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Effect of mid-dose efavirenz concentrations and *CYP2B6* genotype on viral suppression in patients on first-line antiretroviral therapy

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ABSTRACT

The therapeutic range for efavirenz plasma concentrations is unclear and some studies found no correlation with viral non-suppression. Efavirenz concentrations are variable, driven in part by polymorphisms in *CYP2B6*. We hypothesised that efavirenz mid-dosing concentrations, together with *CYP2B6* metaboliser genotype, could predict viral non-suppression. Participants starting first-line efavirenz-based antiretroviral therapy were monitored for 48 weeks. HIV-RNA and efavirenz mid-dose interval concentrations were determined at Weeks 16 and 48. *CYP2B6* metaboliser genotype status was determined by 516G→T and 983T→C polymorphisms. Cox proportional hazards modelling was used to predict viral non-suppression and to determine the most predictive efavirenz mid-dosing concentration threshold. In total, 180 participants were included. Median efavirenz concentrations were 2.3 mg/L (IQR 1.6–4.6 mg/L) and 2.2 mg/L (IQR 1.5–3.9 mg/L) at Weeks 16 and 48, respectively. Moreover, 49 (27.2%), 84 (46.7%) and 39 (21.7%) participants had extensive, intermediate or slow *CYP2B6* metaboliser genotype, respectively. Log_2 efavirenz concentrations [adjusted hazard ratio (aHR) = 0.77, 95% CI 0.67–0.89] and baseline CD4 cell count (aHR = 0.994, 95% CI 0.989–0.998), but not *CYP2B6* genotype, were predictive of viral non-suppression. For every doubling of efavirenz concentration there was a 23% decrease in the hazard of non-suppression. A threshold of 0.7 mg/L was found to be the efavirenz mid-dosing concentration that was most predictive of non-suppression. Mid-dosing efavirenz concentrations are predictive of viral non-suppression, but the currently recommended lower therapeutic limit (1 mg/L) is higher than our finding. Knowledge of *CYP2B6* metaboliser genotype is not required for prediction of virological outcomes.

1. Introduction

Efavirenz is a good candidate for therapeutic drug monitoring (TDM) because there are reliable assays, its plasma concentrations are characterised by high inter-individual variability, and low concentrations have been linked with viral non-suppression and high concentrations with toxicity [1–3]. However, the relationship between efavirenz concentrations and viral suppression has not always been consistent in studies, perhaps due to the rapid development of high-level resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs), thus efavirenz TDM is not routinely recommended [1–6].

The high inter-individual variability of efavirenz concentrations is explained in part by polymorphisms in *CYP2B6*, the gene that encodes the cytochrome P450 (CYP) enzyme CYP2B6 [7–9]. The prevalence of genetic slow metabolisers is high in sub-Saharan African populations [7]. Metaboliser genotype status (ultraslow, slow, intermediate or extensive) did not impact virological outcomes in a recent analysis of pooled studies conducted by the AIDS Clinical Trials Group (ACTG), but the impact of genotype status on virological failure has not been fully explored in a South African population [10].

The lower limit of the currently recommended therapeutic range (1–4 mg/L) for efavirenz is controversial [6,11,12]. Marzolini et al reported that mid-dose efavirenz drug concentrations of <1 mg/L were associated with increased rates of virological failure [11]. Whilst pharmacokinetic data from the 2NN (double nonnucleoside) study suggested an increase in virological failure with trough concentrations of <1.1 mg/L, the authors did not recommend using this

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cut-off value to predict virological outcomes as the sensitivity was low [12]. Recently published data from the ENCORE1 study noted that only a small proportion of those failing treatment had mid-dosing efavirenz concentrations of <1.0 mg/L [6].

We hypothesised that mid-dosing interval efavirenz drug concentrations, together with knowledge of *CYP2B6* metaboliser genotype, would be predictive of virological outcome in a sub-Saharan African population starting first-line antiretroviral therapy (ART). We also examined the lower threshold concentration of efavirenz for therapeutic benefit.

2. Methods

2.1. Participants, setting and standard of care

Participants were recruited at the Hannan Crusaid Treatment Centre (HCTC), a large outpatient ART centre in Cape Town, South Africa. The cohort included ART-naïve adults and adolescents who were eligible if they had their own mobile phone and were willing to sign a written informed consent form.

All those entering the treatment programme at HCTC received three group counsellor-driven treatment literacy sessions prior to commencing NNRTI-based ART [13,14]. They were also visited at home by a community care worker to confirm their address and home circumstances. Those with a raised viral load or low adherence based on a count of tablet returns (<90%) received a stepped-up adherence package, including tailored counselling, monthly drug dispensing and further home visits. Participants were traced by phone call and home visit if they were >4 weeks late for a clinic visit.

2.2. Sub-study design

The parent study was a randomised controlled trial over 48 weeks investigating adherence to ART and has been described in detail elsewhere [15]. Participants also had the option of joining a non-randomised voluntary pharmacokinetic and pharmacogenetic sub-study, which required additional blood sampling.

2.3. Visits and sampling

Sub-study visits included screen, baseline, and Weeks 16 and 48. Visits were timed to coincide with booked clinic visits to minimise inconvenience. Participants were reimbursed for local travel (R20 or ca. US$2) at each visit and were offered a gift of a T-shirt, bag or mug valued at R80 (ca. US$8) or less for each on-study visit.

Demographic and psychosocial details were collected at screen. Prescribed ART was recorded at baseline (Week 0). Weight and current ART were confirmed at all visits. Blood was drawn for CD4 cell count (BD FACSCount™; Becton Dickinson, Franklin Lakes, NJ) and HIV-1 viral load (HIV-1 RNA 3.0 assay®; Bayer Healthcare, Leverkusen, Germany) at screen and at Weeks 16 and 48. At Weeks 16 and 48, for those who gave additional consent, blood was drawn in a lithium heparin tube for mid-dosing interval efavirenz concentrations, in the window between 9 h and 16 h after self-reported efavirenz intake time, and in an ethylene diamine tetra-acetic acid (EDTA) tube for *CYP2B6* pharmacogenetic analysis.

At Weeks 16 and 48, most blood samples for efavirenz concentration and viral load were drawn on the same date. However, in a number of participants, viral load measurements were obtained up to 4 weeks before the scheduled pharmacokinetic visit (as part of standard of care) or afterwards (when the measurement had to be repeated due to issues with the measuring procedure). Samples were kept cold (4 °C) until transfer to the laboratory within 2–3 h of blood draw.

2.4. Pharmacokinetic analyses

Samples were centrifuged at 3500 rpm for 10 min and plasma was pipetted into cryovials that were labelled and frozen at –80 °C. Samples were analysed for efavirenz concentrations using a validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) method.

2.5. Pharmacogenetic analyses

Samples were centrifuged at 3000 rpm for 30 min. The white blood cell layer (buffy coat) was transferred to a labelled cryovial and was frozen at –80 °C. Three *CYP2B6* single nucleotide polymorphisms (SNPs) previously associated with efavirenz concentrations were chosen and analysed: rs3745274 (516G→T); rs28399499 (983T→C); and rs4803419 (15582C→T). Genomic DNA was extracted from 100 μL of stored buffy coat, was re-suspended in a total volume of 300 μL of lysis buffer and 30 μL of proteinase K from the Maxwell®16 LEV Blood DNA Kit (Promega, Southampton, UK) and was incubated at 57 °C for 30 min at 1000 rpm. DNA was extracted according to the manufacturer's instructions on a Maxwell® Automated Extraction Platform (Promega) and was eluted in 100 μL of elution buffer.

The quantity and quality of extracted DNA were determined using a Qubit® DNA BR Assay Kit (Molecular Probes; Life Technologies, Carlsbad, CA) and a Qubit® 2.0 Fluorometer (Invitrogen; Life Technologies) according to the manufacturer's instructions. Once the quantity of DNA was determined, it was diluted to 20 ng/μL using sterile nuclease-free water and then 1 μL was aliquoted into one well per sample in a 96-well plate (Life Technologies, Beijing, China). DNA samples were left at room temperature for 12 h to lyophilise.

Amplification and genotyping of each participant for the presence of SNPs in their *CYP2B6* gene were performed using fluorescent-labelled minor groove binding (MGB) allele-specific probes (Applied Biosystems, Foster City, CA). Participants were genotyped for *CYP2B6* (516G→T, rs3745274; 983T→C, rs28399499; and 15582C→T, rs4803419) using 1 μL of lyophilised DNA and 1× *Taq*Man® Universal PCR Master Mix, No AmpErase® UNG (Applied Biosystems) to a total volume of 12.5 μL. Cycling conditions consisted of an initial enzyme activation step of 95 °C for 10 min, followed by a denaturation step of 95 °C for 15 s and a combined annealing and extension step of 60 °C for 1 min. All amplification reactions were performed on a ViiA™ 7 Real-Time PCR System (Applied Biosystems).

A simplified version of Holzinger et al's metaboliser status classifications was used, as used by Dooley et al [8,16]. Each individual was classified as an ultraslow, slow, intermediate or extensive metaboliser. The effect of 516GT|983TC SNP vector was tested as four metabolic subcategories: extensive metabolisers, 516GG|983TT; intermediate metabolisers, 516GT|983TT or 516GG|983TC; slow metabolisers, 516TT|983TT or 516GT|983TC; and ultraslow metabolisers, all participants 983CC irrelevant of 516G→T genotype [8,16,17].

2.6. Study outcome

The outcome of interest was viral load at Week 16 or Week 48.

2.7. Statistical analysis

Descriptive statistics were used to summarise the baseline characteristics of the participant group and the mid-dosing interval efavirenz concentrations using Stata v.13.0 (Stata Corp., College Station, TX).

2.7.1. Prediction of virological outcome

The change in the relative risk of viraemia was estimated using a Cox proportional hazards regression model (Andersen–Gill

repeated outcomes framework) with Efron approximation and interval censoring using the software R with package survival [18–22]. Each time interval ran from the preceding to the current viral load measurement. Viral loads were converted into dichotomised outcome: an event (classified as non-suppression) was defined as viraemia >400 copies/mL at Week 16 and viraemia >40 copies/mL at Week 48. The following variables were tested for their effect on change in hazard of viral non-suppression: efavirenz concentrations; age; sex; baseline CD4 count; baseline viral load ($\log_{10}$ transformed); and metaboliser status.

Due to some pharmacokinetic and viral load samples falling before or after the planned sampling window at Weeks 16 and 48, time censoring was used. For samples scheduled for Week 16, measurements taken between Weeks 12 and 20 from treatment start were included in the analysis; for Week 48, samples falling between Weeks 32 and 64 were analysed. Mid-dose efavirenz plasma concentrations were matched with viral load measurements taken on the same day or the next closest measurement within the time censoring interval.

Missing categorical covariates were imputed as the population mode, and missing continuous covariates were imputed as the population median. A sensitivity analysis was conducted to test the effect of these imputations by dropping all participants with imputed covariate values.

All variables were tested for their effect on the risk of viral non-suppression in a univariate analysis and were included a priori in the full multivariate model. A backward elimination process was performed starting with the covariate with the least significant *P*-value until all remaining predictors had $P < 0.05$ (final model).

Subsequently, the threshold of efavirenz mid-dosing concentration that was the most predictive of an increased risk of viral non-suppression (at levels described above) was derived as previously proposed by Bienczak et al [23]. Briefly, the threshold was selected by comparing the Akaike information criterion (AIC) values generated by Cox proportional hazard regression models testing efavirenz concentration dichotomised at different cut-off values. The AIC value was profiled by testing all models using concentration cut-offs between 0.1 mg/L and 5 mg/L in increments of 0.005 mg/L. The cut-off resulting in the lowest AIC value was chosen as the desired threshold, since this corresponds to the dichotomisation of efavirenz concentrations that is most predictive of virological non-suppression. The robustness of the estimated threshold was confirmed using a re-simulation approach: the original data set was re-simulated 500 times introducing a normally distributed random error on the detected concentrations, and the estimation procedure for the best cut-off value was repeated on each of the re-simulated data sets. The magnitude of the error was set to the unexplained residual variability estimated in the population pharmacokinetic model by Dooley et al (additive error = 0.0846 mg/L, proportional error = 9.31%) [16]. The results of the re-simulation procedure were used to derive the 90% confidence interval (CI) on the concentration threshold (5th–95th percentiles of the values estimated from the 500 re-simulated data sets).

The positive predictive value (PPV) (i.e. proportion of samples with exposure below the threshold not suppressed) and negative predictive value (NPV) (i.e. proportion of samples with exposure above the threshold that were suppressed) were calculated and compared for the derived exposure threshold and 1 mg/L [11,24].

3. Results

3.1. Baseline characteristics and genotypes

Of the 230 individuals enrolled into the parent study, 180 had matched efavirenz mid-dose concentrations and viral load data: 25 at Week 16 only, 54 at Week 48 only and 101 at both time points. Baseline characteristics are detailed in Table 1. The majority of this population was female. A total of 336 mid-dose efavirenz level samples were available from the 180 individuals, comprising 170 at Week 16 and 166 at Week 48.

CYP2B6 genotype frequencies and metaboliser types are presented in Table 1. In this sub-Saharan African population, more than 20% of the cohort had slow or ultraslow metaboliser status.

3.2. Efavirenz concentrations

Fig. 1 describes efavirenz concentrations by metaboliser genotype and visit week using all efavirenz concentrations available. Whilst the median (interquartile range) concentrations overall for each visit were within the recommended therapeutic range (1–4 mg/L), those with ultraslow and slow efavirenz metaboliser genotypes had higher median efavirenz concentrations throughout the study than those with extensive or intermediate metaboliser genotype (Fig. 1; Supplementary Table S1).

At Weeks 16 and 48, a total of 10 (5.9%) and 13 (7.8%) participants, respectively, had efavirenz concentrations <1 mg/L, the majority of whom had extensive and intermediate metaboliser genotypes. At Weeks 16 and 48, a total of 43 (25.3%) and 33 (19.9%) participants, respectively, had concentrations >4 mg/L, the majority of whom had slow or ultraslow metaboliser genotype.

3.3. Virological outcomes

At Week 16, 118 (93.7%) of 126 participants had a viral load of ≤400 copies/mL and 8 (6.3%) had a viral load >400 copies/mL. At Week 48, 137 (88.4%) of 155 participants had a viral load of ≤40 copies/mL and 18 (11.6%) had a viral load >40 copies/mL.

3.4. Virological outcome model

Of the 180 participants, 101 contributed measurements at both time points, 25 only at Week 16 and 54 only at Week 48. A total of 281 matched viral load and plasma efavirenz mid-dose concentrations were analysed (126 at Week 16 and 155 at Week 48). The only categorical covariate with missing values was the metaboliser status, which was imputed in 10 patients as intermediate (i.e. the population mode). A sensitivity analysis was conducted by dropping all participants with the missing values and revealed that the imputation had no significant effect on the results.

The results of univariate and multivariate analyses using the Cox proportional hazards model are presented in Table 2. Systemic exposure to efavirenz expressed as $\log_2$ mid-dose concentration and baseline CD4 cell count proved to be the most significant predictors of the risk of viral non-suppression. The use of log-transformed efavirenz concentrations provided a better model fit than the use of the original values (results not shown) and it estimated a 23% decrease in the hazard of viral non-suppression ($P = 0.0005$) for every doubling in drug concentration (corresponding to one unit increase in $\log_2$ scale). Similarly, for every 50 cell increase in baseline CD4 cell count there was a 31.5% reduction in the hazard of non-suppression ($P = 0.0018$).

There was a trend towards an increased risk of viremia for participants with a higher baseline viral load (for every 10-fold increase in the baseline viral load there was an 83% higher hazard of non-suppression; $P = 0.07$). No significant effect was detected for age, sex and metaboliser genotype status.

All tested variables were included a priori in a 'full' multivariate model, which found similar associations to the univariate analysis. After adjusting for the effect of other covariates, the trend towards increased risk of non-suppression for slow versus extensive combined with intermediate metabolisers increased, but still did not achieve statistical significance.

Table 1
Baseline characteristics of the cohort used in the analyses.

Variable	Cohort with both efavirenz TDM and VL data		
	Baseline	Week 16	Week 48
Number	180	126	155
Female sex [*n* (%)]	118 (65.6)	79 (62.7)	103 (66.5)
Age (years) [median (IQR)]	32.8 (27.4–40.7)	33.4 (28.1–41.4)	34.7 (28.8–42.6)
Weight (kg) [median (IQR)]	67.0 (58.4–79.8)	67.4 (59.1–79.1)	69.5 (59.0–80.0)
WHO HIV disease stage [*n* (%)]			
1	68 (37.8)		
2	36 (20.0)		
3	57 (31.7)		
4	19 (10.6)		
CD4 count (cells/mm^3) [median (IQR)]	229 (129–287)		
Log_{10} VL (copies/mL) [median (IQR)]	4.9 (4.4–5.4)		
VL >400 copies/mL (Week 16) or >40 copies/mL (Week 48) [*n* (%)]	180 (100)	8 (6.3)	18 (11.6)
Genotype *CYP2B6* 516G→T [*n* (%)]			
GG	75 (41.7)	46 (36.5)	69 (44.5)
GT	73 (40.6)	56 (44.4)	63 (40.6)
TT	24 (13.3)	18 (14.3)	21 (13.5)
Missing	8 (4.4)	6 (4.8)	2 (1.3)
Genotype *CYP2B6* 983T→C [*n* (%)]			
TT	131 (72.8)	94 (74.6)	116 (74.8)
TC	40 (22.2)	26 (20.6)	36 (23.2)
CC	1 (0.6)	0 (0.0)	1 (0.6)
Missing	8 (4.4)	6 (4.8)	2 (1.3)
Genotype *CYP2B6* 15582C→T [*n* (%)]			
CC	144 (80.0)	103 (81.7)	126 (81.3)
CT	27 (15.0)	17 (13.5)	26 (16.8)
TT	1 (0.6)	0 (0.0)	1 (0.6)
Missing	8 (4.4)	6 (4.8)	2 (1.3)
Metaboliser genotype [*n* (%)]			
Extensive	49 (27.2)	31 (24.6)	44 (28.4)
Intermediate	84 (46.7)	60 (47.6)	76 (49.0)
Slow	38 (21.1)	29 (23.0)	32 (20.6)
Ultraslow	1 (0.6)	0 (0.0)	1 (0.6)
Missing	8 (4.4)	6 (4.8)	2 (1.3)

TDM, therapeutic drug monitoring; VL, viral load; IQR, interquartile range; WHO, World Health Organization; HIV, human immunodeficiency virus.

In the final multivariate model, higher efavirenz concentration and higher CD4 count were both associated with decreased risk of viral non-suppression. The analysis was repeated excluding participants with imputed covariate values, with no change to the associations observed.

3.5. Threshold of mid-dose efavirenz concentration for prediction of non-suppression

Dichotomised efavirenz concentration was then tested to identify the most predictive cut-off value. Fig. 2a presents the profiling of the model AIC values when using efavirenz concentration cut-off values between 0.4 mg/L and 4 mg/L, whilst Fig. 2b shows the distribution of the estimates obtained with the re-simulation procedure. The model with the lowest AIC value used a cut-off between 0.63 mg/L and 0.74 mg/L (90% CI 0.24–1.56 mg/L), so the value of 0.7 mg/L was selected. Observations with efavirenz mid-dose concentrations below this threshold had 4.43 times greater hazard of virological failure (95% CI 1.58–12.3; $P = 0.004$).

The PPV was 33.3% for the 0.7 mg/L cut-off and 21.0% for the 1 mg/L cut-off; the NPVs were 91.8% and 91.6%, respectively.

The procedure was repeated using dichotomised concentrations in the multivariate model including the effect of baseline CD4 count and produced a similar value (results not shown).

4. Discussion

We found that mid-dosing interval efavirenz concentrations significantly predicted virological outcomes. The model showed that the most predictive cut-off value for viral suppression was ca. 0.7 mg/L, which is lower than the currently recommended lower limit of 1 mg/L. Lower baseline CD4 cell counts were also predictive of poor virological outcome.

The proportion of individuals in this South African cohort with heterozygous or homozygous variants in *CYP2B6* was similar to others reported from this community [16,17,24]. More than 20% of this sub-Saharan population was slower metabolisers, compared with only 3% noted in people of Caucasian descent [25]. The higher efavirenz concentrations found with slower metaboliser genotypes were similar to those from other groups [6]. However, *CYP2B6* metaboliser genotype alone did not predict virological outcomes.

When testing the effect of efavirenz mid-dose concentration without dichotomising, the model found a 23% decrease in risk of virological failure for every doubling of efavirenz concentration. The use of log-transformed concentrations suggests that relative changes in efavirenz plasma levels (a fold increase), as opposed to absolute changes (an increase of 1 mg/L), are more robust predictors of reduction in risk of non-suppression, as previously reported by Bienczak et al [23].

This analysis raises the question of whether the lower limit of the currently recommended therapeutic range (1 mg/L) is too high [11,12]. The ENCORE1 study has shown equivalent virological outcomes with a 400 mg dose of efavirenz compared with the standard 600 mg dose, despite significantly lower efavirenz exposure [6]. Only a small proportion of those with efavirenz concentrations <0.7 mg/L or <1 mg/L failed in this study (4 of 12 patients and 4 of 19 patients, respectively). This corresponds to similar NPVs for both thresholds. Based on the comparison of AIC values of the selection of models with dichotomised thresholds, the cut-off of 0.7 mg/L was most predictive of an increased risk of non-suppression and it provided a higher PPV. However, the small number of failures observed in our cohort and the wide confidence interval for the new

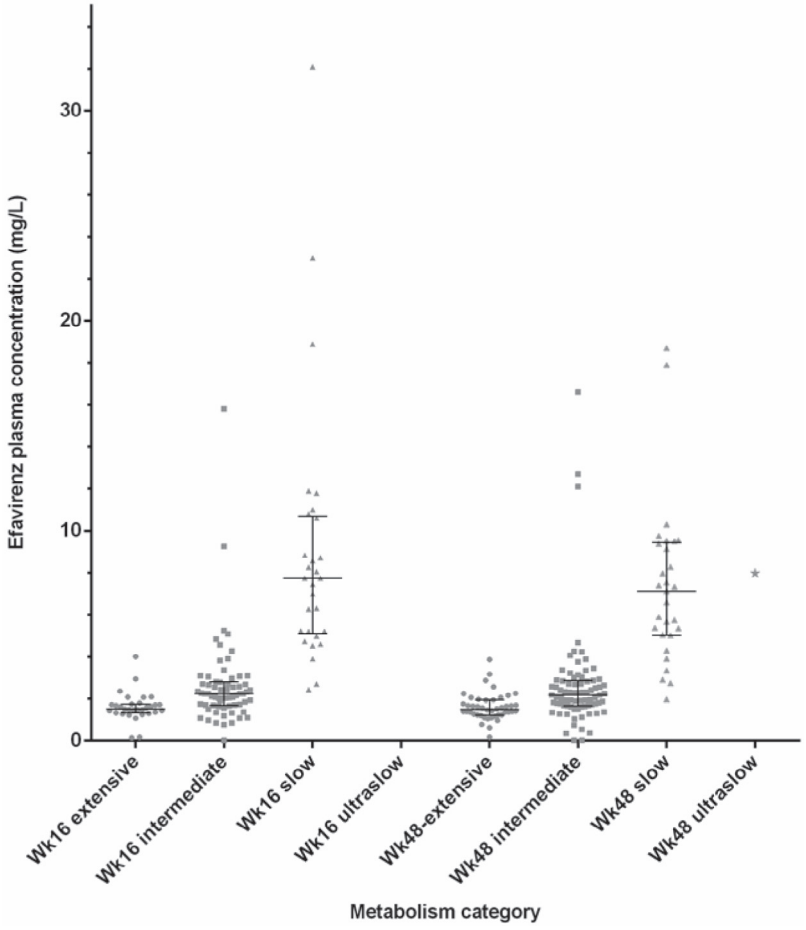

Fig. 1. Median (interquartile range) efavirenz concentrations by visit week and metaboliser genotype.

threshold indicate that further studies with larger sample size are needed to assess whether these findings are robust.

Previous studies have shown that *CYP2B6* metaboliser genotypes, which have a marked impact on efavirenz concentration, are not associated with failure [10]. The likely explanation for the lack of correlation between *CYP2B6* metaboliser genotype and virological outcome in the current study and in the pooled ACTG studies is that other factors, notably adherence, are more important determinants of efavirenz concentrations.

Participants with lower CD4 cell count at baseline had a significantly increased hazard of virological non-suppression. Participants with high baseline viraemia also tended towards poorer virological outcomes, but this did not reach statistical significance in our model. Our data support earlier commencement of ART.

This study has several limitations. Timing of the efavirenz dose was not observed, although most patients reported taking their medication in the evening. The timing of viral loads was not under the control of the study staff and resulted in a large number of efavirenz samples being excluded from the analysis because they fell outside our time windows. As the focus of this study was on virological outcomes, we did not collect adverse event data and could not assess the impact of metaboliser genotype or high efavirenz concentrations on drug-related toxicity. A strength of this study is that the statistical approach used to establish the efavirenz concentration cut-off most predictive of non-suppression objectively analysed all possible dichotomised thresholds in the whole range of observations, instead of analysing only pre-defined cut-offs.

In summary, we have shown that in an ART-naïve cohort, efavirenz mid-dosing interval concentrations at Weeks 16 and 48 predict virological outcome. In addition, we confirm that knowledge of an individual's metaboliser genotype is not per se predictive of viral non-suppression. This analysis identified a threshold for

Table 2
Results of the Cox proportional hazards univariate and multivariate analyses.

Parameter	Univariate model		Full multivariate model		Final multivariate model	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Log_2 efavirenz concentration (mg/L)	0.76 (0.64–0.91)	0.0035	0.78 (0.68–0.90)	0.0005	0.77 (0.67–0.89)	0.0005
Baseline log_{10} VL	1.83 (0.94–3.53)	0.0734	1.40 (0.82–2.37)	0.2182		
Baseline CD4 count	0.9935 (0.9895–0.9976)	0.0019	0.9945 (0.9908–0.9983)	0.0040	0.9937 (0.9898–0.9977)	0.0018
Age (years)	0.9639 (0.9111–1.0200)	0.2010	0.9534 (0.8908–1.0204)	0.1682		
Sex (ref. M)	0.5884 (0.2588–1.3380)	0.2060	0.5289 (0.2090–1.3388)	0.1789		
Metabolic status (ref. FM + IM)	1.07 (0.43–2.65)	0.8810	1.78 (0.61–5.21)	0.2891		

HR, hazard ratio; CI, confidence interval; VL, viral load; FM, fast metaboliser; IM, intermediate metaboliser.

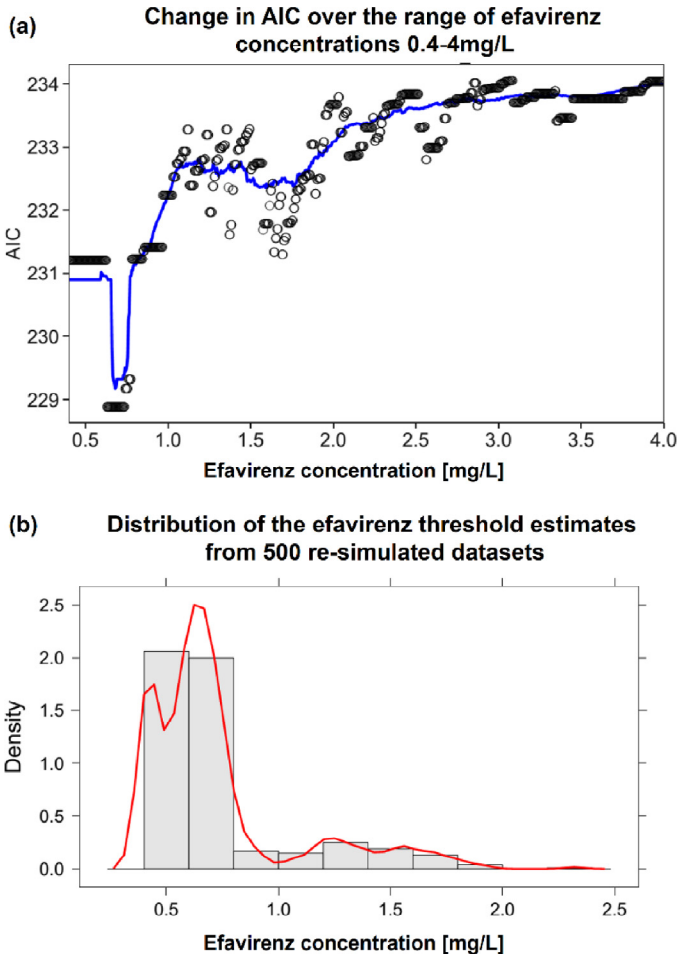

Fig. 2. (a) Comparison of the Akaike information criterion (AIC) values in models with dichotomised efavirenz mid-dose concentrations using cut-off values ranging from 0.4 mg/L to 4 mg/L in increments of 0.005 mg/L. The dots represent the AIC values for each tested concentration cut-off generated from the original data set, whilst the blue line is the median AIC value for tested concentration cut-offs from 500 re-simulated data sets. (b) Distribution of optimal cut-off values obtained from the 500 re-simulated data sets. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

efavirenz therapeutic range lower than currently recommended. Although the findings require confirmation, they suggest that efavirenz TDM using a revised cut-off of 0.7 mg/L may be of use in a routine clinical setting to identify patients at risk of virological failure.

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Competing interests: None declared.

Ethical approval: The study was approved by the University of Cape Town Research Ethics Committee (Cape Town, South Africa) [reference no. 158/2012]. The Chair is Professor Marc Blockman (marc.blockman@uct.ac.za). Each participant provided written informed consent. The trial was registered in the Pan African Clinical Trials Registry [no. PACTR201311000641402].

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2016.03.017.

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