The Association between Cigarette Smoking and Efavirenz Plasma Concentration using the Population Pharmacokinetic Approach

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Abstract

Aim

Efavirenz is still widely used as the preferred first-line antiretroviral agent in the middle- and low-income countries, including Malaysia. The efavirenz population pharmacokinetic profile among HIV-positive smokers is still unknown. We aimed to assess the association of smoking with efavirenz and the differences in HIV clinical outcomes.

Methods

A total of 154 stable HIV-positive patients on efavirenz in northern Malaysia were recruited with a sparse sampling for this multicentre prospective cohort study. The association between smoking and efavirenz pharmacokinetic parameters was determined using the non-linear mixed-effect model (NONMEM). A mixture model of clearance was adopted to describe the metaboliser status because genetic data is unavailable. The effect of smoking on HIV clinical markers (CD4, CD4 / CD8 ratio and viral blips) for at least two years after the antiretroviral initiation was also investigated.

Results

Our data were best fitted with a one-compartment mixture model with first-order absorption without lag time. Smoking significantly associated with higher clearance (CL/F) (β = 1.39; 95% confidence interval (CI): 1.07 to 1.91), while weight affected both CL/F and volume (V/F). From the mixture model, 20% of patients were in the slow clearance group, which mimic the genotype distribution of slow metaboliser. An efavirenz dose reduction is not recommended for smokers ≥60kg with normal metabolism rate. Smoking significantly associated with slower normalisation of CD4 and CD4 / CD8 ratio.

Conclusion

HIV-positive smokers presented with significantly higher efavirenz clearance and unfavourable clinical outcomes. Close monitoring of adherence and clinical response among smokers is warranted.

Keywords

smoking; efavirenz; plasma concentration; population pharmacokinetics; mixture model

Introduction

Efavirenz was mainly metabolised by cytochrome P (CYP) 2B6.[1] In vitro studies proposed that cigarette smoke induces CYP 2B6 through human constitutive androstane receptor (hCAR)[2] and pregnane X receptor (PXR).[3] The expression of brain CYP2B6 activity among smokers was also higher[4], which may lead to lower efavirenz concentration among this population. As compared with the total adult population, there is a higher prevalence of smokers among people living with human immunodeficiency virus (PLHIV).[5],[6] To date, most of the studies reported the effects of smoking on efavirenz as incidental findings.[7]–[12] Only one study in Serbia demonstrated that smoking significantly associated with lower efavirenz plasma concentration regardless of genetic constitution.[7] There was no data of head-to-head comparison in efavirenz concentration between smokers and non-smokers. Furthermore, none in the literature incorporates smoking status as a covariate that influences the population pharmacokinetic (Pop PK) properties of efavirenz.[13]–[22]

CYP 2B6 genetic polymorphism appeared to be a predominant covariate which affects efavirenz concentration significantly in 10 out of 16 studies in the literature review.[7]–[9],[15]–[19],[21],[22] However, genetic testing is not readily and widely available in resource-limited settings, including Malaysia. Therefore, individualising efavirenz therapy by taking into consideration the influence of genotype among local PLHIV may not be feasible. Thus, an alternative way of quantifying the efavirenz pharmacokinetic profile while minimising the inter- and intra- patients' variabilities and confounding effects, such as genetic factor, is required.

The neuropsychiatric adverse effects of efavirenz can be severe enough to cause treatment discontinuation.[23] There is a higher risk of central nervous system (CNS) side effects among patients with efavirenz plasma concentration above 4 mg/L.[24] However, efavirenz is still widely used as the preferred first-line antiretroviral agent in the middle- and low- income countries, including Malaysia.[25] Efavirenz dose of 400mg daily was reported to reduce efavirenz toxicities,[18] and was non-inferior to the usual 600mg daily dose in adult HIV-positive patients.[26]–[29] Nevertheless, none of the previous studies evaluated the drug response of efavirenz dose reduction among HIV-positive smokers.

Therapeutic drug monitoring (TDM) has been shown as a useful tool in optimising efavirenz therapy, especially among patients with inadequate dose or drug toxicities.[28],[30] Nonetheless, efavirenz is usually taken at bedtime and trough level sampling for TDM is not convenient for outpatients.[31] Hence, by applying the Bayesian forecasting method to Pop PK model development allows the prediction of the efavirenz steady-state trough concentration (Cp_{ss}), which may reduce bias and improve precision compared to the conventional approach.

The prevalence of smokers among Malaysian PLHIV is unknown. Moreover, no previous studies describe efavirenz concentration and its variability in this population. Hence, we reported a prospective cohort study on the association of cigarette smoking with efavirenz pharmacokinetic properties among PLHIV in northern Malaysia, using the non-linear mixed-effects modelling approach. The dose-response relationship of efavirenz Cp_{ss}, as well as the association between smoking status and HIV clinical markers (CD4, CD4 / CD8 ratio and viral blips), were also studied.

Methods

Study design

This multicentre prospective cohort study involved adult HIV-positive patients recruited from the infectious disease (ID) outpatient clinics in Hospital Sultanah Bahiyah and Hospital Kulim in Kedah,

Malaysia. Recruitment was done from September 2019 to September 2020 but was paused from 18th March to 9th June 2020 due to the movement control order (MCO). All participants were followed up for at least 24 months after highly active antiretroviral therapy (HAART) initiation.

Participants and clinical data

The diagnosis of HIV-positive status is based on repeatedly reactive results from anti-HIV antibody testing using microparticle enzyme immunoassay (MEIA). All the participants were supplied with Efavir® (Cipla) throughout the study period, which is fully subsidised by the Malaysian government. Smoking status was retrieved from the record of regular social history taking by the attending physician and later being confirmed again during a face-to-face interview session with patient. The assessment of smoking status was done more than once throughout the study period to minimise the selection bias.

The viral load in this study were measured using real-time polymerase chain reaction (PCR), and the limit of detection was 20 copies/ml. Values <20 copies/ml were defined as undetectable. Low-level viraemia of ≥20 and <1000 copies/ml was counted as a viral blip and was categorised into transient, recurrent or persistent type. Viral blip is used as the study endpoint because baseline viral load testing is not a routine practice in the study sites. Persistent viral blip is a detectable viral load for two consecutive readings with at least one month apart. CD4 count is a longitudinal data which was measured using immunofluorescence assay. CD4 counts were compared at 0 months, 6 months, 12 months, 18 months, 24 months, 36 months, and 60 months post-HAART initiation between smokers and non-smokers. Low CD4/CD8 ratio (<1) was shown to be independently associated with non-AIDS related morbidities.[32]–[34] CD4/CD8 ratio has a slower recovery rate than CD4. Thus, it was compared only at 0 months, 12 months, 24 months, and 60 months after HAART commencement. Causality assessment of suspected adverse drug reactions (ADR) was done using the World Health Organization - Uppsala Monitoring Centre (WHO-UMC) Causality Assessment System.[35]

Inclusion and exclusion criteria

HIV-positive patients who fulfilled these criteria: (1) aged between 18 - 65 years old, (2) received HAART for at least one year and efavirenz for at least one month, and on tenofovir disoproxil fumarate, emtricitabine and efavirenz as their current HAART regime (3) had normal liver (liver enzyme <40 U/L) and renal profile (estimated glomerular filtration rate >60 ml/min/1.73m^2), (4) had suppressed viral load (<20 copies/ml) and CD4 count >200 cells/µl, were eligible to participate in this study. Patients with active opportunistic infections, or receiving medications with significant drug-drug interactions with efavirenz were excluded. The participation will be terminated if there are changes in the patient's treatment regimen or if the patient becomes pregnant.

Sample size

The sample size was estimated using the Fleiss sample size calculation method for cohort studies.[36] The two-sided significance level was set at 0.05, and the power of the analysis was set at 80%. The prevalence of smokers among the HIV population was approximately 40%.[5] From the preliminary data, 30% of smokers and 10% of non-smokers have trough efavirenz Cp_{ss} of less than 1 mg/L. Therefore, the calculated total sample size for this study was 125 participants. For Pop PK modelling,

a minimum of two pharmacokinetic data from each participant is required,[37] and at least a sample size of 50 individuals is sufficient to estimate the parameters precisely (95% confidence interval, 50% precision level, power of 0.8).[38]

Efavirenz blood sampling and assay

Blood samples were collected at 8 to 20 hours post-dose for better correlation of efavirenz plasma concentration and 24-hour area under the curve (AUC).[31],[39] There was no pre-determined sampling time for all patients. Each participant was numbered with a study code for confidentiality. The treating physicians and other staffs in the clinic were not aware of the patients' coding. For each recruited participant, 3 ml whole blood was collected in ethylenediaminetetraacetic acid (EDTA) tube. Blood samples were then centrifuged at 3000*g* for 10 minutes within six hours of collection to obtain the plasma. The plasmas were placed in a water bath at 56°C for 30 minutes for virus inactivation. Sample extraction was done by protein precipitation using iced cold acetonitrile. The plasma was then analysed using reversed-phase high-performance liquid chromatography (RP-HPLC) according to a validated protocol.[40]

Population pharmacokinetic (PK) analysis

The population pharmacokinetic model of efavirenz was built using the non-linear mixed-effects modelling (NONMEM) software (version 7.4.4).[41] All the diagnostic plots were created using R (version 3.6.3)[42] and RStudio (version 1.2.5033),[43] with the R package '*nonmem2R*' (version 0.2.1), '*xpose4*' (version 4.7.0)[44] and '*ggplot2*' (version 3.3.2). Perl-speaks-NONMEM (PsN) (version 5.0.0)[45] was used for model evaluation and automated procedures. The first-order conditional estimation method with interaction (FOCEi) was used for model fitting. Natural log-transformed concentration was used as the dependent variable (DV).

Base model development

One- or two-compartment model, with or without absorption lag time, were attempted for the base model. In this study, efavirenz was assumed to have a first-order absorption with first-order elimination based on the previous literature.[16],[22] The parameters estimated include oral clearance (CL/F) and apparent volume of distribution (V/F). The absorption rate constant (k_a) was fixed at 0.7 h^{-1} because the efavirenz blood sample during the absorption phase was not available. According to the literature, by using a fixed k_a value from 0.3 to 3 h^{-1}, there were no significant differences in the other estimated parameters.[22] Furthermore, a mixture model was also tested to mimic the metaboliser status. The $MIXTURE function allows NONMEM to compute the probability of subpopulations based on CL/F. Therefore, if a participant has a low CL/F, it may indicate that the participant is a slow metaboliser. The interpatient variability in PK parameters was modelled in a log-normal distribution with exponential variance model as shown in Equation 1. θ_{xi} is the pharmacokinetic parameter '*x*' of the *i*th individual; θ_x is the fixed effect population parameter estimate; η_{xi} is the inter-individual variability for parameter '*x*' in individual '*i*' drawn from a normal distribution with a mean of zero and variance of ω^2.

$$\theta_{xi} = \theta_x \cdot \exp^{(\eta_{xi})} \qquad \text{(Equation 1)}$$

Proportional, additive and combined proportional and additive residual error models were explored to describe the residual variability. The minimal objective function value (OFV), which is proportional to minus twice the natural log-likelihood (-2LL) of the data, was used as a goodness-of-fit metric. A decrease of 3.84 in OFV corresponds to a statistically significant difference between models (p = 0.05, χ^2 distribution, one degree of freedom (Δdf)). Residual plots were also examined.

Covariate model development

Once the appropriate structural model was established, the following covariates were explored: smoking status, gender, age, weight, hepatitis co-infection status, and duration of efavirenz therapy. The relationships between covariates were examined for independence. Dichotomous covariates were introduced as a linear additive model (Equation 2), and continuous variables were modelled using a power model with normalised covariate (Equation 3).

$$\theta_{xi} = \theta_x + \theta_{COV} \times COV_i \quad \text{(Equation 2)}$$

$$\theta_{xi} = \theta_x \times \left(\frac{COV_i}{COV_{median}}\right)^{\theta_{COV}} \quad \text{(Equation 3)}$$

θ_{xi} and θ_x were defined as described previously. In Equation 2, θ_{COV} is the coefficient of the value for the dichotomous covariate COV_i, which equals to 0 or 1. In Equation 3, COV_i is the value of the covariate for the ith individual; COV_{median} is the median value of the covariate in the population dataset; θ_{COV} is the exponent describing the covariate effect. For weight as a covariate, an allometric model was applied to CL/F and V/F using a reference value (WT_{ref}) of 70kg in Equation 3 above instead of the median of the dataset and fixing the exponent to 0.75 for CL/F and 1 for V/F. [46],[47]

Exploratory plots were used to assess the relationship between covariates and individual predicted pharmacokinetic parameters. All the relevant covariates were added into the base model to form a full covariate model.[48] The best-fitting covariate model was obtained using the Akaike Information Criterion (AIC). The model with the smallest AIC value or significant changes in OFV from the base model (p = 0.001, χ^2 distribution, one degree of freedom (Δdf)) would be considered as the best fitting model.[49] Additionally, a posterior distribution of the covariate effect plot was sketched to evaluate the clinical relevance of covariates. Any covariate with a point estimate and 95% confidence interval (CI) of more than ±20% from reference is deemed to be clinically significant.[17]

Internal model evaluation was performed with visual predictive checks (VPC) of 1000 simulations. The observed data points have to fall within the prediction intervals (80%) of the simulated data for better correlation.[47] Bootstrapping of the final model with 500 replicates was carried out using PsN. Each parameter estimate was compared with the 90% CI of the bootstrap result.

A simulation study was executed to investigate the dosing regimen scenarios of 400mg versus 600mg daily among smokers with different weight and metaboliser status. The 80% prediction intervals of the simulated efavirenz concentrations for each category were plotted.

Other statistical analyses

Apart from the Pop PK model development using NONMEM, all the other statistical tests were performed using IBM SPSS Statistics (version 24). Viral blips between smokers and non-smokers were analysed using the Chi-square test. The differences of CD4 count and CD4/CD8 ratio between smokers and non-smokers were evaluated using a linear mixed model with heterogeneous first-order autoregressive covariance structure.[50]–[52] The time interval between CD4 count measurement was unequal for most of the participants.

Results

Descriptive results

A total of 154 participants were recruited, and 202 efavirenz concentrations were available from 138 participants (Table 1). Four samples (2%) were below the limit of detection due to non-adherence. Hence, 198 levels were included in data analysis, with 60 patients had two data points. The prevalence of smoking was 45% (n = 69), which included 14% (n = 22) of ex-smokers. The smoking status of the participants was consistent throughout the study. On average, the viral load of the participants took seven months after HAART initiation (interquartile range (IQR): 7) to become undetectable, while the first viral blip occurred around 17 months (IQR: 7). Among patients with hepatitis co-infection, 56% (n = 20) were co-infected with hepatitis C. Participant with ADR was noted to have a lower median efavirenz concentration, but no significant association was found (1.47 vs 1.68 mg/L; p = 0.092). The efavirenz-related ADR experienced by the participants mainly consisted of dizziness (n = 35; 57%) and drowsiness (n = 19; 31%).

Chromatographic results

The calibration curve obtained for efavirenz was linear in the range of 0.5 to 16 mg/L. The equation of the calibration curve was $y = 129.66x - 0.0257$, $R^2 = 0.9999$. The limit of detection (LOD) calculated was 0.04 mg/L, while the lower limit of quantification (LLOQ) was 0.13 mg/L.[53] The intraday and interday precision's relative standard deviation (RSD) was 3.75% and 4.09%. The accuracy and recovery rates were 99.7% and 97.4% respectively.

Pharmacokinetic model

A one-compartment mixture model with first-order absorption without lag time, with an additive residual error model, best described our data. The incorporation of the mixture model into the base model was able to reduce the OFV for 27.529 (Δdf = 2; p <0.001). Duration of efavirenz therapy highly correlated with age (r = 0.35; p <0.001) and weight (r = -0.23; p <0.001), thus, it was excluded from the model.

Weight significantly associated with both CL/F and V/F, while smokers with or without hepatitis had higher CL/F. When the $OMEGA BLOCK was used, the ETA-V/F was approaching zero. Hence, the ETA-V/F was fixed at zero for the covariate model to converge successfully. The summary of the parameter estimates in both base and final model was as demonstrated in Table 2. The parameter estimates of the final model fell within the 90% CI of the bootstrapping results, which indicated a good-fitting model. The goodness-of-fit plot of the final model was as shown in Figure 1, and the posterior distribution plot of clinical significance was in Figure 2.

In the simulations, the observed data was within the 80% prediction interval of the predictions denoting a good fit of the model. Smokers had significantly lower efavirenz trough Cp_{ss} than non-smokers (1.64 mg/L versus 2.15 mg/L; p <0.001). Only 18.2% of smokers would have a trough Cp_{ss} below 1 mg/L when receiving 600mg daily efavirenz. However, if it is given at 400mg daily, 40.3% of smokers would have a trough Cp_{ss} below the therapeutic range as opposed to only 25.7% among non-smokers (p <0.001). Among smokers weigh 60kg and above, 57.1% of them on 400mg efavirenz will have a trough level lower than the therapeutic range, while it happened in only 21.4% of those on 600mg (Figure 3). Therefore, dose reduction of efavirenz to 400mg is not recommended for smokers ≥60kg with normal metabolism rate. Besides that, cumulative pack-year and cigarette quantity had no association with efavirenz Cp_{ss}.

HIV clinical markers

The prevalence of viral blips was not significantly different between smokers and non-smokers (p = 0.166), as well as for efavirenz trough Cp_{ss} (p = 0.725). Only patients with nadir CD4 count <200 cells/µl significantly associated with viral blips (p = 0.014). In terms of frequency of viral blips, more smokers had recurrent or persistent viral blips (p = 0.021). A significant difference in CD4 count was found between smokers and non-smokers up to 24 months after HAART initiation (p = 0.026) (Figure 4). Heavy smokers (≥20 cigarettes/day) presented with slower recovery of CD4 count (p = 0.005). There was no association between mean CD4 count and efavirenz Cp_{ss} within 24 months of HAART initiation (p = 0.813). Moreover, the normalisation of the CD4 / CD8 ratio was slower than CD4 (Figure 5). There was a distinct difference in CD4 / CD8 ratio between smokers and non-smokers even at 60 months post-HAART (p <0.001) (Table 3). The gap began to diminish when the duration of HAART approaching 15 years. No association was found between CD4 / CD8 ratio and efavirenz Cp_{ss} (p = 0.088).

Discussion

The parameter estimates of our model were comparable and within the range of the values reported in previous literature (CL/F: 0.5 L/h to 19.6 L/h; V/F: 102 L to 293 L).[13]–[22] Besides weight, no other covariates could effectively address the variation of V/F, which resulted in not much reduction in relative standard error (%RSE) from the base model. The lack of genotype data might be one of the reasons too. The aggregation of CL/F subpopulation by mixture model in this study might not be congruent with the actual genotype data. In the literature, the population of PLHIV on efavirenz was commonly specified into three different genotype polymorphisms (extensive, intermediate, slow) which accounted for the difference in CL/F.[18],[21] However, an attempt of modelling our participants into three subpopulations did not converge, which might be due to inadequate samples of the individuals with intermediate CL/F. Nevertheless, the percentage of individuals in the slow CL/F group (subpopulation 2) (20%) was in agreement with the CYP2B6*6 distribution among the Malaysian population.[54]

Since there was still no consensus on the actual minimum effective concentration (MEC) of efavirenz,[55] we used the cut-off point of 1 mg/L, as by most of the literature.[56] Our study has a longer follow-up period of 24 months compared with 12 months in most of the literature, but yet to prove any significant association between efavirenz Cp_{ss} and viral blips. Patients who were presented with persistent viral blips had efavirenz Cp_{ss} of 0.55 to 1.26 mg/L, which is in accordance with the data of a study in China.[57] From this observation, we proposed that efavirenz might exhibit a time-

dependent efficacy profile. Furthermore, persistently low or fluctuating efavirenz concentrations resulted in the development of resistance.[58],[59] Therefore, it is advisable to maintain efavirenz concentration above the MEC at all time throughout the treatment period.

Following the results of our simulation data, dose reduction of efavirenz to 400mg was predicted to be safe, except for smokers with normal metabolism rate, especially those who weigh more than 60kg. In institutions with limited resources, where genetic data was not available, single efavirenz Cp_{ss} is speculated to be sufficient to identify the presence of slow metabolism. As shown in Figure 3, there was a distinctive difference in the range of efavirenz Cp_{ss} between slow CL/F and normal CL/F population. Once the metabolism and smoking status is known, efavirenz dose adjustment can be made safely. Clinical management with the application of TDM or genotype testing if possible was proven to be more cost-effective.[60],[61]

On the other hand, there were contradicting reports regarding the association of hepatitis status with efavirenz concentration.[62]–[65] Considering 23% (n = 36) of our patients co-infected with hepatitis, the effect on efavirenz concentration might be confounding. Therefore, we also investigated the influence of hepatitis on efavirenz among smokers and non-smokers. As a result, we found no association between hepatitis status and efavirenz concentration in both smokers and non-smokers. Our observation was in agreement with the findings of a genetic study.[66] The expression of CYP2B6 had no significant changes among hepatitis patients, which indicated that the metabolism rate of efavirenz does not decrease with hepatitis co-infection. Hence, we can conclude that smoking significantly reduces efavirenz concentration regardless of hepatitis status.

While numerous works of literature had described a higher baseline viral load and failure of viral suppression among smokers,[67]–[71] our study was the first that elucidate the effect of smoking and efavirenz Cp_{ss} on viral blips. Given the slower recovery of CD4 and CD4 / CD8 ratio among smokers, the clinical progress of HIV smokers should be monitored closely, especially during the first two years of HAART. HAART should be initiated immediately as soon as after HIV diagnosis if no contraindication.[72] Within two years of starting HAART, more frequent viral load and CD4 test should be carried out for smokers until viral suppression is achieved and maintained, while adherence should be assessed at every encounter with the health care providers.

Limitations

Sparse and random samplings of efavirenz concentration were the main limitations of our study. For the sake of convenience of the study participants, blood sampling was taken place in an outpatient setting, which cause the pharmacokinetic profile of the absorption and distribution phase could not be captured. Nevertheless, population pharmacokinetic modelling using sparse data is possible and had been described before in the literature.[17],[73],[74] With the overlay of the COVID-19 pandemic, participants could not present for further blood sampling. Patients who did not turn up in the clinic were not recruited, which could also be a source of selection bias. Efavirenz concentration of ex-smokers before quit smoking was not available. Therefore, we could not describe the effect of smoking cessation on efavirenz. Only limited participants were electronic cigarette users; hence, the data was not presented in this study.

Conclusions

Smoking significantly associated with higher clearance and lower Cp_{ss} of efavirenz. Smokers also presented with slower CD4 count and CD4/CD8 ratio recovery. Optimised therapy and behavioural interventions for smoking cessation need to be enforced to achieve better HIV treatment outcome. Application of TDM may contribute to the evaluation of antiretroviral response, medication adherence, and dosage adjustment. With population pharmacokinetic modelling, antiretroviral dosing regimen can be optimised without extensive blood sampling. Mixture model can be a useful approach when genetic data is not available. Currently, there was still no adoption of pharmacometrics in clinical practice in Malaysia. A centralised research institution with TDM service of antiretroviral agents could be set up in future.

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Contributors

CNK was responsible for study conception and design, collecting and analysing the data and drafting the first manuscript. SNH participated in concept development, reviewed and supported the analyses, reviewed and edited the manuscript. SMSG reviewed and supported the analyses, reviewed and edited the manuscript. AHK participated in material preparation, review and edited the manuscript. WEJ and LLL contributed ideas to the clinical aspect of the data and reviewed the manuscript. All authors read and approved the final manuscript.

Declarations

Compliance with ethical standards

This study has been granted ethical approval from the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (ID: NMRR-18-445-40064) and the Human Research Ethics Committee (JEPeM), Universiti Sains Malaysia (Code: USM/JEPeM/20030178), which complies with the Declaration of Helsinki. Informed consent was obtained from all the participants on the day of recruitment by the principal investigator.

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No funding was received for this study. All authors declare that they have no conflicts of interest or financial relationships relevant to this article to disclose.

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Table 1 Descriptive statistics of demographic and clinical data of participants.

Variables	Total (*n* = 154)
	n (%) or Mean (SD)
Smoking status	
Never smoked	85 (55.2)
Ever smoked	69 (44.8)
Cumulative pack-year[a]	10 (17.9)
Efavirenz trough steady-state concentration, mg/L[a]	1.7 (1.49)
Age, years	42 (11.1)
Weight, kg	60.7 (13.07)
Gender	
Male	106 (68.8)
Female	48 (31.2)
Ethnic	
Malay	94 (61.0)
Chinese	40 (26.0)
Indian	14 (9.1)
Others	6 (3.9)
Mode of transmission	
Heterosexual	87 (56.5)
Homosexual / Bisexual	38 (24.7)
Intravenous drug use	12 (7.8)
Blood transfusion / Vertical transmission	3 (1.9)
Others / Unknown	14 (9.1)
Alcohol drinker	
No	135 (87.7)
Occasional	13 (8.4)
Regular (at least once a week)	6 (3.9)
Comorbidities	
No comorbidity	83 (53.9)
Hepatitis B or C	36 (23.4)
Other comorbidities	35 (22.7)
Adverse drug reactions	
Yes	61 (39.6)
No	93 (60.4)
Nadir CD4 count, cells/µl[a]	120 (243)

Viral blips	
Yes	30 (19.5)
No	124 (80.5)
Duration of HAART, months[a]	56.5 (84)
Duration of efavirenz, months[a]	48 (70)

Note: [a]Presented as median (interquartile range); *$p < 0.05$. SD: standard deviation.

Table 2 Parameter estimates of base model and final covariate model.

Parameter	Base model		Final model	
	Estimate	RSE (%)	Estimate	RSE (%)
OFV	-143.006		-159.539	
CL/F Subpopulation 1, L/h	8.50	6.0	9.88	4.6
CL/F Subpopulation 2, L/h	2.17	13.8	2.26	12.5
V/F, L	159.52	29.9	260.92	29.2
k_a, h^{-1}	0.7 FIX	-	0.7 FIX	-
%CV ETA-CL/F	39.46	27.4	30.12	18.4
ETA Covariance	0.1612	59.4	0 FIX	-
%CV ETA-V/F	55.47	68.8	0 FIX	-
SD RUV	0.14	18.2	0.14	18.7
Probability of Subpopulation 1	0.86	4.2	0.80	4.9
Smoking status on CL/F	-	-	1.39	25.0
Weight on CL/F	-	-	0.75 FIX	-
Weight on V/F	-	-	1.00 FIX	-

CL/F: oral clearance; CV: coefficient of variation; ETA: interindividual random effect; k_a: absorption rate constant; OFV: objective function value; RSE: relative standard error; RUV: residual unexplained variability; SD: standard deviation; V/F: apparent volume of distribution.

Table 3 Mean CD4 count and CD4 / CD8 ratio of smokers versus non-smokers over time.

Time point	Ever smoked CD4, cells/µl Mean (SE)	Never smoked CD4, cells/µl Mean (SE)	*p* value	Ever smoked CD4 / CD8 ratio Mean (SE)	Never smoked CD4 / CD8 ratio Mean (SE)	*p* value
Nadir	128.59 (19.10)	196.28 (16.74)	0.012*	0.16 (0.033)	0.25 (0.031)	0.081
6 months	172.91 (17.87)	242.26 (16.23)	0.005*	-	-	-
12 months	261.02 (18.09)	334.30 (16.49)	0.003*	0.17 (0.027)	0.28 (0.026)	0.006*
18 months	314.97 (18.52)	387.51 (17.18)	0.005*	-	-	-
24 months	377.02 (18.04)	431.28 (16.06)	0.026*	0.37 (0.029)	0.50 (0.028)	0.003*
36 months	401.54 (17.31)	434.88 (15.41)	0.152	-	-	-
60 months	429.86 (16.56)	459.82 (14.83)	0.179	0.46 (0.024)	0.60 (0.023)	0.000*

Note: *$p < 0.05$; SE: standard error.

Figure 1 Goodness-of-fit plots of final model.

Figure 2 Posterior distribution plot of covariates effects. (CL/F: oral clearance; V/F: volume of distribution).

Figure 3 Simulation plot of 600mg versus 400mg oral efavirenz in smokers with weight 60 – 80kg.

Figure 4 Comparison of CD4 count over 24 months between smokers and non-smokers.

Figure 5 Comparison of CD4/CD8 ratio over 60 months between smokers and non-smokers.