

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Cerebrospinal Fluid Inhibitory Quotients of Antiretroviral Drugs in HIV-positive Patients are Associated with Compartmental Viral Control.

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/158854> since 2016-06-08T22:14:29Z

Published version:

DOI:10.1093/cid/ciu773

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

This is the author's final version of the contribution published as:

Calcagno A;Simiele M;Alberione MC;Bracchi M;Marinaro L;Ecclesia S;Di Perri G;D'Avolio A;Bonora S. Cerebrospinal Fluid Inhibitory Quotients of Antiretroviral Drugs in HIV-positive Patients are Associated with Compartmental Viral Control.. CLINICAL INFECTIOUS DISEASES. 60 (2) pp: 311-317.
DOI: 10.1093/cid/ciu773

The publisher's version is available at:

<http://cid.oxfordjournals.org/lookup/doi/10.1093/cid/ciu773>

When citing, please refer to the published version.

Link to this full text:

<http://hdl.handle.net/2318/158854>

Cerebrospinal Fluid Inhibitory Quotients of Antiretroviral Drugs in HIV-Infected Patients Are Associated With Compartmental Viral Control

Andrea Calcagno, Marco Simiele, Maria Chiara Alberione, Margherita Bracchi, Letizia Marinaro, Sara Ecclesia, Giovanni Di Perri, Antonio D'Avolio*, Stefano Bonora*

* A. D. and S. B. contributed equally to this work.

Abstract

Background. Despite the efficacy of highly active antiretroviral treatment (HAART), a large proportion of human immunodeficiency virus (HIV)-infected patients may develop moderate neurocognitive impairment. Antiretroviral drug passage into the central nervous system may be relevant for preventing and treating HIV-associated neurocognitive disorder; nevertheless, clear cerebrospinal fluid (CSF) pharmacodynamic targets are not known.

Methods. HAART-treated adults with wild-type HIV were prospectively enrolled. CSF concentrations (measured by mass spectrophotometric methods) and inhibitory quotients (CSF concentrations divided by in vitro 50% and 95% inhibitory concentrations) were compared among different drugs and related to CSF HIV RNA levels. CSF escape was defined as CSF HIV RNA >50 copies/mL despite contemporary plasma HIV RNA below that threshold.

Results. One hundred twenty-seven patients (91 male [71.7%], 93 white [73.2%], with a median age of 46 years [interquartile range, 40.5–54.5 years]) provided 174 paired CSF and plasma samples. Twice-daily darunavir, once-daily darunavir, and efavirenz had the highest CSF 95% inhibitory quotients (18.5, 8.2, and 6.4, respectively). Higher nadir CD4 cell count ($P = .01$) and plasma HIV RNA <50 copies/mL ($P < .001$) were independent predictors of controlled CSF HIV RNA. Optimal drug exposure (CSF detectable drugs and 95% inhibitory quotient >1) was protective for CSF escape ($P = .01$).

Conclusions. Cerebrospinal fluid 95% inhibitory quotients may be used to compare antiretroviral drug compartmental exposure; they deserve longitudinal studies to assess the adequacy of CSF drug concentrations in treated HIV-infected patients.

Human immunodeficiency virus (HIV) replication in the central nervous system (CNS) occurs a few days after patients have been infected: the uncontrolled viral production leads to perivascular macrophages, microglia, and astrocyte infection, thus eventually leading to neuronal damage [1]. The widespread use of highly active antiretroviral treatment has led to a dramatic decrease in HIV-associated opportunistic diseases including dementia; nevertheless, minor neurocognitive impairments are still highly prevalent [2]. Whereas differential antiretrovirals' penetration into cerebrospinal fluid (CSF) and CNS has been associated with a reduced probability of compartmental HIV replication, its impact on the course and severity of HIV-associated neurocognitive disorder (HAND) is still debated. A large cooperative study by the group has categorized antiretrovirals according to their CNS penetration effectiveness (CPE) score; the higher the score, the lower the risk of detectable HIV RNA in CSF [3, 4]. In any case, comparison of different drugs' penetration into the CNS is difficult given the high interpatient variability in drug exposure and the lack of standardized target concentrations. In several studies, CSF drug exposure was evaluated by referring to the in vitro 50% inhibitory concentration (IC50); however, the use of IC50 as a reference is still debated due to potential selection of resistant variants (at such concentrations, 50% of virions are still theoretically able to replicate). For this reason, 95% and 99% inhibitory concentrations (IC95 and IC99, respectively) have been suggested as a more appropriate threshold without clinical confirmation. Judging the adequacy of

raltegravir CSF exposure, for example, has given conflicting results in 3 different studies; patients' characteristics as well as different target concentrations may explain such discordance [5–7]. Acosta and colleagues recently used a large dataset to validate in vitro inhibitory concentrations of several antiretroviral drugs, thus providing a new and standardized approach to the field [8]. Furthermore, inhibitory quotients have been suggested as an integration of drug exposure and viral susceptibility; they were associated with virological efficacy in protease inhibitor (PI) treatment-naïve (plasma concentrations divided by ICs) and treatment-experienced HIV-infected patients (plasma concentrations divided by different measures of resistance) [9, 10].

The primary objective of this study was to compare CSF 50% and 95% inhibitory quotients (IQ50 and IQ95, respectively) of several antiretroviral drugs in patients not harboring resistance-associated mutations. The secondary objective was to investigate relationship between CSF concentrations above the IC50 and IC95 and viral control in the CSF.

METHODS

Adults on combination antiretroviral therapy undergoing a lumbar puncture for either clinical reasons or specific research protocols were prospectively included. Exclusion criteria were the presence of opportunistic infections or neoplasias, the presence plasma or cerebrospinal fluid of major resistance-associated mutations, or the concomitant use of drugs known to cause significant drug–drug interactions (rifampicin, proton pump inhibitors with atazanavir, etc). This protocol was approved by the Ethics Committee, and patients provided written informed consent.

All patients were admitted to the infectious disease ward at least 24 hours before the procedure, and antiretroviral drugs were administered under direct observation. Paired plasma and CSF samples were withdrawn at 12–14 hours (twice-daily administered compounds) or 21–27 hours (once-daily administered) after drug intake; no more than 15 minutes elapsed between CSF and plasma withdrawal. Antiretroviral concentrations were determined by a validated high-performance liquid chromatography coupled with mass spectrometry method with the lower limits of quantification (LLOQ) reported elsewhere [11]. Wild-type in vitro inhibitory quotients were derived from Acosta et al; these were obtained from clinical isolates and then corrected for protein binding (derived from in vitro experiments) [8]. Inhibitory quotients of PIs, nonnucleoside reverse transcriptase inhibitors (NNRTIs), and raltegravir were calculated as the ratio between CSF concentration and IC50 and IC95 (not corrected for protein binding); adequate exposure was considered if IQ95 >1. For nucleoside reverse transcriptase inhibitors (NRTIs) and maraviroc (MVC), detectability in CSF above the LLOQ was considered to be adequate concentration, due to the unfeasibility of measuring active intracellular moieties (extracellular concentrations may not reflect intracellular exposure and activity) and uncertainty in inhibitory concentration values, respectively. Accordingly, optimal CSF regimen exposure was defined as IQ95 >1 and detectability of CSF concentration of all drugs contained in the regimen.

HIV RNA levels in plasma and CSF were quantified through real-time polymerase chain reaction Cobas AmpliPrep/Cobas TaqMan (Roche HIV-1 version 2.0) with a limit of quantification of 20 copies/mL. CSF escape was defined as CSF HIV RNA >50 copies/mL with plasma HIV RNA <50 copies/mL.

Data are presented as numbers (percentages) or medians (interquartile ranges); nonparametric tests were used to analyze and compare variables. Univariate analysis (Mann–Whitney and χ^2 tests) were performed; variables with P values <.10 were incorporated into multivariate logistic regression analysis (with backward

elimination). Statistical significance was defined as a 2-sided P value of .05. All statistical analyses were performed with SPSS version 20.0 (IBM Corporation, Armonk, New York).

RESULTS

One hundred seventy-four CSF and plasma samples were available from 127 patients. Patients were mostly male (91 [71.7%]) and white (93 [73.2%]), with a median age of 46 years (interquartile range [IQR], 40.5–54.5 years) and a median body mass index of 22.2 kg/m² (IQR, 20–25.2 kg/m²). Hepatitis C virus and hepatitis B virus (HBV)-related chronic hepatitis were diagnosed in 33 (26%) and 14 patients (11%), respectively; 13 patients (10.2%) presented with liver cirrhosis. Lumbar punctures were performed in asymptomatic patients (either in the context of longitudinal studies or asymptomatic patients with <100 CD4 cells/μL: 63 [49.6%]) for the diagnosis of HAND (30 [23.6%]) or other neurological conditions (22 [17.3%]), including epilepsy (5 [3.9%]), neuropathies (5 [3.9%]), and myelopathies (4 [3.9%]) or JC virus–negative leukoencephalopathy (12 [9.4%]).

HIV infection had been diagnosed a median of 13.7 years previously (IQR, 2.8–19 years); the median current and nadir CD4+ T-lymphocyte counts were 321 cells/μL (IQR, 145–575 cells/μL) and 108 cells/μL (IQR, 29–210 cells/μL), respectively. Seventy patients had plasma HIV RNA <20 copies/mL and 14 patients (11%) between 20 and 50 copies/mL; the patients with uncontrolled viral load (42 [33.1%]) had a median value of 274 copies/mL (IQR, 89–5500 copies/mL).

Antiretroviral regimens were mainly a combination of 2 NRTIs plus a third drug (80 [63%])—including a protease inhibitor (59 [73.7%]), an NNRTI (13 [16.2%]), or an integrase inhibitor (9 [11.2%])—complex regimens (25 [19.7%]) NRTI-sparing regimens (22 [17.3%]) as described in Supplementary Table 1. Median CPE score was 7 (IQR, 6–8; range, 3–15).

CSF concentrations, CSF-to-plasma ratios, and the IQ50 and IQ95 are reported in Table 1. The results there reported suggest a high variability among different drugs, with coefficients of variation ranging from 86% (raltegravir) to 176% (atazanavir); the percentage of samples below IC50, between IC50 and IC95, and above IC95 are depicted in Figure 1A, and the IQ95 is shown in Figure 1B. Three samples of patients receiving nevirapine showed concentrations of 290 ng/mL, 4250 ng/mL, and 1 that was undetectable both in CSF and in plasma. Three of 10 samples of patients receiving MVC showed undetectable concentrations; in those that were detectable, the median CSF concentration and CSF-to-plasma ratio were 3 ng/mL (range, 2–9) and 2.6% (range, 0.3%–7.7%), respectively. Comparing drugs, an intraclass hierarchy of drug exposures above IC95 was observed: among PIs, darunavir (both once and twice daily) had the highest IQ95 compared with lopinavir and atazanavir (Kruskal–Wallis test, $P < .001$), whereas among NNRTIs, efavirenz showed the highest IQ95 (Kruskal–Wallis test, $P < .001$). Although no significant difference was noted in IQ95 between atazanavir boosted with ritonavir (/r) and lopinavir/r (Mann–Whitney test, $P = .53$), unboosted atazanavir had the lowest IQ95 (Mann–Whitney test, $P = .03$ vs lopinavir/r and $P = .05$ vs atazanavir/r).

Table 1. Cerebrospinal Fluid Parameters

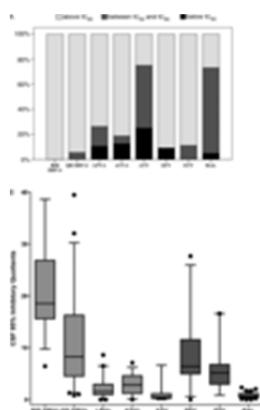
Drug	Samples, No.	CSF PK Median, ng/mL (CV) [IQR]	CSF-to-Plasma Ratio, Median (IQR)	Undetectable Samples, No. (%)	WT IC ₅₀ , ng/mL	IQ ₅₀ , Median (IQR)	WT IC ₉₅ , ng/mL	IQ ₉₅ , Median (IQR)
QD DRV/r	36	15.7 (128%) [8.5–30.9]	0.5% (0.3–0.9)	0 (0)	0.4	39.2 (21.4–77.3)	1.9	8.2 (4.5–16.3)
BID DRV/r	18	35.2 (48%) [29.6–51]	0.8% (0.5–1)	0 (0)	0.4	88 (74–127)	1.9	18.5 (15.6–26.8)
LPV/r	18	26 (96.4%) [16–50]	0.3% (0.1–0.6)	3 (1.7)	3.1	8.4 (5.2–	17	1.5 (0.9–

Drug	Samples, No.	CSF PK Median, ng/mL (CV) [IQR]	CSF-to-Plasma Ratio, Median (IQR)	Undetectable Samples, No. (%)	WT IC ₅₀ , ng/mL	IQ ₅₀ , Median (IQR)	WT IC ₉₅ , ng/mL	IQ ₉₅ , Median (IQR)
ATV/r	16	18.1 (75.5%) [7.5–30]	1.3% (0.6–1.4)	1 (6.25)	1.7	16.1 (10.7 (4.4–17.5)	6.5	2.8 (1.1–4.5)
ATV	8	2.8 (176%) [1.5–7.3]	1.4% (0.6–3.4)	1 (12.5)	1.7	1.7 (0.9–4.3)	6.5	0.4 (0.2–1.1)
EFV	11	30 (80%) [23.3–54.3]	1.3% (0.8–1.6)	0 (0)	1.3	23.1 (17.9–41.8)	4.7	6.4 (5.0–11.5)
ETV	9	18 (79%) [10.3–20.6]	2.7% (1.9–3.7)	0 (0)	0.9	20 (11.4–26.2)	3.5	5.1 (2.9–6.7)
RAL	41	30 (68%) [17.5–51.5]	17% (1.8–33.8)	2 (4.9)	3.6	8.3 (4.9–14.3)	44	0.7 (0.4–1.2)

CSF concentrations, number of samples with levels below the limit of detection, wild-type IC₅₀ and IC₉₅, and IQ₉₅ of protease inhibitors, nonnucleoside reverse transcriptase inhibitors, and RAL are shown. Drugs with <5 available samples were not included in the table: nevirapine (3 samples), fosamprenavir (2 samples), tipranavir (2 samples).

Abbreviations: ATV, unboosted atazanavir; ATV/r, boosted atazanavir; BID DRV/r, darunavir twice daily; CSF, cerebrospinal fluid; CV, coefficient of variation; EFV, efavirenz, ETV, etravirine; IC₅₀, 50% inhibitory concentration; IC₉₅, 95% inhibitory concentration; IQ₉₅, 95% inhibitory quotient; IQR, interquartile range; LPV, lopinavir, PK, pharmacokinetics; QD DRV/r, darunavir once daily; RAL, raltegravir; WT, wild type.

Figure 1.



Cerebrospinal fluid (CSF) drug concentrations according to the 50% and 95% inhibitory concentrations (IC₅₀ and IC₉₅, respectively). A, Prevalence of CSF drug concentrations below IC₅₀, between IC₅₀ and IC₉₅, and above IC₉₅ according to different third drugs. B, CSF 95% inhibitory quotient (IQ₉₅) according to different drugs. Horizontal lines represent median values, boxes show interquartile ranges, and black dots are single-sample outliers. Abbreviations: ATV, unboosted atazanavir; ATV/r, boosted atazanavir; BID DRV/r, darunavir twice daily; EFV, efavirenz; ETV, etravirine; LPV, lopinavir; QD DRV/r, darunavir once daily; RAL, raltegravir.

Twenty-one samples (12.1%) presented NRTI CSF concentrations below the LLOQ: 17 samples (tenofovir) and 4 samples (both tenofovir and emtricitabine) presented undetectable concentrations. Sixty-four patients (50.4%) showed optimal CSF drug exposure (IQ₉₅ >1 and detectability of CSF concentration of all drugs contained in the regimen).

CSF HIV RNA was <20 copies/mL, between 20 and 50 copies/mL, or >50 copies/mL in 58 (45.7%), 13 (10.2%), and 56 (44.1%) patients, respectively. In patients with plasma HIV RNA <50 copies/mL (n = 84), CSF escape was noted in 24 subjects (28.6%).

Factors associated with CSF HIV RNA <50 copies/mL and with CSF escape are shown in Table 2; patients with optimal CSF exposure had a higher probability of CSF HIV RNA <50 copies/mL (71.4% vs 47.4%; odds ratio [OR], 2.78 [95% confidence interval {CI}, 1.31–5.91]; P = .007). At multivariate analysis, nadir CD4 cell count (adjusted OR [AOR], 2.04 [95% CI, 1.18–3.54]; P = .01) and plasma HIV RNA <50 copies/mL (AOR, 8.14 [95% CI, 2.67–24.76]; P < .001) were independently associated with CSF HIV RNA <50 copies/mL; optimal regimen CSF exposure (all drugs in the regimen with IQ95 >1 or detectable CSF concentration) was borderline significant (AOR, 2.36 [95% CI, .85–6.29]; P = .09).

Table 2. Factors Associated With Cerebrospinal Fluid (CSF) HIV RNA <50 Copies/mL and With CSF Escape, in Univariate and Multivariate Analyses

Variable	CSF HIV RNA <50 Copies/mL (n = 127)					CSF Escape in Patients With Plasma VL <50 Copies/mL (n = 84)				
	Univariate		Multivariate			Univariate		Multivariate		
	P Value	OR	P Value	AOR	95% CI	P Value	OR	P Value	AOR	95% CI
Male sex	.9461
Age (10 y increase)	.8048
HCV Ab positivity	.2170
HBsAg positivity	.04	0.26	.1006	5.3	.66
Previous syphilis	.2215
CPE score (per 1-point increase)	.3144
Nadir CD4 count (per 100 cells/μL increase)	.0901	2.04	1.18–3.54	.35
Current CD4 count (per 100 cells/μL increase)	.012932
Plasma VL <50 copies/mL	<.01	6.1	<.01	8.14	2.67–24.76	NA	NA	NA	NA	NA
Duration of VL <50 copies/mL (per 6-mo increase)	.5652
CSF Conc >IC ₉₅	.01	2.5501	0.28
Detectable CSF NRTIs or MVC	.09	2.3616
Optimal CSF regimen exposure	<.01	2.78	.0901	0.28	.01	0.28	.11–.76

Optimal CSF regimen exposure defined as 95% inhibitory quotient >1 detectability of CSF concentration of all drugs contained in the regimen. Abbreviations: Ab, antibody; AOR, adjusted odds ratio; CI, confidence interval; Conc, concentration; CPE, cerebrospinal fluid penetration effectiveness; CSF, cerebrospinal fluid; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IC₉₅, 95% inhibitory concentration; MVC, maraviroc; NA, not applicable; NRTI, nucleoside reverse transcriptase inhibitor; OR, odds ratio; VL, viral load.

In patients with plasma viral load <50 copies/mL (n = 84), univariate analysis optimal CSF drug exposure was associated with a decreased probability of CSF escape (17% vs 42.1%; OR, 0.28 [95% CI, .10–.76]; P = .011); in multivariate analysis, optimal CSF exposure was protective for CSF escape (AOR, 0.28 [95% CI, .11–.76]; P = .01). In neither analysis was CSF exposure above the IC50 associated with CSF viral control.

DISCUSSION

In this study, the CSF 95% inhibitory quotients of several antiretrovirals were compared, showing that the 2 drugs with the highest IQ95 were darunavir (twice and once daily) and efavirenz. We were also able to show that optimal CSF exposure of all components of the regimen was associated with a reduced probability of CSF escape.

Several attempts have been made at classifying antiretrovirals according to their rate of CNS penetration and activity. The most complete and recent study suggested a CPE score (from 1 to 4, with 4 being the most penetrating/active drugs), which was subsequently validated in other cohorts [3, 4, 12, 13]. Nevertheless, for most of the compounds, pharmacodynamic data are not available and the score relies only on biochemical and pharmacokinetic properties. Published studies do evaluate antiretrovirals' CSF exposure, mostly by referring to the IC50. However, low CSF concentrations generally are reached by antiretrovirals, and concentrations are usually in the range of IC50 conversely to what is observed in plasma, where antiretrovirals generally reach levels several times higher than the IC50 [14]. Levels of drug exposure above the IC50 but still below the IC95, in fact, could lead to suboptimal viral inhibition and incomplete therapeutic response. Therefore, the use of standardized IC95, as defined by a recent study, seems more adequate than IC50, even considering the low protein levels in CSF. Among PIs (Figure 1), darunavir showed the highest IQ95 both given once and twice daily: the influence of dosing, previously reported by our group, did not change this observation [15]. Boosted atazanavir and lopinavir have comparable IQ95, in contrast to previous studies using IC50 in which lopinavir showed better CSF exposure, whereas unboosted atazanavir was associated with lower levels as already reported [16]. Among NNRTIs (Figure 1), efavirenz and etravirine had high IQ95, confirming previous results, although unexpected CSF protein binding may be taken into account [17]. Samples with nevirapine were unfortunately too few for further analysis. Raltegravir recipients showed a low IQ95 due to 73.2% of concentrations below the IC95, confirming previous results [5–7]: however, raltegravir's well-known inter- and inpatient variability, as well the lack of a clear pharmacokinetic/pharmacodynamic relationship, does not allow a definitive definition of its CSF exposure adequacy [18].

The herein-confirmed variability in drug exposure and CSF-to-plasma ratios (ranging from 0% to 33.8%) could question the usefulness of measuring drug concentrations in the CSF. However, as 12.1% and 4% of the samples had undetectable concentrations of NRTIs or third drugs, respectively, it may be useful to monitor drug exposure in patients with HAND and/or CSF escape. In the 2 case series reporting patients with symptomatic CSF escape, in fact, some subjects had undetectable CSF levels, thus suggesting limited drug entry into the CSF/CNS [19, 20]. Given the impact of blood-brain permeability on some drug CSF exposure, it is possible that a subset of subjects may have a very limited passage, thus influencing compartmental drug activity [5, 7, 21]; data from the CHARTER group using CSF-to-albumin ratios in patients with HAND seem to confirm this hypothesis [22].

In our study, the most important predictors of cerebrospinal viral control were, as expected, the immunovirological characteristics: nadir CD4 cell count and plasma HIV RNA <50 copies/mL. The association of chronic HBV infection with worse CSF viral control has not been reported before, and is not confirmed by multivariate analysis; it may reflect a selection bias or the larger use of tenofovir as an anti-HIV/HBV drug. These conditions suggesting optimal viral control and low reservoir size have been constantly associated with compartmental viral control and with the risk of HAND [23]. However, in our patients with suppressed plasma viral load, having adequate CSF levels of all drugs in the regimen according to our definition was associated with a reduced risk of CSF escape; this may potentially affect the possible interventions in patients with compartmental viral replication. A high proportion of patients with CSF escape was observed in this cohort (28.6%); the inclusion of patients with HAND, with very low CD4 nadir, and with JC virus–negative leukoencephalopathy may explain the difference with previously published studies [24].

Some of the limitations of this study must be considered, such as the cross-sectional design, the heterogeneity of included subjects, the limited number of patients with fully controlled plasma viral load,

the possible bias introduced by performing lumbar punctures for clinical reasons, and the unavailability of free-drug concentrations.

In conclusion, CSF drug IQ95 seems to be a new and reliable tool to study drug exposure in the CSF of patients with HIV. In the clinical setting, IQ95 deserves further prospective evaluation to study the adequacy of CSF concentrations in successfully treated HIV-infected patients.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online: (<http://cid.oxfordjournals.org.offcampus.dam.unito.it>).

References

1. Gannon P, Khan MZ, Kolson DL. Current understanding of HIV-associated neurocognitive disorders pathogenesis. *Curr Opin Neurol* 2011;24:275-83.
2. Clifford DB, Ances BM. HIV-associated neurocognitive disorder. *Lancet Infect Dis* 2013;13:976-86.
3. Hammond ER, Crum RM, Treisman GJ, et al. The cerebrospinal fluid HIV risk score for assessing central nervous system activity in persons with HIV. *Am J Epidemiol* 2014;180:297-307.
4. Ciccarelli N, Fabbiani M, Colafigli M, et al. Revised central nervous system neuropenetration-effectiveness score is associated with cognitive disorders in HIV-infected patients with controlled plasma viraemia. *Antivir Ther* 2013;18:153-60.
5. Yilmaz A, Gisslén M, Spudich S, et al. Raltegravir cerebrospinal fluid concentrations in HIV-1 infection. *PLoS One* 2009;4:e6877.
6. Croteau D, Letendre S, Best BM, et al. Total raltegravir concentrations in cerebrospinal fluid exceed the 50-percent inhibitory concentration for wild-type HIV-1. *Antimicrob Agents Chemother* 2010;54:5156-60.
7. Calcagno A, Cusato J, Simiele M, et al. High interpatient variability of raltegravir CSF concentrations in HIV-positive patients: a pharmacogenetic analysis. *J Antimicrob Chemother* 2014;69:241-5.
8. Acosta EP, Limoli KL, Trinh L, et al. Novel method to assess antiretroviral target trough concentrations using in vitro susceptibility data. *Antimicrob Agents Chemother* 2012;56:5938-45.
9. Zhu L, Liao S, Child M, et al. Pharmacokinetics and inhibitory quotient of atazanavir/ritonavir versus lopinavir/ritonavir in HIV-infected, treatment-naive patients who participated in the CASTLE study. *J Antimicrob Chemother* 2012;67:465-8.
10. Gonzalez de Requena D, Bonora S, Viganò O, et al. Comparative evaluation of seven resistance interpretation algorithms and their derived genotypic inhibitory quotients for the prediction of 48 week virological response to darunavir-based salvage regimens. *J Antimicrob Chemother* 2011;66:192-200.
11. D'Avolio A, Siccardi M, Sciandra M, et al. HPLC-MS method for the simultaneous quantification of the new HIV protease inhibitor darunavir, and 11 other antiretroviral agents in plasma of HIV-infected patients. *J Chromatogr B Analyt Technol Biomed Life Sci* 2007;859:234-40.
12. Cusini A, Vernazza PL, Yerly S, et al. Higher CNS penetration-effectiveness of long-term combination antiretroviral therapy is associated with better HIV-1 viral suppression in cerebrospinal fluid. *J Acquir Immune Defic Syndr* 2013;62:28-35.
13. Vassallo M, Durant J, Biscay V, et al. Can high central nervous system penetrating antiretroviral regimens protect against the onset of HIV-associated neurocognitive disorders? *AIDS* 2014;28:493-501.

14. Yilmaz A, Price R, Gisslen M. Antiretroviral drug treatment of CNS HIV-1 infection. *J Antimicrob Chemother* 2012;67:299-311.
15. Calcagno A, Yilmaz A, Cusato J, et al. Determinants of darunavir cerebrospinal fluid concentrations: impact of once-daily dosing and pharmacogenetics. *AIDS* 2012;26:1529-33.
16. Best BM, Letendre SL, Brigid E, et al. Low atazanavir concentrations in cerebrospinal fluid. *AIDS* 2009;23:83-7.
17. Nguyen A, Rossi S, Croteau D, et al. Etravirine in CSF is highly protein bound. *J Antimicrob Chemother* 2013;68:1161-8.
18. Siccardi M, D'Avolio A, Rodriguez-Novoa S, et al. Inpatient and outpatient pharmacokinetic variability of raltegravir in the clinical setting. *Ther Drug Monit* 2012;34:232-5.
19. Canestri A, Lescurer FX, Jaureguiberry S, et al. Discordance between cerebral spinal fluid and plasma HIV replication in patients with neurological symptoms who are receiving suppressive antiretroviral therapy. *Clin Infect Dis* 2010;50:773-8.
20. Peluso MJ, Ferretti F, Peterson J, et al. Cerebrospinal fluid HIV escape associated with progressive neurologic dysfunction in patients on antiretroviral therapy with well controlled plasma viral load. *AIDS* 2012;26:1765-74.
21. Calcagno A, Bonora S, Simiele M, et al. Tenofovir and emtricitabine cerebrospinal fluid-to-plasma ratios correlate to the extent of blood-brain barrier damage. *AIDS* 2011;25:1437-9.
22. Letendre S, Croteau D, Ellis RJ, et al. CSF-serum albumin ratios are associated with neurocognitive impairment in antiretroviral-treated people with HIV. 18th Conference on Retroviruses and Opportunistic Infections; 27 February–2 March 2011. Boston, MA; Abstract 408.
23. Valcour VG, Ananworanich J, Aagsald M, et al. HIV DNA reservoir increases risk for cognitive disorders in cART-naive patients. *PLoS One* 2013;8:e70164.
24. Eden A, Fuchs D, Hagberg L, et al. HIV-1 viral escape in cerebrospinal fluid of subjects on suppressive antiretroviral treatment. *J Infect Dis* 2010;202:1819-25.