-**	**-**	**4.09 (3.52 – 6.54)**
Simulation (*n* = 10)	8.94	67.12	3.95	2.07 (1.41 – 3.45)
Predicted/Observed	-	-	-	0.51

IQR = interquartile range; EFV = efavirenz; PK = pharmacokinetic; T_3 = third trimester: 37 weeks; CL/F = oral clearance; AUC = area under the plasma concentration time curve; C_{max} = maximum plasma concentration; C_{min} = EFV trough concentration.

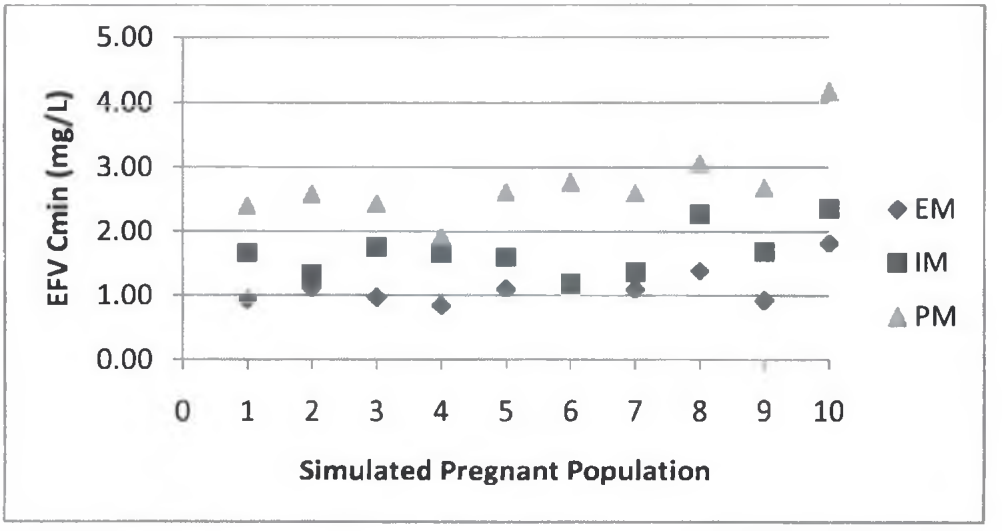
As reflected in Table 4, observed data for C_{min} were extracted from Dooley *et al.* [18]. No additional PK parameters (such as CL/F, AUC, and C_{max}), stratified by phenotype, during T_3, were reported by Dooley *et al.* [18]. The predicted C_{min} values accurately recovered the observed values in EM, IM and PM in T_3 [18]. Sub-therapeutic EFV concentrations were observed in EM, during T_3 [18, 19]. Predicted median C_{min} in EM, during T_3, were below the therapeutic threshold of 1.0 mg l^{-1}.

Recovery of the clinical data [4, 18] by the pregnancy model suggested that it was suitable for application to predict EFV PK changes during pregnancy based on *CYP2B6* metaboliser status. Predicted mean C_{min} concentrations, during the three trimesters of pregnancy, are shown in Figure 5 and the impact of *CYP2B6* phenotypes on EFV PK parameters during T_1, T_2 and T_3 of pregnancy is shown in Table 5.

(A)



(B)



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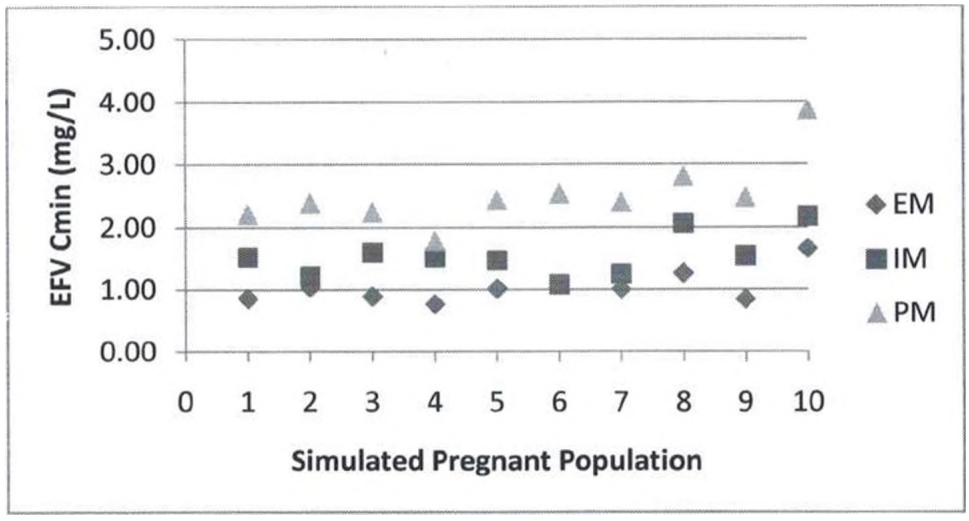

Figure 5

Simulated mean Efavirenz (EFV) trough concentrations (C_{min}) for extensive *CYP2B6* metabolisers (EM), intermediate *CYP2B6* metabolisers (IM) and poor *CYP2B6* metabolisers (PM), during T_1 (A), T_2 (B) and T_3 (C) of pregnancy.

Table 5

Effect of *CYP2B6* polymorphism on mean EFV PK during pregnancy based on the predicted EFV PK for T_1, T_2 and T_3.

THIRD TRIMESTER: 37 WEEKS				
PK parameter	**CL/F (l h^{-1})**	**AUC (mg h l^{-1})**	**C_{max} (mg l^{-1})**	**C_{min} (mg l^{-1})**
Simulation EM (***n* = 100**)	18.38	32.64	2.32	0.99 (0.60 – 1.53)
Simulation IM (***n* = 100**)	13.92	43.12	2.83	1.21 (0.81 – 2.03)
Simulation PM (***n* = 100**)	9.31	64.46	3.77	2.54 (1.81 – 3.88)
PM vs. EM: % change	49.35 %	- 97.49 %	- 62.50 %	- 156.57 %
IM vs. EM: % change	24.27 %	- 32.11 %	- 21. 98 %	- 22.22 %
SECOND TRIMESTER: 22 WEEKS				
PK parameter	**CL/F (l h^{-1})**	**AUC (mg h l^{-1})**	**C_{max} (mg l^{-1})**	**C_{min} (mg l^{-1})**
Simulation EM (***n* = 100**)	16.68	35.97	2.55	1.13 (0.67 – 1.73)
Simulation IM (***n* = 100**)	12.66	47.38	3.11	1.37 (0.91– 2.32)
Simulation PM (***n* = 100**)	8.57	70.05	4.09	2.90 (2.04 – 4.27)
PM vs. EM: % change	48.62 %	- 94.75 %	- 60.39 %	- 156.64 %
IM vs. EM: % change	24.10 %	- 31.72 %	- 21.96 %	- 21.24 %

FIRST TRIMESTER: 10 WEEKS				
PK parameter	**CL/F ($l\ h^{-1}$)**	**AUC ($mg\ h\ l^{-1}$)**	**C_{max} ($mg\ l^{-1}$)**	**C_{min} ($mg\ l^{-1}$)**
Simulation EM (***n* = 100**)	14.78	40.60	2.89	1.19 (0.71 – 1.85)
Simulation IM (***n* = 100**)	11.22	53.48	3.52	1.46 (0.96 – 2.47)
Simulation PM (***n* = 100**)	7.61	78.81	4.62	3.13 (2.18 – 4.60)
PM vs. EM: % change	48.51 %	- 94.11 %	- 59.86 %	- 163.03 %
IM vs. EM: % change	24.09 %	- 31.72 %	- 21.80 %	- 22.69 %

Data are geometric mean. EFV = efavirenz; PK = pharmacokinetic; CL/F = oral clearance; AUC = area under the plasma concentration time curve; C_{max} = maximum plasma concentration; C_{min} = EFV trough concentration; EM = extensive *CYP2B6* metabolisers; IM = intermediate *CYP2B6* metabolisers; PM = poor *CYP2B6* metabolisers.

PM vs. EM percentage change was calculated as follows:

$$\frac{EM - PM}{EM} x\ 100$$

IM vs. EM percentage change was calculated as follows:

$$\frac{EM - IM}{EM} x\ 100$$

The PBPK model was used to predict the extent to which EFV PKs were affected, by *CYP2B6* phenotype during each trimester of pregnancy (Table 5). During T_3, EFV CL/F was 49.35 % higher in EM, compared to PM (Table 5). EFV AUC, during T_3, was 97.49 % lower, in EM, compared to PM (Table 5). EFV CL/F in EM, during T_3, increased by 19.59 %, when compared to T_1 and increased by 9.05 % when compared to T_2 (Table 6). EFV AUC, in EM, during T_3 decreased by 7.45 % in T_2 and by 12.65 % in T_3 (Table 6). Predictions showed that during T_3, 50 % EM had EFV trough concentrations (C_{min}) below the accepted effective concentration of 1.0 mg l^{-1}. During T_1 and T_2, 45 % of EM experienced sub-therapeutic EFV plasma concentrations. The percentage of IM who had C_{min} values below the therapeutic range were 27.14 % in T_1, 30.71 % in T_2 and 41.43 % in T_3. Predictions of PM during T_1 did not demonstrate any sub-therapeutic EFV plasma concentrations and only 3.33 % of PM experienced sub-therapeutic C_{min} during T_2 and T_3.

Table 6

Percentage change in EFV PK during pregnancy in extensive, intermediate and poor *CYP2B6* metabolisers, based on simulated mean data reflected in Table 5.

EXTENSIVE *CYP2B6* METABOLIZERS				
	CL/F ($l\ h^{-1}$)	**AUC ($mg\ h\ l^{-1}$)**	**C_{max} ($mg\ l^{-1}$)**	**C_{min} ($mg\ l^{-1}$)**
T_1 vs. T_3: % change	19.59	- 24.39	- 24.57	- 20.20
T_2 vs. T_3: % change	9.25	- 10.20	- 9.91	- 14.14
INTERMEDIATE *CYP2B6* METABOLIZERS				
	CL/F ($l\ h^{-1}$)	**AUC ($mg\ h\ l^{-1}$)**	**C_{max} ($mg\ l^{-1}$)**	**C_{min} ($mg\ l^{-1}$)**
T_1 vs. T_3: % change	19.40	- 24.03	- 24.38	- 20.66
T_2 vs. T_3: % change	9.05	- 9.88	- 9.90	- 13.22
POOR *CYP2B6* METABOLIZERS				
	CL/F ($l\ h^{-1}$)	**AUC ($mg\ h\ l^{-1}$)**	**C_{max} ($mg\ l^{-1}$)**	**C_{min} ($mg\ l^{-1}$)**
T_1 vs. T_3: % change	18.26	- 22.26	- 22.55	- 23.23
T_2 vs. T_3: % change	7.95	- 8.67	- 8.49	- 14.17

EFV = efavirenz; PK = pharmacokinetic; T_1 = first trimester: 10 weeks; T_2 = second trimester: 22 weeks; T_3 = third trimester: 37 weeks; CL/F = oral clearance; AUC = area under the plasma concentration time curve; C_{max} = maximum plasma concentration; C_{min} = EFV trough concentration.

T_1 vs. T_3: percentage change was calculated as follows:

$$\frac{T3 - T1}{T3} \; x \; 100$$

T_2 vs. T_3 percentage change was calculated as follows:

$$\frac{T3 - T2}{T3} \; x \; 100$$

DISCUSSION

The PBPK model developed in this study was able to successfully predict EFV exposure, associated with *CYP2B6* EM, IM and PM phenotypes, following administration of a single dose of EFV (600 mg), as well as multiple doses, in non-pregnant, Caucasian subjects, as well as pregnant subjects in the third trimester of pregnancy.

Predicted single-dose (Table 2) and multiple dose (Table 3) EFV PKs demonstrated higher CL/F and lower AUC, C_{min} and C_{max} in EM when compared to PM, as observed clinically [4, 9, 10, 15, 16]. As with the observations made in two studies [4, 16] higher CL/F was predicted in IM compared with PM. However, no significant difference was observed in EFV exposure or CL/F between IM and PM, in a study conducted by Kwara *et al.* [15]. It is uncertain whether co-medication can explain this difference since the patients in the study by Kwara and co-workers [15] had both HIV and TB. In addition, the patients in the Kwara *et al.* [15] study were of African origin.

Differences in the predicted PKs in the third trimester of pregnancy in EM, IM and PM were similar to those observed in the clinical studies [16, 17, 19]. Increased CL/F of EFV in EM resulted in C_{min} values, at steady-state, that were very close to or below the therapeutic EFV C_{min} of 1.0 mg l^{-1} [19]. The findings are in accordance with the results reported by Dickinson *et al.* [16] who suggest that decreased C_{min} values in EM exposed patients, carrying the *CYP2B6* 516G→G phenotype (EM), to the risk of virological failure [16].

Olagunju *et al.* [4] reported a significant increase in CL/F in pregnant patients compared with postpartum patients [4]. The study conducted by Cressey *et al.* [17] also demonstrated increased EFV CL/F and reduced exposure during pregnancy, particularly during T_3 [17]. *CYP2B6* induction during pregnancy is the likely explanation for the observed phenotype-dependent differences in the magnitude of pregnancy-induced changes [4]. Weight increases, synonymous with the progression of pregnancy have also been reported to increase the CL/F of EFV [18].

The verified PBPK model was extrapolated to a pregnant population to simulate the PK profiles of EFV, during T_1 and T_2 for each phenotype. The predicted EFV PK demonstrated a progressive increase in CL/F and decrease in exposure (AUC, C_{min} and C_{max}) from T_1 to T_3 for all phenotypes, with the most significant increase in CL/F occurring in EM in T_3. The highest EFV CL/F (18.38 l h^{-1}) and lowest EFV AUC (32.64 mg h l^{-1}) were predicted in EM during T_3. Studies conducted by Olagunju *et al.* [4] and Dooley *et al.* [18] also demonstrated significantly lower AUC and C_{min} in EM during T_3 and sub-therapeutic EFV C_{min}, (below 1.0 mg l^{-1}) in pregnant women with the *CYP2B6* 516G→G phenotype (EM), during T_3 [4, 18].

C_{min} did not fall below the therapeutic range, in PM, during T_1 and a very small percentage of PM demonstrated sub-therapeutic C_{min}, during T_2 and T_3. The percentage of IM, with C_{min} below the therapeutic threshold, increased through the progression of pregnancy ($T_1 – T_3$), but not to the same extent as EM. As such, the standard daily dose of 600mg appears to be adequate for the IM and PM phenotypes. EM experienced sub-therapeutic concentrations of EFV throughout pregnancy; particularly during T_3 (50 % of patients), and thus it can be seen that dosage adjustments may be required in EM during T_3.

Pharmacogenetic testing may be a useful tool for optimising EFV dosage strategies during pregnancy. Dose adjustments must be carefully considered in pregnant populations and the altered drug PKs in the maternal body need to be understood in order to achieve the desired therapeutic effectiveness of drug treatment in pregnant women [3]. Considering the clinical and ethical concerns associated with clinical trials in a pregnant population, PBPK models are useful for predicting expected PK changes and the corresponding need for dosage adjustments. The results of this study lend support to the recommendation that increased EFV dosage regimens could be considered for pregnant women with EM status.

A limitation of this study was that the simulations were performed in a Caucasian pregnant population. Extrapolation of the model to other ethnic groups may be useful. A further limitation to the study was identified in that the study focused on the

CYP2B6 polymorphism in position 516G→T and *CYP2B6* mutations at positions 785A→G, 983C→T and 1459C→T were not explored. Recommendations for improvement on this study design would include the development of an African pregnancy population that accounts for differences in demographics, physiology, biochemistry and metabolism between the ethnic groups [30]. Further clinical studies focusing on the verification of the predictions made in this study, especially for T_1 and T_2, would be useful.

Furthermore the capability of the predictive model can be extended to evaluate untested drug dosages, drug-drug interactions and metabolic interactions associated with enzyme polymorphisms. The applicability of PBPK models to therapeutics can be further enhanced by combination with pharmacodynamics models [31].

In conclusion, this study demonstrates that PBPK modelling can be used to successfully predict the PK profiles of drugs in a pregnant population, based on CYP metaboliser phenotype. Such predictions are useful in identifying patient sub-groups who may require dosage adjustments.

REFERENCES

1. Shisana O, Rehle T, Simbayi LC, Zuma K, Jooste S, Zungu N, *et al.* South African national HIV prevalence, incidence and behaviour survey, 2012. Cape Town, HSRC Press: 2014.

2. Acosta. EP, Zorrilla C, Van Dyke R, Bardeguez A, Smith E, Hughes M, *et al.* Pediatric AIDS Clinical Trials Group 386 Protocol Team. Pharmacokinetics of saquinavir-SGC in HIV-infected pregnant women. HIV Clin Trials 2001; 2: 460 – 465.

3. Mirochnick M, Capparelli E. Pharmacokinetics of antiretrovirals in pregnant women. Clin Pharmacokinet 2004; 43: 1071 – 1087.

4. Olagunju A, Bolaji O, Amara A, Else L, Okafor O, Adejuyigbe E, *et al.* Pharmacogenetics of pregnancy-induced changes in efavirenz pharmacokinetics. Clin Pharmacol Ther2015; 97(3): 298 – 306.

5. Ke AB, Rostami-Hodjegan A, Zhao P, Unadkat JD. Pharmacometrics in pregnancy: an unmet need. Annu Rev PharmacolToxicol 2014; 54: 53 – 69.

6. Buckoreelall K, Cressey TR, King JR. Pharmacokinetic optimization of antiretroviral therapy in pregnancy. Clin Pharmacokinet 2012; 51: 639 – 659.

7. Meintjes G, Maartens G, Boulle A, Conradie F, Goemaere E, Hefer E, *et al.* Guidelines for antiretroviral therapy in adults, by the southern African HIV clinicians society. SAJHIVMED 2012; 13: 114 – 133.

8. Rekić D, Röshammar D, Mukonzo J, Ashton M. *In silico* prediction of efavirenz and rifampicin drug-drug interaction considering weight and CYP2B6 phenotype. Br J Clin Pharmacol 2010; 71: 536 – 543.

9. Xu C, Quinney SK, Guo Y, Hall SD, Li L, Desta Z. CYP2B6 pharmacogenetics-based *in vitro-in vivo* extrapolation of efavirenz clearance by physiologically based pharmacokinetic modelling. Drug Metab Dispos 2013; 41: 2004 – 2011.

10. Haas DW, Gebretsadik T, Mayo G, Menon UN, Acosta EP, Shintani A, *et al.* Associations between CYP2B6 polymorphisms and pharmacokinetics after a single dose of nevirapine or efavirenz in African Americans. J Infect Dis 2009; 199: 872 – 880.

11. Nyakutira C, Röshammar D, Chigutsa E, Chonzi P, Ashton M, Nhachi C, *et al.* High prevalence of the CYP2B6 516G→T(*6) variant and effect on the population pharmacokinetics of efavirenz in HIV/AIDS outpatients in Zimbabwe. Eur J Clin Pharmacol 2008; 64: 357 – 365.

12. Siccardi M, Almond L, Schipani A, Csajka C, Marzolini C, Wyen C, *et al.* Pharmacokinetic and pharmacodynamic analysis of efavirenz dose reduction using an *in vitro-in vivo* extrapolation model. Clin Pharmacol Ther 2012; 92: 494 – 502.

13. Naidoo P, Chetty VV, Chetty M. Impact of CYP polymorphisms, ethnicity and sex differences in metabolism on dosing strategies: the case of efavirenz. Eur J Clin Pharmacol 2014; 70(4): 379 – 389.

14. Cabrera SE, Santos D, Valverde MP, Domínguez-Gil, González F, Luna G, *et al.* Influence of the cytochrome P450 2B6 genotype on population pharmacokinetics of efavirenz in human immunodeficiency virus patients. Antimicrob Agents Chemother 2009; 53: 2791 – 2798.

15. Kwara A, Lartey M, Sagoe KW, Xexemeku F, Kenu E, Oliver-Commey J, *et al.* Pharmacokinetics of efavirenz when co-administered with rifampin in TB/HIV co-infected patients: pharmacogenetic effect of CYP2B6 variation. J Clin Pharmacol 2008; 48: 1032 – 1040.

16. Dickinson L, Amin J, Else L, Boffito M, Egan D, Owen A, Khoo S, *et al.* Pharmacokineticandpharmacodynamiccomparisonofonce-dailyefavirenz(400 mg vs. 600 mg) in treatment-naïve HIV-infected patients: results of the ENCORE1 study. Clin Pharmacol Ther 2015; 98(4): 406 – 416.

17. Cressey TR, Stek A, Capparelli E, Bowonwatanuwong C, Prommas S, Sirivatanapa P, *et al.* Efavirenz pharmacokinetics during the third trimester of pregnancy and postpartum. J Acquir Immune Defic Syndr 2012; 59: 245 – 252.

18. Dooley KE, Denti P, Martinson N, Cohn S, Mashabela F, Hoffmann J, *et* al. Pharmacokinetics of efavirenz and treatment of HIV-1 among pregnant women with and without tuberculosis co-infection. J Infect Dis 2015; 211: 197 – 205.

19. Marzolini C, Telenti A, Decosterd LA, Greub G, Biollaz J, Buclin T. Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. AIDS 2001; 15: 71–75.

20. Kostewicz ES, Aarons L, Bergstrand M, Bolger MB, Galetin A, Hatley O, *et al.* PBPK Models for the Prediction of In Vivo Performance of Oral Dosage Forms. Eur J Pharm Sci 2014; 57: 300 – 321.

21. Ke AB, Nallani SC, Zhao P, Rostami-Hodjegan A, Unadkat JD. A PBPK model to predict the disposition of CYP3A-metabolized drugs in pregnant women: verification and discerning the site of CYP3A induction. CPT Pharmacomet Syst Pharmacol 2012; 1: e3.

22. De Sousa Mendes M, Hirt D, Urien S, Valade E, Bouazza N, Foissac F, *et al.* Physiologically-based pharmacokinetic modelling of renally excreted antiretroviral drugs in pregnant women. Br J ClinPharmacol 2015; 80: 1031 – 1041.

23. Ke AB, Nallani SC, Zhao P, Rostami-Hodjegan A, Isoherranen N, Unadkat JD. A PBPK model to predict disposition of CYP2D6 CYP1A2 metabolized drugs in pregnant women. Drug Metab Dispos 2013; 43: 801 – 813.

24. Gaohua L, Abduljalil K, Jamei M, Johnson TN, Rostami-Hodjegan A. A pregnancy physiologically based pharmacokinetic (p-PBPK) model for disposition of drugs metabolized by CYP1A2, CYP2D6 and CYP3A4: PBPK for pregnancy with time-varying physiological parameters. Br J Clin Pharmacol 2012; 74: 873 – 885.

25. Lang T, Klein K, Fischer J, Nüssler AK, Neuhaus P, Hofmann U,Eichelbaum M, *et al.* Extensive genetic polymorphism in the human CYP2B6 gene with impact on expression and function in human liver. Pharmacogenetics 2001; 11: 399 – 415.

26. Jamei M, Marciniak S, Feng K, Barnett A, Tucker G, Rostami-Hodjegan A. The simcyp population-based ADME simulator. Expert Opin Drug Metab Toxicol 2009; 5: 211 – 223.

27. Ke AB, Nallani SC, Zhao P, Rostami-Hodjegan A, Unadkat JD. Expansion of a PBPK model to predict disposition in pregnant women of drugs cleared via multiple CYP enzymes, including CYP2B6, CYP2C9 and CYP2C19: PBPK prediction of PK changes during pregnancy. Br J ClinPharmacol 2014; 77: 554 – 570.

28. Ke A, Barter Z, Rowland-Yeo K and Almond L. Towards a Best Practice Approach in PBPK Modelling: Case Example of Developing a Unified Efavirenz Model Accounting for Induction of CYPs 3A4 and 2B6. CPT Pharmacometrics Syst Pharmacol 2016; 5(7): 367 – 376.

29. Abduljalil K, Cain T, Humphries H, Rostami-Hodjegan A. Deciding on success criteria for predictability of pharmacokinetic parameters from *in vitro* studies: an analysis based on *in vivo* observations. Drug MetabDispos 2014; 42: 1478 – 1484.

30. Burger D, van der Heiden I, la Porte C, van der Ende M, Groeneveld P, Richter C, *et al.* Interpatient variability in the pharmacokinetics of the HIV non-nucleoside reverse transcriptase inhibitor efavirenz: the effect of gender, race and *CYP2B6* polymorphism. Br J ClinPharmacol 2006; 61(2): 148 – 154.

31. Chetty M, Rose RH, Abduljalil K, Patel N, Lu G, Cain T, *et al.* Applications of linking PBPK and PD models to predict the impact of genotypic variability, formulation differences, differences in target binding capacity and target site drug concentrations on drug responses and variability. Front Pharmacol 2014; 26;5:258

APPENDICES

Table 5: Calculations

Table 6: Calculations

Waiver of Ethical Clearance (W-CJ-160829-1)

Approval for Change of Title

Table 5

Effect of *CYP2B6* polymorphism on mean EFV PK during pregnancy based on the predicted mean EFV PK for T_1, T_2 and T_3.

THIRD TRIMESTER: 37 WEEKS				
PK parameter	**CL/F (l h^{-1})**	**AUC (mg h l^{-1})**	**C_{max} (mg l^{-1})**	**C_{min} (mg l^{-1})**
Simulation EM (***n* = 100**)	18.38	32.64	2.32	0.99 (0.60 – 1.53)
Simulation IM (***n* = 100**)	13.92	43.12	2.83	1.21 (0.81 – 2.03)
Simulation PM (***n* = 100**)	9.31	64.46	3.77	2.54 (1.81 – 3.88)
PM vs. EM: % change	$\frac{18.38-9.31}{18.38} x\ 100 = 49.35$	$\frac{32.64-64.46}{32.64} x\ 100 = -97.49$	$\frac{2.32-3.77}{2.32} x\ 100 = -62.50$	$\frac{0.99-2.54}{0.99} x\ 100 = -156.57$
IM vs. EM: % change	$\frac{18.38-13.92}{18.38} x\ 100 = 24.27$	$\frac{32.64-43.12}{32.64} x\ 100 = -32.11$	$\frac{2.32-2.83}{2.32} x\ 100 = -21.98$	$\frac{0.99-1.21}{0.99} x\ 100 = -22.22$
SECOND TRIMESTER: 22 WEEKS				
PK parameter	**CL/F (l h^{-1})**	**AUC (mg h l^{-1})**	**C_{max} (mg l^{-1})**	**C_{min} (mg l^{-1})**
Simulation EM (***n* = 100**)	16.68	35.97	2.55	1.13 (0.67 – 1.73)
Simulation IM (***n* = 100**)	12.66	47.38	3.11	1.37 (0.91– 2.32)
Simulation PM (***n* = 100**)	8.57	70.05	4.09	2.90 (2.04 – 4.27)

PM vs. EM: % change	$\frac{16.68 - 8.57}{16.68} x\ 100 = 48.62$	$\frac{35.97 - 70.05}{35.97} x\ 100 = -94.75$	$\frac{2.55 - 4.09}{2.55} x\ 100 = -60.39$	$\frac{1.13 - 2.90}{1.13} x\ 100 = -156.64$
IM vs. EM: % change	$\frac{16.68 - 12.66}{16.68} x\ 100 = 24.10$	$\frac{35.97 - 47.38}{35.97} x\ 100 = -31.72$	$\frac{2.55 - 3.11}{2.55} x\ 100 = -21.96$	$\frac{1.13 - 1.37}{1.13} x\ 100 = -21.24$
FIRST TRIMESTER: 10 WEEKS				
PK parameter	**CL/F (l h^{-1})**	**AUC (mg h l^{-1})**	**C_{max} (mg l^{-1})**	**C_{min} (mg l^{-1})**
Simulation EM (***n* = 100**)	14.78	40.60	2.89	1.19 (0.71 – 1.85)
Simulation IM (***n* = 100**)	11.22	53.48	3.52	1.46 (0.96 – 2.47)
Simulation PM (***n* = 100**)	7.61	78.81	4.62	3.13 (2.18 – 4.60)
PM vs. EM: % change	$\frac{14.78 - 7.61}{14.78} x\ 100 = 48.51$	$\frac{40.60 - 78.81}{40.60} x\ 100 = -94.11$	$\frac{2.89 - 4.62}{2.89} x\ 100 = -59.86$	$\frac{1.19 - 3.13}{1.19} x\ 100 = -163.03$
IM vs. EM: % change	$\frac{14.78 - 11.22}{14.78} x\ 100 = 24.09$	$\frac{40.60 - 53.48}{40.60} x\ 100 = -31.72$	$\frac{2.89 - 3.52}{2.89} x\ 100 = -21.80$	$\frac{1.19 - 1.46}{1.19} x\ 100 = -22.69$

EFV = efavirenz; PK = pharmacokinetic; CL/F = oral clearance; AUC = area under the plasma concentration time curve; C_{max} = maximum plasma concentration; C_{min} = EFV trough concentration; EM = extensive CYP2B6 metabolisers; IM = intermediate CYP2B6 metabolisers; PM = poor CYP2B6 metabolisers.

PM vs. EM percentage change was calculated as follows:

$$\frac{EM - PM}{EM} x\ 100$$

IM vs. EM percentage change was calculated as follows:

$$\frac{EM - IM}{EM} x\ 100$$

Table 6

Percentage change in EFV PK during pregnancy in extensive, intermediate and poor *CYP2B6* metabolisers, based on simulated mean data reflected in Table 5.

EXTENSIVE *CYP2B6* METABOLIZERS				
PK parameter	**CL/F (l h^{-1})**	**AUC (mg h l^{-1})**	**C_{max} (mg l^{-1})**	**C_{min} (mg l^{-1})**
T_1 vs. T_3: % change	$\frac{18.38-14.78}{18.38} x\ 100 = 19.59$	$\frac{32.64-40.60}{32.64} x\ 100 = -24.39$	$\frac{2.32-2.89}{2.32} x\ 100 = -24.57$	$\frac{0.99-1.19}{0.99} x\ 100 = -20.20$
T_2 vs. T_3: % change	$\frac{18.38-16.68}{18.38} x\ 100 = 9.25$	$\frac{32.64-35.97}{32.64} x\ 100 = -10.20$	$\frac{2.32-2.55}{2.32} x\ 100 = -9.91$	$\frac{0.99-1.31}{0.99} x\ 100 = -14.14$
INTERMEDIATE *CYP2B6* METABOLIZERS				
PK parameter	**CL/F (l h^{-1})**	**AUC (mg h l^{-1})**	**C_{max} (mg l^{-1})**	**C_{min} (mg l^{-1})**
T_1 vs. T_3: % change	$\frac{13.92-11.22}{13.92} x\ 100 = 19.40$	$\frac{43.12-53.48}{43.12} x\ 100 = -24.03$	$\frac{2.83-3.52}{2.83} x\ 100 = -24.38$	$\frac{1.21-1.46}{1.21} x\ 100 = -20.66$
T_2 vs. T_3: % change	$\frac{13.92-12.66}{13.92} x\ 100 = 9.05$	$\frac{43.12-47.38}{43.12} x\ 100 = -9.88$	$\frac{2.83-3.11}{2.83} x\ 100 = -9.90$	$\frac{1.21-1.37}{1.21} x\ 100 = -13.22$
POOR CYP2B6 METABOLIZERS				
PK parameter	**CL/F (l h^{-1})**	**AUC (mg h l^{-1})**	**C_{max} (mg l^{-1})**	**C_{min} (mg l^{-1})**
T_1 vs. T_3: % change	$\frac{9.31-7.61}{9.31} x\ 100 = 18.26$	$\frac{64.46-78.81}{64.46} x\ 100 = -22.26$	$\frac{3.77-4.62}{3.77} x\ 100 = -22.55$	$\frac{2.54-3.13}{2.54} x\ 100 = -23.23$
T_2 vs. T_3: % change	$\frac{9.31-8.57}{9.31} x\ 100 = 7.95$	$\frac{64.46-70.05}{64.46} x\ 100 = -8.67$	$\frac{3.77-4.09}{3.77} x\ 100 = -8.49$	$\frac{2.54-2.90}{2.54} x\ 100 = -14.17.$

EFV = efavirenz; PK = pharmacokinetic; T_1 = first trimester: 10 weeks; T_2 = second trimester: 22 weeks; T_3 = third trimester: 37 weeks; CL/F = oral clearance; AUC = area under the plasma concentration time curve; C_{max} = maximum plasma concentration; C_{min} = EFV trough concentration.

T_1 vs. T_3: percentage change was calculated as follows:

$$\frac{T3 - T1}{T3} \; x \; 100$$

T_2 vs. T_3 percentage change was calculated as follows:

$$\frac{T3 - T2}{T3} \; x \; 100$$

Human Research Ethics Committee (Medical)

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Ref: W-CJ-160829-1.(Title change) 29/08/2016

Original Ref: W-CJ-151106-4.

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigator: **Andrea Julsing (student no 765092)**

Project title: **A physiologically based pharmacokinetic model to characterise the association between CYP2B6 polymorphisms and Efavirenz pharmacokinetics in pregnancy.**

Reason: This study uses information in the public domain. There are no human participants.

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