**Comprehensive Pharmacokinetic, Pharmacodynamic and Pharmacogenetic Evaluation of Once Daily Efavirenz 400 mg and 600 mg in Treatment-Naïve HIV-Infected Patients at 96 Weeks: Results of the ENCORE1 Study**

Laura Dickinson1, Janaki Amin2, Laura Else1, Marta Boffito3, Deirdre Egan1, Andrew Owen1, Saye Khoo1, David Back1, Catherine Orrell4, Amanda Clarke5, Marcelo Losso6, Praphan Phanuphak5, Dianne Carey2, David A Cooper2, Sean Emery2, Rebekah Puls2,  
on behalf of the ENCORE1 study group

1 Department of Molecular & Clinical Pharmacology, University of Liverpool, Liverpool, UK; 2 The Kirby Institute, UNSW Australia, Sydney, Australia; 3 Chelsea & Westminster Foundation Trust, London, UK; 4 Desmond Tutu HIV Foundation, Cape Town, South Africa; 5 HIV-NAT Thai Red Cross AIDS Research Center, Bangkok, Thailand; 6 Hospital Ramos Mejía, Buenos Aires, Argentina

**Correspondence:** Laura Dickinson, Department of Molecular & Clinical Pharmacology, University of Liverpool, Block H, First Floor, 70 Pembroke Place,

Liverpool, L69 3GF, UK

Telephone: +44 (0) 151 794 5553, Fax: +44 (0) 151 794 5656 [laurad@liv.ac.uk](mailto:laurad@liv.ac.uk)

**Alternative**

**correspondence:** Dianne Carey, The Kirby Institute, UNSW Australia, Sydney, Australia.

[Dcarey@kirby.unsw.edu.au](mailto:Dcarey@kirby.unsw.edu.au)

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**Key Points**

Despite concerns regarding lower plasma concentrations obtained with efavirenz 400 mg (EFV400) compared to 600 mg (EFV600) in ENCORE1, virological efficacy was not compromised at 96 weeks [HIV-RNA (pVL) <200 copies/mL: 97% vs. 99%; p=0.091]. Achieving pVL <200 copies/mL at 96 weeks was not associated with the selection of single nucleotide polymorphisms (SNP; *CYP2B6,* *CYP2A6*, *CYP3A4*, *NR1I3*, *NR1I2*, *ABCB1*) assessed.

EFV-related adverse events and discontinuations due to these events were increased with dose but the higher rate of EFV-related adverse events for EFV600 was not associated with the SNPs investigated. CNS adverse events were not driven by EFV dose or concentrations however, *CYP2B6* 15582CT/TT and *ABCB1* 3435TT carriers were at higher risk (46% and 131%, respectively) of CNS-related adverse events compared to 35% lower risk in *CYP2B6* 983TC/CC patients. Possession of the *CYP2B6* 516GT and TT variants and *CYP2A6*\*9B CA/AA carriers were associated with higher risk of overall EFV discontinuation (80%, 166%, 100%, respectively) whereas *NR1I2* 63396TT carriers were at decreased risk (22%).

ENCORE1 questions the validity of the currently accepted minimum effective concentration (MEC) of 1.0 mg/L. The proportions of patients with pVL ≥200 copies/mL was not significantly different between those with model predicted EFV C12 (mid-dosing interval concentration) above or below 1.0 mg/L [2% (11/557) *vs.* 11% (2/18); *p*=0.059; note that 2/20 patients with C12 <1.0 mg/L had missing pVL at 96 weeks]. Although a threshold concentration is clinically useful, the acceptable ROC criteria associated with a range of C12 cut-offs (0.47-0.76 mg/L) for pVL <200 copies/mL at 96 weeks suggests a single target value is not statistically valid.

**Abstract**

**Background**: ENCORE1 demonstrated non-inferiority of daily efavirenz 400 mg (EFV400) versus 600 mg (EFV600) to 96 weeks in treatment-naïve, HIV-infected adults but concerns regarding lower EFV400 concentrations remained. Therefore, relationships between EFV pharmacokinetics (PK) and key genetic polymorphisms with 96 week efficacy and safety were investigated.

**Methods**: Relationships between EFV PK parameters and single nucleotide polymorphisms (SNP; *CYP2B6,* *CYP2A6*, *CYP3A4*, *NR1I3*, *NR1I2*, *ABCB1*) with plasma HIV-RNA (pVL) <200 copies/mL and EFV discontinuation and adverse events at 96 weeks were explored. ROC analysis evaluated the predictability of mid-dose interval (C12) cut-offs and 96 week pVL.

**Results**: A total of 606 patients (32% female; 37% African, 33% Asian; n=311 EFV400, n=295 EFV600) were included. EFV PK parameters including C12 were not associated with pVL <200 copies/mL at 96 weeks [OR (95% CI): 5.25 (0.41-67.90); *p*=0.204]. Lower risk of CNS-related adverse events was associated with *CYP2B6* 983TC/CC [OR (95% CI): 0.35, 0.15-0.81; *p*=0.015] and higher risk with *CYP2B6* 15582CT/TT and *ABCB1* 3435TT [1.46, 1.02-2.09; *p*=0.040, 2.31, 1.33-4.02; *p*=0.003]. Discontinuation due to adverse events (clinician decision) was independently associated with dose [OR (95% CI): 2.54 (1.19-5.43); *p*=0.016]. C12 between 0.47-0.76 mg/L provided sensitivity/specificity >90% (100%/92.3%-98.9%/92.3%) for achieving pVL <200 copies/mL at 96 weeks.

**Conclusions**: Higher rate of EFV-related adverse events and discontinuations due to them for EFV600 were not driven by polymorphisms assessed. Although a single threshold concentration associated with HIV suppression may be clinically useful it was not viable for ENCORE1. Implementation of EFV400 would improve toxicity management whilst maintaining efficacy.

**Introduction**

Antiretroviral dose reduction is an ongoing area of debate, focusing on advantages of reduced adverse events and treatment costs versus the potential risk of higher rates of virological failure.

Efavirenz (600 mg once daily), the mainstay of combination antiretroviral therapy in resource-limited settings [1], was selected as a potential candidate for dose reduction based on early clinical data that observed similar short-term efficacy with lower efavirenz doses (200 and 400 mg once daily [2]). These data and the principle that successful antiretroviral dose reduction can cut medication costs and allow greater treatment coverage, was the impetus behind the design and implementation of the ENCORE1 trial. ENCORE1, a multi-centre, double-blind, placebo-controlled trial, demonstrated non-inferiority of reduced dose efavirenz (400 mg once daily; EFV400) with the standard dose (600 mg once daily; EFV600) in treatment-naïve, HIV-infected adults at 48 weeks [3] and was sustained to 96 weeks [4].

Important concerns regarding the impact of lower concentrations with EFV400 and overall influence of key genetic factors on pharmacokinetics (PK) were recently addressed for the 48 week outcome data [5]. Here we present the final EFV PK-pharmacodynamic (PD) and pharmacogenetic cross-sectional analysis of ENCORE1 at 96 weeks.

**Methods**

*Patients*

ENCORE1 study design (to 48 and 96 weeks) has been described in detail previously [4, 3]. ENCORE1 was a randomised, double-blind, placebo-controlled trial in treatment-naïve, HIV-infected individuals ≥16 years recruited from 38 study sites across Africa, Asia, South America, Europe and Oceania. Patients were randomised to EFV400 or EFV600 with tenofovir/emtricitabine (Truvada®, 300/200mg) administered once daily. The study was granted ethical and regulatory approval and written informed consent was obtained from all participants.

*Sampling and Pharmacokinetics*

The ENCORE1 PK sampling scheme has been reported previously [5]. Random, single blood samples were collected at weeks 4 and 12 of therapy (between 8-16 hours post-dose) and intensive sampling was also carried out in a sub-group of patients (n=46) between weeks 4 and 8 [pre-dose (0 hour), 2, 4, 8, 12, 16 and 24 hours post-dose]. EFV plasma concentrations were quantified by a validated high-performance tandem mass spectrometry (HPLC-MS/MS) method [6] and non-linear mixed effects modelling was applied to the data (NONMEM v. 7.2, ICON Development Solutions, Ellicott City, MD, USA [7]) to determine EFV PK parameters in each patient at each sampling occasion. The impact of patient demographics and SNPs (see below) on EFV concentrations was evaluated as part of the modelling process [5]. Derived PK parameters including area under the concentration-time curve over the 24 hour dosing interval (AUC0-24), maximum concentration (Cmax), trough concentration 24 hours post-dose (C24) and concentration 12 hours post-dose representing the mid-dose interval concentration (C12) were determined for each sampling occasion and the mean calculated for each patient. Standard modelling practises were applied and the procedures have recently been described in detail [5].

*Genotyping*

Single nucleotide polymorphisms, *CYP2B6* 516 G>T (rs3745274), *CYP2B6* 983 T>C (rs28399499), *CYP2B6* 15582C>T (rs4803419), *CYP2A6*\*9B (rs8192726), *CYP2A6*\*17 (rs28399454), *CYP3A4*\*22 (rs35599367), *NR1I3* 540C>T (rs2307424) and *NR1I3* 1089T>C (rs3003596) were genotyped previously [5]. Additionally, *ABCB1* 3435C>T (rs1045642), *NR1I2* 63396C>T (rs2472677) and *NR1I2* 7635A>G (rs6785049)were genotyped using real-time PCR allelic discrimination assays for the present analysis (C\_7586657\_20, C26079845\_10 and C\_29280426\_10, respectively; Applied Biosystems, Foster City, CA, USA) as previously described [8, 9].

*PK-PD Analysis: relationships with virological and safety endpoints*

The primary PD endpoint was the proportion of patients with plasma HIV RNA (pVL) <200 copies/mL at 96 weeks by randomised dose (Fisher’s exact test). Patients without a viral load measurement at 96 weeks were excluded from the analysis. Relationships between pVL <200 copies/mL at 96 weeks and log transformed model predicted EFV AUC0-24, Cmax, C24, and C12 was performed by logistic regression.

Safety endpoints consisted of EFV discontinuation and adverse events. Overall discontinuation was defined as interruption in EFV treatment for more than 30 days. Adverse events were categorised as EFV-related defined in the Stocrin® Product Information [10] and EFV-related according to clinician decision. Additionally, CNS adverse events (as a subset of adverse events) defined in the Stocrin® Product Information (including abnormal dreams, anxiety, dizziness, headache, impaired concentration, insomnia and somnolence [10]) and treatment cessation due to EFV-related adverse events (clinician decision) were also assessed.

Differences in proportions of each safety endpoint by EFV dose were assessed by Pearson’s Chi-Square. Geometric mean ratio (GMR, 90% CI) was calculated to compare PK parameters between those who did or did not stop therapy and/or experience adverse events. Differences were considered significant if the CI did not cross 1.

*Pharmacogenetics: relationships with virological and safety endpoints*

Differences in proportions of pVL <200 copies/mL at 96 weeks for each genetic polymorphism and pVL ≥200 copies/mL at week 96 stratified for metaboliser status (extensive, intermediate, slow; based on *CYP2B6* 516G>T/986T>C/*CYP2A6*\*9B/\*17 composite genotype as previously reported [5]) and dose were assessed by Fisher’s exact test.

Evaluation of relationships between overall discontinuation with SNPs and EFV-related adverse events (Stocrin® Product Information) and dose and SNPs was performed by Cox regression adjusted *a priori* for potential confounders (e.g. age, sex). Post-hoc exploratory analysis of the crude association of dose and SNPs with CNS-related adverse effects, EFV-related adverse events (clinician decision) and treatment cessation due to EFV-related adverse event (clinician decision) was undertaken using logistic regression or Cox regression as appropriate.

*Evaluation of the recommended minimum effective concentration (MEC, 1.0 mg/L)*

Differences in the proportions of patients with model predicted EFV C12 below and above the recommended MEC of 1.0 mg/L [11] stratified by pVL (<200 copies/mL vs. ≥200 copies/mL) was determined by Fisher’s Exact test. A ROC analysis was also performed to investigate the predictability of mid-dose interval concentration (C12) cut-offs and achieving pVL <200copies/mL at 96 weeks. Patients with pVL missing at 96 weeks were excluded from the analysis.

Statistical analyses were performed using SPSS (v. 21, IBM, New York, USA).

**Results**

*Patients and Pharmacokinetics*

Six hundred and thirty patients received at least one dose of EFV as part of ENCORE1 [4]; 606 (32% female) were included in the previously described population PK model [5] and the present analyses (Fig. 1a). Median (range) age, weight, baseline (week 0) pVL and CD4 cell count were 35 years (18-69), 65 kg (39-148), 56803 copies/mL (162-10000000) and 270 cell/mm3, respectively. Patients identified as African (37%), Asian (33%), Hispanic (17%), Caucasian (13%) and Aboriginal/Torres Straits Islander (ATSI; 0.2%) and 51% and 49% were randomised to EFV400 (n=311) and EFV600 (n=295), respectively.

Subsequent to PK model development [5] three additional SNPs were genotyped (*ABCB1* 3435C>T, *NR1I2* 63396C>T, *NR1I2* 7635A>G) to complete the panel selected for ENCORE1. Upon assessment in the model as covariates they were found not to have a significant impact on EFV apparent oral clearance (CL/F). The PK parameters therefore did not alter from the previous 48 week analysis and were carried forward to the 96 week analyses. The final model included baseline weight and *CYP2B6* 516G>T/983T>C/*CYP2A6*\*9B/\*17 composite genotype as significant covariates [5]. Predicted EFV PK parameters stratified by dose and by dose and metaboliser status (extensive, intermediate, slow; based on *CYP2B6* 516G>T/983T>C/*CYP2A6*\*9B/\*17 composite genotype) as presented for the 48 week analysis are summarised (Online Resource 1 & 2, respectively).

*Genotyping*

Genotyping was possible in 595 patients, and of the 606 included in the analysis, 32 did not have a genotyping sample (Fig. 1b). Amplification failed in three patients for *NR1I2* 63396C>T and *NR1I2* 7635A>G. Depending on the SNP, PK and genetic data were available for between 570-574 patients (Fig. 1b). Genotype frequencies summarised by ethnicity are shown (Table 1; Caucasian, Hispanic and ATSI were combined for consistency with the 48 week analysis [5]); all were in Hardy-Weinberg equilibrium with the exception of *NR1I2* 7635A>G, however this was rectified when stratified by ethnicity.

*PK-PD Analysis: relationships with virological and safety endpoints*

At 96 weeks, 97% and 99% were <200 copies/mL for EFV400 and EFV600, respectively (*p*=0.091; 98% pVL <200 copies/mL overall); 2% (n=13) had a detectable pVL ≥200 copies/mL and 5% (n=31) of pVL were unavailable.

Following univariable logistic regression no relationships were observed between achieving pVL <200 copies/mL at 96 weeks and log transformed EFV PK parameters [logAUC0-24 odds ratio (OR; 95% CI): 4.20 (0.31-57.77); *p*=0.283, logCmax OR (95% CI): 1.87 (0.11-32.50); p=0.667, logC24 OR (95% CI): 4.17 (0.70-24.94); *p*=0.118 and logC12 OR (95% CI): 5.25 (0.41-67.90); *p*=0.204].

Eleven percent (n=34) and 13% (n=39) of patients discontinued EFV400 and EFV600, respectively [*p*=0.395; 73/606 (12%)] and amongst those that discontinued, median (range) time to discontinuation was 36 weeks (2-90). Significantly higher proportions of EFV600 patients experienced EFV-related adverse events than EFV400 (Stocrin® Product Information: 73% *vs.* 66%; *p*=0.043, clinician decision: 46% *vs.* 38%; *p*=0.048) and more stopped therapy due to adverse events judged by a clinician (8% *vs.* 3%; *p*=0.019). CNS adverse events were similar between doses (42% EFV400 *vs.* 46% EFV600; *p*=0.287).

Model derived AUC0-24, Cmax and C12 were significantly lower in those that did not discontinue therapy or stop due to EFV-related adverse events (clinician decision). EFV Cmax was significantly reduced in those that did not experience EFV-related adverse events (Stocrin® Product Information or clinician decision). PK parameters were not significantly different between those that did and did not have CNS adverse events (Table 2).

*Pharmacogenetics: relationships with virological and safety endpoints*

None of the SNPs assessed were associated with achieving pVL <200 copies/mL (Table 3). Proportions of patients with pVL ≥200 copies/mL at 96 weeks stratified by metaboliser status were similar between doses (EFV400 *vs*. EFV600 extensive: 3% *vs*. 1%; *p*=0.624, intermediate: 4% *vs*. 2%; *p*=0.281, slow: 5% *vs*. 0%; *p*=0.504).

Following adjustment for age, sex and dose and stratifying by country, *CYP2B6* 516GT, TT and *CYP2A6*\*9B heterozygote or homozygous variant (CA or AA) patients had a 80%, 166% and 100% increased risk of overall discontinuation, respectively whereas *NR1I2* 63396TT carriers were at reduced risk of 22% (Table 4). Upon multivariable Cox regression analysis, dose or SNPs were not associated with EFV-related adverse events (Stocrin® Product Information or clinician decision) following adjustment, however a greater risk of stopping due to EFV-related adverse events by clinician decision was observed with EFV600 compared to EFV400 [odds ratio (OR; 95% CI): 2.54 (1.19-5.43); *p*=0.016]. A decreased risk of CNS adverse events (Stocrin® Product Information) was associated with *CYP2B6* 983TC or CC carriers [OR (95% CI): 0.30 (0.12-0.75); *p*=0.010] but an increased risk in patients with *CYP2B6* 15582CT or TT and *ABCB1* 3435TT carriers was observed [OR (95% CI): 1.59 (1.11-2.27); *p*=0.011 and OR (95% CI): 2.14 (1.25-3.67); *p*=0.006, respectively].

*Evaluation of the recommended minimum effective concentration (MEC, 1.0 mg/L)*

The proportions of patients with pVL ≥200 copies/mL was not significantly different between those with model predicted EFV C12 above or below 1.0 mg/L (2% *vs.* 11%; *p*=0.059). Fourteen and six patients had predicted C12 below the recommended MEC for EFV400 and EFV600, respectively, but only one patient in each randomised arm was not suppressed below 200 copies/mL at 96 weeks. EFV C12 and metaboliser status were 0.77 mg/L, extensive metaboliser (EFV400) and 0.38 mg/L, intermediate metaboliser (EFV600; 2 viral load measurements were unavailable) in these two patients (Online Resource 3). The ranges of predicted C12 stratified by metaboliser status of the 10 (EFV400) and three patients (EFV600) with pVL ≥200 copies/mL at 96 weeks (n=13 total) were, EFV400: 0.77-3.65 mg/L (extensive, n=3), 1.45-3.38 mg/L (intermediate, n=5), 3.0 mg/L and 6.10 mg/L (slow, n=2); EFV600: 2.19 mg/L (extensive, n=1), 0.38 mg/L and 3.02 mg/L (intermediate, n=2).

The ROC curve lay generally along the line of unity between Sensitivity and 1-Specificity suggesting the analysis was informative to an extent. The sensitivity/specificity of using C12 of 1.0 mg/L (currently recommended MEC) for achieving pVL <200 copies/mL at 96 weeks was 97.1%/84.6% with a likelihood ratio (LR) of 6. Acceptable ROC criteria were generated for a number of C12 values suggesting a range of potential cut-offs, for example C12 between 0.47-0.76 mg/L provided sensitivity/specificity >90% (100%/92.3% to 98.9%/92.3%) with LR of 13.

**Discussion**

ENCORE1 included a genetically and geographically diverse population of patients thus providing an important dataset for thorough investigation of EFV PK-PD and pharmacogenetic relationships with clinical outcome and adverse events. EFV concentrations have previously been associated with virus suppression [12, 11]; however, this was not confirmed in ENCORE1. Relationships between model derived PK parameters and achieving pVL <200 copies/mL at 96 weeks (cross-sectional assessment) were not significant. Although significant associations were observed with pVL <200 copies/mL at the 48 week cross-sectional analysis (but confidence intervals were wide) [5], both analyses should be interpreted cautiously given only 16/593 (3%) and 13/575 (2%) of patients had pVL ≥200 copies/mL at 48 and 96 weeks, respectively. Furthermore, the PK was performed between 4-12 weeks and the association may have been lost for the more distal assessment at 96 weeks. Moreover, similar to the 48 week analysis [5], none of the SNPs assessed showed a significant association with virological control at 96 weeks. This is in agreement with previous studies in which *CYP2B6* polymorphisms in particular did not predict virological failure in HIV patients with differential or self-reported poor adherence [13, 14]. Given the low proportion of failures in ENCORE1, the study lacked adequate power to fully evaluate the impact of selected SNPs on HIV suppression. However, a genome-wide association study conducted by Lehmann and colleagues was able to detect genotypic relative risk of ∼80% power for polymorphisms with strong individual effects, but no associations with failure were observed even when adherence subgroups were considered [14].

Possession of homozygous wild type *CYP2B6* 15582C>T/516G>T/983T>C (CC/GG/TT) is predictive of EFV C24 in the lowest concentration stratum [15] and concerns have grown as to whether this population of individuals would be at increased risk of virological failure, particularly when receiving EFV400. This genotype was not predictive of failure in patients receiving the standard EFV dose [14] and of 47 ENCORE1 patients randomised to EFV400 with this genotype; only one had a detectable pVL ≥200 copies/mL at 96 weeks. Individual mean predicted EFV C24 was 2.79 mg/L in this patient and well above the median of 0.82 mg/L for this genotype group.

A previously defined MEC of 1.0 mg/L is often quoted as a therapeutic cut-off for EFV mid-dosing interval concentrations [12, 11]. However, this value was obtained in an era of less potent antiretroviral therapy with lamivudine, zidovudine, nelfinavir and amprenavir most commonly co-administered with efavirenz [12, 11]. The validity of a threshold concentration for virological failure has also been disputed due to low sensitivity of the predictive value particularly in adherent patients [16]. ENCORE1 provided an opportunity to investigate the plausibility of the widely implemented MEC. We chose to evaluate the threshold using the final 96 week pVL data rather than 48 weeks as this may be more representative of patients on long-term therapy. Assessment of the MEC was based on C12 (representing mid-dose interval concentrations) instead of C24 to remain consistent with the original publication by Marzolini et al [11]. However it is important to note that with only 2% of patients with pVL ≥200 copies/mL at 96 weeks, a robust interrogation of the MEC is limited and care must be taken not to infer too much from the analysis. A range of C12 cut-offs (representing mid-dosing interval concentration) with acceptable sensitivity and specificity criteria were obtained by ROC analysis, suggesting a single threshold value is not statistically valid. Also, the proportion of patients with detectable viral load ≥200 copies/mL at 96 weeks was not significantly different between patients with predicted C12 below or above 1.0 mg/L with a similar lack of association for C24 (data not shown). However, this analysis should be interpreted cautiously given the limited failures and that PK data obtained following 4-12 weeks of therapy may not reflect concentrations at 96 weeks. Although, EFV concentrations below the currently accepted MEC had better sensitivity/specificity for achieving 96 week pVL <200 copies/mL, suggesting adherence is an important driver of virological suppression at 96 weeks in ENCORE1 patients. Self-reported adherence was documented at weeks 4, 48 and 96 and was greater than 90% in both treatment arms, which is generally consistent with findings observing optimal treatment response with adherence ≥95% by pill count [17]. Unfortunately, the adherence data collected as part of ENCORE1 were not sensitive enough to determine impact on clinical outcome.

Rates of overall discontinuation increased from 7% at 48 weeks [5] to 12% at 96 weeks but were similar for both EFV doses and comparable to previous reports [10, 18, 19]. EFV concentrations influenced by metabolic and nuclear receptor polymorphisms but not dose were significantly associated with discontinuation. In contrast to the 48 week analysis, carriers of both *CYP2B6* 516GT or TT variants were at increased risk due to higher EFV concentrations along with *CYP2A6*\*9B CA/AA. For the 48 week analysis, *CYP2B6* 516GT was not associated with discontinuation [5], however at 96 weeks discontinuations had increased potentially altering the statistical association. Possession of *NR1I2* 63396TT lowered the risk of discontinuation by 22% but was not assessed at 48 weeks and inclusion in the multivariable model at 96 weeks may also speak to the disparity in relationships observed with overall discontinuation at 48 and 96 weeks. Pregnane X receptor (PXR, NR1I2) regulates basal CYP3A4 expression and *NR1I2* 63396C>T has been linked to altered expression of PXR and activity of CYP3A4 [20]. Homozygosity for the *NR1I2* 63396C>T variant has been associated with increased oral clearance and subtherapeutic trough concentrations of unboosted atazanavir [21, 22], and although CYP3A4 is a minor route of EFV metabolism decreased risk of discontinuation in *NR1I2* 63396TT patients may be a consequence of lower concentrations resulting from increased metabolism.

CNS adverse events at 96 weeks (as outlined in the Stocrin® Product Information) were not associated with EFV dose or plasma concentrations. The primary metabolite produced by CYP2B6 metabolism, 8-hydroxy-efavirenz (8OH-EFV) [23], has been identified *in vitro* as a contributing factor to toxicity in rat neuronal cultures [24] and potentially 8OH-EFV, rather than the parent compound is a causative agent of CNS adverse events. Indeed, in ENCORE1 patients a lower risk of CNS adverse events at 96 weeks (and similarly at 48 weeks [5]) was observed in *CYP2B6* 983TC/CC carriers, in which CYP2B6 metabolism is impeded, generating less 8OH-EFV thus providing a protective effect. Conversely, *ABCB1* 3435TT markedly increased the risk of experiencing CNS adverse events by 131% compared to wild-type (CC). This is in general consensus with a previous ACTG study that reported a relationship between *ABCB1* 3435TT (with *ABCB1* 2677G>T) and failure of EFV-containing regimens due to toxicity [25]. *ABCB1* encodes the multidrug efflux transporter p-glycoprotein, which is present at various physiological sites including the blood-brain-barrier [26, 27], where it limits entry of compounds including drugs into the CNS. Furthermore, *ABCB1* 3435TT has been associated with decreased p-glycoprotein expression [28]. EFV is not transported by p-glycoprotein [29, 30] but it is currently unknown whether EFV metabolites, such as 8OH-EFV are substrates. We hypothesise that if 8OH-EFV is a substrate, patients possessing the *ABCB1* 3435TT variant would be at greater risk of CNS toxicity as a result of reduced efflux at the blood-brain-barrier.

Concerns regarding EFV-induced toxicities and discontinuations due to them have recently led to alterations in HIV treatment guidelines in the UK and US, replacing EFV with intergrase inhibitor-based (raltegravir, dolutegravir, elvitegravir-cobicistat) or boosted darunavir or atazanavir-contaning regimens as preferred first-line treatment for therapy-naïve adults [31, 32]. Although recommended as an alternative agent in developed countries, EFV remains the first-line option for treatment-naïve patients in resource-limited settings due to lack of availability of newer compounds [1]. Lower rates of EFV-related adverse events (Stocrin® Product Information and clinician decision) were experienced with EFV400 compared to EFV600. Moreover, EFV600 was independently associated with 154% higher risk of stopping due to EFV-related adverse events (clinician decision). Improved tolerability of EFV400 would therefore prove beneficial, lowering discontinuations and preserving future treatment-options for longer.

EFV plays a key role in the treatment of HIV/tuberculosis (TB) co-infection [1] and is a recommended option for HIV-infected pregnant women [33, 34]. Rifampicin and isoniazid, essential components of TB therapy, are known to alter the EFV metabolic pathway through potent induction of CYP2B6 and CYP3A4 and inhibition of CYP2A6, respectively [35, 36]. However, adequate HIV suppression has been observed in HIV/TB patients receiving EFV600 in the presence of TB medications [37]. Differential effects of rifampicin on CYP2B6 induction according to genotype have been reported with greater effects observed in those with fully functional CYP2B6 leading to lower EFV concentrations in the presence of rifampicin compared to EFV alone [38], potentially placing these patients at higher risk of failure. The impact of TB therapy on EFV400 has not been studied and PK-PD data are important before considering EFV dose reduction in this patient population.

EFV PK-PD data during pregnancy and post-partum are increasing. Some studies suggest little clinical impact of pregnancy on EFV PK [39, 40] however others have reported increased CL/F, particularly in extensive metabolisers [35, 41], but cases of mother-to-child transmission were rare [35]. In the absence of clinical evidence, EFV dose reduction in this distinct population is not recommended however a clinical study to investigate the PK of EFV400 during pregnancy is planned in virologically suppressed (pVL <50 copies/mL), HIV-infected women stable on EFV600 [ClinicalTrials.gov Identifier: NCT02499874 [42]].

ENCORE1 has demonstrated successful antiretroviral dose reduction, striking a balance between sustained virological responses with fewer adverse events. Although a threshold concentration may be clinically valuable it was not associated with HIV suppression in ENCORE1 patients and may be of questionable use in resource-limited settings were routine drug measurement is not performed. Implementation of EFV dose reduction to 400 mg once daily would improve toxicity management whilst maintaining durable efficacy and reduce drug costs allowing greater treatment coverage. Potentially the savings made could also aid funding of other public health initiatives such as HIV prevention and education strategies.

**Compliance with Ethical Standards**

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**References**

1. World Health Organisation. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Recommendations for a public health approach. 2013. <http://appswhoint/iris/bitstream/10665/85321/1/9789241505727_engpdf?ua=1>. Accessed 12 Aug 2015.

2. Haas DW, Seekins D, Cooper R, Gallant JE, Tashima K, Hicks C et al. A phase II, double-bliind, placebo-controlled, dose ranging study to assess the antiretroviral activity and safety of efavirenz in combination with open-label zidovudine with lamivudine at 36 weeks [DMP 266-005]. The XII International AIDS Conference, 1998, Geneva, Switzerland. Abstract 22334.

3. Puls R, Amin J, Losso M, Phanuphak P, Nwizu C, Orrell C et al. Efficacy of 400 mg efavirenz versus standard 600 mg dose in HIV-infected, antiretroviral-naive adults (ENCORE1): a randomised, double-blind, placebo-controlled, non-inferiority trial. Lancet. 2014;383(9927):1474-82.

4. ENCORE1 Study Group. Efficacy and safety of efavirenz 400 mg daily versus 600 mg daily: 96-week data from the randomised, double-blind, placebo-controlled, non-inferiority ENCORE1 study. Lancet Infect Dis. 2015;15(7):793-802.

5. Dickinson L, Amin J, Else L, Boffito M, Egan D, Owen A et al. Pharmacokinetic and Pharmacodynamic Comparison of Once Daily Efavirenz (400mg Versus 600mg) in Treatment-Naive HIV-Infected Patients: Results of the ENCORE1 Study. Clin Pharmacol Ther. 2015. doi:10.1002/cpt.156.

6. Amara AB, Tjia J, Dutton J, Else LJ, Back DJ, Khoo S. Development and validation of a HPLC-MS/MS assay to quantify the antiretroviral (ARV) drug, efavirenz and its major metabolites in plasma. British Mass Spectrometry Society Meeting, 2011, Cardiff, UK. Abstract BMS S11-1240.

7. Beal S, Sheiner LB. NONMEM Users Guide. ICON Development Soluntions, Ellicott City, Maryland, USA. 1989-1998.

8. Olagunju A, Schipani A, Siccardi M, Egan D, Khoo S, Back D et al. CYP3A4\*22 (c.522-191 C>T; rs35599367) is associated with lopinavir pharmacokinetics in HIV-positive adults. Pharmacogenet Genomics. 2014;24(9):459-63.

9. Olagunju A, Siccardi M, Amara A, Jevtovic D, Kusic J, Owen A et al. CYP2B6 516G>T (rs3745274) and smoking status are associated with efavirenz plasma concentration in a Serbian cohort of HIV patients. Ther Drug Monit. 2014;36(6):734-8.

10. Merck Sharp & Dohme (Australia). STOCRIN® (efavirenz) tablets, capsules and oral solution Product Information. 2015 <http://securehealthlinksnetau/content/msd/picfm?product=mkpstocr>. Accessed 12 Aug 2015.

11. Marzolini C, Telenti A, Decosterd LA, Greub G, Biollaz J, Buclin T. Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. AIDS. 2001;15(1):71-5.

12. Csajka C, Marzolini C, Fattinger K, Decosterd LA, Fellay J, Telenti A et al. Population pharmacokinetics and effects of efavirenz in patients with human immunodeficiency virus infection. Clin Pharmacol Ther. 2003;73(1):20-30.

13. Glass TR, Rotger M, Telenti A, Decosterd L, Csajka C, Bucher HC et al. Determinants of sustained viral suppression in HIV-infected patients with self-reported poor adherence to antiretroviral therapy. PLoS One. 2012;7(1):e29186.

14. Lehmann DS, Ribaudo HJ, Daar ES, Gulick RM, Haubrich RH, Robbins GK et al. Genome-wide association study of virologic response with efavirenz-containing or abacavir-containing regimens in AIDS clinical trials group protocols. Pharmacogenet Genomics. 2015;25(2):51-9.

15. Holzinger ER, Grady B, Ritchie MD, Ribaudo HJ, Acosta EP, Morse GD et al. Genome-wide association study of plasma efavirenz pharmacokinetics in AIDS Clinical Trials Group protocols implicates several CYP2B6 variants. Pharmacogenet Genomics. 2012;22(12):858-67.

16. Leth FV, Kappelhoff BS, Johnson D, Losso MH, Boron-Kaczmarska A, Saag MS et al. Pharmacokinetic parameters of nevirapine and efavirenz in relation to antiretroviral efficacy. AIDS Res Hum Retroviruses. 2006;22(3):232-9.

17. Shalit P, Cohen C, Mills A, Bredeek F, Crofoot G, Nguyen T et al. Elvitegravir/cobicistat/emtricitabine/tenofovir DF demonstrates comparable efficacy to efavirenz/emtricitabine/tenofovir DF in subjects with adherence < 95%. 7th IAS Conference on HIV Pathogenesis and Treatment, 2013, Kuala Lumpur, Malaysia. Abstract TuPE293.

18. Gallant JE, DeJesus E, Arribas JR, Pozniak AL, Gazzard B, Campo RE et al. Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. N Engl J Med. 2006;354(3):251-60.

19. Wyen C, Hendra H, Siccardi M, Platten M, Jaeger H, Harrer T et al. Cytochrome P450 2B6 (CYP2B6) and constitutive androstane receptor (CAR) polymorphisms are associated with early discontinuation of efavirenz-containing regimens. J Antimicrob Chemother. 2011;66(9):2092-8.

20. Lamba J, Lamba V, Strom S, Venkataramanan R, Schuetz E. Novel single nucleotide polymorphisms in the promoter and intron 1 of human pregnane X receptor/NR1I2 and their association with CYP3A4 expression. Drug Metab Dispos. 2008;36(1):169-81.

21. Schipani A, Siccardi M, D'Avolio A, Baietto L, Simiele M, Bonora S et al. Population pharmacokinetic modeling of the association between 63396C->T pregnane X receptor polymorphism and unboosted atazanavir clearance. Antimicrob Agents Chemother. 2010;54(12):5242-50.

22. Siccardi M, D'Avolio A, Baietto L, Gibbons S, Sciandra M, Colucci D et al. Association of a single-nucleotide polymorphism in the pregnane X receptor (PXR 63396C-->T) with reduced concentrations of unboosted atazanavir. Clin Infect Dis. 2008;47(9):1222-5.

23. Ward BA, Gorski JC, Jones DR, Hall SD, Flockhart DA, Desta Z. The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. J Pharmacol Exp Ther. 2003;306(1):287-300.

24. Tovar-y-Romo LB, Bumpus NN, Pomerantz D, Avery LB, Sacktor N, McArthur JC et al. Dendritic spine injury induced by the 8-hydroxy metabolite of efavirenz. J Pharmacol Exp Ther. 2012;343(3):696-703.

25. Motsinger AA, Ritchie MD, Shafer RW, Robbins GK, Morse GD, Labbe L et al. Multilocus genetic interactions and response to efavirenz-containing regimens: an adult AIDS clinical trials group study. Pharmacogenet Genomics. 2006;16(11):837-45.

26. Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR et al. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. Proc Natl Acad Sci USA. 1989;86(2):695-8.

27. Kim RB, Fromm MF, Wandel C, Leake B, Wood AJ, Roden DM et al. The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. J Clin Invest. 1998;101(2):289-94.

28. Marzolini C, Paus E, Buclin T, Kim RB. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. Clin Pharmacol Ther. 2004;75(1):13-33.

29. Dirson G, Fernandez C, Hindlet P, Roux F, German-Fattal M, Gimenez F et al. Efavirenz does not interact with the ABCB1 transporter at the blood-brain barrier. Pharm Res. 2006;23(7):1525-32.

30. Stormer E, von Moltke LL, Perloff MD, Greenblatt DJ. Differential modulation of P-glycoprotein expression and activity by non-nucleoside HIV-1 reverse transcriptase inhibitors in cell culture. Pharm Res. 2002;19(7):1038-45.

31. British HIV Association (BHIVA). BHIVA guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy. 2015. <http://wwwbhivaorg/documents/Guidelines/Treatment/consultation/150621-BHIVA-Treatment-GL-Final-draft-for-consultationpdf>. Accessed 12 Aug 2015.

32. DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. 2015. <http://wwwaidsinfonihgov/ContentFiles/AdultandAdolescentGLpdf>. Accessed 12 Aug 2015.

33. World Health Organisation. Antiretroviral drugs for treating pregnant women and preventing HIV infection in infants: recommendations for a public health approach. 2010. <http://whqlibdocwhoint/publications/2010/9789241599818_engpdf?ua=1>. Accessed 12 Aug 2015.

34. World Health Organisation. Technical update on treatment optimization. Use of efavirenz during pregnancy: a public health perspective. 2012. <http://appswhoint/iris/bitstream/10665/70920/1/9789241503792_engpdf?ua=1>. Accessed 12 Aug 2015.

35. Dooley KE, Denti P, Martinson N, Cohn S, Mashabela F, Hoffmann J et al. Pharmacokinetics of efavirenz and treatment of HIV-1 among pregnant women with and without tuberculosis coinfection. J Infect Dis. 2015;211(2):197-205.

36. Rae JM, Johnson MD, Lippman ME, Flockhart DA. Rifampin is a selective, pleiotropic inducer of drug metabolism genes in human hepatocytes: studies with cDNA and oligonucleotide expression arrays. J Pharmacol Exp Ther. 2001;299(3):849-57.

37. Luetkemeyer AF, Rosenkranz SL, Lu D, Marzan F, Ive P, Hogg E et al. Relationship between weight, efavirenz exposure, and virologic suppression in HIV-infected patients on rifampin-based tuberculosis treatment in the AIDS Clinical Trials Group A5221 STRIDE Study. Clin Infecti Dis. 2013;57(4):586-93.

38. Ngaimisi E, Mugusi S, Minzi O, Sasi P, Riedel KD, Suda A et al. Effect of rifampicin and CYP2B6 genotype on long-term efavirenz autoinduction and plasma exposure in HIV patients with or without tuberculosis. Clin Pharmacol Ther. 2011;90(3):406-13.

39. Bartelink IH, Savic RM, Mwesigwa J, Achan J, Clark T, Plenty A et al. Pharmacokinetics of lopinavir/ritonavir and efavirenz in food insecure HIV-infected pregnant and breastfeeding women in Tororo, Uganda. J Clin Pharmacol. 2014;54(2):121-32.

40. Cressey TR, Stek A, Capparelli E, Bowonwatanuwong C, Prommas S, Sirivatanapa P et al. Efavirenz pharmacokinetics during the third trimester of pregnancy and postpartum. J Acquir Immune Defic Syndr. 2012;59(3):245-52.

41. Olagunju A, Bolaji O, Amara A, Else L, Okafor O, Adejuyigbe E et al. Pharmacogenetics of pregnancy-induced changes in efavirenz pharmacokinetics. Clin Pharmacol Ther. 2015;97(3):298-306.

42. St. Stephen's AIDS Trust. SSAT063 - Pharmacokinetics of efavirenz 400 mg once daily during pregnancy in HIV-1 infected women. 2015. https://clinicaltrialsgov/ct2/show/NCT02499874?term=efavirenz+pregnancy&rank=2. Accessed 12 Aug 2015.

**Figure Legends**

**Fig. 1** Flow diagram summarising (**a**) the data included in the population pharmacokinetic model and (**b**) genetic data available for analysis

**Table 1** Genotype frequencies stratified by ethnicity in patients included in the ENCORE1 96 week pharmacokinetic/pharmacodynamics and pharmacogenetic analysis (n=606).

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Number of patients [n (%)]** | | |
| **SNP** | **Caucasian (n=179)a** | **Asian (n=201)** | **African (n=226)** |
| *CYP2B6* 516G>T |  |  |  |
| GG | 88 (49.2) | 80 (39.8) | 85 (37.6) |
| GT | 68 (38.0) | 97 (48.3) | 97 (42.9) |
| TT | 10 (5.6) | 18 (9.0) | 31 (13.7) |
| Missing | 13 (7.3) | 6 (3.0) | 13 (5.8) |
| *CYP2B6* 983T>C |  |  |  |
| TT | 164 (91.6) | 195 (97.0) | 176 (77.9) |
| TC | 2 (1.1) | 0 (0.0) | 34 (15.0) |
| CC | 0 (0.0) | 0 (0.0) | 3 (1.3) |
| Missing | 13 (7.3) | 6 (3.0) | 13 (5.8) |
| *CYP2B6* 15582C>T |  |  |  |
| CC | 68 (38.0) | 80 (39.8) | 172 (76.0) |
| CT | 82 (45.8) | 101 (50.2) | 39 (15.0) |
| TT | 16 (8.9) | 13 (6.5) | 2 (0.9) |
| Missing | 13 (7.3) | 7 (3.5) | 13 (5.8) |
| *CYP2A6*\*9B |  |  |  |
| CC | 148 (82.7) | 144 (71.6) | 174 (77.0) |
| CA | 18 (10.1) | 37 (18.4) | 35 (15.5) |
| AA | 0 (0.0) | 11 (5.5) | 3 (1.3) |
| Missing | 13 (7.3) | 9 (4.5) | 14 (6.2) |
| *CYP2A6*\*17 |  |  |  |
| CC | 158 (88.3) | 184 (91.5) | 172 (76.1) |
| CT | 8 (4.5) | 9 (4.5) | 38 (16.8) |
| TT | 0 (0.0) | 0 (0.0) | 3 (1.3) |
| Missing | 13 (7.3) | 8 (4.0) | 13 (5.8) |
| *CYP3A4*\*22 |  |  |  |
| GG | 64 (35.8) | 42 (20.9) | 179 (79.2) |
| GA | 75 (41.9) | 96 (47.8) | 34 (15.0) |
| AA | 27 (15.0) | 57 (28.4) | 0 (0.0) |
| Missing | 13 (7.3) | 6 (3.0) | 13 (5.8) |
| *NR1I3* 540C>T |  |  |  |
| CC | 58 (32.4) | 40 (20.0) | 55 (24.3) |
| CT | 81 (45.1) | 90 (44.8) | 106 (46.9) |
| TT | 27 (15.1) | 65 (32.3) | 52 (23.0) |
| Missing | 13 (7.3) | 6 (3.0) | 13 (5.8) |
| *NR1I3* 1089T>C |  |  |  |
| TT | 149 (83.2) | 186 (92.5) | 210 (92.9) |
| TC | 17 (9.5) | 8 (4.0) | 3 (1.3) |
| CC | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Missing | 13 (7.3) | 7 (3.5) | 13 (5.8) |
| *ABCB1* 3435C>T |  |  |  |
| CC | 40 (22.3) | 52 (25.9) | 167 (73.9) |
| CT | 89 (49.7) | 104 (51.7) | 45 (19.9) |
| TT | 37 (20.7) | 39 (19.4) | 1 (0.4) |
| Missing | 13 (7.3) | 6 (3.0) | 13 (5.8) |
| *NR1I2* 63396C>T |  |  |  |
| CC | 39 (21.9) | 23 (11.4) | 87 (38.5) |
| CT | 81 (45.3) | 105 (52.2) | 107 (47.3) |
| TT | 45 (25.1) | 65 (32.3) | 19 (8.4) |
| Missing | 14 (7.8) | 8 (4.0) | 13 (5.8) |
| *NR1I2* 7635A>G |  |  |  |
| AA | 45 (25.1) | 84 (41.8) | 182 (80.5) |
| AG | 75 (41.9) | 92 (45.8) | 27 (11.9) |
| GG | 46 (25.7) | 18 (9.0) | 2 (0.9) |
| Missing | 13 (7.3) | 7 (3.5) | 15 (6.6) |

SNP: single nucleotide polymorphism

aCaucasian, Hispanic and Aboriginal/Torres Strait Islander combined for consistency with the 48 week analysis.

**Table 2** Differences in mean individual predicted pharmacokinetic parameters for safety endpoints, assessed by calculation of geometric mean ratios (GMR) and 90% CI (n=605a).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **GMR (90% CI)b** | | | | |
| **Parameter** | **Overall discontinuation** | **Adverse event (Stocrin PI)** | **CNS adverse event (Stocrin PI)** | **Adverse event (clinician decision)** | **Stopping due to adverse event (clinician decision)** |
| AUC0-24 | 0.85 (0.76-0.95) | 0.93 (0.86-1.01) | 0.94 (0.88-1.02) | 0.93 (0.87-1.00) | 0.78 (0.67-0.92) |
| Cmax | 0.84 (0.77-0.93) | 0.92 (0.86-0.99) | 0.94 (0.88-1.00) | 0.93 (0.87-0.99) | 0.77 (0.67-0.88) |
| C24 | 0.86 (0.74-1.01) | 0.94 (0.85-1.05) | 0.95 (0.86-1.05) | 0.94 (0.85-1.04) | 0.85 (0.68-1.06) |
| C12 | 0.86 (0.71-0.96) | 0.94 (0.86-1.02) | 0.95 (0.88-1.02) | 0.93 (0.87-1.01) | 0.81 (0.69-0.95) |

a n=1 patient excluded; received 800 mg efavirenz during pharmacokinetic sampling

b no event/event

PI: Product Information; CI: confidence interval; AUC0-24: area under the curve over 24 hours; Cmax: maximum concentration; C24: trough concentration 24 hours post-dose; C12: concentration 12 hours post-dose representing the mid-dose interval concentration

**Table 3** Summary of the relationships between achieving plasma viral load <200 copies/mL at week 96 of therapy and single nucleotide polymorphisms (data analysed by Fisher’s exact test).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | | **Viral load [n/N (%)]** | |  |
| **Single nucleotide polymorphism** | | | **<200 copies/mL** | **≥200 copies/mL** | ***p* value** |
| *CYP2B6* 516G>T | | GG | 238/243 (97.9) | 5/243 (2.1) | 0.420 |
|  | | GT | 242/249 (97.2) | 7/249 (2.8) |
|  | | TT | 52/54 (96.3) | 2/54 (3.7) |
| *CYP2B6* 983T>C | | TT | 500/513 (97.5) | 13/513 (2.5) | 1.000 |
|  | | TC/CC | 33/33 (100) | 0/33 (0.0) |
| *CYP2B6* 15582C>T | | CC | 294/301 (97.7) | 7/301 (2.3) | 1.000 |
|  | | CT/TT | 238/244 (97.5) | 6/244 (2.5) |
| *CYP2A6*\*9B | | CC | 440/450 (97.8) | 10/450 (2.2) | 0.470 |
|  | | CA/AA | 89/92 (96.7) | 3/92 (3.3) |
| *CYP2A6*\*17 | | CC | 477/488 (97.7) | 11/488 (2.3) | 0.634 |
|  | | CT/TT | 54/56 (96.4) | 2/56 (3.6) |
| *NR1I3* 540C>T | | CC | 258/265 (97.4) | 7/265 (2.6) | 0.324 |
|  | | CT | 192/198 (97.0) | 6/198 (3.0) |
|  | | TT | 83/83 (100) | 0/83 (0.0) |
| *NR1I3* 1089T>C | | TT | 140/143 (97.9) | 3/143 (2.1) | 0.718 |
|  | | TC | 258/266 (97.0) | 8/266 (3.0) |
|  | | CC | 135/137 (98.5) | 2/137 (1.5) |
| *CYP3A4*\*22 | | GG | 506/518 (97.7) | 12/518 (2.3) | 0.487 |
|  | | GA | 26/27 (96.3) | 1/27 (3.7) |
| *ABCB1* 3435C>T | | CC | 232/239 (97.1) | 7/239 (2.9) | 0.797 |
|  | | CT | 227/232 (97.8) | 5/232 (2.2) |
|  | | TT | 74/75 (98.7) | 1/75 (1.3) |
| *NR1I2* 63396 C>T | | CC | 135/139 (97.1) | 4/139 (2.9) | 0.462 |
|  | | CT | 269/277 (97.1) | 8/277 (2.9) |
|  | | TT | 126/127 (99.2) | 1/127 (0.8) |
| *NR1I2* 7635A>G | | GG | 283/292 (96.9) | 9/292 (3.1) | 0.610 |
|  | | GA | 183/186 (98.4) | 3/186 (1.6) |
|  | | AA | 64/65 (98.5) | 1/65 (1.5) |

**Table 4** Cox regression assessing the relationship between overall discontinuation of efavirenz once daily and *CYP2B6*, *CYP2A6*, *CYP3A4*, *ABCB1*, *NR1I3*, *NR1I2* polymorphisms.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | |  |  |  |  | **Univariable Cox regression** | | | **Multivariable Cox regressiona** | | | **Multivariable Cox regressionb** | | |
| **Single nucleotide polymorphism** | | **Event** | **no Event** | **Total** | **%** | ***p* value** | **HR** | **95% CI** | ***p* value** | **HR** | **95% CI** | ***p* value** | **HR** | **95% CI** |
| *CYP2B6* 516G>T | GG | 22 | 231 | 253 | 8.7 | 0.034 |  |  | 0.030 |  |  | 0.025 |  |  |
|  | GT | 33 | 228 | 261 | 12.6 | 0.154 | 1.48 | 0.86-2.54 | 0.162 | 1.47 | 0.86-2.53 | 0.047 | 1.80 | 1.01-3.21 |
|  | TT | 12 | 47 | 59 | 20.3 | 0.010 | 2.53 | 1.25-5.12 | 0.008 | 2.58 | 1.28-5.22 | 0.010 | 2.66 | 1.26-5.60 |
| *CYP2B6* 983T>C | TT | 59 | 475 | 534 | 11.0 |  |  |  |  |  |  |  |  |  |
|  | TC/CC | 8 | 31 | 39 | 20.5 | 0.082 | 1.93 | 0.92-4.03 |  |  |  |  |  |  |
| *CYP2B6* 15582C>T | CC | 42 | 277 | 319 | 13.2 |  |  |  |  |  |  |  |  |  |
|  | CT/TT | 25 | 228 | 253 | 9.9 | 0.212 | 0.73 | 0.45-1.20 |  |  |  |  |  |  |
| *CYP2A6*\*9B | CC | 48 | 417 | 465 | 10.3 |  |  |  |  |  |  |  |  |  |
|  | CA/AA | 19 | 85 | 104 | 18.3 | 0.024 | 1.85 | 1.09-3.14 | 0.012 | 1.98 | 1.16-3.38 | 0.016 | 2.00 | 1.14-3.52 |
| *CYP2A6*\*17 | CC | 63 | 450 | 513 | 12.3 |  |  |  |  |  |  |  |  |  |
|  | CT/TT | 4 | 54 | 58 | 6.9 | 0.240 | 0.55 | 0.20-1.50 |  |  |  |  |  |  |
| *NR1I3* 540C>T | CC | 44 | 241 | 285 | 15.4 | 0.023 |  |  |  |  |  |  |  |  |
|  | CT | 16 | 188 | 204 | 7.8 | 0.013 | 0.49 | 0.27-0.86 |  |  |  |  |  |  |
|  | TT | 7 | 77 | 84 | 8.3 | 0.098 | 0.51 | 0.23-1.13 |  |  |  |  |  |  |
| *NR1I3* 1089T>C | TT | 20 | 133 | 153 | 13.1 | 0.837 |  |  |  |  |  |  |  |  |
|  | TC | 31 | 245 | 276 | 11.2 | 0.595 | 0.86 | 0.49-1.51 |  |  |  |  |  |  |
|  | CC | 16 | 128 | 144 | 11.1 | 0.613 | 0.84 | 0.44-1.63 |  |  |  |  |  |  |
| *CYP3A4*\*22 | GG | 61 | 483 | 544 | 11.2 |  |  |  |  |  |  |  |  |  |
|  | GA | 5 | 23 | 28 | 17.9 | 0.284 | 1.65 | 0.66-4.10 |  |  |  |  |  |  |
| *ABCB1* 3435C>T | CC | 41 | 217 | 258 | 15.9 | 0.017 |  |  |  |  |  |  |  |  |
|  | CT | 21 | 217 | 238 | 8.8 | 0.017 | 0.53 | 0.31-0.89 |  |  |  |  |  |  |
|  | TT | 5 | 72 | 77 | 6.5 | 0.046 | 0.39 | 0.15-0.98 |  |  |  |  |  |  |
| *NR1I2* 63396 C>T | CC | 23 | 126 | 149 | 15.4 | 0.008 |  |  | 0.006 |  |  | 0.018 |  |  |
|  | CT | 40 | 252 | 292 | 13.7 | 0.635 | 0.88 | 0.53-1.48 | 0.666 | 0.89 | 0.53-1.49 | 0.970 | 1.01 | 0.59-1.72 |
|  | TT | 4 | 125 | 129 | 3.1 | 0.002 | 0.19 | 0.07-0.54 | 0.002 | 0.18 | 0.06-0.52 | 0.008 | 0.22 | 0.07-0.67 |
| *NR1I2* 7635A>G | GG | 40 | 270 | 310 | 12.9 | 0.607 |  |  |  |  |  |  |  |  |
|  | GA | 21 | 173 | 194 | 10.8 | 0.478 | 0.83 | 0.49-1.40 |  |  |  |  |  |  |
|  | AA | 5 | 61 | 66 | 7.6 | 0.402 | 0.69 | 0.29-1.63 |  |  |  |  |  |  |

a forwards likelihood ratio; b adjusted for dose, age, sex; stratified by country; CI: confidence interval; HR: hazard ratio