Title

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A mechanism-based population pharmacokinetic analysis assessing the feasibility of efavirenz dose reduction to 400 mg in pregnant women.

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Abstract:

BACKGROUND:

Reducing the dose of efavirenz may improve safety, reduce costs, and increase access for patients with HIV infection. According to the World Health Organization, for universal roll-out, a similar dosing strategy for all patient populations is desirable. It remains unknown whether the 400mg daily dose is adequate during pregnancy.

METHODS:

We developed a mechanistic population pharmacokinetic model using pooled data from seven studies (1968 samples, 774 collected during pregnancy). Total and free efavirenz exposure ($AUC_{0\text{-}24h}$ and C_{12}) were predicted for 400mg (reduced) and 600mg (standard) doses in pregnant and non-pregnant women.

RESULTS:

With 400mg, median (IQR) efavirenz total $AUC_{0\text{-}24}$ and C_{12} during third trimester were 92% and 88% of values among non-pregnant women, respectively. Median free efavirenz C_{12} and $AUC_{0\text{-}24}$ were predicted to increase during pregnancy by 12% and 17%, respectively.

CONCLUSIONS:

It was predicted that reduced-dose efavirenz provides adequate exposure during pregnancy.

INTRODUCTION

In the past twenty years, the development of effective and safe interventions for the prevention of mother-to-child transmission (PMTCT) of HIV-1 has been one of the great successes in global and public health. (1) New HIV infections among children have decreased by 58% since 2000, and 73% of HIV-positive pregnant women had access to antiretroviral therapy in 2014. Currently, lifelong treatment for all pregnant and breastfeeding women living with HIV, regardless of CD4 cell count or World Health Organization (WHO) clinical stage is now recommended in WHO antiretroviral treatment guidelines.(2) In parts of the world where HIV is most prevalent, the antiretroviral drug efavirenz is a key component of antiretroviral treatment and PMTCT of HIV. This is due to its excellent antiviral potency, long-term efficacy, once-daily dosing, generic availability and substantial data demonstrating its efficacy and safety during pregnancy. (3)

To date, the standard 600mg efavirenz dose has been approved by regulatory authorities such as the FDA and recommended by major HIV treatment guidleines. (4, 5) However, t~~T~~here has been global interest in reducing the standard efavirenz dose, in part to avoid drug toxicities, but largely to reduce cost. (6) A 33% dose reduction may translate into three-year cost savings of up to US$336 million (7), which could be critical in the efforts to advance universal access to antiretroviral therapy for HIV-infected individuals. The ENCORE1 study was performed to assess the efficacy of a reduced-dose efavirenz (400mg once-daily (QD)) versus standard of care (600 mg QD). In this study, conducted in non-pregnant, treatment-naïve adults, reduced-dose efavirenz was non-inferior to the standard dose in terms of virologic response. (8)

Lower efavirenz doses will inevitably lead to lower efavirenz exposures. Efavirenz mid-dose interval (MDI) concentrations lower than 0.7-1 mg/L have been associated with virological failure. (9, 10) Although the reductions in exposure seen with 400 mg efavirenz QD versus 600 mg were not clinically-important in non-pregnant adults, the pharmacokinetics of antiretroviral drugs may be altered as a result of pregnancy-induced changes in anatomy and physiology (e.g. body composition, gastrointestinal function, protein plasma concentration, and metabolic activity), leading to a higher risk of sub-therapeutic exposures in that population. (11) This, in turn, may lead to treatment failure, emergence of drug-resistance, and mother-to-child transmission of HIV. (11) Thus, it is essential to get

the drug dosing right in pregnant women. Efavirenz is highly albumin bound (>99%) and primarily metabolized by the hepatic cytochrome 2B6 enzyme (CYP2B6). (4) Consequently, pregnancy-induced alterations in plasma albumin concentrations or hepatic enzyme activities could change the pharmacokinetics. (12) In fact, sSeveral studies have investigated the impact of pregnancy on the pharmacokinetics of efavirenz 600 mg QD. Although most studies found reduced efavirenz exposure during pregnancy compared to postpartum for the 600 mg QD regimen, the reductions were modest and unlikely to be clinically relevant. (13-15) However, to date no studies have been conducted to assess the adequacy of drug exposures with a 400 mg dose in pregnancy.

The WHO strives to recommend a limited formulary of preferred treatment options that is applicable across all patient populations, and this knowledge gap regarding low-dose efavirenz pharmacokinetics during pregnancy is an important barrier towards universal roll-out of reduced-dose efavirenz. (6) As it is pivotal to bridge this knowledge gap, we performed a mechanistic pharmacokinetic analysis of efavirenz in pregnant and non-pregnant women to assess the adequacy of efavirenz exposure when reducing the efavirenz dose.

RESULTS

In addition to the well-stirred liver model, a 2-compartment disposition model with first-order elimination and absorption through three absorption transit compartments best described the data (Figure 1). Inter-individual variability was included for CL_{int}/F and MAT. Inter-occasion variability was included for F. The residual error structure was proportional. We explored separate error models for different studies, but the changes were minor and did not result in changes in parameter estimates. Hence this strategy was abandoned. Overall, no indication of bias was observed.

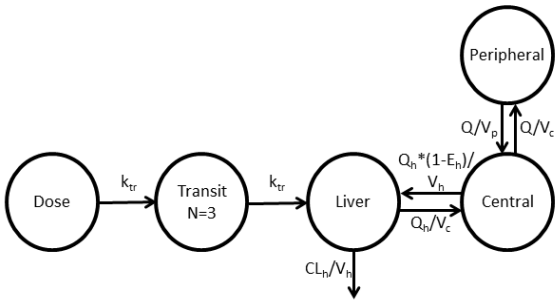

Figure 1. Final structural model. Efavirenz is absorbed through 3 transit compartments into the liver compartment, based on 4 identical first-order rate constants (k_{tr}). For the first-pass through the liver a fraction of the efavirenz amount is extracted (E_h) and cleared, the fraction of the amount remaining ($1-E_h$) reaches the systemic circulation and becomes available for redistribution into the peripheral compartment. Efavirenz recirculates from the central compartment to the liver with a flow equivalent to liver plasma flow (Q_h), and at each pass the liver extracts a further fraction (E_h).

Initially the mixture population frequencies were estimated. This led to model instability, and stochastic simulation and estimation showed that the population frequencies of the mixture could not be numerically identified. Therefore, population frequencies were fixed to 14, 36 and 50% for the SM, IM and EM, based on available data on race or region combined with known prevalence of the CYP2B6 genotypes (c.516G>T) (ΔOFV -309; $p<0.001$). (16, 17) Efavirenz has properties related to auto-induction, but this could not be identified because almost all data available contained information at steady-state only. (4) Final population estimates are shown in Table 2.

Table 2. Final parameter estimates

Parameter	Parameter estimate	RSE (%)	RSE (%) from SIR
MAT (h) MAT (h) pregnant	2.12 1.67	(7) (2)	(7) (4)
CL_{int}/F (L/h)[$] - Poor - Intermediate - Extensive	 1380 3340 4580	 (6) (8) (6)	 (7) (6) (5)
V_c/F (L)[$]	133	(7)	(6)
V_p/F (L)[$]	390	(5)	(6)
Q/F (L/h)[$]	35	(7)	(7)
F (%) relative to non-pregnant	116	(5)	(4)
IIV CL_{int}/F (%)	32	(7)	(14)
IIV MAT (%)	44	(8)	(15)
IOV F (%)	24	(4)	(12)
Proportional residual error (%)	18	(1)	(5)

[$]The values refer to a typical individual of 70kg. MAT, mean absorption time (3 transit compartments); CL_{int}/F, intrinsic clearance; V_c/F, central volume of distribution; V_p/F, peripheral volume of distribution; Q/F, inter-compartmental clearance; F, relative bioavailability. IIV, inter-individual variability; IOV, inter-occasion variability; SIR, sampling importance resampling; RSE, relative standard error.

Based on the fixed mechanistic relations that we incorporated *a priori* the pregnancy-related decrease in albumin concentration over gestational age led to an increase in the fraction of unbound EFV. In turn, this led to an increased apparent hepatic efavirenz clearance over gestational age. The *a priori* implementation of this relationship was accompanied by a ΔOFV of -53. With univariate testing of pregnancy on all pharmacokinetic parameters, associations were found for V_c (ΔOFV -22; p<0.001), F (ΔOFV -15; p<0.001), and MAT (ΔOFV -35; p<0.001). Forward inclusion and stepwise elimination led to the inclusion of parameter-pregnancy relationships for MAT and F (total ΔOFV -49; p<0.001). Standard goodness-of-fit plots of the final model indicated no bias in the structural model or unaccounted heterogeneity in the data (Figure 2). A pcVPC stratified for pregnancy based on 500 samples is shown in Figure 3. The pcVPC indicated that the model has internal predictive value in terms of both structural and stochastic model components. The pcVPC stratified for pregnancy based on 500 samples for the external model evaluation is shown in Figure 4. This visual diagnostic indicated that the model developed based on the data from studies 2 to 7 adequately described the data from study 1. This was further supported by the evaluation of the observations NPDE based on 2500

samples, as the null hypothesis (a N(0,1) distribution) could not be rejected based on the three statistics specified in the method section, using a 10% significance level (P>0.1). This indicated that besides internal predictive performance, the developed model has adequate external predictive performance and, altogether, qualified the model for further use in the simulation phase of this study.

An *a posteriori* power evaluation using Monte Carlo Mapped Power (available in PsN), based on the number of paired (pregnant versus non-pregnant) observations available in our dataset, indicated >80% power to detect pregnancy covariate effects (≥20%) for all structural model parameters, except for those associated with the peripheral compartment (data not shown). (18)

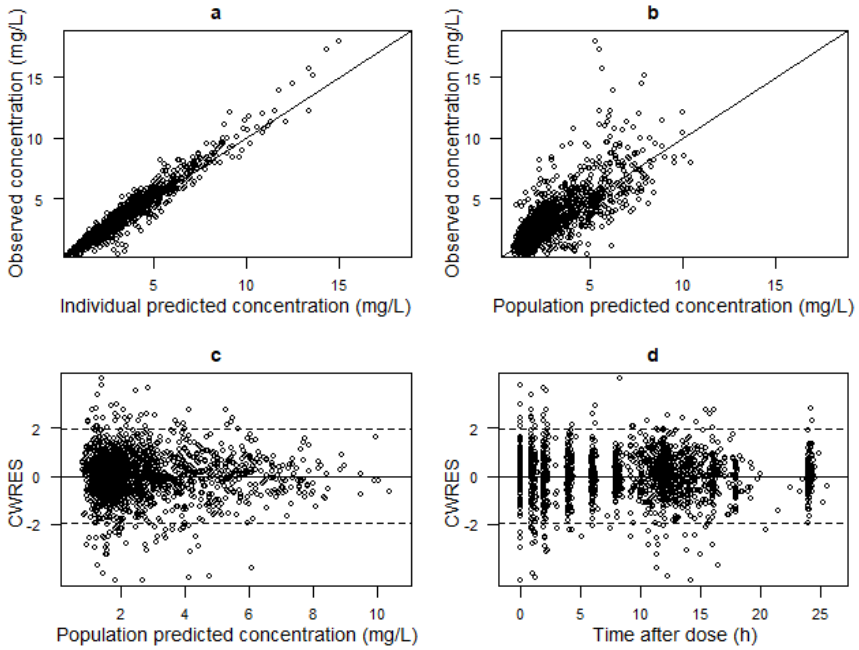

Figure 2. Standard goodness-of-fit plots for the final model: a.) observed concentration versus individual predicted concentration around the line of unity; b.) observed concentration versus population predicted concentration around the line of unity; c.) conditional weighted residual (CWRES) versus population predicted concentrations; d.) conditional weighted residual versus time after dose. The dotted lines represent the 95% limits of the assumed CWRES distribution (i.e. 0 ± 1.96).

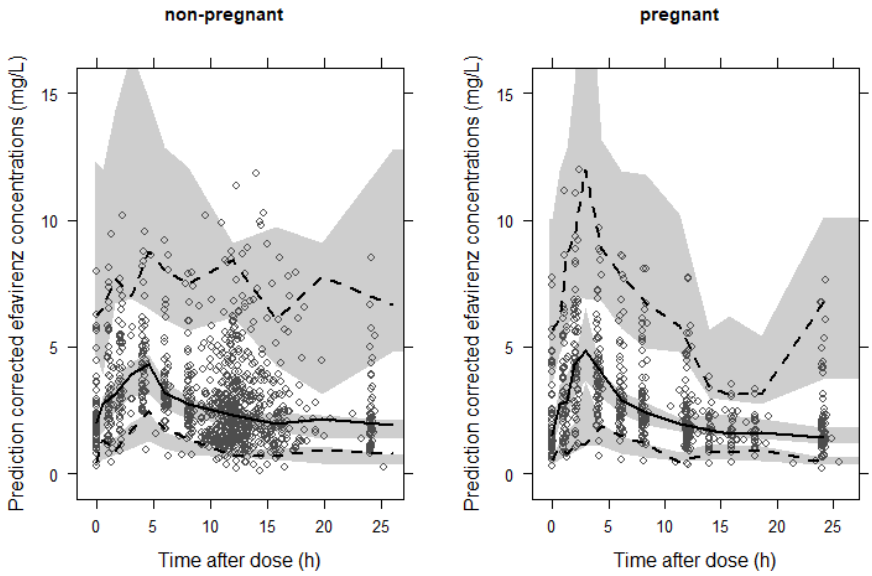

Figure 3. pcVPC of final model for efavirenz 600 mg stratified for pregnancy. The observations are indicated by the open circles. The median (continuous line) and 5th and 95th percentiles (dashed line) of the observations are shown, as well as the confidence interval around the median and 5th and 95th percentiles of the simulated data (grey shaded areas).

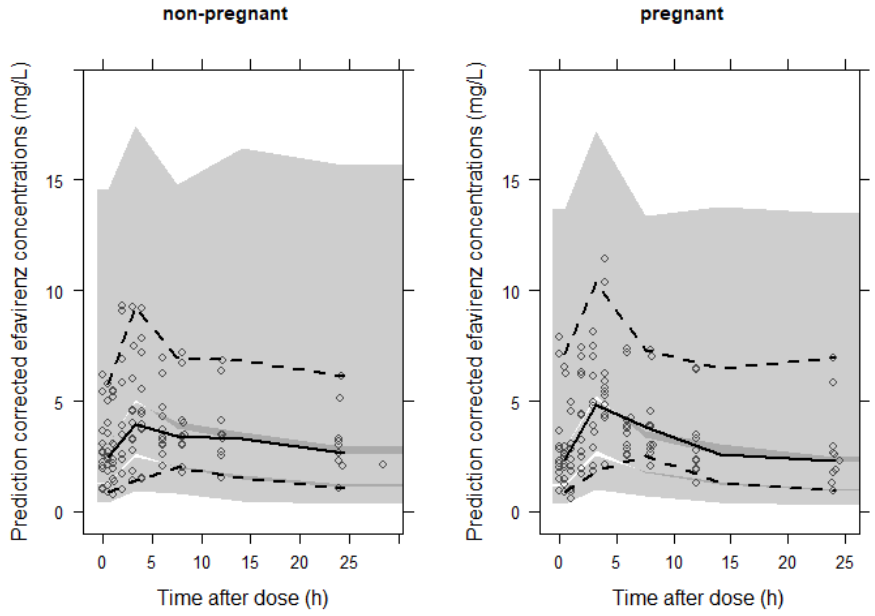

Figure 4. pcVPC of final model describing external data from study 1, stratified for pregnancy. The observations are indicated by the open circles. The median (continuous line) and 5th and 95th percentiles (dashed line) of the observations are shown, as well as the confidence interval around the median and 5th and 95th percentiles of the simulated data (grey shaded areas).

The simulated total EFV steady-state pharmacokinetic parameters ($AUC_{0\text{-}24}$ and C_{12}) following oral administration of efavirenz 600 mg and 400 mg QD are shown in Table 3, stratified for pregnancy, as well as metabolizer status. During third trimester of pregnancy median $AUC_{0\text{-}24}$ and C_{12} over all phenotypes were 91% and 87% when compared to non-pregnant women, respectively. The simulated total C_{12} during pregnancy compared to non-pregnant women, stratified by phenotype, are plotted in Figure 5A. More sub-therapeutic C_{12} were predicted during third trimester of pregnancy as compared to non-pregnant women for all phenotypes, except the poor metabolizers. The percentage of ~~Following efavirenz 600 mg QD administration to non-pregnant women 0%, 3% and 9% of~~ total C_{12} ~~were~~ below 0.7 mg/L or 1 mg/L for SM, IM, and EM, are reported in Table 3. ~~The simulated total C_{12} during pregnancy compared to non-pregnant women, stratified by phenotype, are plotted in Figure 5A. respectively. Following efavirenz 600 mg QD administration to women during third trimester of pregnancy, 0%, 7% and 23% had a simulated total C_{12} below 1 mg/L for SM, IM, and EM, respectively. Of non-pregnant women, 0%, 15% and 41% had a total C_{12} below 1 mg/L following administration of efavirenz 400 mg QD, for SM, IM, and EM, respectively. Simulated total C_{12} following efavirenz 400 mg QD were below 1 mg/L during third trimester of pregnancy in 0%, 23%, and 53% of women for SM, IM and EM, respectively.~~

~~**Table 3.** Median (IQR) total efavirenz exposure ($AUC_{0\text{-}24}$ and C_{12}) and the percentage of simulated C_{12} below 1 and 0.7 mg/L, following administration of efavirenz 400 mg and 600 mg QD to pregnant (third trimester) and non-pregnant women, stratified for metabolizer status.~~

~~more sub-therapeutic C_{12} were predicted during third trimester of pregnancy as compared to non-pregnant wome~~

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Table 3. Median (IQR) total efavirenz exposure (AUC_{0-24} and C_{12}) and the percentage of simulated C_{12} below 1 and 0.7 mg/L, following administration of efavirenz 400 mg and 600 mg QD to pregnant (third trimester) and non-pregnant women, stratified for metabolizer status.

Parameter	PM	IM	EM
Non-pregnant			
Efavirenz 600 mg QD			
AUC (mg/h*L)	154 (121-194)	63 (50-80)	46 (37-61)
C_{12} (mg/L)	6.1 (4.6-7.9)	2.4 (1.8-3.2)	1.7 (1.2-2.3)
C_{12} < 1 mg/L	0%	3%	9%
C_{12} < 0.7 mg/L	0%	0%	2%
Efavirenz 400 mg QD			
AUC (mg/h*L)	103 (81-130)	42 (33-54)	31 (24-41)
C_{12} (mg/L)	4.1 (3.1-5.2)	1.6 (1.2-2.1)	1.1 (0.81-1.5)
C_{12} < 1 mg/L	0%	15%	41%
C_{12} < 0.7 mg/L	0%	4%	14%
Pregnant, third trimester			
Efavirenz 600 mg QD			
AUC (mg/h*L)	140 (110-177)	57 (45-73)	42 (33-56)
C_{12} (mg/L)	5.4 (4.1-7.0)	2.1 (1.6-2.8)	1.4 (1.0-2.0)
C_{12} < 1 mg/L	0%	7%	23%
C_{12} < 0.7 mg/L	0%	1%	5%
Efavirenz 400 mg QD			
AUC (mg/h*L)	93 (73-118)	38 (30-49)	28 (22-37)
C_{12} (mg/L)	3.9 (2.7-4.7)	1.4 (1.1-1.9)	1.0 (0.69-1.4)
C_{12} < 1 mg/L	0%	23%	53%
C_{12} < 0.7 mg/L	0%	8%	26%

PM, poor metabolizer; IM, intermediate metabolizer; EM, extensive metabolizer

The simulated free C_{12} concentrations, based on the individual predicted fraction unbound, though, were not lowered by pregnancy. Instead, the median free efavirenz C_{12} concentrations is predicted to be increased during pregnancy by 11% (Figure 5B). Overall, median free efavirenz exposure ($AUC_{0-24,free}$) is predicted to be 15% higher during pregnancy.

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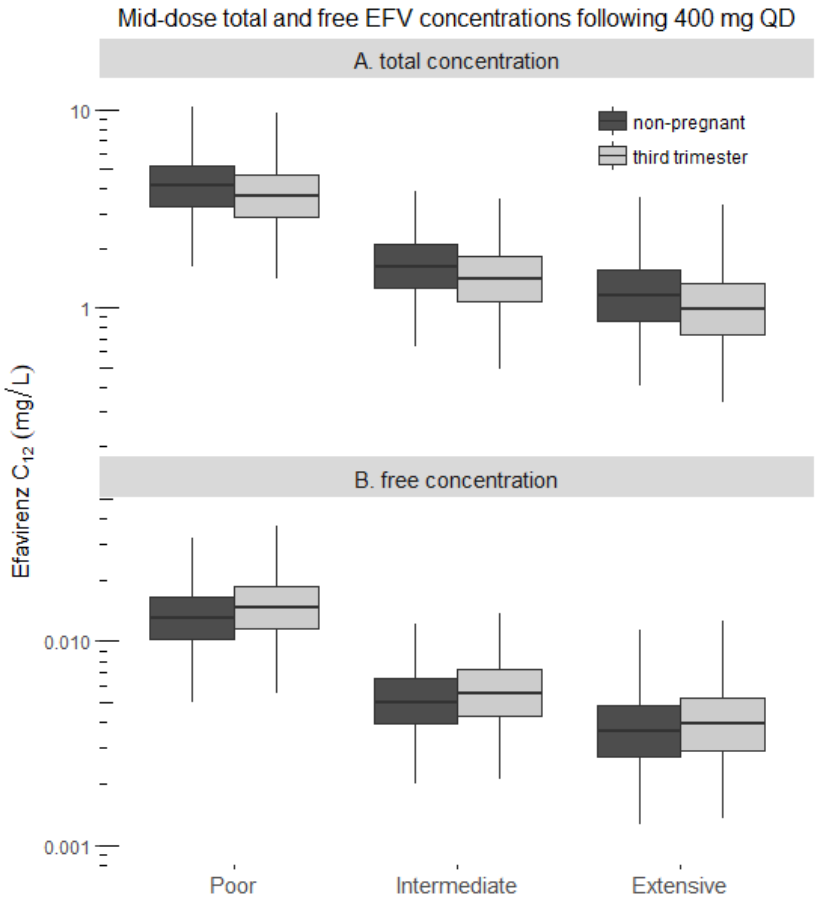

Figure 5. Simulated total (A) and free (B) concentrations following administration of 400 mg efavirenz QD during third trimester of pregnancy and for non-pregnant women, stratified by metabolizer status.

DISCUSSION

In this study we found a modest effect of pregnancy on the efavirenz total $AUC_{0\text{-}24}$ and C_{12}, a 9% and 13% reduction during third trimester of pregnancy compared to non-pregnant women, respectively. However, fortunately, the predicted free efavirenz exposure was not decreased during pregnancy. This indicates that any decrease in total efavirenz concentrations following 400 mg QD, is unlikely to be clinically relevant since only the free efavirenz concentration is available for the pharmacological effect at the site of action.

Achieving adequate efavirenz exposure during pregnancy is essential to prevent treatment failure, selection of drug-resistance and prevention of MTCT of HIV. (11) Previous pharmacokinetic studies have indicated that pregnancy-related effects on the standard efavirenz 600 mg QD regimen are limited and of minor clinical relevance (13, 14). In the current study, for the newly proposed efavirenz 400 mg QD regimen, an increase in the proportion of women having sub-therapeutic total drug concentrations was predicted during third trimester of pregnancy. Efavirenz C_{12} below 0.7 mg/L was predicted for 19% of women with EM metabolizer status during third trimester of pregnancy as compared to 9% for non-pregnant women. Although for efavirenz 400 mg QD the rate of C_{12} below 0.7 mg/L was predicted to be twice as high during third trimester of pregnancy, the difference is mostly restricted to the EM subpopulation and, in absolute terms, is small (median C_{12} of 1.0 vs. 1.1 mg/L).

Importantly, because efavirenz is highly albumin-bound (>99%) and only the free concentrations (at the target site) are related to the pharmacological effects, conclusions solely based on total concentrations may be biased. Ideally the free efavirenz concentrations during pregnancy would be measured but no such data were available for modeling and we relied on model predictions to distinguish between total and free efavirenz concentrations. As no additional pregnancy-related covariate effects on hepatic clearance were identified, the increase in hepatic clearance during pregnancy can be primarily ascribed to the pregnancy-related increase in fraction unbound. Physiologically, this indicates the absence of significant and relevant pregnancy-induced efavirenz biotransformation, such as induction of the major efavirenz metabolizing enzyme CYP2B6. Although pregnancy-related induction of CYP2B6 has been suggested based on *in vitro* assays, to date this has not been confirmed *in vivo*. (19) Since efavirenz has a low extraction ratio, changes in fraction

unbound would not be expected to alter free efavirenz concentrations. (20) Consequently, the predicted free efavirenz concentrations were not decreased during the third trimester of pregnancy. Even a slight increase in free efavirenz exposure (C_{12} and AUC_{0-24}) was predicted. This was related to alterations in efavirenz relative bioavailability and mean absorption time during pregnancy. Physiologically, this could be ascribed to relatively low efavirenz solubility and expected bioavailability (40-45%; absolute bioavailability never determined, also implicating that the estimates of intrinsic clearance should be interpreted as the apparent intrinsic clearance). (21) Reduced small intestine motility in pregnant women could increase the incomplete efavirenz absorption and maintain higher intestinal concentration gradients. (12) Additionally, increased blood flow to the gastro-intestinal tract resulting from increased cardiac output during pregnancy may result in an increased absorption rate and decreased mean absorption time. (22) This has been previously observed in population pharmacokinetic analysis. (23)

A major strength of the current study was the availability of the largest set of efavirenz pharmacokinetic data from pregnant and non-pregnant women compiled to date. Although overall there exists consensus that pregnancy-related changes in efavirenz 600 mg QD pharmacokinetics are of minor clinical relevance, this was not at all a clear case for efavirenz 400 mg QD. (6) For a model-based investigation of the efavirenz dose reduction to 400 mg QD in pregnancy, accurate identification of the pregnancy-related effects on primary pharmacokinetic parameters was essential. Given that efavirenz pharmacokinetics are highly variable and the effects of pregnancy are relatively small, a large sample size is needed for sufficient power to detect these effects. (13) Smaller studies with sometimes less informative design may not have been capable to identify these effects, but pooling the data from multiple sources allowed us to investigate these effects with higher statistical power.

Pooling data also comes at a cost as it may introduce bias related to inter-study differences. For example, a large part of data was from studies with cross-over design (i.e. intra-subject comparison). (13, 14, 24, 25) The postpartum assessment served as the control for the non-pregnant situation, and although this design provides a powerful intra-subject comparison, it can be questioned to what extent pregnancy-induced physiological processes have normalized during the early post-partum period, and, further, the timing of the postpartum assessment may vary between studies. Fortunately, in our study,

post-partum samples were mostly taken between 4 and 6 weeks after delivery, and previous work indicated that this time span is sufficient for relevant physiological processes to normalize, and no remaining effects on pharmacokinetics have been observed, allowing us to pool these data with other datasets from non-pregnant women. (26). The impact of such inter-study differences was monitored by means of stepwise integration of data from different sources and continued goodness-of-fit evaluation. Because the number of studies included in this analysis was still limited, we did not include inter-study variability. (27)

Another strength of this study is its mechanism-based nature. Where purely empirical modeling of total concentrations would have led us to the conclusion that the pregnancy-related effects on efavirenz 400 mg QD are modest and probably not relevant, our mechanism-based approach allowed us to take inferences one step further. Namely, our analysis suggests that even if exposure in terms of total concentrations may be affected, free concentrations are unlikely to be decreased and free efavirenz exposure following 400 mg QD is, thus, sufficient during pregnancy. To reach such a conclusion, it was of paramount importance to ensure that the incorporated mechanistic information was valid and reasonable. To ensure that the inclusion of mechanistic information relied on evidence and quality, ~~(and were not just added willy-nilly to the model during the model-building process)~~ we pre-specified all mechanistic information to be included in the model. Additionally, as opposed to full 'bottom up' physiologically-based pharmacokinetic models, the mechanistic model development was still informed by a large clinical dataset. This allowed us to statistically test the mechanistic relations included and prevented us from enforcing effects that were absent in the (clinical) data. For example, the pregnancy-related change in fraction unbound increased hepatic efavirenz clearance. Though seemingly more complex, this is basically a time-varying parameter-covariate relationship between gestational age and hepatic clearance, through predicted albumin levels and fraction unbound. If for some reason the relationship between gestational age and hepatic clearance had been non-existent or in the opposite direction, this would have been picked up during the covariate testing of pregnancy on hepatic clearance.

Limitations of this study were that pharmacodynamic data were not available (e.g. viral load) from the vast majority of the studies included. This limited our ability to assess the exposure-response

relationship in this particular population. Consequently, we relied on target concentrations for efavirenz established in previous pharmacokinetic-pharmacodynamic analyses. A long standing efavirenz target total drug concentration is 1 mg/L. (9) In the ENCORE1 study however, the lower 400 mg QD dose was non-inferior to standard 600 mg dose despite more observed sub-therapeutic exposure defined as <1 mg/L. (28) This indicates that this threshold is not fully evidence-based and most likely conservative. Therefore, we used the lower target concentration of 0.7 mg/L for evaluation of the simulated C_{12} that has been proposed recently. (10) Importantly, there is no free drug target for efavirenz, yet the concentration of pharmacologically-available drug is likely what drives treatment response. (20) Another limitation is that data on individual CYP2B6 genotype were available only from one study. (14) Still, we were able to differentiate between metabolic phenotypes using the mixture model. (29) As mentioned previously, free efavirenz concentrations were not determined. Also, the individual plasma albumin concentrations were not available, and we relied on predicted population albumin concentration based on gestational age for the prediction of free efavirenz concentrations.

To conclude, our model predicts a modest decrease in total efavirenz exposure during the third trimester of pregnancy. For efavirenz 400 mg QD this decrease seems of minor clinical relevance. Moreover, the model predicted free, pharmacologically active, efavirenz exposure was not decreased. Currently, a prospective pharmacokinetic study with the reduced-dose efavirenz in pregnant women is being conducted (NCT02499874). Ideally, this study includes measurements of free efavirenz as well as serum albumin concentrations . When the outcomes of this trial ~~Once~~ confirme~~sd *in-vivo*, these~~ our findings, ~~suggest that~~ the proposed dose reduction to 400mg EFV can be extended to pregnant women as well.

METHODS

We conducted a mechanism-based population pharmacokinetic analysis. In such analyses some elements of the model are fixed based on available physiological and mechanistic information, as in physiologically-based pharmacokinetic modeling. Other elements of the model, that can be obtained from the data, are estimated using the population approach. This has been referred to as the 'middle-out' approach. (30) One of the main advantages is that such models provide a rationale to extrapolate to special populations such as pregnancy, based on pregnancy-related physiology. (31) Additionally,

418 the outcomes may point the way to further studies, provide deeper mechanistic understanding, and
419 allow for mechanistic inferences. (32)
420
421 *General workflow*
422 In short, the modeling process consisted of the following steps; 1.) review of efavirenz
423 pharmacokinetics and relevant physiology-related changes during pregnancy, 2.) select mechanistic
424 information to include in the modeling process and develop the plan of analysis, 3.) collect and pool
425 data for analysis, 4.) develop a population pharmacokinetic model using non-linear mixed effects
426 modeling, including covariate analysis, informed by step 2, 5.) model evaluation and qualification for
427 the purpose of this study, 6.) apply model to investigate exposure with the efavirenz 400 mg dose
428 through simulation.
429
430 *Pharmacokinetic data*
431 Data from six studies (studies 2-7; Table 1) that included pregnant and non-pregnant women taking
432 efavirenz were pooled. The datasets were pooled sequentially. Data from non-pregnant women were
433 added first to evaluate the general structural and stochastic aspects of the model. Next, data from
434 pregnant women were added to incorporate the pregnancy-related covariate effects into the model. At
435 each step the structural model was re-evaluated and the effect of pregnancy was implemented and
436 investigated.
437
438 In total, 1968 plasma samples were available from 258 women, of which 774 samples were taken
439 during pregnancy (n=142). Women using potentially interacting concomitant medicines (e.g. rifampicin
440 or isoniazid) were excluded. (14) All except five of the patients included received the standard 600 mg
441 efavirenz QD. Patient characteristics for each study are summarized in Table 1.
442
443 *Mechanistic information used for pharmacokinetic modeling*
444 Based on a review of published efavirenz pharmacokinetic data and relevant pregnancy-related
445 changes in physiology, we took into account the following considerations and made the following
446 decisions prior to the modeling process. This was pre-specified in an analysis plan that was circulated
447 to all coauthors involved.

Efavirenz is primarily metabolized by the liver and <1% is renally excreted as unchanged drug. (4) To account for the relationship between hepatic systemic and first-pass metabolism, we implemented a well-stirred liver model [eq.1&2].(33)

$$CL_{hep}/F = Q_{hep,plasma} * E_h \qquad [\text{eq. } 1]$$

$$E_h = \frac{CL_{int,hep} \cdot f_u}{Q_{hep,plasma} + CL_{int,hep} \cdot f_u} \qquad [\text{eq. } 2]$$

Apparent hepatic clearance (CL_{hep}/F; F = bioavailability) is expressed as a function of hepatic plasma flow ($Q_{hep,plasma}$) and hepatic extraction ratio (E_h). E_h is defined as a function of apparent intrinsic hepatic clearance ($CL_{int,hep}/F$), and fraction unbound (f_u). With regards to $CL_{int,hep}/F$ (i.e. enzyme pool), cytochrome P450 2B6 genetic polymorphisms have a clinically relevant impact on the extent of efavirenz biotransformation. (34) Therefore, we assumed three subpopulations (metabolic phenotypes): poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM). If individual CYP2B6 genotype was available, the women were assigned to a subpopulation based on the classification proposed in Dooley et al. (14) Additionally, pregnancy can induce enzymatic pathways, but the available evidence was not sufficiently convincing to, *a priori,* assume pregnancy-related induction of CYP2B6. (22)

Since efavirenz is highly albumin-bound (>99%), changes in albumin plasma concentrations can result in relatively large differences in f_u and, consequently, CL_{hep}/F. (35) This has been previously observed for other drugs. (36) Another known factor affecting CL_{hep}/F during pregnancy is an increased $Q_{hep,plasma}$. This is related to a decrease in hematocrit (Ht) during pregnancy. (22) Additionally, cardiac output is higher during pregnancy, potentially translating into an increased hepatic blood flow (Q_{hep}). Based on the current body of literature, however, we could not describe the magnitude or relevance of changes in Q_{hep} during pregnancy and, therefore, this was not included and fixed to the literature values (109 L/h) for non-pregnant women. (22, 37) Pregnancy-induced increase in $Q_{hep,plasma}$ [eq.3] and decrease in f_u [eq.4] were included *a priori* using the following relations:

$$Q_{hep,plasma} = (1 - Ht) \cdot Q_{hep} \qquad [\text{eq. } 3]$$

$$f_u = \frac{k_D}{(k_D + [P])} \qquad [\text{eq. } 4]$$

Efavirenz protein (albumin)-binding dissociation constant (k_D) was fixed to the *in vitro* literature value, 2.05 µM. (35) For efavirenz, the range of free concentrations encountered *in vivo* is much lower than the k_D. (38) This implies linear binding and a fraction unbound independent of the free efavirenz concentration. (20) Polynomial relations describing the relationship between gestational age (GA) and albumin concentrations (P) [eq.5] as well as Ht [eq.6] were used to predict pregnancy-induced changes in f_u and $Q_{hep,plasma}$, respectively, on a population level.(22, 38)

$$[P(\mu M)] = \frac{(45.8 - 0.1775 \cdot GA - 0.0033 \cdot GA^2)}{0.07} \quad [\text{eq. } 5]$$

$$[Ht(v/v\ \%)] = 39.1 - 0.0544 \cdot GA - 0.0021 \cdot GA^2 \quad [\text{eq. } 6]$$

Population pharmacokinetic analysis

Data were analyzed using NONMEM® 7.3.0 (ICON Development Solutions, Hanover, MD, USA). The first-order conditional estimation method was used with eta–epsilon interaction. We used Pirana 2.9.1 (http://www.pirana-software.com) as an interface for NONMEM to structure and document model development (39); R version 3.2.2 (with Rstudio interface version 1.0.136) for data preparation, and graphical visualization and evaluation; and Perl Speaks Nonmem 4.6.0 for automation of a diverse range of processes related to model development. (40)

Several population pharmacokinetic models have been developed for efavirenz, but most were purely empirical and not based on data from pregnant women. A model developed previously by Dooley et al. (14) was both semi-mechanistic and based on data from pregnant women. Hence this model was suitable as a starting point for further development. For the structural model, including the well-stirred liver model, we tested 1 to 3-compartmental distribution. Models tested to describe absorption included zero- and first-order processes and implementation of transit compartments to describe a gradual onset of absorption. The transit rate constant (k_{tr}) for the transit compartments was estimated and the mean absorption time (MAT) was calculated based on equation 7,

$$k_{tr} = (n + 1)/MAT \quad [\text{eq. } 7]$$

where *n* equals the number of transit compartments. (41) Because no data were available that allowed estimation of absolute bioavailability the typical value of bioavailability was fixed to 1. For the

estimation of model parameters we assumed log-normal distributions for the inter-individual variability (IIV) and inter-occasion variability (IOV) according to the equation 7,

$$\theta_i = \theta \cdot e^{(\eta_i)} \quad [\text{eq. } 8]$$

where θ_i is the individual parameter value, θ is the typical population value, and η_i is the random effect (IIV or IOV) drawn from a normal distribution with mean 0 and variance ω^2. Different residual error models with additive, proportional, and combined error structures were tested.

To account for body weight-induced changes in pharmacokinetics *a priori*, all flow parameters and volumes were scaled to a total non-pregnant body weight of 70 kg according to allometric theory. The allometric exponents were fixed to ¾ for flow parameters and 1 for volumes of distribution. (42, 43)

Structured covariate analysis

Pregnancy was tested as covariate (dichotomous) on all model parameters using a forward inclusion and backward elimination approach. The covariate selection was based on scientific and physiological plausibility and on maximum likelihood statistics (quantified by the objective function value [OFV]) with a 5% significance level (dOFV> -3.84) applied for likelihood ratio testing of nested models. Backward elimination was based on a 1% significance level (dOFV > -6.64). The Akaike information criterion was used for comparison of non-nested models.

Handling of missing covariates and data below lower limit of quantification

Only one study included data for participant height. Consequently, we did not explore and test the relation between model parameters and body size descriptors other than weight (e.g. fat-free mass).

Data on CYP2B6 genotype in our population were limited (18%). A mixture model was implemented to account for the multi-modal distribution of CL_{int}/F as a result of CYP2B6 polymorphisms by imputing the missing CYP2B6-related phenotypes; poor (PM), intermediate (IM) and extensive (EM) metabolizers. Subjects with missing genotype were assigned to the mixture (subpopulation) with the highest individual probability. (29)

The number of plasma concentrations below the lower limit of quantification (LLOQ) for each individual study was very low (<1%). This is mainly because the LLOQ was generally much lower than the

concentrations clinically observed. Given the limited amount of data below LLOQ, these data were ignored. For a description of the methods of bioanalysis we refer to the primary study reports (Table 1).

Model evaluation and qualification

We evaluated precision in parameter estimates and standard goodness-of-fit plots. For the final model, parameter uncertainty was obtained from the default covariance step in NONMEM as well as the sampling importance resampling (SIR) procedure. (44) To further evaluate and qualify the model for simulation we used prediction corrected visual predictive checks (pcVPC). (27) pcVPCs aim to adjust for the variability related to the fixed effects. In case of a model including a mixture, prediction correction cannot be done in a standard way, since there can be one population prediction for each subpopulation to which the subject can be assigned. To account for this, we employed a strategy proposed previously for nevirapine. (27) Additionally, we conducted an external model evaluation in line with best practice to further qualify ~~the model for simulation~~the developed model. For this, data on file from study 1 were used (details in Table 1). External model performance was visually evaluated based on pcVPC and statistically based on the observations NPDE, under the null hypothesis that the model developed based on studies 2 to 7 (learning) adequately describes the data from study 1: the NPDE follow a N(0,1) distribution. This hypothesis was tested based on three statistics as proposed by Brendel at al.: 1.) a student t-test, to test whether the mean is significantly different from 0; 2.) a Fisher test for variance, to test whether the variance is significantly different from 1; 3.) a Shapiro–Wilks test, to test whether the distribution is significantly different from a normal distribution. (45, 46)

Simulation

The final model was used to simulate efavirenz concentrations for women during third trimester of pregnancy and non-pregnant women. Third trimester of pregnancy was chosen since the risks of mother-to-child transmission are highest during late pregnancy and labor. (47) Also, absolute differences in pharmacokinetics are expected to be highest during third trimester. Simulations (500x for each phenotype) were performed for efavirenz 400 mg and 600 mg QD, assuming linear pharmacokinetics over this dosing range.(4) Bodyweights used for simulation were randomly drawn from a log-normal distribution with geometric mean ± geometric standard deviation of 62±1.3 kg,

based on the distribution found in our data. Gestational age during third trimester of pregnancy was drawn from a normal distribution with mean±sd of 34±2.3 weeks, based on the distribution found in our data. Secondary steady-state pharmacokinetic parameters of total and free concentrations at steady state ($AUC_{0\text{-}24h}$ and C_{12}) were derived. The C_{12} were then compared to the suggested mid-dose target concentrations for efavirenz pharmacotherapy, 1 mg/L (9), and more recently, 0.7 mg/L (10).

Study highlights

What is the current knowledge on the topic?

Reduced-dose efavirenz (400mg) is non-inferior to standard-dose efavirenz (600mg) for HIV treatment, and may be less toxic Dose reduction can lower costs, facilitating universal treatment access.

What question did this study address?

According to the World Health Organization, for universal roll-out, a similar dosing strategy for all patient populations is desirable. Pregnancy impacts efavirenz pharmacokinetics. Is efavirenz exposure with the reduced-dose adequate for pregnant women?

What this study adds to our knowledge?

Pregnancy is associated with a minimal decrease in total efavirenz exposure, but predicted free (pharmacologically active) exposure is not decreased. Reduced-dose efavirenz likely provides adequate efavirenz exposure during pregnancy.

How this might change clinical pharmacology or translational science?

Inferences based on mechanistic pharmacokinetic models can have high impact, in this case supporting the universal roll-out of reduced-dose efavirenz, including among pregnant women. Reduced toxicity, lower cost, and increased universal access to antiretroviral treatment may result.

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Author contributions

SS Wrote Manuscript, Designed Research, Performed Research, Analyzed Data

RtH Wrote Manuscript, Designed Research, Performed Research, Analyzed Data

ACC Wrote Manuscript, Designed Research, Performed Research

ADRH Designed Research, Performed Research

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KED Wrote Manuscript, Designed Research, Performed Research

EC Designed Research, Performed Research, Analyzed Data

BMB Designed Research, Performed Research,

TRC Wrote Manuscript, Designed Research, Performed Research,

RG Wrote Manuscript, Performed Research

FGMR Wrote Manuscript, Performed Research

MM Designed Research, Performed Research

DMB Designed Research, Performed Research

626 **Table 1.** Patient and study characteristics summarized by study (reference).

	Study 1 (24)	Study 2 (48)	Study 3 (49)	Study 4 (50)	Study 5 (13)	Study 6 (25)	Study 7 (14)
Number or patients	14	1091	25	172	25	27	97
Number of patients included	11	129	7	14	25	26	46
Number of samples - Pregnant - Not pregnant	 110 109	 NA 541	 NA 77	 NA 23	 224 199	 317 199	 123 46
Median (range) gestational age at sampling times	34 (32 – 36)	NA	NA	NA	34 (29 – 38)	29 (21 – 37)	37 (33 – 39)
Sampling design (h postdose)	Rich cross-over: 0 (pre-dose),0.5,1,2,3,4,6,8,12,24	Sparse: mid-dose	Rich: 0(pre-dose),1, 2,3,4,6,8,10,12,16,24	Sparse: mid-dose	Rich cross-over: 0 (pre-dose),1,2,4,6,8,12,24	Rich cross-over: 0 (pre-dose),1,2,4,6,8,12,24	Sparse cross-over: mid-dose
Lower limit of quantification (mg/L)	0.05	0.05	0.05	0.01	0.03	0.03	0.02
Median (range) weight - Second trimester - Third trimester - Not pregnant	 NA 69 (45 – 124; n=11) 76 (50 – 132; n=11)	 NA NA 60 (40 – 100)	 NA NA 53 (46 – 64)	 NA NA 60 (49 – 71)	 78 (69 – 89; n=3) 69 (40 – 130; n=25) 63 (37 – 125; n=25)	 83 (54 – 129; n=14) 80 (55 – 128; n=26) 74 (47 – 126; n=25)	 NA 72 (52 – 112; n=33) 67 (42 – 105; n=39)
CYP2B6 Phenotype - Poor metabolizer - Intermediate metabolizer - Extensive metabolizer	Not determined	Not determined	Not determined	Not determined	Not determined	Not determined	1 not determined 10 25 26
Efavirenz dose	600 mg	600 mg	600 mg	600 mg (300 mg; n=1, 400 mg; n=1)	600 mg (800 mg; n=3)	600 mg	600 mg
Population	100% Black	Mixed international (Thai, South Africa, South America, Western Countries)	100% Black	100% Caucasian	84% Thai, 16% Caucasian	56% Hispanic, 4%Unknown, 40% Non-Hispanic	100% Black

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Supplementary figure of NPDE external evaluation. Quantile–quantile plot of NPDE versus the expected standard normal distribution (upper left). Histogram of NPDE with the density of the standard normal distribution overlayed (upper right). Scatterplot of NPDE versus time after dose (lower left). Scatterplot of NPDE versus predicted concentrations (lower right).