CYP2B6 GENOTYPE BASED EFAVIRENZ DOSE RECOMMENDATIONS DURING RIFAMPICIN-BASED ANTITUBERCULOSIS CO-TREATMENT FOR A SUB-SAHARAN AFRICA POPULATION

Article *in* British Medical Journal Global Health · February 2017

DOI: 10.1136/bmjgh-2016-000260.102

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Pharmacogenomics

CYP2B6 genotype-based efavirenz dose recommendations during rifampicin-based antituberculosis cotreatment for a sub-Saharan Africa population

Aim: To assess genotype effect on efavirenz (EFV) pharmacokinetics, treatment outcomes and provide genotype-based EFV doses recommendations during for tuberculosis (TB)-HIV-1 cotreatment. **Materials & methods:** EFV concentrations from 158 HIV-TB co-infected patients treated with EFV/lamivudine/zidovidine and rifampicin were analyzed. Genotype and CD4 and viral load data were analyzed using a population PK model. **Results:** Simulated AUCs for 600 mg EFV dose were 1.2- and 2.4-times greater than the product label for Ugandans in general and *CYP2B6*6/*6* genotypes respectively. EFV daily doses of 450 and 250 mg for Ugandans and *CYP2B6*6/*6* genotypes, respectively, yielded simulated exposures comparable to the product label. **Conclusions:** Around 450 and 250 mg daily doses might meet EFV dosing needs of HIV-TB infected Ugandans in general and *CYP2B6*6/*6* genotypes, respectively.

First draft submitted: 27 March 2015; Accepted for publication: 26 January 2016; Published online: 4 April 2016

Keywords: African population • cotreatment • dose recommendations • efavirenz • HIV-TB

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Efavirenz (EFV) is currently the recommended choice non-nucleoside reverse transcriptase inhibitor (NNRTI) for HIV patients, particularly during cotreatment with rifampicin [1]. As a result, EFV has been extensively used as part of antiretroviral therapy (ART). Its use in sub-Saharan Africa (SSA) has further scaled up following the recent WHO recommendation of EFV-based ART during pregnancy for prevention of mother to child transmission of HIV. However, as a result of pharmacogenetic variations, EFV exhibits wide between population pharmacokinetic variability [2]. Significant gene-dependent drug–drug pharmacokinetic interactions between EFV and rifampicin result in variation in exposure and possible differences in dose requirements occur [3].

EFV is primarily metabolized to 8-hydroxyefavirenz mainly by CYP2B6 and to a lesser extent by CYP3A [4]. CYP2A6 mediated 7-hydroxylation of EFV constitutes the secondary metabolic pathway [5,6]. Importance of CYP2A6 [6,7] and CYP3A [8] for EFV metabolism particularly in CYP2B6 slow metabolizers is described. P-glycoprotein, encoded by *ABCB1*, is the major efflux transporter at the blood–brain barrier that limits entry into the CNS for a large number of drugs, and probably contributes to patient-to-patient variability in response to CNS pharmacotherapy [9]. Although *in vitro* and animal studies report that P-glycoprotein as not the main cellular transporter protein for EFV [10], several studies in African HIV patients reported association of genetic variation in *ABCB1* with EFV exposure and/or treatment outcomes. Associations between *ABCB1* c.4036A>G with higher plasma EFV concentrations in Ugandan HIV patients was first reported by our group [11], a finding latter confirmed in South African [12], Ethiopians, Tanzanians [2] and other non-African populations [13]. Association of *ABCB1* c.3435C>T

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variation with CD4-cell recovery after EFV therapy initiation has also been reported [13,14]. Accordingly P-glycoprotein may have a role in EFV cellular transport. Alternatively the functional genetic variants in *ABCB1* might be in strong linkage disequilibrium with other SNPs located in another gene(s) relevant for EFV disposition and hence my serve as tag SNP. All of the major EFV metabolizing enzymes and *ABCB1* are genetically polymorphic with their functional genetic variants exhibiting wide population frequency differences probably leading to variations in dosing requirements [15].

Rifampicin is a potent inducer of many genes coding for drug metabolizing enzymes and transporters, including CYP3A, CYP2B6 and the drug efflux pump P-glycoprotein, encoded by *ABCB1* [16,17]. Previous studies conducted mainly in white population reported a 22–26% reduction of EFV plasma concentrations during co-administration with rifampicin due to enzyme induction [18,19]. As a result, a suggestion to increase EFV dose by 30% during concomitant rifampicin-based anti-TB therapy was made [19,20]. However more recent studies, conducted in SSAs populations, reported neither significant differences in EFV concentrations with or without rifampicin-based anti-TB cotreatment [21–24], nor significant difference in virologic response between HIV patients treated with EFV in presence or absence of rifampicin [21,25,26]. While the magnitude of rifampicin effect on both EFV exposure and treatment outcome seem to respectively depend upon genotype and allelic frequency at individual and population levels, no dose requirement studies have been conducted to this effect. We constructed a population pharmacokinetic–pharmacogenetic model using steady state EFV concentrations in HIV-TB co-infected patients receiving rifampicin-based anti-TB therapy to: describe genetic effects on EFV steady state pharmacokinetics during cotreatment with rifampicin, estimate the population pharmacokinetic parameters for EFV exposure and used simulations to determine an optimal EFV dose for HIV-TB co-infected Ugandans and *CYP2B6* and *ABCB*1 genotypes during rifampicin cotreatment.

Subjects & methods

The current study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from Makerere University College of Health Sciences Institutional Review Board and The Uganda National Council for Science and Technology. Each participant gave written informed consent. A total of 1216 EFV concentration data points collected from 158 HIV-TB co-infected patients (76 females) over 252 days following initiation of EFV-based HAART were used for the PK analysis. HIV treatment constituted an oral daily dose of 600 mg EFV (Stocrin®; Merck, Sharpe & Dohme, NJ, USA) plus zidovidine/lamivudine (150/300 mg). Participants also received ethambutol/isoniazid/rifampicin/pyrazinamide for 2 months followed by 4 months of isoniazid and rifampicin combination therapy for TB. Anti-TB treatment was initiated 2–8 weeks before ART. In addition, subjects received prophylactic trimethoprim/sulfamethoxazole treatment. Mid-dose EFV plasma concentration samples (11–18 h after the last dose) were collected on about eight different occasions per subject over the study period. CD4 counts and HIV–1 RNA cells/ml measures were performed at baseline and months 3 and 6. *CYP2B6* (**6* & **11*), *CYP2A6*9*, *CYP3A5 (*3, *6* & **7*) and *ABCB1* (c.4046A>G and c.3435C>T) genotype analysis was performed for all participants.

Bio-analysis

EFV pharmacokinetic analysis

Blood samples were collected into EDTA tubes and prepared for analysis by centrifugation at 3000 rpm for 10 min and stored at -70°C until HPLC analysis was performed as described previously [11]. Plasma EFV was determined by reverse phase HPLC with UV detection. The HPLC instrument, Agilent series 1100, consisted of a column compartment G1316A, Degasser G132A, Quat pump G1311A and an auto-sampler ALS, G1329A and G1315B diode array detector. An Ace3C18, 3 µm 50 × 30 mm (Advanced Chromatography Technologies, Aberdeen, Scotland) column was used. EFV (99.9%), supplied by the WHO Collaborating center for chemical reference substances through Apoteket AB (Stockholm, Sweden) was used as the standard. The retention time for EFV was 2. Forty-two minutes as detected at UV–VIS 1, 210 nm, UV–VIS 2, 220 nm. This method was linear, with a within-day coefficient of variation of 3.2, 3.3 and 5.1% at concentrations of 2.0 mM (n = 17), 8.0 mM (n = 17) and 20 mM (n = 16), respectively, and a between-day coefficient of variation of 4.1% (n = 50).

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes using QIAamp DNA Maxi Kit (QIAGEN GmbH, Hilden, Germany). All participants were genotyped for *CYP2B6*6* and **11*, *CYP3A5*3*,**6* and **7* and *ABCB1* (3435CT and rs3842), *CYP2A6*9*. SNP selections, apart from ABCB1 (3435C>T), was based in their role in EFV pharmacokinetics according to our previous report [11]. *ABCB1* 3435C>T was selected on basis of previous conflicting reports on its role in pharmacokinetics and pharmacodynamics

of ART. Allelic discrimination reactions were performed using TaqMan® (Applied Biosystems, CA, USA) genotyping assays: (C___7586657_20 for *ABCB1* 3435C>T, C___7817765_60, for *ABCB1 rs3842T>C*, C__29560333_20, for *CYP2B6* 516G>T [*CYP2B6*6*], for *CYP2B6* 136A>G [*CYP2B6*11*], C__26201809_30 for *CYP3A5* 6986A>G [*CYP3A5*3*], C__30203950_10 for *CYP3A5*14690G>A [*CYP3A5*6*]), C__32287188_10 for *CYP3A5* g.27131_27132insT (*CYP3A5*7*) and C_30634332_10 for *CYP2A6 -48T>G* (*CYP2A6*9*) on ABI 7500 FAST (Applied Biosystems, CA, USA). The final volume for each reaction was 10 µl, consisting of 2× TaqMan Universal PCR Master Mix (Applied Biosystems), 20× drug metabolizing genotype assay mix and 10 ng genomic DNA. The PCR profile consisted of an initial step at 50°C for 2 min and 50 cycles with 95°C for 10 min and 92°C for 15 s.

Data analysis

Pharmacokinetic model development

A population PK model of EFV was built using nonlinear mixed-effect modeling (NONMEM™, version 7.3.0, ICON plc, Dublin, Ireland). The software packages ggplot2 (Version 9.3.1), PsN 3.4.2 and R (Version 3.0.1) were used for dataset construction, graphical and statistical analysis. Pharmacokinetic parameter estimates were obtained using first-order conditional method with interaction (FOCEI). A one-compartment model with first-order absorption and elimination specified in NONMEM by the ADVAN2 and TRANS2 subroutines was fitted to Log-transformed EFV plasma concentrations. Log transformed concentrations were used in order to stabilize the model and improve the efficiency of parameter estimation. Estimated fixed-effect PK parameters included the apparent clearance (CL/F), relative bioavailability (F1) between ABCB1 groups and the apparent distribution volume (V/F). Model discrimination was based on relative objective function values (OFV), precision of parameter estimates and goodness-of-fit plots. Interindividual variability (IIV) was included on Cl/F and V/F with exponential error models. Residual error was described with a proportional error model.

Covariate analysis

Covariate analysis was performed using a forward-selection (α = 0.05) followed by backward elimination (α = 0.01) method. Gender, baseline body weight and pharmacogenetic covariates including *CYP2B6* (**6* and **11*), *CYP3A5* and *ABCB1* (c.4046A>G and c.3435C>T) were tested for significance of effect on absorption coefficient (KA), oral clearance (CL/F) and volume of distribution (V/F) parameter. Each covariate-parameter relationship was first tested in a univariate manner. Covariates with one, two and three degrees of freedom were included in the forward selection if they reduced the OFV by at least 3.84, 5.99 and 7.81, respectively, corresponding to a p-value of <0.05 for a χ^2 distribution. The full covariate model was reached when addition of further covariate-parameter relationships did not decrease the OFV to the specified criteria. The covariate-parameter relationships were re-examined in the backward deletion step in a manner similar to the forward inclusion step but reversed and with a more conservative significance level of α = 0.01. *ABCB1* c.4046A>G was included as a covariate on relative bioavailability (F1) based on previous findings, reported elsewhere [11].

Estimates of Exposures

For each patient, EFV trough concentrations and areas under the curve were derived from the estimated individual pharmacokinetic parameter estimates as shown in Equations 1 & 2, respectively.

$$C_{24\,h} = \frac{F1 * DOSE * KA}{V(KA - K)}\left[\frac{e^{-24K}}{1 - e^{-24k}} - \frac{e^{-24KA}}{1 - e^{-24KA}}\right]$$

$$AUC = \frac{F1 * DOSE}{CL}$$

Typical group values of F1 and empiric Bayesian estimates of clearance were used in the computation of AUC. The doses needed to achieve comparable exposure in the different population subgroups were calculated using Equation 3.

$$AUC = \frac{AUC_2 * DOSE_1}{AUC_1}$$

Pharmacokinetic simulations

The PK model was used to simulate 1000 datasets of 158 patients each, with the same *CYP2B6* and *ABCB1* c.4046A>G genotype frequency as the original dataset. Fixed and random model effects parameters were set equal to the reduced (final) PK model, given the data. EFV exposure profiles for doses of 200, 250, 300, 450 and 600 mg were simulated for individual *CYP2B6*6, CYP2B6*11* and *ABCB1* (c.4046A>G) genotypes as well each of the 18 possible combinations thereof and their frequencies in the study population. EFV trough concentrations were calculated for each simulated individual and summary statistics are presented.

PK/PD associations

Efficacy was measured in terms of immunological recovery (change between baseline and last measured CD4 counts or CD4 counts on days 84, 168 and >200)

Table 1. List of variant alleles with the respective rs number, position in cDNA or genomic DNA and observed SNP frequencies in HIV-tuberculosis co-infected study participants (n = 158).

Gene	Position	rs number	Allele	Protein	Observed frequency (%)
CYP2B6	c.516G>T	rs3745274	*CYP2B6*6*	Q172H	54.8
	c.136A>G	rs35303484	*CYP2B6*11*	M46V	12.7
CYP3A5	g.6986A>G	rs776746	*CYP3A5*3*	Splicing defect	18.2
	g.14690G>A	–	*CYP3A5*6*	Splicing defect	17.2
	g.27131–27132insT	rs241303343	*CYP3A5*7*	346 frame shift	18.4
ABCB1	c.3435T/C	rs1045642	–	Ile1145Ile	12.1
	c.4036A/G	rs3842	–	3′ UTR	17.8
CYP2A6	-48T>G	rs28399433	*CYP2A6*9*	TATA Box	11.1

and virologic decay to below detection or <40 copies per milliliter by day 84. Correlations between C_{24} and/or AUC and efficacy were explored graphically.

Results

Overall the pharmacokinetic dataset contained log transformed 1216 EFV concentration values collected from 158 HIV/AIDS patients, 76 of them females, over days 252 of daily cotreatment with EFV-based HAART and rifampicin-based anti-TB treatment. Mean (standard deviation [SD]) bodyweight and age were 50.3 (7.97) kg, and 32.4 (6.88) years, respectively. The baseline mean (SD) serum albumin, alanine aminotransferase, urea and estimated serum creatinine were 2.6 (0.66) g/dl, 26.88 (23.18) u/l, 4.09 (2.67) mMol/l and 71.15 (33.63) µmMol/l, respectively. Other baseline characteristics and dose relevant genotype information on study subjects are summarized in Table 1. The population allelic frequencies of SNPs without implications for EFV dose modification including *CYP3A5* (**3*, **6* & **7*) and *ABCB1* c.3435C>T did not differ from the findings of our previous study [11].

A one-compartment model with first-order absorption described our data well, as is presented in Figure 1. The effects and statistical importance

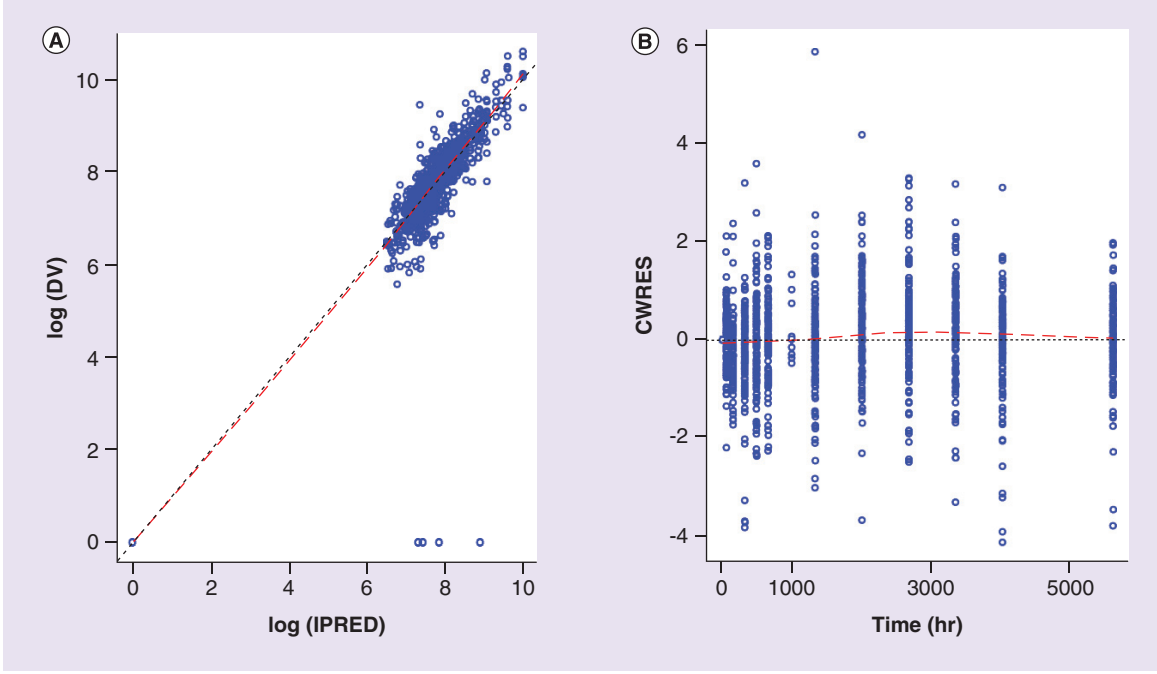

Figure 1. Individual predicted efavirenz concentrations versus observed concentrations (goodness of fit) and weighted residuals versus time plots demonstrates a good fit of all time point concentration data by the model. Missing observations are output as zeros in NONMEM tables and are represented by four isolated data points at the bottom of the log(DV) versus the log(IPRED) plot.

Table 2. Summary of significant factors in the covariate analysis; forward inclusion (α = 0.05) followed by backward elimination (α = 0.01).

Covariate relationship	Change in objective function value	p-value
Forward inclusion		
*CYP2B6*6*-CL	25.01063	0.000004
*CYP2B6*11*-CL	20.368	0.000006
ABCB1 c.3435C>T-CL	7.1201	0.028437
Backward elimination		
*CYP2B6*11*-CL, *CYP2B6*6*-CL	51.363	<0.001

of covariates identified in the study population on pharmacokinetic parameter estimates are depicted in Table 2. The final model pharmacokinetic parameters are reported in Table 3. Notably, EFV postinduction CL/F was 2.5- and 1.7-fold lower in *CYP2B6*6/*6* and *CYP2B6*1*/6* compared *CYP2B6*1/*1*. EFV post-induction CL/F was also dependent upon *CYP2B6*11* genotypes with **1/*1* exhibiting 1.3-times CL/F compared with **1/*11*. A 23% increase in F1 was observed for *ABCB1* c.4046AG plus c.4046GG variants.

The estimated overall mean trough concentration ($C_{24\ h}$) value was 2.69 mg/dl compared with the recommended efficacy threshold of 1 mg/dl. It was 6-, 4.1- and 3.5-fold higher than the recommended efficacy threshold of 1 mg/dl for *CYP2B6*6* homozygous, *CYP2B6*11* heterozygous and *ABCB1* c.4046AG plus GG variants, respectively. No correlation was observed between viral decay and $C_{24\ h}$ (Figure 2). The genotype stratified mean estimates of EFV $C_{24\ h}$ and the area under the curve following 600 mg once daily dosing are presented in Table 4, Mean estimates of $C_{24\ h}$ at simulated daily doses of 200, 250, 300, 450 and 600 mg according to each genotype and different genotype combinations are presented in Tables 5 & 6, respectively. The estimated population mean AUC was 1.2-fold greater than the target product label AUC of 58.084 × 10^3 µg/l·h. Compared to the product label AUC, a 2.4-, 1.74-, 1.2- and 1.4-fold increase was associated with *CYP2B6* (**6/*6*), (**1/*6*), and participants carrying at least one allele for *CYP2B6*11* and *ABCB1* c.4046A>G, respectively. As in our previous study, findings from this study demonstrate that a comparable target AUC is achievable with daily EFV dosing of 450 mg, for Ugandan population although the mean $C_{24\ h}$ at this dosing level was 1.9-times the threshold $C_{24\ h}$. For *CYP2B6*6* homozygous individuals, an AUC comparable to the target product label was achievable with a daily EFV dose of 250 mg while the mean $C_{24\ h}$

Table 3. Final model pharmacokinetic parameters.

	Parameter	SE (%)
KA	0. 03	14
V(l)	116	36
CL(l/h)- *CYP2B6*6*(**6/*6*) and *CYP2B6*11*(**11/*11*)	6.27	15
CL(l/h)- *CYP2B6*11*(**1/*11*)	9.26	17
CL(l/h)- *CYP2B6*6*(**1/*6*)	12.48	33
CL(l/h)- *CYP2B6*6*(**1/*1*)	16	7
F1- *ABCB1* c.4036 A/G (1)	1 FIXED	NA
F1- *ABCB1* c.4036 A/G (2 & 3)	1.23	57
OMEGAS		
IIV–V	0.2	29
11V–CL	0.29	7
RV-proportional	0.1	4

Due to low population allele frequency heterozygous and homozygous ABCB1 c.4036 A/G were grouped together and designated as: ABCB1 c.4036 A/G (2 & 3) in the table.
CL: Clearance; F1: Apparent bioavailability; KA: Absorption constant; RV: Residual variability; SE: Standard error; V: Volume of distribution.

Table 4. Mean efavirenz trough concentrations (C24 h) and area under the curve NONMEM estimate values for the entire study population, *ABCB1* c.4046A>G and *CYP2B6* (**6* and **11*) genotypes.

Genotype	Mean trough concentrations and AUC	
	Trough concentration/mg/dl	AUC 10^3/μg/l h
*Cyp2B6*6* (**1/*1*)	2.06	55.6
*Cyp2B6*6* (**1/*6*)	2.74	100.8
*Cyp2B6*6* (**6/*6*)	6.38	141.6
*Cyp2B6*11* (**1/*1*)	2.21	58.3
*Cyp2B6*11* (**1/*11*)	4.10	70.8
ABCB1 c. 4046A>G (GG)	2.35	64.0
ABCB1 c. 4046A>G (AG)	3.35	78.4
ABCB1 c. 4046A>G (AA)	3.49	79.1
ALL	2.69	69.0

ALL: All study participants; AUC: Area under the curve.

was 2.3 mg/dl (Table 6). By scaling exposures, EFV daily doses of 265 mg would be expected to achieve similar plasma exposure in individuals homozygous to *CYP2B6*6* as reported in the EFV product label. Similarly, adjustments to a 467 mg daily dose of EFV would provide the typical Ugandan HIV-1 infected adult with exposure equal to the mean AUC in the drug label. Since these specific dose amounts are not achievable with marketed formulations, we simulated exposures for EFV daily doses of 200, 250, 300, 450 and 600 mg. Corresponding $C_{24\ h}$ for the Ugandan population, different genotypes and combinations are presented in Tables 5 & 6.

Pharmacodynamic evaluations

Baseline mean (SD) $\log_{10}$ HIV RNA copies per mL and CD4 counts per ml (IQR) were 11.67(1.41) and 86.77 (20–135), respectively. Mean (SD) change from baseline CD4 counts at days 84, 168 and after 200 days of EFV-based ART was 93.7 (87.2), 154.3 (83.0) and 206.1 (104.5), respectively, while mean CD4 change by last time of measurement was 177.9 (101.2). Fourteen of the participants (8. 9%) had at least one HIV RNA > 40 copies/ml after 84 days of ART. These results did not depict any association between drug concentrations and rate of HIV viral decay (Figure 2). Additionally,

Table 5. Mean (standard deviation) of simulated trough concentrations (mg/dl) by genotype at different efavirenz doses.

Genotype	N	Mean (SD) of simulated C24 h (mg/dl) by genotype at different EFV doses				
		600 mg	450 mg	300 mg	250 mg	200 mg
CYP2B6*6						
*1/*1	79,768	2. 04 (1.5)	1. 53 (1.12)	1. 02 (0.75)	0. 85 (0.62)	0. 68 (0.5)
*1/*6	65,100	2. 66 (1.93)	1. 99 (1.45)	1. 33 (0.97)	1. 11 (0.81)	0. 89 (0.64)
*6/*6	13,132	5. 57 (3.94)	4. 18 (2.96)	2. 79 (1.97)	2. 32 (1.64)	1. 86 (1.31)
CYP2B6*11						
*1/*1	118,084	2.15 (1.67)	1.61 (1.25)	1.08 (0.83)	0.9 (0.70)	0.72 (0.56)
*1/*11	39,916	3.89 (2.95)	2.92 (2.22)	1.94 (1.48)	1.62 (1.23)	1.3 (0.98)
ABRS						
0	103,164	2.4 (2.01)	1.8 (1.51)	1.20 (1.01)	1 (0.84)	0.8 (0.67)
1	50,826	2.94 (2.47)	2.21 (1.86)	1.47 (1.24)	1. 23 (1.03)	0.98 (0.82)
2	2986	2.97 (2.53)	2.23 (1.9)	1.49 (1.27)	1. 24 (1.05)	0.99 (0.84)
Missing	1024	3.07 (2.71)	2.3 (2.03)	1.53 (1.35)	1. 28 (1.13)	1.02 (0.90)
ALL	158,000	2.59 (2.2)	1.94 (1.65)	1.30 (1.10)	1. 08 (0.92)	0.86 (0.73)

ALL: All study participants; EFV: Efavirenz: N: Number iterations for individual genotypes based upon their individual population frequencies, SD: Standard deviation.

Table 6. Mean simulated efavirenz trough concentrations by genotype combinations at daily dose levels ranging from 200 to 600 mg

Genotype combinations			N	Mean (SD) of simulated efavirenz trough concentrations (mg/dl) at different dose levels by the different genotype combinations				
*CYP2B6*6*	*CYP2B6*11*	*ABCB1* c.4046A		200 mg C24 h	250 mg C24 h	300 mg C24 h	450 mg C24 h	600 mg C24 h
**1/*1*	**1/*1*	0	38,867	0.52 (0.34)	0.66 (0.42)	0.79 (0.51)	1.18 (0.76)	1.57 (1.02)
**1/*1*	**1/*1*	1	19,057	0.64 (0.41)	0.8 (0.51)	0.96 (0.61)	1.44 (0.92)	1.92 (1.22)
**1/*1*	**1/*1*	2	1181	0.66 (0.42)	0.82 (0.53)	0.99 (0.63)	1.48 (0.95)	1.97 (1.27)
**1/*1*	**1/*11*	0	13,235	0.95 (0.59)	1.18 (0.74)	1.42 (0.89)	2.13 (1.33)	2.84 (1.77)
**1/*1*	**1/*11*	1	6604	1.16 (0.73)	1.45 (0.91)	1.74 (1.1)	2.61 (1.64)	3.48 (2.19)
**1/*1*	**1/*11*	2	337	1.2 (0.76)	1.5 (0.94)	1.8 (1.13)	2.7 (1.7)	3.6 (2.27)
**1/*6*	**1/*1*	0	31,948	0.68 (0.43)	0.85 (0.54)	1.03 (0.65)	1.54 (0.97)	2.05 (1.3)
**1/*6*	**1/*1*	1	15,595	0.84 (0.54)	1.04 (0.67)	1.25 (0.8)	1.88 (1.2)	2.51 (1.61)
**1/*6*	**1/*1*	2	918	0.86 (0.55)	1.07 (0.69)	1.29 (0.83)	1.93 (1.24)	2.58 (1.65)
**1/*6*	**1/*11*	0	10,540	1.23 (0.75)	1.54 (0.94)	1.85 (1.12)	2.77 (1.69)	3.69 (2.25)
**1/*6*	**1/*11*	1	5343	1.53 (0.96)	1.91 (1.2)	2.29 (1.44)	3.44 (2.16)	4.59 (2.88)
**1/*6*	**1/*11*	2	319	1.49 (0.98)	1.87 (1.22)	2.24 (1.47)	3.36 (2.2)	4.48 (2.93)
**6/*6*	**1/*1*	0	6422	1.43 (0.88)	1.79 (1.1)	2.15 (1.32)	3.23 (1.99)	4.3 (2.65)
**6/*6*	**1/*1*	1	3138	1.75 (1.07)	2.19 (1.34)	2.63 (1.61)	3.94 (2.42)	5.25 (3.22)
**6/*6*	**1/*1*	2	175	1.83 (1.17)	2.28 (1.46)	2.74 (1.76)	4.11 (2.63)	5.48 (3.51)
**6/*6*	**1/*11*	0	2152	2.6 (1.58)	3.25 (1.97)	3.9 (2.37)	5.85 (3.55)	7.8 (4.74)
**6/*6*	**1/*11*	1	1089	3.07 (1.89)	3.84 (2.36)	4.6 (2.83)	6.91 (4.25)	9.21 (5.66)
**6/*6*	**1/*11*	2	56	3.48 (1.85)	4.36 (2.31)	5.23 (2.77)	7.84 (4.15)	10.45 (5.54)

N: Number iterations for individual genotypes based upon their individual population frequencies, SD: Standard deviation.

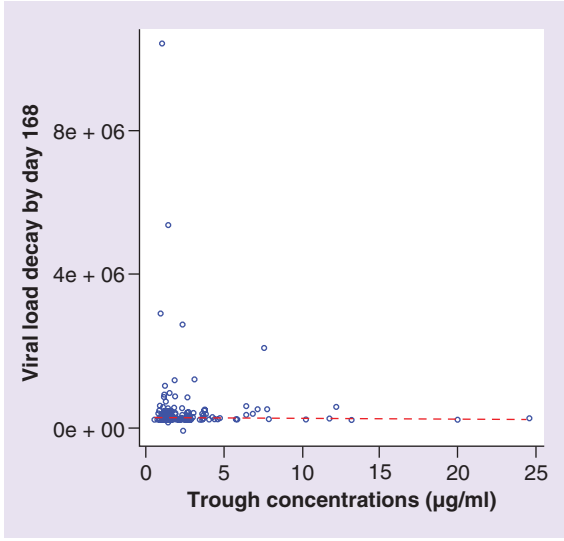

Figure 2. Lack of correlation between virological decay and trough concentrations indicating that trough efavirenz concentrations achieved in the study population might be far greater than the threshold required C_{trough}.

the mean $C_{24\ h}$ and AUCs of individuals with HIV RNA >40 copies/ml after 84 days of ART were 4.42 mg/dl and 113.70 × 10^3 µg/l·h, respectively.

Discussion

EFV exhibits extensive interethnic pharmacokinetic variability that is dependent on host-genetic factors. As a result of their possible effects on EFV metabolizing enzymes and ABCB1, rifamycins might exhibit gene and or ethnic dependent effects on EFV pharmacokinetics and consequent variation in dosing requirements during cotreatment with the two drugs. Based on pharmacogenetics, we predicted 450 and 250 mg daily EFV doses as optimal for adult HIV-TB co-infected Ugandans and *CYP2B6*6* homozygous individuals receiving rifampicin cotreatment, respectively. In agreement with our previous reports [21,27], genetic makeup rather than rifampicin cotreatment may predict EFV dosing needs most significantly. The 450 mg daily EFV dose recommended for adult HIV-TB co-infected Ugandans receiving rifampicin co-treatment is similar to our previous dosing recommendations for the same population without rifampicin cotreatment [15] and is in agreement with several studies indicating variability in EFV pharmacokinetics as largely dependent upon *CYP2B6*6* genotype [21,27–31]. Reduced EFV metabolism in individuals either homozygous or heterozygous for *CYP2B6*6* ultimately results in increased plasma exposure to the drug with higher likelihood of EFV CNS related symptoms [29]). The black race has been associated with higher plasma EFV exposure [32,33]. Consistent with these observations and our previous report [15], the current study reports a 1.2-fold higher AUC among HIV-infected Ugandans receiving rifampicin cotreatment than the target product label AUC. In the present study, we also report a mean $C_{24\ h}$ of 2.7 mg/dl at the standard 600 mg daily EFV dose compared with the recommended efficacy threshold of 1 mg/dl. This 2.7-fold higher trough concentration offers a probable explanation for lack of association between viral decay and EFV exposure in the current study. The overall greater EFV plasma exposure is explained by the significant role of *CYP2B6*6* polymorphism, whose allele frequency of 55% among Ugandans [11], on EFV pharmacokinetics. Indeed, the current study demonstrated high plasma exposure with consequent lower EFV dose requirements among *CYP2B6*6* homozygous individuals receiving rifampicin cotreatment. While we previously predicted 300 mg daily EFV dose as optimal for HIV-1 infected *CYP2B6*6* homozygous Ugandans, the 250 mg daily dose predicted during rifampicin cotreatment in the same population implies greater plasma exposure when the two drugs are co-administered. This is in conformity with findings by Habtewold *et al.*, Cohen *et al.* and Ramachandran *et al.* who also reported greater EFV plasma exposure during rifampicin cotreatment in *CYP2B6*6* homozygous variants [21,23,24].

A higher frequency of EFV associated CNS symptoms that are possibly due to supra-therapeutic drug exposure has been reported among Ugandans and other African populations. The results of this study have clinical relevance in attempting to improve compliance by limiting the occurrence of adverse events. Our EFV dose adjustment recommendations are further supported by a clinical case report of *CYP2B6*6* heterozygous patient who successfully attained viral suppression and sustained it for more than 18 months on EFV dose of 400 mg daily, as well as the findings of other studies [34–37]. Additionally, successful HIV viral suppression has been demonstrated at EFV doses of 400 mg and 200 mg daily among patients that exhibited supra-therapeutic plasma concentrations following 600 mg daily EFV dose [35–38]. The question of whether the product label target AUC of 5.8 × 10^3 µg/l·h and the widely published 1.0 mg/dl $C_{24\ h}$ constitute the most appropriate reference measures EFV plasma exposure is important, but serves as a best available guideline in the absence of a PK/PD model based target.

In addition to the long follow-up period of up to 252 days, the pharmacodynamic evaluation component further strengthens the dose adjustment recommendations by the current study. Fourteen (8.9%) of patients did not achieve viral suppression to below

detection by day 84 of treatment, however, the lack of association between drug exposure and day 84 viral loads, as well as their greater mean $C_{24\,h}$ and AUCs (4.4- and twofold, respectively) suggest other possible causes may be responsible for the virologic failure. Erratic adherence and intrinsic viral resistance previously reported [39] are two factors possibly responsible for failure to achieve viral suppression to below detection in this particular study.

Findings from this study have extensive application for most of the SSA region, which is characterized by high frequency of the defective *CYP2B6*6* variant alleles [33,34]. EFV is used to treatment millions of people worldwide. Its use is even now expanding to treat HIV infected pregnant women for prevention of mother to child transmission of HIV. Apparently the current reference dose of EFV in SSA may expose patients, including pregnant women, to unnecessary EFV plasma concentration dependent adverse events such as CNS [29] and liver toxicity [40–42] without any additional efficacy benefit. Furthermore, EFV dose reduction might result in reduction of treatment costs per patient and a consequent increase in access to ART. In summary, based on this population pharmacokinetic analysis and simulation study, we propose a *CYP2B6* genotype based EFV dosage adjustment in Ugandan and African population in general.

Conclusion

Our recommendation of 450 and 250 mg daily doses are anticipated to meet the EFV treatment needs of HIV-1 infected Ugandans in general, and for individuals homozygous for *CYP2B6*6* homozygous individuals receiving rifampicin cotreatment, respectively.

Future perspective

Using population PK/PD/PGx modeling and simulations of data obtained from HIV patients without TB co-infection, we previously reported the current standard EFV 600 mg/day adult dose is unnecessarily high and suggested a dose reduction by a third for HIV patients without TB co-infection [15]. Independent randomized clinical trials confirmed the noninferiority of 400 mg daily EFV dose compared with the standard 600 mg daily dose [36,37].

In the current study, we performed a similar study in a different patient cohort, namely TB-HIV co-infected patients receiving rifampicin-based anti-TB cotreatment. We report that 450 and 250 mg daily doses are optimal to meet the EFV treatment needs of HIV-1 infected Ugandans in general and for individuals homozygous for *CYP2B6*6*, respectively. A randomized placebo noninferiority clinical trial comparing the safety and efficacy of a reduced EFV dose versus the 600 mg daily EFV dose in HIV patients with active TB receiving rifampicin-based anti-TB treatment is urgently needed to confirm our population PK/PD based EFV dose recommendation.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial

Executive summary

Background

- Plasma efavirenz (EFV) exposure is mainly influenced by *CYP2B6* genotype.
- Rifampicin, a potent CYP enzyme and transporter proteins, lowers plasma EFV concentration in Caucasians and Asian population, but no such effect was observed in Africans.
- Using population PK/PD studies, we previously reported that the standard EFV 600 mg/day adult dose is unnecessarily high and suggested a dose reduction by one quarter for HIV patients without TB co-infection.
- Optimal EFV dose prediction for HIV-TB co-infected patients receiving concomitant rifampicin-based anti-TB therapy has not yet been investigated.

Patients & methods

- We performed population pharmacokinetic and pharmacodynamics modeling and simulation study to identify optimal EFV predicted dose during rifampicin-based anti-TB cotherapy for an African population and for CYP2B6 slow metabolizers.

Results

- EFV plasma exposure was mainly influenced by *CYP2B6* genotype but not rifampicin-based anti-TB therapy.
- Simulated AUCs for 600 mg EFV dose were 1.20- and 2.4-times greater than the product label for Ugandans in generals and *CYP2B6*6/*6* genotypes, respectively.
- EFV daily doses of 450 and 250 mg for Ugandans and *CYP2B6*6/*6* genotypes respectively yielded simulated exposures comparable to the product label.

Conclusion

- Around 450 and 250 mg daily doses might meet EFV dosing needs of HIV-TB infected Ugandans in general and *CYP2B6*6/*6* genotypes, respectively.

interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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