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ORIGINAL RESEARCH ARTICLE

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

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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. Receiver operating characteristic curve analysis evaluated the predictability of mid-dose interval (C_{12}) cutoffs 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 C_{12}, were not associated with pVL <200 copies/mL at 96 weeks (odds ratio [OR] 5.25, 95 % confidence interval [CI] 0.41–67.90, p = 0.204). Lower risk of CNS-related adverse events was associated with *CYP2B6* 983TC/CC (OR 0.35, 95 % CI 0.15–0.81, p = 0.015) and higher risk was associated with *CYP2B6* 15582CT/TT and *ABCB1* 3435TT (OR 1.46, 95 % CI 1.02–2.09, p = 0.040; OR 2.31, 95 % CI 1.33–4.02, p = 0.003, respectively). Discontinuation due to adverse events (clinician decision) was independently associated with dose (OR 2.54, 95 % CI 1.19–5.43, p = 0.016). C_{12} between 0.47 and 0.76 mg/L provided sensitivity/specificity >90 % (100 %/92.3 to 98.9 %/92.3 %) for achieving pVL <200 copies/mL at 96 weeks.

Conclusions A higher rate of EFV-related adverse events and discontinuations due to these events 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 still maintaining good efficacy.

On behalf of the ENCORE1 Study Group.

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Key Points

Despite concerns regarding lower plasma concentrations obtained with efavirenz 400 mg (EFV400) compared with 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 with 35 % lower risk in *CYP2B6* 983TC/CC patients. Possession of the *CYP2B6* 516GT and TT variants and *CYP2A6**9B CA/AA carriers was associated with a higher risk of overall EFV discontinuation (80, 166 and 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 C_{12} (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 C_{12} <1.0 mg/L had missing pVL at 96 weeks). Although a threshold concentration is clinically useful, the acceptable receiver operating characteristic criteria associated with a range of C_{12} cutoffs (0.47–0.76 mg/L) for pVL <200 copies/mL at 96 weeks suggests a single target value is not statistically valid.

1 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 (EFV; 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 EFV 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 multicentre, double-blind, placebo-controlled trial, demonstrated non-inferiority of reduced-dose EFV (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]. In this study, we present the final EFV PK-pharmacodynamic (PD) and pharmacogenetic cross-sectional analysis of ENCORE1 at 96 weeks.

2 Methods

2.1 Patients

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

2.2 Sampling and Pharmacokinetics (PK)

The ENCORE1 PK sampling scheme has been previously reported [5]. Random, single blood samples were collected at weeks 4 and 12 of therapy (between 8 and 16 h post-dose) and intensive sampling was also carried out in a subgroup of patients ($n = 46$) between weeks 4 and 8 (pre-dose [0 h], 2, 4, 8, 12, 16 and 24 h post-dose). EFV plasma concentrations were quantified by a validated high-performance liquid chromatography–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 single nucleotide polymorphisms (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-h dosing interval (AUC_{24}), maximum concentration (C_{max}), trough concentration 24 h post-dose (C_{24}) and concentration 12 h post-dose representing the mid-dose interval concentration (C_{12}) were determined for each sampling occasion, and the mean for each patient was calculated. Standard modelling practices were applied, with the procedures recently being described in detail [5].

2.3 Genotyping

The SNPs *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 previously genotyped [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].

2.4 Pharmacokinetic–Pharmacodynamic (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 AUC_{24}, C_{max}, C_{24}, and C_{12} were 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 using Pearson's Chi-square test. Geometric mean ratio (GMR; 90 % confidence interval [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.

2.5 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 using 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 using 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.

2.6 Evaluation of the Recommended Minimum Effective Concentration (MEC, 1.0 mg/L)

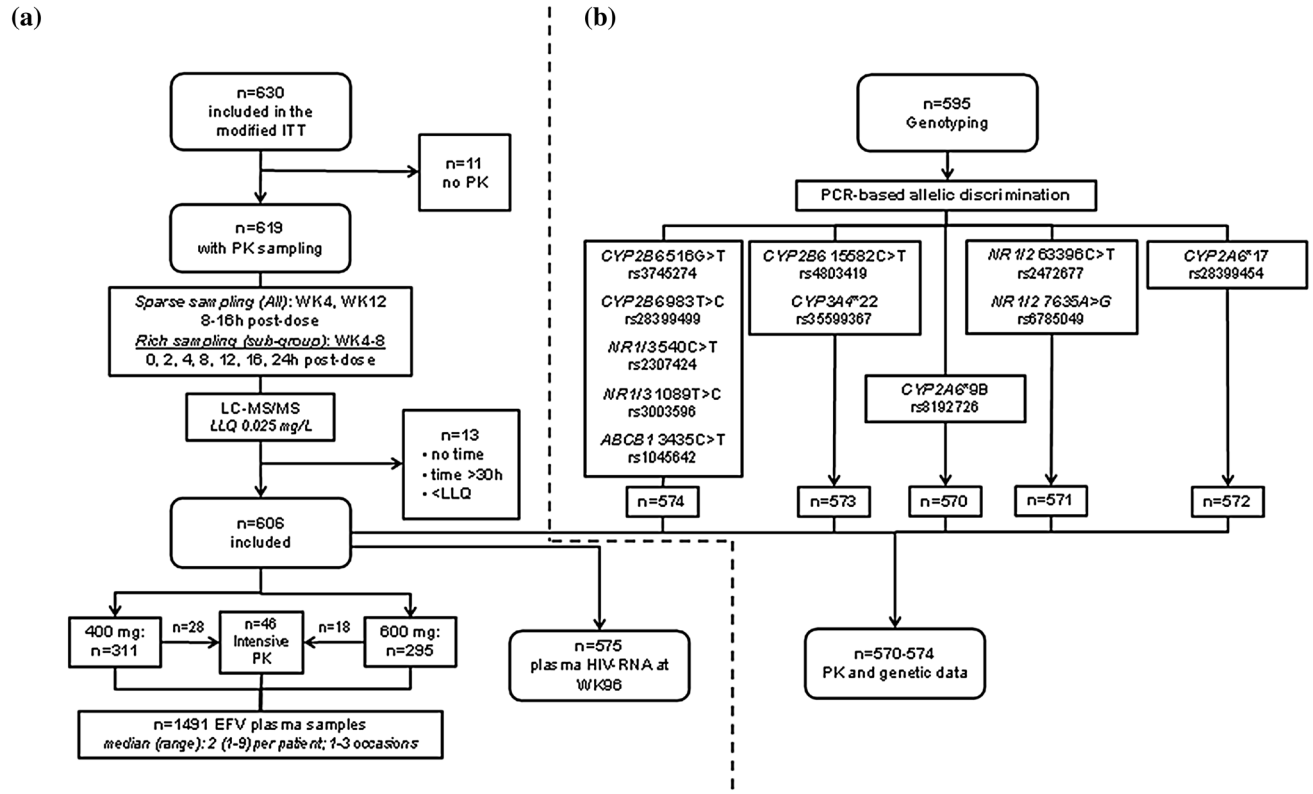
Differences in the proportions of patients with model-predicted EFV C_{12} below and above the recommended minimum effect concentration (MEC) of 1.0 mg/L [11] stratified by pVL (<200 copies/mL vs. ≥200 copies/mL) was determined using Fisher's exact test. A receiver operating characteristic (ROC) analysis was also performed to investigate the predictability of mid-dose interval concentration (C_{12}) cutoffs and achieving pVL <200 copies/mL at 96 weeks. Patients with pVL missing at 96 weeks were excluded from the analysis.

Statistical analyses were performed using SPSS version 21 (IBM Corporation, Armonk, NY, USA).

3 Results

3.1 Patients and PK

Overall, 630 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



EFV: efavirenz; PK: pharmacokinetics; WK: week; LLQ: lower limit of quantification; ITT: intent to treat

Fig. 1 Flow diagram summarising **a** the data included in the population pharmacokinetic model and **b** genetic data available for analysis. *EFV* efavirenz, *PK* pharmacokinetics, *LLQ* lower limit of quantification, *ITT* intention to treat, *LC–MS/MS* liquid chromatography–tandem mass spectrometry, *PCR* polymerase chain reaction, *WK* week

(18–69), 65 kg (39–148), 56,803 copies/mL (162–10,000,000) and 270 cells/mm^3, respectively. Patients identified themselves as African (37 %), Asian (33 %), Hispanic (17 %), Caucasian (13 %) and Aboriginal/Torres Strait 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). Therefore, the PK parameters did not alter from the previous 48-week analysis and were carried forward to the 96-week analysis. 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 and 2, respectively).

3.2 Genotyping

Genotyping was possible in 595 patients, and of the 606 patients 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 and 574 patients (Fig. 1b). Genotype frequencies summarised by ethnicity are shown in 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.

3.3 PK–PD Analysis: Relationships with Virological and Safety Endpoints

At 96 weeks, 97 and 99 % of patients were <200 copies/mL for EFV400 and EFV600, respectively (p = 0.091;

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

SNP	Number of patients [n (%)]		
	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)

Table 1 continued

SNP	Number of patients [*n* (%)]		
	Caucasian (*n* = 179)[a]	Asian (*n* = 201)	African (*n* = 226)
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

[a] Caucasian, Hispanic and Aboriginal/Torres Strait Islander combined for consistency with the 48-week analysis

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 (logAUC_{24} odds ratio [OR] 4.20, 95 % CI 0.31–57.77, $p = 0.283$; logC_{max} OR 1.87, 95 % CI 0.11–32.50, $p = 0.667$; logC_{24} OR 4.17, 95 % CI 0.70–24.94, $p = 0.118$; and logC_{12} OR 5.25, 95 % CI 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 who 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 AUC_{24}, C_{max} and C_{12} were significantly lower in those who did not discontinue therapy or stop due to EFV-related adverse events (clinician decision), and EFV C_{max} was significantly reduced in those who did not experience EFV-related adverse events (Stocrin® product information or clinician decision). PK parameters were not significantly different between those who did and did not have CNS adverse events (Table 2).

3.4 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

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

Parameter	GMR (90 % CI)[b]				
	Overall discontinuation	Adverse event (Stocrin PI)	CNS adverse event (Stocrin PI)	Adverse event (clinician decision)	Stopping due to adverse event (clinician decision)
AUC_{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)
C_{max}	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)
C_{24}	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)
C_{12}	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)

GMRs geometric mean ratios, *PI* product information, *CI* confidence interval, AUC_{24} area under the curve over 24 h, C_{max} maximum concentration, C_{24} trough concentration 24 h post-dose, C_{12} concentration 12 h post-dose representing the mid-dose interval concentration

[a] *n* = one patient excluded; received efavirenz 800 mg during pharmacokinetic sampling

[b] No event/event

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)

Single nucleotide polymorphism	Viral load [*n/N* (%)] <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)	

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 an 80, 166 and 100 % increased risk of overall discontinuation, respectively, whereas *NR1I2* 63396TT carriers had a 22 % reduced risk (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 with EFV400 (OR 2.54, 95 % CI 1.19–5.43, $p = 0.016$). A decreased risk of CNS

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

Single nucleotide polymorphism	Event	No event	Total	%	Univariable Cox regression			Multivariable Cox regression[a]			Multivariable Cox regression[b]		
					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						

CI confidence interval, *HR* hazard ratio

[a] Forwards likelihood ratio

[b] Adjusted for dose, age, sex; stratified by country

adverse events (Stocrin® product information) was associated with *CYP2B6* 983TC or CC carriers (OR 0.30, 95 % CI 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 1.59, 95 % CI 1.11–2.27, $p = 0.011$; and OR 2.14, 95 % CI 1.25–3.67, $p = 0.006$, respectively).

3.5 Evaluation of the Recommended MEC (1.0 mg/L)

The proportions of patients with pVL ≥200 copies/mL was not significantly different between those with model-predicted EFV C_{12} above or below 1.0 mg/L (2 vs. 11 %, $p = 0.059$). Fourteen and six patients had predicted C_{12} 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. In these two patients, EFV C_{12} and metaboliser status were 0.77 mg/L and extensive metaboliser (EFV400), and 0.38 mg/L and intermediate metaboliser (EFV600; two viral load measurements were unavailable) [Online Resource 3]. The ranges of predicted C_{12} stratified by metaboliser status of the ten (EFV400) and three patients (EFV600) with pVL ≥200 copies/mL at 96 weeks ($n = 13$ total) were 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, $n = 2$) for EFV400; and 2.19 mg/L (extensive, $n = 1$), 0.38 mg/L and 3.02 mg/L (intermediate, $n = 2$) for EFV600.

Generally, the ROC curve lay along the line of unity between sensitivity and 1-specificity, suggesting the analysis was informative to an extent. The sensitivity/specificity of using C_{12} 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 C_{12} values, suggesting a range of potential cutoffs; for example, C_{12} between 0.47 and 0.76 mg/L provided sensitivity/specificity >90 % (100 %/92.3 % to 98.9 %/92.3 %) with an LR of 13.

4 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 [11, 12]; 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 CIs were wide) [5], both analyses should be interpreted cautiously, given only 16/593 (3 %) and 13/575 (2 %) patients had pVL ≥200 copies/mL at 48 and 96 weeks, respectively. Furthermore, the PK was performed between 4 and 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 a genotypic relative risk of approximately 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 C_{24} 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 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 C_{24} 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 cutoff for EFV mid-dosing interval concentrations [11, 12]; however, this value was obtained in an era of less potent antiretroviral therapy, with lamivudine, zidovudine, nelfinavir and amprenavir most commonly coadministered with EFV [11, 12]. 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 receiving long-term therapy. Assessment of the MEC was based on C_{12} (representing mid-dose interval concentrations) instead of C_{24} in order 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 C_{12} cutoffs (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 C_{12} below or above 1.0 mg/L with a similar lack of association for C_{24} (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. Nonetheless, 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 >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 with 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-hydroxyefavirenz (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 and thus providing a protective effect. Conversely, *ABCB1* 3435TT markedly increased the risk of experiencing CNS adverse events by 131 % compared with wild-type (CC). This is in general consensus with a previous AIDS Clinical Trials Group (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 these toxicities have recently led to alterations in HIV treatment guidelines in the UK and US, replacing EFV with integrase inhibitor-based (raltegravir, dolutegravir, elvitegravir–cobicistat) regimens, or boosted darunavir- or atazanavir-containing regimens as the 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 the 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 with EFV600. Moreover, EFV600 was independently associated with a 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 with EFV alone [38] and 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 necessary 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 [42].

5 Conclusions

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 where 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 would 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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All authors had full access to the study data and agreed to submit for publication. The corresponding author had final responsibility for the decision to submit for publication. No medical writers were used and no agency made any payments for writing. Neither the funding agency nor pharmaceutical companies supporting the trial played any role in the collection, analysis, interpretation or reporting of these data.
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