J Antimicrob Chemother 2017; **72**: 200–204
doi:10.1093/jac/dkw403 Advance Access publication 21 September 2016

Journal of Antimicrobial Chemotherapy

Impact of efavirenz pharmacokinetics and pharmacogenomics on neuropsychological performance in older HIV-infected patients

Uriel Sandkovsky[1]*, Anthony T. Podany[2], Courtney V. Fletcher[1,2], Andrew Owen[3], Angela Felton-Coleman[1], Lee C. Winchester[2], Kevin Robertson[4] and Susan Swindells[1]

[1]Division of Infectious Diseases, University of Nebraska Medical Center, Omaha, NE, USA; [2]Department of Pharmacy Practice, College of Pharmacy, University of Nebraska Medical Center, Omaha, NE, USA; [3]Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, UK; [4]Department of Neurology, University of North Carolina, Chapel Hill, NC, USA

*Corresponding author. Tel: +1-402-559-8339; Fax: +1-402-559-5581; E-mail: usandkovsky@unmc.edu

Received 3 June 2016; returned 28 July 2016; revised 16 August 2016; accepted 26 August 2016

Background: Pharmacokinetics (PK) and pharmacodynamics of efavirenz and its 8-hydroxy metabolite (8-OH-efavirenz) have not been robustly evaluated in older HIV-infected persons.

Objectives: We investigated relationships between neuropsychological (NP) performance and efavirenz and 8-OH-efavirenz PK in HIV-infected individuals >50 years of age.

Methods: A cross-sectional study of HIV-infected adults on an efavirenz-containing regimen. The 12 and 18 h post-dose plasma efavirenz and 8-OH-efavirenz were quantified. *CYP2B6* polymorphisms were investigated. Participants underwent neuropsychological tests; surveys were used for depression, sleep quality and anxiety. We investigated potential correlations of efavirenz and 8-OH-efavirenz plasma concentrations with NP performance, sleep, depression, anxiety and *CYP2B6* polymorphisms.

Results: Thirty participants (24 men and 6 women) with mean age 57 years (range 50–68). Plasma efavirenz concentrations did not correlate with NP performance; however, higher plasma 8-OH-efavirenz correlated with better learning ($P=0.002$), language ($P=0.002$) and total NP *z*-scores ($P=0.003$). No correlation was seen for efavirenz or 8-OH-efavirenz with sleep, anxiety or depression. Median 12 and 18 h efavirenz plasma concentrations were 1967 ng/mL (IQR 1476–2394) and 1676 ng/mL (IQR 1120–2062), respectively. Median 12 and 18 h 8-OH-efavirenz plasma concentrations were 378 ng/mL (IQR 223–589) and 384 ng/mL (IQR 216–621), respectively. *CYP2B6* G516T was associated with significantly higher plasma efavirenz at 12 and 18 h ($P=0.02$) but not worse NP function.

Conclusions: Better neurocognitive functioning was associated with higher 8-OH-efavirenz but not efavirenz plasma concentrations. No correlation was observed with sleep or depression. These findings point to a need for greater understanding of the metabolic profile of efavirenz and 8-OH-efavirenz in plasma and the CNS and relationships with antiviral effect and neurotoxicity.

Introduction

The US CDC estimates that up to 15% of newly diagnosed cases of HIV infection are among people aged ≥50 years. By 2016, more than one-half of all HIV-infected individuals in the USA will be aged >50 years, not only from new cases but the greatly increased lifespan attributed to ART.[1] Assessments of antiretroviral pharmacokinetics (PK) in older HIV-infected patients are sparse and there are no specific dosing guidelines for older patients in contrast to the general geriatric population.[2] Studies in older populations demonstrate decrements in liver metabolism and renal clearance, which may require dosage adjustments for drugs eliminated by the kidney. Additionally, decreased bioavailability due to changes in drug transporters alter the PK of many drugs in older populations.[3,4] In a relevant study of older HIV-infected patients, trough lopinavir concentrations from 44 subjects showed that older age was associated with higher lopinavir concentrations.[3] In another study of 51 patients receiving darunavir, a univariate analysis determined that every 10 years of age lowered the clearance of darunavir by 19%.[5] Importantly, efavirenz has been sparsely evaluated in older patients,[6] even though this is one of the most commonly prescribed antiretroviral agents.[7,8]

CNS side effects associated with efavirenz are common and for this reason it is recommended that the drug be taken at bedtime.[9] Neuropsychological (NP) performance and symptoms associated

with efavirenz use were carefully evaluated in the ACTG study 5097. In this study, individuals on efavirenz experienced an increased rate of bad dreams and had an increased rate of neurological symptoms that correlated with plasma concentrations of efavirenz, but only during the first week of treatment. No differences were seen in depression or anxiety between those on efavirenz and those on other regimens. Six percent of patients discontinued efavirenz as a result of neuropsychiatric side effects.[10] Pharmacogenetics (PG) may also play an important role in efavirenz exposure in the older population as previous studies have shown correlations between both efavirenz concentrations and CNS adverse effects and CYP2B6 polymorphisms in adults.[11,12]

Efavirenz is primarily metabolized in the liver. Clearance of efavirenz occurs predominantly via CYP2B6 to an 8-OH-efavirenz metabolite.[13–16] Additionally, there is a subsequent pathway via CYP2A6 to a 7-OH-efavirenz metabolite.[13–15] The 8-OH-efavirenz metabolite was found to induce direct neuronal toxicity and death via increased Ca^{2+} influx *in vitro*.[17] CYP2B6 polymorphisms have been associated with higher plasma concentrations of efavirenz. Subsequently, CYP2B6 polymorphisms have been observed in patients with increased frequency of efavirenz-related side effects and are associated with drug discontinuation.[11,12] In addition to CYP2B6, early treatment discontinuation of efavirenz was also associated with a polymorphism in the constitutive androstane receptor.[16]

The aim of this study was to investigate steady-state PK of efavirenz, in older HIV-infected patients, to correlate efavirenz and 8-OH-efavirenz drug exposure with NP performance and to explore the role of CYP2B6 polymorphisms in efavirenz metabolism in patients >50 years of age.

Patients and methods

A cross-sectional study was conducted at the University of Nebraska Medical Center. Entry criteria were a diagnosis of HIV disease, >50 years of age and on an efavirenz-containing regimen for >12 weeks. Participants were required to be able to provide written informed consent and to complete the questionnaires in English, as not all of the NP screens and questionnaires have been validated in other languages. Exclusion criteria were recent intercurrent acute infection, active psychiatric illness, active neurological disease, current delirium or intoxication and active drug or alcohol abuse.

Participant medical records were accessed to obtain demographic and medical history data, hepatitis C virus (HCV) status, CD4+ T cell count and HIV viral load when available within ≤3 months of study entry. Participants underwent a validated NP battery to evaluate multiple domains most affected by HIV disease including executive function, motor skills, verbal learning, memory and speed of processing. The specific instruments used were: the Wide Range Achievement Test 4 (WRAT-4) Reading Test; Timed Gait; Hopkins Verbal Learning Test—Revised; Trailmaking A and B; Grooved Pegboard; the Wechsler Adult Intelligence Scale (WAIS-3) Digit Symbol; Verbal or Letter Fluency; and the Stroop Interference Task. Normative standards were used, which are corrected for age, education, sex and ethnicity as appropriate. The validated questionnaires administered were the Center for Epidemiologic Studies Depression Scale (CES-D) to assess for depression and the Pittsburgh Sleep Quality Index to assess for sleep.

Participants were asked to record the time of efavirenz dosing the night before and plasma samples were collected 12 and 18 h post-dose for measurement of efavirenz and 8-OH-efavirenz concentrations. The sampling schedule was chosen as efavirenz is typically dosed daily in the evening and PK/pharmacodynamic relationships have been established for mid-dosing interval efavirenz concentrations, including risk of both CNS side effects and virological failure.[18] In addition, whole blood was collected for PG analysis. Efavirenz and its 8-OH metabolite concentrations were quantified via LC-MS/MS[19,20] in the Antiviral Pharmacology Laboratory of the University of Nebraska Medical Center.

PG analyses were performed at the University of Liverpool. Genotyping was conducted by real-time PCR-based allelic discrimination using standard methodology.[16] The genes of interest include genes coding for proteins involved in phase I metabolism (*CYP2B6*, *CYP3A4*, *CYP2D6* and *CYP2A6*), phase II metabolism (*UGT2B7*) and factors of transcriptional regulation of drug disposition (PXR and *CAR*).

Table 1. Patient characteristics, median 12 and 18 h efavirenz and 8-OH-efavirenz concentrations and correlations between efavirenz and 8-OH-efavirenz concentrations and neuropsychological tests

Variable	Value	Range (IQR)
Median age	57	50–68
Median time on ART, years	11.25	1–22.8 (10.92)
Median CD4 cells/mm^3	657	145–2062 (463)
Nadir CD4 cells/mm^3	259	7–769 (342)
HIV RNA <20 copies/mL	30[a]	—
Hepatitis C antibody	3[b]	—
PK determination (median), ng/mL		
12 h EFV	1967	IQR 1476–2394
18 h EFV	1676	IQR 1120–2062
12 h 8-OH-EFV	378	IQR 223–589
18 h 8-OH-EFV	384	IQR 216–621

	Efavirenz		8-OH-efavirenz		
NP test	12 h post	18 h post	12 h post	18 h post	*P* value[c]
Total *z*	0.04	0.09	0.38	0.52	0.003
Motor	0.13	0.15	0.20	0.37	0.044
Learning	0.1	0.11	0.52	0.61	0.002
Memory	0.05	0.13	0.27	0.2	0.24
Language	0.05	0.08	0.5	0.53	0.002
Executive	−0.03	−0.05	0.16	0.29	0.12
Speed	0.11	−0.02	0.31	0.42	0.02
Fluency	0.24	0.24	0.14	0.24	0.2

P values considered significant if <0.01. PK, pharmacokinetics; EFV, efavirenz; 8-OH-EFV, 8-hydroxy efavirenz.
[a]Twenty-six participants had HIV RNA <20 copies/mL and four had HIV RNA <100 copies/mL, but all four had HIV RNA <20 copies/mL on subsequent measurements.
[b]Three participants had positive HCV antibodies, all had undetectable HCV viraemia at the time of study. Two participants had been previously treated for HCV and one had spontaneously cleared viraemia.
[c]Adjusted with Bonferroni correction.

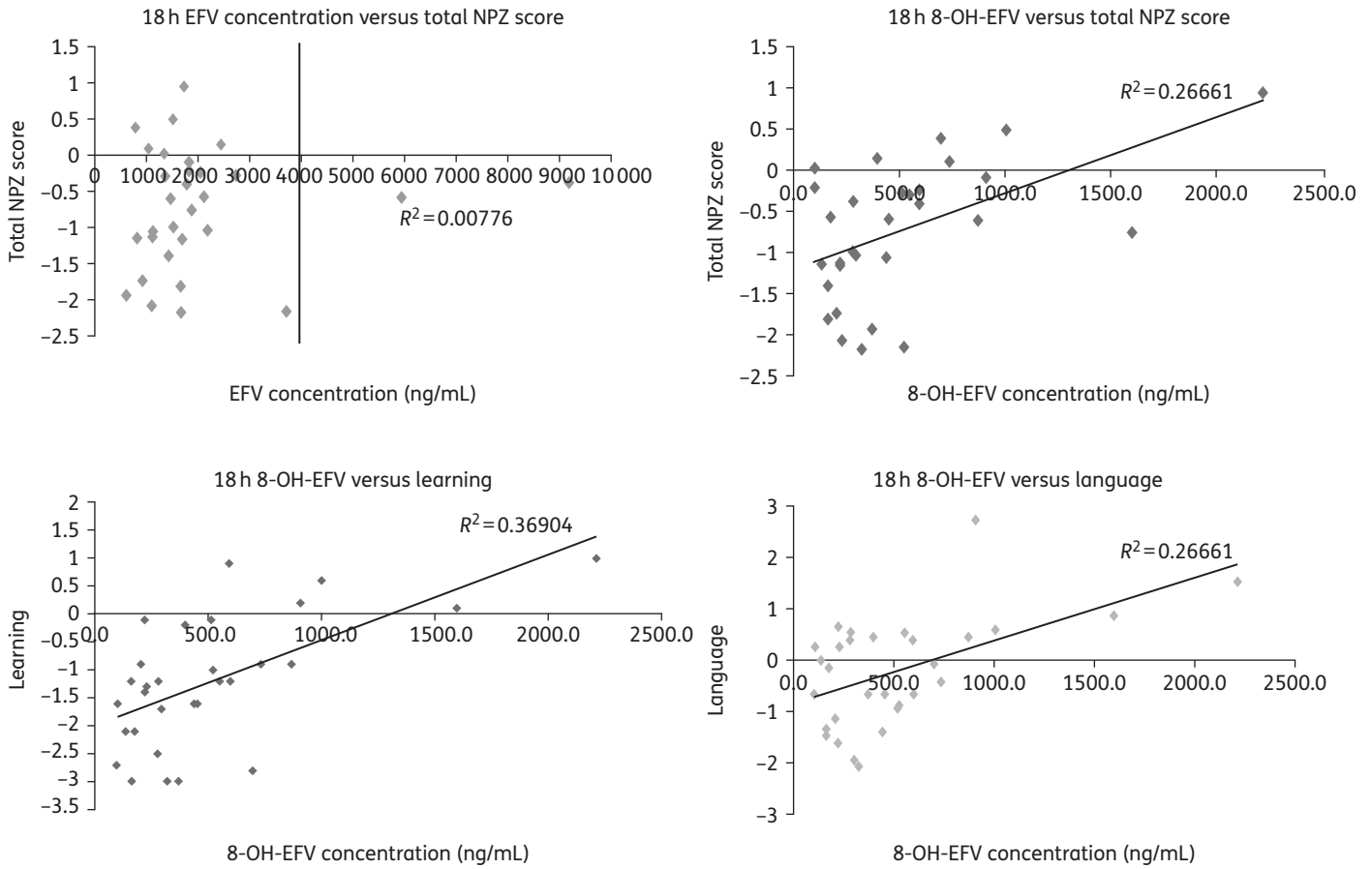

Figure 1. Correlation of efavirenz (EFV) and 8-OH-EFV concentrations and neuropsychological performance. Not all comparisons are included.

Ethics

The study was approved by the University of Nebraska Institutional Review Board (IRB no. 209-13-FB). All study patients provided signed informed consent.

Statistical analysis

Participant characteristics and outcomes were descriptively summarized using medians, range and proportions. The median and IQR were used to report drug and metabolite concentrations. Raw NP scores were standardized to *z*-scores (NPZ) in order to adjust for age and education. Pearson's correlation test was used to examine correlations of plasma efavirenz and 8-OH-efavirenz concentrations with NP test results. Bonferroni correction was applied for tests with multiple comparisons. A *P* value <0.01 (Bonferroni correction) was used to define significance for correlations between efavirenz and 8-OH-efavirenz and NP test results.

Results

Thirty participants were recruited. There were 24 men and 6 women; 22 white and 8 black.

Patient characteristics and median 12 and 18 h efavirenz and 8-OH-efavirenz concentrations are summarized in Table 1. When looking at NP performance, 21 participants scored less than −1 in two domains and 12 overall had composite NPZ scores below −1. Plasma concentrations of efavirenz did not correlate with NP performance (Table 1 and Figure 1). Conversely, higher 8-OH-efavirenz plasma concentrations at 12 and 18 h correlated with better learning (P=0.002), language (P=0.002) and total NPZ scores (P=0.003) (Table 1 and Figure 1).

Twenty-one participants had poor sleep quality (score >5 on Pittsburgh Sleep Quality Index) with a median score of 7.5 (range 1–16; IQR=6). The median CES-D score was 15. Fourteen participants had increased risk of clinical depression (CES-D score >16). There was no significant difference in the median 12 and 18 h efavirenz or 8-OH-efavirenz concentrations between participants with or without depression or between those with poor or better sleep scores. Neither higher or lower efavirenz nor 8-OH-efavirenz plasma concentrations correlated with sleep disturbance, anxiety or depression. No correlation was seen between 8-OH-efavirenz concentrations and age of participants.

Three participants were homozygous for the *CYP2B6* G516T polymorphism; two of the three had elevated 12 h (5634 and 8962 ng/mL) and 18 h (5944 and 9194 ng/mL) plasma efavirenz concentrations while the remaining participant had efavirenz concentrations within normal range (12 h=2562 and 18 h=2114 ng/mL).

Presence of the *CYP2B6* G516T polymorphism was associated with significantly higher concentrations of efavirenz at 12 and 18 h (P=0.02), but did not correlate with worse NP function.

Discussion

The findings of our study were unexpected. As the 8-OH metabolite of efavirenz has been shown to be neurotoxic *in vitro*, we expected worse NP function in patients with higher 8-OH-efavirenz concentrations. In contrast, we found that better neurocognitive function was associated with both higher 12 and 18 h 8-OH-efavirenz plasma concentrations. These findings may be explained by previous clinical data that have correlated higher efavirenz concentrations with greater risk of CNS side effects.[18] Patients with faster clearance of plasma efavirenz could potentially have higher 8-OH-efavirenz concentrations and lower efavirenz concentrations by nature of clearing efavirenz more quickly, thus leading to lower efavirenz exposure and ultimately less severe CNS adverse effects. Winston *et al.*[21] found a positive correlation between increased frequency of efavirenz-related side effects and CSF 8-OH-efavirenz exposure. Interestingly, there was no correlation between plasma efavirenz or 8-OH-efavirenz and CSF 8-OH-efavirenz concentrations and the authors have proposed that higher exposure to 8-OH-efavirenz in CSF may be related to saturation effects or local efavirenz metabolic pathways in the blood–brain barrier.[21] In contrast to the findings by Johnson *et al.*,[22] where abnormal NP performance was found in a group of HIV-negative participants after a single dose of efavirenz, no correlation was found between higher or lower efavirenz concentrations and NP performance in our cohort. Although this can be explained by differences between the population studied by Johnson *et al.*[22] and the patients in our study, who could have self-selected for tolerance of efavirenz CNS effects as they all came into the study with a long history of efavirenz treatment. We were not able to replicate the findings by Gallego *et al.*,[23] where higher efavirenz plasma concentrations were seen in those with insomnia or reduced sleep efficiency, but we must note that mean efavirenz concentrations were higher than in our study. NP performance in our cohort showed a negatively skewed curve; moreover, two-thirds had NPZ scores of less than −1 in two or more domains, which would classify them as being impaired. If we consider total NPZ scores, 12 participants would be classified as impaired.[24] This is consistent with NP performance of other chronically HIV-infected patients.[6]

Limitations of this study include that it was a single-centre study with a small sample size. Moreover, the cohort used in our study had long-standing HIV disease and long history of exposure to efavirenz-containing regimens. These attributes may have rendered our cohort less likely to report NP issues related to efavirenz use. Additionally, we did not have a control group or CSF samples for efavirenz or 8-OH-efavirenz determination.

Although the PK of 8-OH-efavirenz has not been extensively studied *in vivo*, it is possible that this metabolite does not cross the blood–brain barrier as effectively as efavirenz. 8-OH-efavirenz concentrations in the CSF would be dependent on both 8-OH-efavirenz penetration into the CSF as well as local metabolism of the parent drug in CSF. If this was true, we would expect to find higher CSF concentrations of 8-OH-efavirenz in those with higher concentrations of efavirenz in the CSF only. This hypothesis may in part explain better function related to higher plasma 8-OH-efavirenz, as less efavirenz would be transported in the CSF and the 8-OH metabolite would be compartmentalized in plasma.[25]

These findings point to a need for greater understanding of the metabolic profile of efavirenz and 8-OH-efavirenz in plasma and the CNS and relationships with antiviral effect and potential neurotoxicity.

Acknowledgements

This work was partially presented at the 22nd Conference on Retroviruses and Opportunistic Infections, Seattle, WA, 23–26 February 2015 as Oral Abstract no. 84. We thank Dr Howard Fox for his ongoing support, Deanna Hansen for administrative support and we also thank the participants for their invaluable contribution in research studies.

Funding

This work was supported by NIH grant P30 MH062261 to Dr Howard Fox.

Transparency declarations

None to declare.

References

1 High KP, Brennan-Ing M, Clifford DB *et al*. HIV and aging: state of knowledge and areas of critical need for research. A report to the NIH Office of AIDS Research by the HIV and Aging Working Group. *J Acquir Immune Defic Syndr* 2012; **60** Suppl 1: S1–18.

2 Hanlon JT, Aspinall SL, Semla TP *et al*. Consensus guidelines for oral dosing of primarily renally cleared medications in older adults. *J Am Geriatr Soc* 2009; **57**: 335–40.

3 Crawford KW, Spritzler J, Kalayjian RC *et al*. Age-related changes in plasma concentrations of the HIV protease inhibitor lopinavir. *AIDS Res Hum Retroviruses* 2010; **26**: 635–43.

4 Hilmer SN, McLachlan AJ, Le Couteur DG. Clinical pharmacology in the geriatric patient. *Fundam Clin Pharmacol* 2007; **21**: 217–30.

5 Dickinson LJA, Garvey L, Watson V *et al*. *Population pharmacokinetic modelling of plasma and intracellular once daily ritonavir-boosted darunavir in HIV-infected patients*. In: *Abstracts of the Twelfth International Workshop on Clinical Pharmacology of HIV Therapy, Miami, 2011*. Abstract O12.

6 Winston A, Arenas-Pinto A, Stohr W *et al*. Neurocognitive function in HIV infected patients on antiretroviral therapy. *PLoS One* 2013; **8**: e61949.

7 WHO. *What's New in HIV Treatment*. http://apps.who.int/iris/bitstream/10665/204347/1/WHO_HIV_2015.44_eng.pdf.

8 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. https://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf.

9 *Sustiva Package Insert*. Bristol-Myers Squibb, Princeton, NJ, USA. http://packageinserts.bms.com/pi/pi_sustiva.pdf.

10 Clifford DB, Evans S, Yang Y *et al*. Long-term impact of efavirenz on neuropsychological performance and symptoms in HIV-infected individuals (ACTG 5097s). *HIV Clin Trials* 2009; **10**: 343–55.

11 Haas DW, Ribaudo HJ, Kim RB *et al*. Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. *AIDS* 2004; **18**: 2391–400.

12 Ribaudo HJ, Haas DW, Tierney C *et al*. Pharmacogenetics of plasma efavirenz exposure after treatment discontinuation: an Adult AIDS Clinical Trials Group study. *Clin Infect Dis* 2006; **42**: 401–7.

13 Avery LB, VanAusdall JL, Hendrix CW *et al*. Compartmentalization and antiviral effect of efavirenz metabolites in blood plasma, seminal plasma, and cerebrospinal fluid. *Drug Metab Dispos* 2013; **41**: 422–9.

14 di Iulio J, Fayet A, Arab-Alameddine M *et al*. In vivo analysis of efavirenz metabolism in individuals with impaired CYP2A6 function. *Pharmacogenet Genomics* 2009; **19**: 300–9.

15 Markwalder JA, Christ DD, Mutlib A *et al*. Synthesis and biological activities of potential metabolites of the non-nucleoside reverse transcriptase inhibitor efavirenz. *Bioorg Med Chem Lett* 2001; **11**: 619–22.

16 Wyen C, Hendra H, Siccardi M *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**: 2092–8.

17 Tovar-y-Romo LB, Bumpus NN, Pomerantz D *et al*. Dendritic spine injury induced by the 8-hydroxy metabolite of efavirenz. *J Pharmacol Exp Ther* 2012; **343**: 696–703.

18 Marzolini C, Telenti A, Decosterd LA *et al*. Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. *AIDS* 2001; **15**: 71–5.

19 Mutlib AE, Chen H, Nemeth GA *et al*. Identification and characterization of efavirenz metabolites by liquid chromatography/mass spectrometry and high field NMR: species differences in the metabolism of efavirenz. *Drug Metab Dispos* 1999; **27**: 1319–33.

20 Winchester L, Sandkovsky U, Fletcher CV *et al*. *Quantification of efavirenz and its 7-hydroxy and 8-hydroxy metabolites in human plasma via LC/MS/MS*. In: *Abstracts of the Annual American Association of Pharmaceutical Scientists Meeting, Orlando, FL, 2015*. Poster R6190. American Association of Pharmaceutical Scientists, Arlington, VA, USA.

21 Winston A, Amin J, Clarke A *et al*. Cerebrospinal fluid exposure of efavirenz and its major metabolites when dosed at 400 mg and 600 mg once daily: a randomized controlled trial. *Clin Infect Dis* 2015; **60**: 1026–32.

22 Johnson DH, Gebretsadik T, Shintani A *et al*. Neuropsychometric correlates of efavirenz pharmacokinetics and pharmacogenetics following a single oral dose. *Br J Clin Pharmacol* 2013; **75**: 997–1006.

23 Gallego L, Barreiro P, del Rio R *et al*. Analyzing sleep abnormalities in HIV-infected patients treated with efavirenz. *Clin Infect Dis* 2004; **38**: 430–2.

24 Arenas-Pinto A, Winston A, Stohr W *et al*. Neurocognitive function in HIV-infected patients: comparison of two methods to define impairment. *PLoS One* 2014; **9**: e103498.

25 Smurzynski M, Wu K, Letendre S *et al*. Effects of central nervous system antiretroviral penetration on cognitive functioning in the ALLRT cohort. *AIDS* 2011; **25**: 357–65.