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Degree of plasma protein binding affects central nervous system (CNS) free drug penetration for the HIV-1 protease inhibitors among patients in an urban HIV clinic.

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By

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B.S., University of North Carolina-Chapel Hill, 2001

M.D., University of North Carolina, 2007

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An abstract of

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Abstract

Degree of plasma protein binding affects central nervous system (CNS) free drug penetration for the HIV-1 protease inhibitors among patients in an urban HIV clinic.

By Cecile Delille

Background: High prevalence of HIV-1 associated neurocognitive disorders (HAND) persists in the highly active antiretroviral therapy (HAART) era. For optimal antiviral effect, adequate antiretroviral penetration into the central nervous system (CNS) is needed. HIV protease inhibitors' (PI) CNS concentration may be limited by high level of plasma protein blinding, as only unbound drug crosses the blood-brain barrier easily. Atazanavir (ATV) and Darunavir (DRV), two prescribed PIs, differ in degree of plasma protein binding, 86% and 95%, respectively, and cerebrospinal fluid (CSF):plasma free drug ratio should be higher for ATV.

Objectives: The primary objective was to compare the CNS penetration, as measured by free drug CSF:plasma trough concentration ratios, between ATV and DRV. Relationships between PI free CSF concentrations and CSF HIV-1 RNA and CSF neopterin were assessed.

Methods: In a cross-sectional study conducted among virologically suppressed adult HIV-infected individuals receiving tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) + ritonavir (RTV)-boosted once daily ATV or DRV for $\geq$ 6 months, paired CSF and plasma was collected at trough times. Free PI concentrations were measured using rapid equilibrium dialysis and liquid chromatography/tandem mass spectrometry. Plasma and CSF HIV-1 RNA and neopterin were measured using Ampliprep/COBAS® Taqman® 2.0 assay (Roche) and enzyme linked immunosorbent assay (ALPCO), respectively.

Results: Thirty subjects (15 per arm) were enrolled. Demographics and comorbidities were comparable between arms. All subjects had normal renal and liver function. CSF: plasma free drug ratio was higher for ATV compared to DRV, 0.38 (95% CL 0.20-0.56) vs 0.065 (95% CL 0.043-0.087), $p<0.0001$. CSF free drug concentrations exceeded protein adjusted wild-type IC_{50} for 13 of 15 subjects in each arm. 13% (2/15) and 26.7% (4/15) in the ATV and DRV arms had detectable (>40 copies/mL) CSF HIV-1 RNA, $p = 0.65$. Mean (95% CL) CSF neopterin levels were within normal range, 1.76 ng/mL(1.38-2.13) for ATV and 1.73 ng/mL (1.53-1.92) for DRV, $p =0.88$.

Conclusions: Higher CSF:plasma free ATV concentration ratio relative to DRV is likely due to lower ATV protein binding. CSF free drug concentrations were not associated with CSF HIV-1 RNA or neopterin, implying that multiple factors play roles in controlling CNS viremia and inflammation.

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INTRODUCTION

According to the Centers for Disease Control and Prevention (CDC), by the end of 2009, there were 1,148,200 persons aged 13 or older living with HIV-1 infection in the United States. The estimated incidence of new HIV-1 infections in 2010 was 47,500, a rate that has remained stable since 2007 [1]. In the mid 1990s, the advent of highly active anti-retroviral therapy (HAART) resulted in sustainable plasma virologic suppression and robust immune reconstitution, leading to a decrease in the rate of opportunistic infections and extension of lifespan for these patients.

Despite these improvements in HIV treatment, HAART has not resulted in complete eradication of the virus from the body. Sanctuary sites exist where penetration of anti-retrovirals remains limited, such as peripheral blood mononuclear cells, the genital tract, gastrointestinal lymphoid tissue, and the central nervous system (CNS) [2]. HIV-1 invades the CNS early after infection via macrophages, monocytes, and dendritic cells [3, 4]. HIV-associated neurocognitive disorders (HAND) can develop, including HIV-associated dementia, mild neurocognitive disorders, and asymptomatic neurocognitive impairment [5]. Over time, HAND has been shown to contribute significantly to morbidity [6-8] and early mortality [9, 10].

Overall prevalence of HAND is no different in the HAART era than the pre-HAART era: 36.2-44.8% [7]. Suboptimal control of HIV in the CNS occurs, even in the context of plasma virologic suppression. Evidence to support this includes documented cases of discordance between the CSF and plasma HIV replication [11, 12], different resistance patterns among the virus in CSF and plasma [13], and persistent inflammation, as measured by CSF neopterin, a marker of macrophage activation, among patients

receiving HAART [14]. Both CSF neopterin and HIV-1 RNA have been found to be predictive markers for development of HIV-associated dementia [15, 16].

Limited CNS penetration by antiretroviral (ARV) regimens is associated with HAND [17-20]. Several factors impact drug CNS penetration, including molecular size, lipophilicity, affinity for efflux pump transporters, and degree of plasma-protein binding [21-23]. Specifically, the protease inhibitor (PI) class of ARV medications may be limited in their CNS penetration due to their large molecular size, affinity for efflux pump transporters, and high degree of plasma protein binding [12, 24-26]. Protein-bound drug cannot leave capillaries, therefore it is the free (unbound) form of the drug that is considered pharmacologically active and crosses the blood-brain and blood-CSF barriers [21]. However, most studies assessing CNS penetration of ARVs have only looked at total (unbound+bound) drug concentrations in CSF rather than free (unbound) drug.

Two of the most commonly used PIs, Atazanavir (ATV) and Darunavir (DRV), are recommended as first line agents in HAART among ARV-naïve individuals [27]. This study investigated whether the difference in degree of plasma protein binding between the two drugs results in a difference in free drug CNS penetration, as measured by their free drug CSF:plasma trough concentration ratios. In addition, relationships between CSF free protease inhibitor concentrations and both CSF HIV-1 RNA and CSF neopterin were assessed. Finally, the proportion of patients with free drug concentrations exceeding the drug specific IC_{50} (intracellular concentration required to achieve 50% inhibition of wild-type virus in vitro) was compared between patients on Atazanavir versus Darunavir.

BACKGROUND

Protease inhibitors (PIs) remain a workhorse of anti-retroviral therapy for HIV-1 infection. In the HIV Outpatient Study (HOPS Cohort), over 50% (n = 499) of ARV-naïve patients were initiated on protease inhibitor based regimens between 2004-2008 [28]. The PIs provide several advantages over other classes of ARVs: they have a high genetic barrier to resistance and can be used in patients with renal dysfunction. Like other major classes of ARVs, several PIs have once daily dosing options, a key component in successful adherence. Currently, two protease inhibitors, Atazanavir (ATV) and Darunavir (DRV), are approved by the Department of Health and Human Services (DHHS) as part of first line regimens for ARV-naïve patients when boosted with Ritonavir (RTV) and given with a backbone of Tenofovir disoproxil fumarate /Emtricitabine (TDF/FTC) [27].

Both Atazanavir and Darunavir have characteristics that may impede CNS penetration: they are large molecules (>500 Daltons) and have known affinity to efflux pump transporters (particularly p-glycoprotein). However, they differ in their degree of plasma protein binding: Atazanavir is only 86% plasma protein bound whereas Darunavir is 95% bound [29, 30]. Lipophilic drugs, such as protease inhibitors, require some degree of binding to circulating proteins, particularly alpha$_1$-acid glycoprotein (AAG). It is primarily the unbound (free) component of a drug that freely crosses the blood-brain and blood-CSF barriers [21]. Therefore, it is reasonable that a drug with lower degree of plasma protein binding (higher proportion of free drug) would better be able to penetrate the CNS. In fact, a study examining total CSF drug levels in an older generation of protease inhibitors found that indinavir, a drug with significantly lower

plasma protein binding than other PIs (61%), achieved the highest concentration of drug in the CSF [31].

In vitro experiments on protease inhibitors have shown that adding physiologic concentrations of AAG into the medium lowers the unbound concentration of drug in the system and reduces anti-HIV activity of the compounds *[32-34]*. This implies that measurement of free drug concentrations of protease inhibitors may be a more accurate representation of effective drug activity than total drug levels.

Despite this, studies examining the CSF drug concentrations of Atazanavir and Darunavir have predominantly studied total drug levels. A study published in 2009 by Best et al found that 19/79 (24%) CSF samples drawn from patients on Atazanavir (boosted by Ritonavir) had total drug concentrations below the assay's limit of detection (5 ng/mL) [35]. Three studies looking specifically at Darunavir concentrations in the CSF have been published within the past 5 years. First, Yilmaz et al found that 14/14 CSF samples of patients on twice daily Darunavir had total drug concentrations that exceeded the IC_{50} for the drug [26]. Next, Calcagno et al compared the CSF total drug trough concentrations in once daily dosing of Darunavir with twice daily dosing and found that the twice daily dosing group had a mean CSF Darunavir trough that was statistically significantly higher than the once daily dosing group, 38.2 ng/mL (30.2-53.2) vs 10.7 ng/mL (6.7-23.0), $p = 0.0004$, with 3 samples in the once daily dosing group below the IC_{50} for the drug [25]. These studies were limited for several reasons: first, except for the Calcagno study, CSF was sampled at various times during the dosing interval. Anti-retrovirals are considered to have time-dependent activity, therefore the concentration at the end of the dosing interval (the trough) must be sufficient to inhibit HIV replication [36]. Measuring drug concentrations prior to the trough paints an incomplete picture of drug activity. In addition, patients being compared were receiving

different background anti-retroviral medications, and adherence to the regimen was questionable. A specific limitation of the Atazanavir study was that the assay's limit of detection was above the estimated IC_{50} of the drug, reported to be anywhere from 1-11 ng/mL [24]. Most importantly, all these studies measured only total drug levels and not free drug levels, which may not accurately reflect drug activity.

The only study measuring free drug concentrations of Darunavir was recently published by Croteau, et al but examined patients receiving twice daily dosing [37]. Their results found that 28/29 CSF free DRV levels exceeded both the IC_{50} and IC_{90} for the drug. Unfortunately, this study was limited because all patients were receiving twice daily (600 mg) dosing of Darunavir, a dosing regimen now only recommended in treatment-experienced patients; these results may not apply to treatment-naïve patients that receive only once daily Darunavir (800 mg daily) under most recent guidelines [27].

In summary, it is unknown whether the difference in degree of plasma protein binding between these two protease inhibitors impacts their free drug CNS penetration, as measured by the CSF:plasma free drug trough concentration ratio. Further, it is unknown whether their CSF free drug trough concentrations exceed the drug-specific IC_{50} or are associated with CSF HIV viremia or inflammation, both predictive markers of HIV-1 dementia [15, 16].

Hypothesis: Protease inhibitors with lower degree of plasma protein binding achieve higher CNS penetration in HIV positive patients, resulting in free drug levels capable of inhibiting viral replication and decreasing inflammation.

AIM 1: To compare the steady state free drug CSF:plasma trough concentration ratio between **Atazanavir,** a drug that is 86% plasma protein-bound, and **Darunavir,** a drug that is 95% plasma protein-bound, among HIV-1 infected patients with history of

plasma virologic suppression (HIV-RNA PCR below limit of detection for at least 6 months).

AIM 2a: To assess the relationship between protease inhibitor free drug CSF concentration and CSF HIV-1 RNA.

AIM 2b: To assess the relationship between protease inhibitor free drug CSF concentration and CSF neopterin, a measure of inflammation.

AIM 3: To compare the proportion of patients in the **Atazanavir** and **Darunavir** groups who attain free CSF drug concentrations that exceed the drug-specific IC_{50}.

METHODS

Study design

A cross-sectional study was conducted at the Grady System-affiliated Infectious Disease Program (IDP) in Atlanta, Georgia from May 2012 through December 2012.

Characteristics of study population

The population of interest included HIV-1 infected individuals with established care at IDP currently receiving a stable protease inhibitor-based regimen of HAART.

Inclusion criteria

1. HIV-1 infection, as documented by any licensed serologic test confirmed by western blot or by a positive plasma HIV-1 RNA performed by any laboratory that has a CLIA certification
2. Age $\geq$ 18 years old
3. Adherent to a HAART regimen consisting of standard doses of (TDF/FTC) + RTV -boosted ATV or DRV for $\geq$ 6 months prior to study entry.
4. Documented adherence to anti-retroviral therapy for $\geq$ 6 months by review of physician and pharmacy records.
5. Able and willing to provide informed consent.
6. Able and willing to undergo venipuncture and lumbar puncture in the clinic during the study visit

Exclusion criteria

1. Pregnancy (by clinical history or positive urine pregnancy test at screening)
2. Currently taking medications with known protease inhibitor interactions.
3. History of seizure or CNS infection within the last 6 months
4. Laboratory values obtained within 90 days prior to study entry:

- Platelet count <100,000/mm^3
- Aspartate aminotransferase (AST), alanine aminotransferase (ALT), or alkaline phosphotase > 5 times the upper limit of normal
- Creatinine clearance < 50 mL/min, as estimated by the Cockcroft-Gault equation

5. Skin infection at site of lumbar puncture
6. Known history of coagulopathy, either inherited or acquired
7. Prothrombin time (PT) > 14 seconds or International Standardized Ratio (INR) > 1.4 at screening visit.
8. Current focal neurologic signs or symptoms, including diplopia, headache, or paresthesias.
9. Presence of ventricular shunt.
10. Receiving investigational drug.

Recruitment and sample collection

Posters explaining the purpose of the research study were placed in clinical areas and hallways of IDP. Eligible patients were contacted via telephone or face-to-face encounters and purpose and time course of study was described. Interested and eligible patients attended one screening visit where informed consent was obtained and the Health Insurance Portability and Accountability Act (HIPAA) was reviewed. During the screening visit, the patient's clinical HIV and medication history was obtained, physical examination and urine pregnancy testing (females) performed, and venipuncture for measurement of prothrombin time/international standardized ratio (PT/INR) completed. If patients met all eligibility criteria and were interested in participating, they returned for one study visit scheduled 24 hours from time of most recent ARV dose (approximate plasma trough time) for paired plasma and CSF collection. Venipuncture

and lumbar puncture were performed to collect plasma and CSF, respectively. Patients received monetary compensation for their time and inconvenience: $50 for screening visit and $250 for study visit.

Sample collection and storage

Blood was collected from brachial venipuncture and centrifuged to isolate the plasma component. Plasma samples were stored at -80°C until analysis. Cerebrospinal fluid was collected via lumbar puncture using sterile technique and then stored at -80°C until analysis.

Measurements

Information on covariates such as demographic characteristics, duration of time on regimen, nadir CD4 count, and comorbidities was collected by review of physician and nursing notes, patient history, and laboratory results both in the electronic medical record and patient paper chart.

AIM 1:

Exposure

The exposure variables of interest were the protease inhibitors ATV and DRV. Duration of time on medications and adherence were measured by review of physician and nursing notes, pharmacy records, and patient history in both the Grady electronic medical record and patient paper charts.

Outcome

The outcome variable was the CSF free drug: plasma trough concentration ratio at steady state for both ATV and DRV.

Steady state occurs when the overall intake of a drug is in dynamic equilibrium with its elimination, approximately 4-5 times the drug's half-life. Steady state is reached in approximately 35 hours for ATV (half-life = 7 hours) and at 75 hours for DRV (half-life = 15 hours) [29, 30]. By including only participants who had been on a stable regimen of these protease inhibitors for a minimum of 6 months, steady state was achieved. Paired CSF and plasma samples were collected at plasma trough times, approximately 24 hours from the participant-reported time of most recent ARV dose.

Free drug concentrations were measured using rapid equilibrium dialysis and high performance liquid chromatography-tandem mass spectrometry [38]. First, to separate drug bound to plasma proteins (albumin and alpha-1 acid glycoprotein) from free (unbound) drug, the Thermo Fisher Scientific Rapid Equilibrium Dialysis (RED) device was used on both plasma and CSF samples. The RED device utilizes disposable inserts made of two side-by-side chambers separated by a vertical chamber of dialysis membrane. A molecular weight cut-off of 8000 Daltons was used in the dialysis membrane. Rapid equilibrium dialysis was achieved after a 5 hour incubation at 37°C on an orbital shaker in the RED device. In validation studies, correlation between plasma protein binding of drug found in the literature and that found on the RED device was strong ($R^2 = 0.90$) [39].

Once free (unbound) drug was isolated, liquid chromatography and tandem mass spectrometry was performed to quantitate free drug concentrations of ATV and DRV in CSF and plasma. The lower and upper limit of detection for ATV and DRV drug concentrations ranged from 0.1 to 1000 ng/mL. Free drug concentration quantification was performed in duplicate and the mean of the two drug levels obtained was reported.

AIM 2a:

Exposure

The exposure variable was the CSF free drug concentrations for ATV or DRV.

Outcome

CSF HIV-1 RNA was measured using the COBAS® Ampliprep/COBAS® Taqman® version 2.0 HIV-1 assay (Roche Molecular Systems, Inc) [40]. This assay isolates HIV-1 RNA and uses reverse transcription (RT) of RNA, Polymerase Chain Reaction (PCR) amplification of the resultant cDNA, and quantitation of HIV-1 in real time. The COBAS® Ampliprep/COBAS® Taqman® version 2.0 HIV-1 assay detects 20 to 10,000,000 copies/mL in plasma, with a sensitivity of 98.3% and specificity of 99.4%. Commercial RT- PCR assays have been shown to successfully quantitate HIV-1 RNA in other biological compartments, including CSF [41]. Because the CSF samples needed to be diluted to achieve proper volume necessary for HIV-1 RNA quantification, the lower limit of detection for the CSF and plasma samples in this study was 40 copies/mL instead of 20 copies/mL.

AIM 2b:

Exposure

The exposure variable was the CSF free drug concentrations for ATV and DRV.

Outcome

The outcome variable was CSF neopterin, a measure of macrophage activation and inflammation. CSF neopterin was quantitated using Enzyme-linked Immunosorbent Assay (ELISA) from ALPCO Diagnostics. Neopterin from participants' plasma and CSF samples compete with neopterin/alkaline phosphotase conjugates for binding to anti-neopterin antibodies on microtitre plates. Next, 4-nitrophenyl phosphate substrate solution is added and starts the enzyme reaction where alkaline phosphotase bound to neopterin catalyses the cleavage of 4-nitrophenyl phosphate, leading to the production of

yellow-4-nitrophenol. The intensity of the color (measured in optic density OD) is inversely proportional to the neopterin concentration in the patient sample. Optical density is measured by a microtitre plate reader at an absorption maximum of 405 nm; results are calculated by plotting optical density versus concentration of neopterin standards [42] and are reported in ng/mL.

AIM 3

Exposure

The exposure variable was free drug CSF concentration of ATV and DRV.

Outcome

The outcome variable being examined was the proportion of participants in each arm whose protease inhibitor free CSF drug concentration exceeded the drug-specific IC_{50}. The mean IC_{50} for ATV and DRV (1.7 ng/mL and 0.4 ng/mL, respectively) was previously derived from standardized in vitro phenotypic drug susceptibility measurements of wild-type clinical isolates tested between 2009-2010, compiled in the Monogram Biosciences database [43]. The isolates were defined as wild type if previously described drug-selected mutations in protease were not detected [44].

Sample size and power calculations

Preliminary data were not available to power the study for the research question. The study was powered to estimate the correlation coefficient between free drug CSF and plasma concentration separately for each group. A sample size of 15 patients for each arm achieves 88% statistical power to detect a difference of 0.70 between the null hypothesis correlation of 0 and the alternative hypothesis correlation of 0.70 using a two sided hypothesis test with a significance level of 0.05.

Analytic plan

Demographic and clinical characteristics were summarized by descriptive statistics. For all categorical variables, differences in proportions between the ATV and DRV groups were examined by Chi Square or Fisher's exact test. Wilcoxon rank sum test was used to compare differences between continuous variables in both groups as the variables did not meet criteria for normality.

AIM 1

The CSF: plasma free drug concentrations ratios were log transformed (natural log) and were compared between the two groups using a two-sided two-sample t test with an alpha level of 0.05.

To investigate other determinants of CSF:plasma free drug concentration ratio, univariate and multivariate linear regression was performed. Variables of interest examined included arm, age, sex, race, years with HIV diagnosis, number of prior anti-retroviral regimens, nadir CD4 count in cells/mcL, number of months on current regimen, and detection of plasma and CSF HIV-1 RNA.

AIM 2a

CSF HIV-1 RNA was dichotomized into undetectable (HIV-1 RNA < 40 copies/mL) and detectable. CSF protease inhibitor free drug concentration was assessed as both a continuous variable and a dichotomized variable (CSF protease inhibitor free drug concentration exceeding drug IC_{50} versus concentration not exceeding drug IC_{50}). The CSF protease inhibitor free drug concentration was dichotomized based on IC_{50} because absolute CSF free drug concentrations cannot be compared between the two groups.

To assess the relationship between CSF free drug concentration and detection of CSF HIV-1 RNA, a point biserial correlation test was performed separately for the Atazanavir and Darunavir groups.

Additionally, determinants of detectable CSF HIV-1 RNA were examined using binary logistic regression. Variables examined as predictors of detectable CSF HIV-1 RNA included study arm, CSF free drug concentration exceeding IC_{50}, age, sex, race, years with HIV diagnosis, number of prior ARV regimens, nadir CD4 count, months on antiretroviral regimen, and detection of plasma HIV-1 RNA.

AIM 2b

To assess the association between CSF neopterin and CSF free protease inhibitor drug concentration, the Spearman rank correlation test was performed separately for the ATV and DRV groups. Univariate and multivariate linear regression was performed to examine determinants of CSF neopterin. Variables examined as predictors of CSF neopterin included study arm, CSF free drug concentration exceeding IC_{50}, age, sex, race, years with HIV diagnosis, number of prior ARV regimens, nadir CD4 count, months on antiretroviral regimen, detection of CSF HIV-1 RNA, and plasma neopterin.

AIM 3

To compare the proportions of participants in each group achieving CSF free protease inhibitor concentrations exceeding the IC_{50}, a Chi Square test was performed.

RESULTS

Thirty HIV-1 infected adults were enrolled at the Infectious Diseases Program (IDP) in Atlanta, Georgia between May 2012 and December 2012. Through IDP pharmacy records, 522 patients were identified as receiving an ATV-containing regimen and 265 were receiving a DRV regimen. Of these, 336 people were screened for the ATV arm; 300 were excluded because they did not meet eligibility criteria, 21 declined, and 15 enrolled. For the Darunavir group, all 265 patients were screened; 242 excluded because they did not meet eligibility criteria, 8 declined, and 15 were enrolled (Figure 1).

In Table 1, demographic and clinical characteristics were summarized. Of the 30 patients enrolled, 23 were male (76.7%), 26 were black (86.7%), and median age was 46.9 years (IQR 37.9-51.9 yrs). Most common risk factors for HIV infection were heterosexual sex (50%) and men who have sex with men (MSM) in 40%. Nadir CD4 count was 62 cells/mcL (IQR 10-128 cells/mcL), and CD4 count within 90 days of enrollment was 307 (IQR 218-450 cells/mcL). Participants had been receiving their current ARV regimens for 21.8 months (IQR 14.1-34.0 months) and reported excellent adherence to medications, (median 0 missed doses in 30 days, IQR 0-1). Consecutive number of months with undetectable plasma HIV-1 RNA was 19.5 (IQR 9.2-26.1).

There were more females in the ATV group than the DRV group (5 vs 3, $p = 0.39$). Other demographic characteristics, including age, racial composition, and HIV risk factors, were similar between the arms. Median nadir CD4 count was statistically significantly different between the two groups: 117 cells/mcL (44-173) in the ATV group and 13 cells/mcL (6-108) in the DRV group, $p = 0.03$. The ATV group also had a higher number of months on current ARV regimen compared to the DRV group: 34.0 months (14.7-40.4) and 18.8 months (13.8 – 23.4), respectively, $p = 0.0102$. However, the CD4 count

within 90 days of enrollment and months with undetectable plasma HIV-1 RNA were similar between them.

Measures of renal and liver function within 90 days of enrollment were comparable for the two groups except for total bilirubin and alkaline phosphotase (Table 2). As expected, participants in the ATV group had a higher median total bilirubin level (1.2, IQR 0.9-2.6) compared to DRV (0.5, IQR 0.4 – 0.6), $p = 7.12 \times 10^{-6}$. Unconjugated bilirubinemia is a known benign side effect of Atazanavir use but is not indicative of liver toxicity. Alkaline phosphotase was higher in the ATV group (79 U/L, IQR 68-99) than the DRV group (61, IQR 51-70), p = 0.0011, but these values were all within normal limits for the assay. Table 3 summarizes comorbidities and past medical history of the participants. Body mass index, co-infection with viral hepatitis, and history of CNS infection were similar between the arms. The ATV group had a greater number of participants with psychiatric disorders and current tobacco use than the DRV group, but these differences were not statistically significant at an alpha level of 0.05. Table 4 illustrates that timing of plasma and CSF sampling relative to ARV dosing approached plasma trough times (approximately 24 hours from last ARV dose) and was similar for both groups.

Results from CSF and plasma sampling are shown in Table 5. Mean CSF:plasma free drug concentration was 0.38 (95% CL 0.20-0.56) for ATV and 0.065 (95% CL 0.043-0.087) for DRV. The log transformed ratios were compared between the ATV and DRV arms using a two-sided two-sample t test and was found to be statistically significantly higher in the ATV arm at an alpha level of 0.05, p <0.0001 (Figure 2). However, both groups had 13/15 (86.7%) patients who achieved CSF free protease inhibitor

concentrations exceeding the drug-specific IC_{50}. CSF and plasma neopterin levels were low and similar between the two groups.

The ATV group had a greater number of people with detectable plasma HIV-1 RNA than DRV (10/15, 66.7% vs 4/15, 26.7%), p = 0.03. However, only 6 of 30 patients had detectable CSF HIV-1 RNA: 2 in the ATV group (13.3%) and 4 in the DRV group (26.7%), p = 0.65. Both patients in the ATV group with detectable CSF HIV-1 RNA also had detectable plasma HIV-1 RNA in contrast to only 1/4 in the DRV arm. All 6 achieved CSF free protease inhibitor concentrations that exceeded their drug-specific IC_{50} (Table 6). Of the 4 patients (2 in each arm) that did not achieve CSF free drug concentrations exceeding the IC_{50} , none had detectable CSF HIV-1 RNA (Table 7).

Table 8 shows the results of univariate analysis evaluating predictors of the log CSF:plasma free drug ratio. In univariate analysis, detectable plasma HIV-1 RNA was found to be a statistically significant predictor of log CSF:plasma free drug concentration ratio at an alpha level of 0.05 (p = 0.019). However, when multivariate analyses were conducted with the variables arm, sex, detectable plasma HIV-1 RNA, and number of months on regimen (Table 9), detectable plasma HIV-1 RNA was no longer significant. Along with arm, sex was kept in the final model (Table 10) because it is a known factor impacting plasma protein binding and was imbalanced between the two arms. Based on the final model, compared to the DRV arm, patients in the ATV arm had a mean log CSF:plasma free drug concentration ratio that was 3.97 times higher ($p<0.0001$). Compared to men, holding arm constant, women had a mean log CSF:plasma free drug concentration that was 1.48 times higher (p = 0.1493).

Figure 3 shows the relationship between CSF free protease inhibitor concentration and detection of CSF HIV-1 RNA (detectable CSF HIV-1 RNA defined as $\geq$ 40 copies/mL). Using point biserial Pearson correlation separately for ATV and DRV, there was no significant association noted for either arm: r = 0.1194, p = 0.6718 for ATV and r = 0.4108, p=0.1282 for DRV. Binary logistic regression was performed to determine whether CSF free drug levels exceeding the IC_{50} predicted detection of CSF HIV-1 RNA, but there was complete separation of variables. Other variables were examined in univariate analysis (Table 11) but none were found to be predictive. As there were only 6 participants with detectable CSF HIV-1 RNA, multivariate logistic regression analysis could not be performed.

Figure 4 illustrates that no association was seen between CSF free protease inhibitor concentration and CSF neopterin for either group, (r = 0.0965 for Atazanavir and r = 0.1413 for Darunavir). In particular, CSF free PI concentration exceeding the $IC_{50,}$ was not found to be a statistically significant predictor of CSF neopterin using linear regression analysis (p = 0.2317). Plasma neopterin and detection of CSF HIV-1 RNA were the best predictors of CSF neopterin levels (Table 12). As plasma neopterin increased by 1 ng/mL, CSF neopterin also increased by 0.627 ng/mL (p <0.0001). Compared to participants with undetectable CSF HIV-1 RNA, those with detectable RNA had CSF neopterin levels that were 0.290 ng/mL higher (p = 0.0525).
Finally, proportion of patients achieving CSF free PI concentrations exceeding the IC_{50} was no different: 86.7% in both groups, as illustrated in Figure 5.

DISCUSSION

The results of this study contribute significantly to the anti-retroviral pharmacology literature by measuring free (unbound) Atazanavir and Darunavir trough concentrations in both CSF and plasma for a population of HIV-1 infected patients on a stable once daily regimen. Prior literature had predominantly reported total (bound+unbound) drug levels drawn at various times during the dosing interval, and on twice daily dosing for Darunavir.

CNS penetration, as measured by the free CSF:plasma drug trough concentration ratio, was higher in patients receiving a regimen of once daily Atazanavir than once daily Darunavir. This statistically significant difference persisted even when sex was added to the model as a confounder. Estrogen can lower degree of plasma protein binding by inducing hepatic glycosylation of alpha-1 acid glycoprotein (AAG) [45], theoretically leading to an increased CSF:plasma free drug ratio. These findings are significant for several reasons. First, the results suggest that degree of plasma protein binding plays a role in predicting CSF free drug penetration. Additionally, in contrast to prior studies, these findings show that ATV achieves measurable and adequate drug trough concentrations in the CSF. Based on these limited prior studies, Atazanavir has been given a lower CNS Penetration Effectiveness (CPE) score than Darunavir. The CPE score is a validated ranking system developed by Letendre et al using clinical effectiveness studies, CSF pharmacology, and chemical properties in a hierarchical manner to create a scoring system for anti-retroviral agents [17]. The findings from this study suggest that degree of plasma protein binding and measurement of CSF free drug concentrations should be weighed more heavily in the CPE score.

Although the CSF:plasma free drug concentration ratio was significantly different between the two protease inhibitors, both groups achieved CSF free drug concentrations that exceeded the IC_{50} for that specific drug (13/15 , 86.7%, in both groups). This indicates that almost all patients on either of these once daily dosing protease inhibitor based regimens maintained the ability to inhibit at least 50% of virus throughout the entire course of the drug's dosing interval. So, while Atazanavir did achieve higher CNS penetration than Darunavir, it is unknown whether the difference in the ratio between these groups would result in clinically significant disparity in incidence or severity of HIV-associated neurocognitive disorders over time. Prospective studies exploring clinical outcomes are required to answer this question.

Contrary to this study's hypothesis, CSF free protease inhibitor concentrations were not associated with detection of CSF HIV-1 RNA. In fact, all patients with detectable CSF HIV-1 RNA achieved drug levels exceeding the IC_{50}. Several explanations are possible. First, the IC_{50} is calculated based on assays using wild-type virus (virus lacking significant mutations concurring resistance to anti-retrovirals). If the virus compartmentalized in the CSF has undergone significant mutations, it is possible that it has acquired resistance to the protease inhibitor, rendering the IC_{50} less useful. To verify this, genotypic sequencing of the HIV-1 RNA needs to be performed on these CSF samples. Alternatively, an association between drug concentration and CSF HIV-1 RNA may exist, but was not captured if CSF HIV-1 RNA levels were below the assay's limit of detection (40 copies/mL).

Mean CSF neopterin was similar in both groups: 1.76 ng/mL (95% CI 1.38-2.13 ng/mL) in the Atazanavir group and 1.73 ng/mL (95% CI 1.53-1.92 ng/mL) in the Darunavir group. These values were still higher than levels found previously in normal healthy

controls, 1.34 ng/mL, SD 0.56 ng/mL (or 5.3 nmol/L, SD 2.2), indicating that some low level of immune activation remains in the setting of long-term HAART. Consistent with prior findings in the literature, both plasma neopterin and detection of CSF HIV-1 RNA were found to be predictors of CSF neopterin [14]. CSF free protease inhibitor concentrations, however, were not associated with CSF neopterin, indicating that drug concentrations may only be one of many factors required to control immune activation. For example, one theory posits that inflammatory cascades, first initiated at the onset of HIV infection, leads to a chronic state of cellular activation despite stabilization by HAART.

There are several limitations of this study. To start, this study utilized cross-sectional analysis with a small sample size. Because of the study design, unmeasured confounders may have been distributed differently between the Atazanavir and Darunavir groups, leading to bias. Also, adherence to medications was measured by review of pharmacy records and patient reporting. Although suppression of plasma viral load within the last 90 days was an objective measure used to confirm recent adherence to HAART, appropriate daily intake of medications could not be confirmed. Similarly, plasma drug trough times were estimated solely by patient-reported history, which is prone to bias given the propensity of patients to forget the exact time they take medications. Finally, CSF measurements of drug concentrations were used as a proxy to the CNS and may not accurately represent penetration into the brain parenchyma.

To summarize, penetration of anti-retroviral medications into several sanctuary sites of the body, including the central nervous system, is limited and proves to be an obstacle in the complete eradication of HIV. Higher degree of plasma protein binding in the protease inhibitor class may limit free drug penetration into the CSF, as seen in this

study. Higher CSF free drug concentrations, however, were not associated with lower CSF viremia or inflammation, implying that adequate CSF penetration is likely only one piece of the puzzle impacting viral control and immune activation.

These study results elicit further questions regarding drug penetration: does degree of plasma protein binding affect penetration of protease inhibitors into other sanctuary sites such as gastrointestinal lymphoid tissue or peripheral blood mononuclear cells? Collaboration with drug discovery and drug development groups is necessary in the future to help design drugs with characteristics that maximize both delivery to sites and efficacy of viral inhibition in the quest of HIV-1 eradication. In addition, these findings suggest that further work must be done in examining the pathogenesis of HIV in the CNS and other compartments, particularly in the presence of anti-retrovirals in order to fully explain ongoing viremia and immune activation.

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TABLES/FIGURES

Figure 1: Summary of recruitment and enrollment for Atazanavir and Darunavir arms

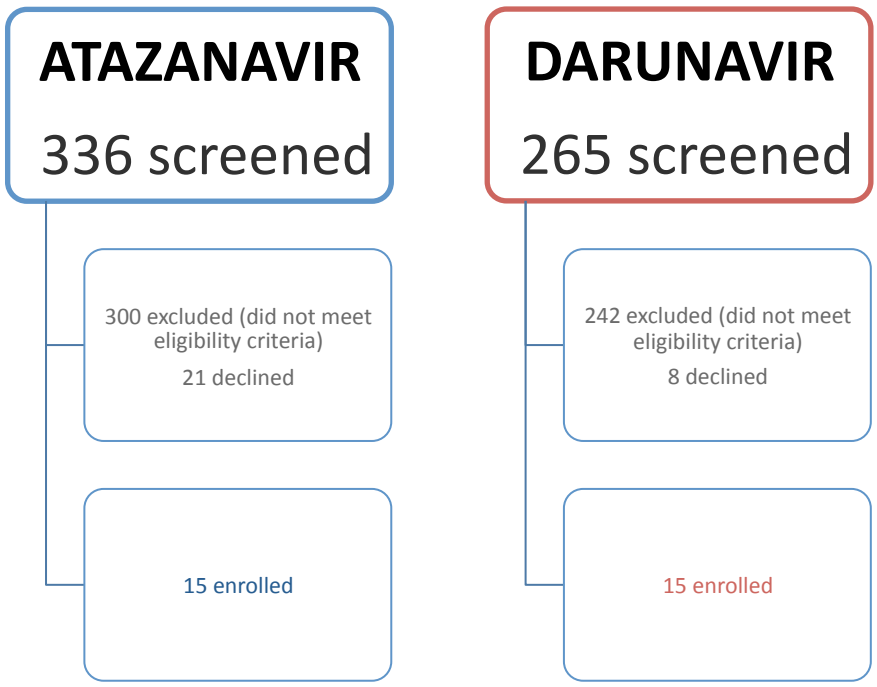


Table 1: Demographic and clinic characteristics at enrollment

	Atazanavir (n = 15) n (%) or median (IQR†)	Darunavir (n = 15) n (%) or median (IQR)	All (n = 30) n (%) or median (IQR)	P value comparing arms
Male sex	10 (66.7)	13 (86.7)	23 (76.7)	0.3898*
Age in years	44.5 (31.8 – 49.8)	49.3 (40.7 – 55.3)	46.9 (37.9 – 51.9)	0.1041
Race				1.0*
Black	13 (86.7)	13 (86.7)	26 (86.7)	
White	2 (13.3)	2 (13.3)	4 (13.3)	
HIV risk factors				0.7055
MSM††	5 (33.3)	7 (46.7)	12 (40)	
Heterosexual sex	8 (53.3)	7 (46.7)	15 (50)	
MSM and heterosexual sex	1 (6.7)	1 (6.7)	2 (6.7)	
Unknown	1 (6.7)	0 (0)	1 (3.3)	
Years since HIV diagnosis	8 (2-12)	9 (6-13)	8.5 (5-12)	0.5868
Nadir CD4 count in cells/mcL	117 (44-173)	13 (6-108)	62 (10-128)	**0.0294**
CD4 within 90 days of enrollment in cells/mcL	318 (222-484)	288 (136-409)	307 (218-450)	0.4864
Number of different ARV† regimens used	1 (1-2)	2 (1-3)	2 (1-2)	0.4767
Months on current ARV regimen	34.0 (14.7-40.4)	18.8 (13.8-23.4)	21.8 (14.1-34.0)	**0.0102**
Missed doses of ARVs in last 30 days	1 (0-1)	0 (0-1)	0 (0-1)	0.4416
Months with undetectable plasma HIV-1 viral load	20.0 (9.2-29.6)	19.1 (8.0-23.9)	19.5 (9.2-26.1)	0.6452

Continuous variables compared between arms using Wilcoxon rank sum test
Categorical variables compared between arms using Chi Square test or * = Fisher's exact test
† IQR = interquartile range, 25th -75th quartile
†† MSM = men who have sex with men
† ARV = anti-retroviral

Table 2: Laboratory findings within 90 days of enrollment

	Atazanavir (n = 15) median (IQR†)	Darunavir (n = 15) median (IQR)	All (n = 30) median (IQR)	P value comparing arms
Total leukocyte count (K/mcL)	5.8 (4.8 - 7.7)	4.8 (4.6 – 5.5)	5.3 (4.7 – 7.2)	0.1037
Lymphocytes (%)	34 (26 - 40)	30 (22 – 44)*	33 (24 – 41)	0.5829
Hemoglobin (gm/dL)	13.9 (12 – 14.8)	13.7 (13 – 14.2)	13.7 (13 – 14.5)	0.9755
Platelets (K/mcL)	207 (188 – 227)	192 (148 – 241)	207 (183 – 227)	0.5873
Creatinine clearance†† (mL/minute)	101 (67 – 119)	100 (90 – 122)	101 (80 – 119)	0.9758
Aspartate aminotransferase (units/L)	20 (14 – 21)	19 (15 – 25)	20 (15 – 24)	0.5179
Alanine aminotransferase (units/mL)	15 (11 – 24)	15 (12 – 20)	15 (12 – 22)	0.6887
Alkaline phosphotase (units/mL)	79 (68 – 99)	61 (51 – 70)	69 (54 – 85)	**0.0011**
Total bilirubin (mg/dL)	1.2 (0.9 – 2.6)	0.5 (0.4 – 0.6)	0.7 (0.5 – 1.4)	**7.117×10^{-6}**
Albumin (gm/dL)	4.3 (4.2 – 4.4)	4.2 (4.1 – 4.4)	4.3 (4.2 – 4.4)	0.5520

Continuous variables compared between arms using Wilcoxon rank sum test
† IQR = interquartile range, 25th -75th quartile
†† Creatinine clearance calculated using the Cockcroft-Gault equation
* Data available for 14/15 participants

Table 3: Comorbidities and past medical history

	Atazanavir (n = 15) n (%) or median (IQR†)	Darunavir (n = 15) n(%) or median (IQR)	All (n = 30) n (%) or median (IQR)	P value comparing arms
Body mass index†† in kg/m^2	25.6 (21.3 – 30.1)	25.0 (23.0 – 28.7)	25.6 (23.0 - 29.0)	0.5740
Co-infected with Hepatitis B or C	1 (6.7)	1 (6.7)	2 (6.7)	1.0*
History of CNS infection > 6 months prior**	2 (13.3)	2 (13.3)	4 (13.3)	1.0*
Dyslipidemia	1 (6.7)	2 (13.3)	3 (10.0)	1.0*
Hypertension	5 (33.3)	5 (33.3)	10 (33.3)	1.0
Psychiatric diagnosis	8 (53.3)	4 (26.7)	12 (40.0)	0.1360
Current tobacco use	7 (46.7)	4 (26.7)	11 (36.7)	0.2557

Continuous variables compared between arms using Wilcoxon rank sum test
Categorical variables compared between arms using Chi Square test or * = Fisher's exact
† IQR = interquartile range, 25th -75th quartile
†† Body mass index calculated by: mass (kg) / (height (m))^2
**CNS = central nervous system

Table 4: Timing of plasma and cerebrospinal fluid (CSF) collection

	Atazanavir (n = 15) median (IQR†)	Darunavir (n = 15) median (IQR)	All (n = 30) median (IQR)	P value comparing arms
Hours between last ARV†† dose and plasma collection	23.8 (23.6 – 24.5)	23.7 (23.2 – 24.2)	23.8 (23.5 – 24.3)	0.1834
Hours between last ARV dose and CSF* collection	24.7 (24.3 – 25.1)	24.4 (23.8 – 24.9)	24.6 (24.2 – 25.0)	0.1907
Minutes between paired plasma and CSF sample collection	35 (30 – 46)	34 (30 – 40)	35 (30 – 45)	0.4776

Continuous variables compared between arms using Wilcoxon rank sum test
† IQR = interquartile range, 25th -75th quartile
†† ARV = anti-retroviral
*CSF = cerebrospinal fluid

Table 5: Plasma and CSF sampling results stratified by arm

	Atazanavir (n = 15) **n (%) or mean (95% CL)**	**Darunavir (n = 15)** **n(%) or mean (95% CL)**	**P value comparing arms**
CSF† free protease inhibitor concentration in ng/mL	10.33 (6.83-13.83)	3.52 (1.85-5.18)	**n/a**
Plasma free protease inhibitor concentration in ng/mL	36.19 (21.44-50.94)	57.23 (36.33-78.13)	n/a
CSF:plasma free protease inhibitor ratio	0.38 (0.20-0.56)	0.065 (0.043-0.087)	n/a
Log CSF:plasma free protease inhibitor ratio	-1.23	-2.68	**<0.0001**
CSF free protease inhibitor concentration exceeding IC_{50}	13 (86.7)	13 (86.7)	1.0000
CSF HIV-1 RNA, detected	2 (13.3)	4 (26.7)	0.6513
Plasma HIV-1 RNA, detected	10 (66.7)	4 (26.7)	**0.0281** *
CSF neopterin in ng/mL	1.76 (1.38-2.13)	1.73 (1.53-1.92)	0.8802
Plasma neopterin in ng/mL	1.98 (1.50-2.45)	1.70 (1.47-1.93)	0.2737

Means of continuous variables compared between arms using two-sided two-sample t test
Categorical variables compared between arms using Chi-Square test, except * Fisher's exact test
† CSF = cerebrospinal fluid

Table 6: Plasma and CSF sampling for patients with detectable CSF HIV-1 RNA

ID	Arm	CSF* HIV-1 RNA (cop/mL)	Plasma HIV-1 RNA (cop/mL)	CSF neopterin (ng/mL	CSF free PI concentration (ng/mL)	CSF:plasm free drug ratio
107	ATV†	320	920	1.41	18.35	0.40
115	ATV	120	240	1.67	6.03	0.19
206	DRV††	50	<40	1.99	2.47	0.09
208	DRV	50	40	2.44	1.34	0.05
211	DRV	60	<40	2.14	12.20	0.09
215	DRV	40	<40	1.94	5.98	0.06

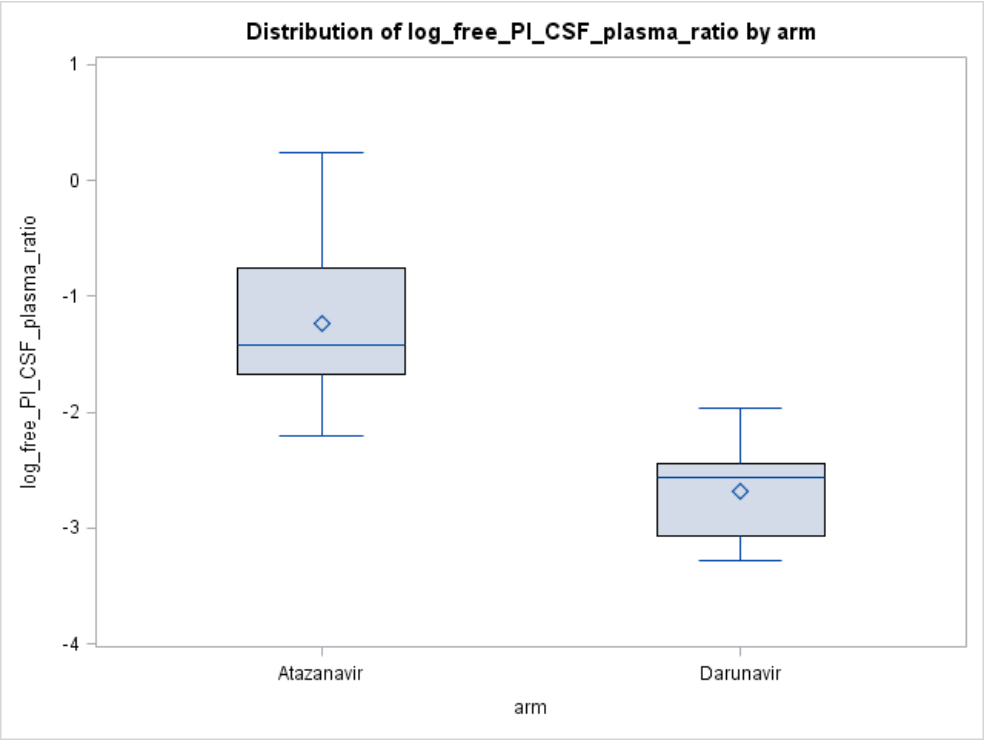
* CSF = cerebrospinal fluid
† ATV = Atazanavir
†† DRV = Darunavir

Table 7: Plasma and CSF sampling of patients with CSF free protease inhibitor concentrations not exceeding drug-specific IC_{50}

ID	Arm	CSF* HIV-1 RNA (cop/mL)	Plasma HIV-1 RNA (cop/mL)	CSF neopterin (ng/mL)	CSF free PI** (ng/mL)	Plasma free PI (ng/mL)	CSF:plasma free PI ratio
110	ATV†	<40	310	1.30	0.66	5.95	0.11
112	ATV	<40	<40	1.44	0.47	1.83	0.26
204	DRV††	<40	6790	1.78	0	113.00	0
207	DRV	<40	<40	1.24	0	0.20	0

*CSF = cerebrospinal fluid
**PI = protease inhibitor
† ATV = Atazanavir
†† DRV = Darunavir
Note: IC_{50} for Atazanavir = 1.7 ng/mL, Darunavir = 0.4 ng/mL

Figure 2: Log free CSF:plasma drug concentration ratio compared by arm



T statistic = 6.39
p = <0.0001

Table 8: Univariate analysis using linear regression for determinants of log free CSF‡:plasma free protease inhibitor trough concentration ratio

Variable	Estimate (ß)	Standard error	e^ ß	95% CL for e^ ß	p value
Arm (Atazanavir = 1, Darunavir = 0)	1.447	0.227	4.250	3.387-5.333	<0.0001
Sex (Females = 2, males =1)	0.716	0.394	2.046	1.380-3.034	0.081
Age in years	-0.020	0.017	0.980	0.964-0.997	0.406
Race (white =2, black = 1)	-0.068	0.517	0.934	0.557-1.567	0.896
Years with HIV diagnosis	-0.013	0.030	0.987	0.958-1.017	0.640
Nadir CD4 count (cells/mcL)	0.003	0.002	1.003	1.001-1.005	0.173
Number of prior ARV† regimens	-0.182	0.127	0.834	0.734-0.946	0.164
Months on current ARV regimen	0.017	0.010	1.017	1.007-1.027	0.105
Plasma HIV-1 RNA (detectable =1, undetectable =0)	0.813	0.326	2.255	1.627-3.124	0.019
CSF HIV-1 RNA (detectable = 1, undetectable =0)	-0.368	0.435	0.692	0.448-1.069	0.406

†ARV = anti-retroviral
‡CSF = cerebrospinal fluid

Table 9: Multivariate analyses using linear regression for determinants of log free CSF‡:plasma free protease inhibitor trough concentration ratio

Variable	Estimate (ß)	Standard error	e^ ß	95% CL for e^ ß	p value
Arm (Atazanavir = 1, Darunavir = 0)	1.389	0.307	4.011	2.951-5.452	0.0002
Sex (Females = 2, males =1)	0.365	0.278	1.441	1.091-1.902	0.202
Plasma HIV-1 RNA (detectable =1, undetectable =0)	0.108	0.280	1.114	0.842-1.474	0.702
Months on current ARV† regimen	-0.003	0.008	0.997	0.989-1.005	0.686

†ARV = anti-retroviral
‡CSF = cerebrospinal fluid

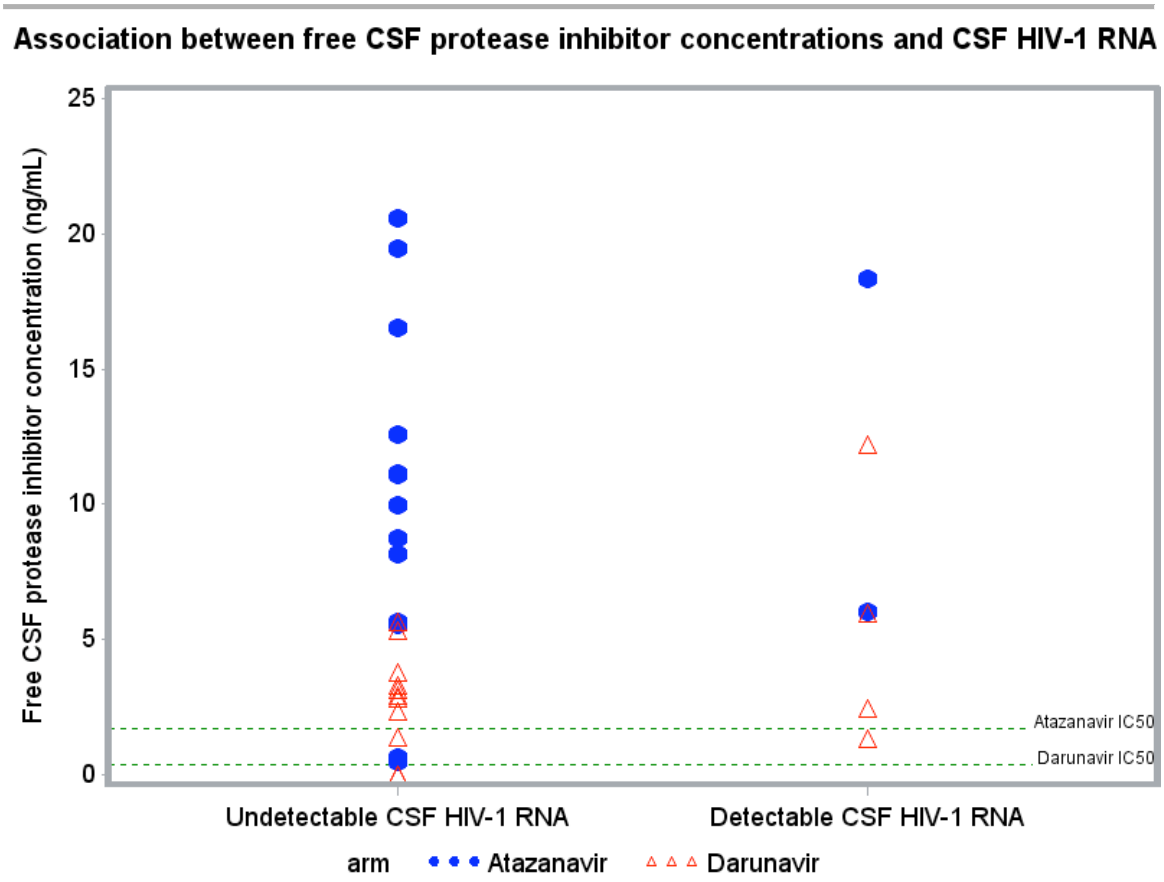
Table 10 : Final predictive model using linear regression for determinants of log free CSF:plasma free protease inhibitor trough concentration ratio

Variable	Estimate (ß)	Standard error	e^ ß	95% CL for e^ ß	p value
Arm (Atazanavir,= 1, Darunavir = 0)	1.378	0.226	3.967	3.165-4.973	<0.0001
Sex (male = 1, female =2)	0.389	0.261	1.476	1.137-1.916	0.1493

Linear regression equation used: $E(\log \text{free CSF:plasma}) = ß_0 + ß_1 * \text{Arm} + ß_2 * \text{sex}$

$R^2 = 0.643$

Figure 3: Association between CSF† free protease inhibitor trough concentration and CSF HIV-1 RNA detection



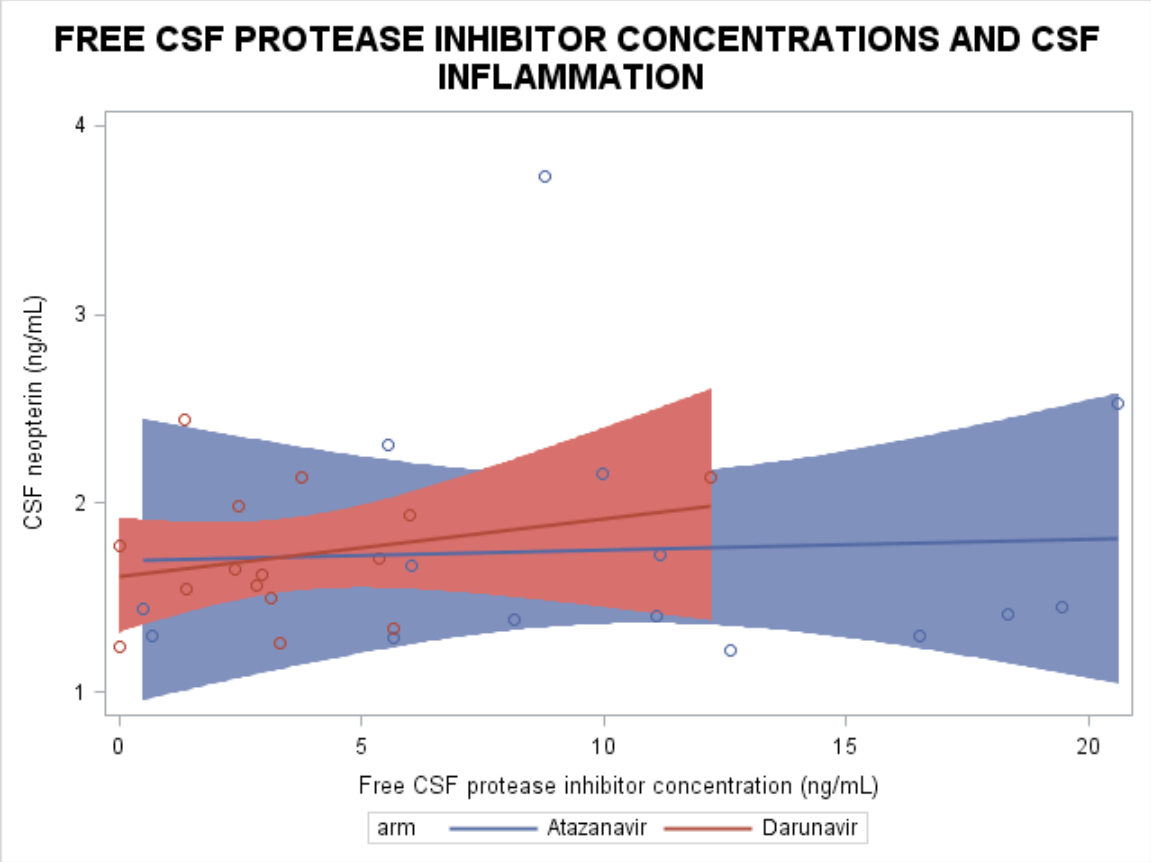
† CSF = cerebrospinal fluid
Point biserial Pearson correlation, r = 0.1194, p = 0.6718 for ATV and r = 0.4108, p=0.1282 for DRV.

Table 11: Univariate analyses using logistic regression for outcome of detectable CSF †HIV-1 RNA

Variable	Odds Ratio	95% Confidence interval	Wald Chi Square	P value
Arm (Atazanavir vs Darunavir)	2.364	0.361-15.455	0.806	0.369
Sex (Females vs Males)	0.600	0.058-6.213	0.184	0.668
Age in years	0.978	0.895-1.068	0.246	0.620
Race (white vs black)	0.714	0.061-8.395	0.072	0.789
Years with HIV diagnosis	1.007	0.871-1.164	0.008	0.930
Nadir CD4 count (cells/mcL)	0.998	0.988-1.009	0.119	0.731
Number of prior ARV† regimens	1.381	0.605-3.151	0.588	0.443
Months on current ARV regimen	0.984	0.935-1.035	0.390	0.532
Plasma HIV-1 RNA (detectable vs undetectable)	0.846	0.141-5.070	0.033	0.855
Free CSF PI†† concentration exceeding IC_{50} vs not exceeding	<0.001*	<0.001->999.99	0.0016	0.969
CSF neopterin in ng/mL	2.126	0.456-9.909	0.922	0.337

†ARV = anti-retroviral
‡CSF = cerebrospinal fluid
††PI = protease inhibitor
*Separation of variables: all patients with detectable CSF HIV-1 RNA had free CSF PI concentrations exceeding the IC_{50}

Figure 4: Association between CSF† neopterin and CSF free protease inhibitor trough concentration



† CSF = cerebrospinal fluid

Spearman rank correlation: Atazanavir, r = 0.0965, p= 0.732; Darunavir, r = 0.1413, p = 0.615

Table 12: Univariate analysis using linear regression for determinants of CSF† neopterin

Variable	Estimate (ß)	Standard error	T value	P value
Arm (Atazanavir = 1, Darunavir = 0)	0.030	0.197	0.15	0.880
Sex (Females = 2, males =1)	0.122	0.232	0.52	0.605
Age in years	0.009	0.010	0.97	0.341
Race (white =2, black = 1)	0.439	0.278	1.58	0.126
Years with HIV diagnosis	-0.014	0.016	-0.92	0.365
Nadir CD4 count (cells/mcL)	-0.0005	0.001	-0.50	0.618
Number of prior ARV† regimens	-0.074	0.070	-1.06	0.299
Months on current ARV‡regimen	0.001	0.006	0.18	0.858
Plasma neopterin in ng/mL	0.619	0.090	6.88	<0.0001
CSF HIV-1 RNA (detectable = 1, undetectable =0)	0.240	0.242	0.99	0.331
Free CSF PI†† concentration(exceeds IC_{50} =1, does not exceed=0)	0.346	0.283	1.22	0.232

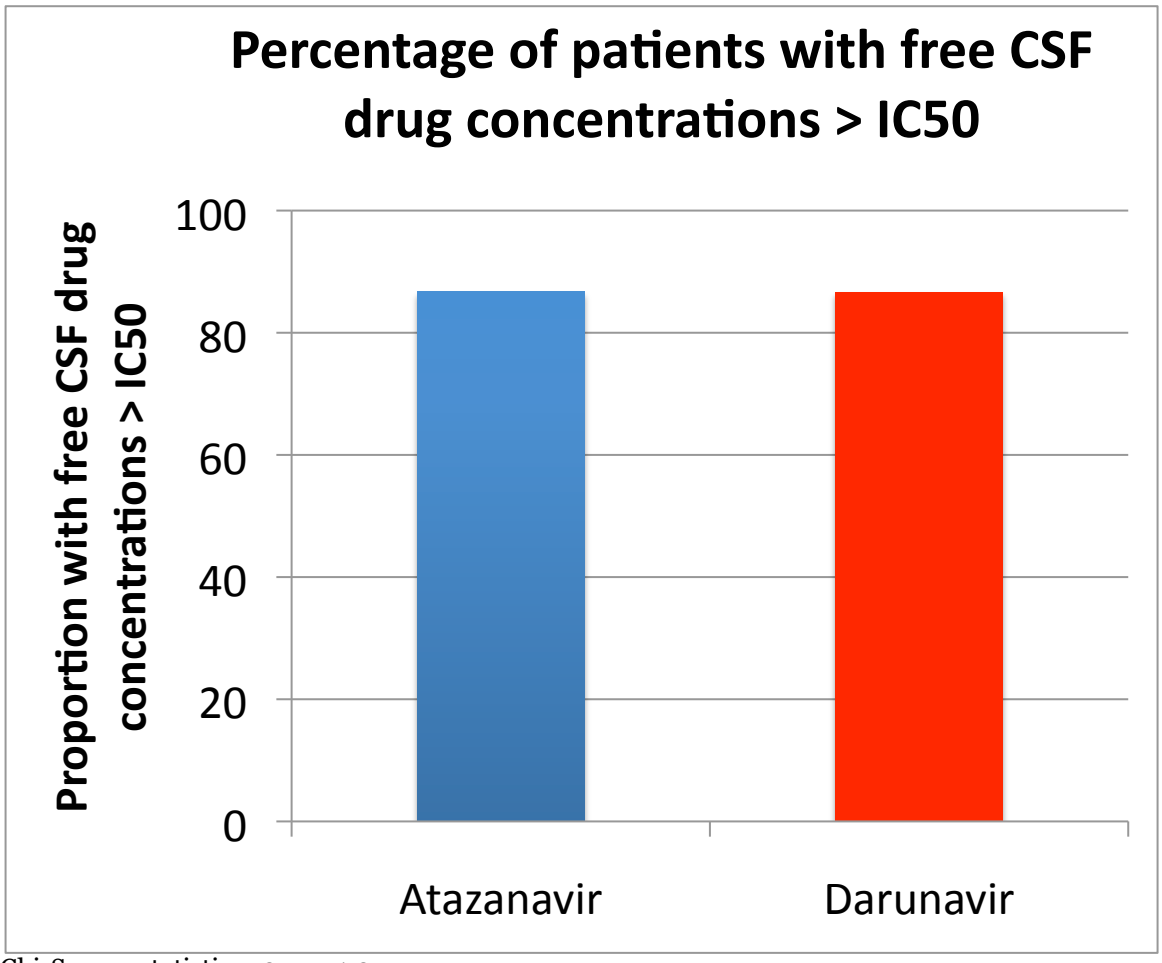
†ARV = anti-retroviral
‡CSF = cerebrospinal fluid
††PI = protease inhibitor

Table 13: Final predictive model using linear regression for determinants of CSF† neopterin

Variable	Estimate (ß)	Standard error	T value	P value
Plasma neopterin (ng/mL)	0.627	0.086	7.34	<0.0001
CSF HIV-1 RNA (1 = detectable, 0 = undetectable)	0.290	0.143	2.03	0.0525

†CSF = cerebrospinal fluid

Figure 5: Proportions of participants with CSF free protease inhibitor trough concentration exceeding IC_{50} compared between arms



Chi-Square statistic = 0, p = 1.0