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Antiretroviral Drug Concentrations in Brain Tissue of Adult Decedents

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Abstract

Objective: Determine concentrations of antiretroviral therapy (ART) drugs in the human brain.

Design: Cohort study of persons with HIV (PWH) who consented to antemortem assessment and postmortem autopsy.

Methods: Eleven PWH who were taking ART at the time of death and had detectable concentrations of at least one ART drug in intracardiac aspirate at autopsy were evaluated. Autopsies were performed within 24 hours of death and brain tissue was stored at -80^{o}C. Concentrations of 11 ART drugs were measured in three brain regions [globus pallidus (GP), cortical gray matter (CGM), white matter (WM)] by high performance liquid chromatography tandem mass spectrometry with a lower limit of quantification of 25 ng/mL.

Results: Participants were mostly men (82%) with a mean age of 40.4 years. Drug concentrations in brain tissue were highly variable and exceeded published concentrations in CSF for several drugs, including for tenofovir, efavirenz, and lopinavir. Drug concentrations correlated most strongly between CGM and GP (rho=0.70) but less well between GP and WM (rho=0.43). Combining all drugs and brain regions (n=89), higher drug concentrations in brain were associated with longer estimated duration of HIV infection (p=0.015), lower HIV RNA in plasma (p=0.0001), lower nadir CD4+ T-cell count (p=0.053), and worse neurocognitive performance (p=0.017).

Conclusions: This is the first analysis of ART drug concentrations in human brain tissue. Concentrations of several drugs in this analysis were similar to published concentrations in CSF but others exceeded published concentrations. The association between higher drug concentrations in the brain and worse neurocognitive performance may indicate ART neurotoxicity.

Keywords: HIV; Antiretroviral Therapy; Brain; Neurotoxicity; Pharmacology.

Introduction

Antiretroviral therapy (ART) can suppress HIV replication below the limit of detection for most persons with HIV. Nevertheless, ART does not eradicate the viral reservoir that persists in lymphocytes and other cells, including those in protected anatomic compartments such as the central nervous system (CNS). ART drug concentrations in cerebrospinal fluid (CSF) provide an estimate of those in brain tissue and are often substantially lower than ART drug concentrations in blood. Multiple factors can influence distribution of ART drugs into the CNS, including the multicellular structure of the blood-brain barrier (BBB) and blood-CSF barrier (BCB), physical and chemical characteristics of the drugs themselves (e.g., fat solubility and protein-binding), and concomitant clinical characteristics (e.g., age).[1] The distribution of many ART drugs into the CNS is not mediated by passive processes that depend on drug physicochemical characteristics alone but can involve drug transporters (e.g., organic cation or anion transporters).[2, 3] Transport occurs in both directions (influx and efflux) and can be affected by concomitant drugs, for example by inhibition or induction of the transporters.[4]

Age-related comorbidities may also affect BBB permeability and drug distribution. These conditions include cerebrovascular disease,[5], diabetes, and neurodegenerative disorders.[6] Conditions that increase the concentration of drug-binding proteins, such as albumin, in the CNS could also reduce the concentration of unbound, active drug. Since the concentrations of drug-binding proteins in the CNS are typically very low, even a small change could substantially alter the already low concentrations of active ART drugs in this protected compartment. This could be important since lower concentrations of ART drugs in CSF appear to correlate with higher HIV RNA levels in CSF.[7, 8]

The clinical value of estimating ART drug concentrations in the CNS continues to be debated, in part because estimation methods have historically been based on drug concentrations in CSF, which may not accurately reflect drug concentrations in the brain. For example, efavirenz (EFV) concentrations are

much lower in CSF than in blood[9] but murine data and physiology-based pharmacokinetic modeling supports that EFV concentrations are much higher in brain tissue than in blood.[10] This report was recently confirmed in non-human primates and extended to include other ART drugs such as tenofovir (TFV), atazanavir (ATV), and maraviroc.[11]

This manuscript reports ART drug concentrations measured in postmortem human brain tissue. While some of the ART drugs used by participants in this project are no longer used in the clinic, the overall finding that ART drug concentrations in brain tissue exceed those in CSF is important. In addition, some of the measured drugs still have clinical relevance. For example, TFV, emtricitabine (FTC), and lamivudine (3TC) continue to be recommended for use in all clinical environments (i.e., in low-, middle-, and high-income countries). TFV and FTC are also used as pre-exposure prophylaxis to prevent HIV infection. As low- and middle-income countries continue to transition to the use of integrase strand transfer inhibitors like dolutegravir, use of older drugs like EFV and ATV continue to be used to treat persons with HIV. Thus, the findings presented here have clinical relevance even though some of the drugs measured in this pilot project are no longer routinely used.

ART drug concentrations in brain tissue are difficult to assess in living humans since brain biopsy is only rarely performed for conditions such as primary CNS lymphoma, which likely alter the BBB. At present, collection of postmortem tissue at autopsy is the only method of obtaining the tissue needed to measure ART drug concentrations in the brain. This method presents challenges such as accurately predicting time-of-death and ensuring that ART is dosed until death. Even with these challenges, measuring ART drug concentrations in human postmortem brain tissue would provide valuable data. To address this, we meaured tissue concentrations of multiple ART drugs in three brain regions from adults who participated in the California NeuroAIDS Tissue Network (https://cntn.hivresearch.ucsd.edu) and died with HIV infection.

Methods

This was an observational retrospective cohort study designed to determine ART drug concentrations in postmortem brain tissue. The initial pool of participants consisted of 24 persons with HIV (PWH) who consented to all study procedures; reported using ART within 6 months of death; did not have a CNS opportunistic condition (e.g., Toxoplasma encephalitis, progressive multifocal encephalopathy); and had brain tissue collected within 24 hours of death (median 14 hours) between 15 March 2000 and 18 November 2006. The research procedures were approved by the Human Research Protections Program at the University of California, San Diego (irb.ucsd.edu).

Since the time of the last antemortem ART dosing was unknown, we screened eligible participants for recent dosing by qualitatively measuring in intracardiac aspirate collected at autopsy an ART drug in their regimen that had a relatively long half-life [e.g., TFV, EFV, ATV, or lopinavir (LPV)]. Eleven of 24 participants had an ART drug detected in intracardiac aspirate. In these participants, ART drug concentrations were measured in three brain regions, globus pallidus (GP), cortical gray matter (CGM), and subcortical white matter (WM). Two participants had brain tissue available from only two regions (CGM and WM).

Brain tissue was kept frozen at -80C until the time of quantitative drug concentration assays, which were performed in duplicate using validated high-performance liquid chromatography tandem mass spectrometry methods with a lower limit of quantification of 25 ng/mL. Two of the 11 participants had ART drug concentrations below the lower limit of quantification in all but one drug-region pair. These participants are included in Table 1 but were excluded from analyses, leaving an analyzable group of nine participants.

These nine participants had HIV RNA measured in antemortem blood and CSF by a commercial reverse transcriptase polymerase chain reaction (RT-PCR) assay with a lower limit of quantification of

50 copies/mL (c/mL). CD4+ T-cells were counted by flow cytometry in a Clinical Laboratory Improvement Amendments (CLIA)-certified clinical laboratory. Neurocognitive performance was assessed within 6 months of death (median 4.6 months) using a comprehensive and standardized battery of tests, as described in detail elsewhere.[12, 13] Briefly, the battery covered seven neurocognitive domains commonly affected by HIV: verbal fluency, executive functioning, processing speed, learning, delayed recall, attention/working memory, and motor skills.[13] Demographically uncorrected scaled scores were converted to T scores that corrected for the influence of age, education, sex, and race/ethnicity. T scores were then converted to a global deficit score (GDS), which quantifies the number and degree of impaired performances throughout the test battery while attaching relatively less significance to normal performances.[14, 15]

Data were described as either medians (interquartile range, IQR) or means (standard deviation, SD), which were calculated using JMP Pro (version 14.3, Cary, NC). Concentrations of each drug in brain tissue were compared to its published concentrations in CSF using the Wilcoxon signed-rank test. The number of participants using each drug was small (range 2-7) so, in some analyses, all drug concentrations were combined to improve power (total number of drug concentrations: 89). The combined concentrations were compared to demographic, disease, and treatment characteristics as well as the three sampled brain regions using Spearman's correlation coefficient and Wilcoxon signed-rank tests. When indicated by inspection of graphical relationships, polynomial statistical tests were also performed. Multivariate linear regression of ART drug concentration was also performed with all models adjusting for drug (e.g., EFV, LPV, TFV) and sampled brain region.

Results

As summarized in Table 1, participants were mostly middle-aged (mean 40.4 years) men (81.8%) of European ancestry (54.5%). At the time of last antemortem assessment, the mean duration of the current

ART regimen was 2.0 years (SD 2.9). The median plasma HIV RNA 2.82 $\log_{10}$ c/mL (IQR 2.03-4.32) and median CD4+ T-cell count was 56 cells/μL (IQR 32.5-120.0). All participants had AIDS at the time of death with a mean estimated duration of HIV disease of 14 years (SD 5.4) and a median nadir CD4+ T-cell count of 13 cells/μL (IQR 7-53). The most common causes of death were respiratory [pneumonia (n=3), respiratory failure (n=1), pulmonary embolism (n=1)]. Other causes were disseminated Kaposi's Sarcoma (n=1) and hepatocellular carcinoma (n=1). Cause of death was not recorded for two participants.

Table 2 summarizes the ART drug concentrations measured in the three brain tissue regions. The table also includes a summary measure of published concentrations of each ART drug in CSF. Among nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), median (IQR) concentrations in ng/mL across all brain regions were 147.9 (80.6-291.8) for TFV, 111.4 (25.0-361.7) for FTC, 63.4 (25.0-271.8) for 3TC, and 25.0 (25.0-174.5) for abacavir (ABC). All stavudine (D4T) concentrations were below the lower limit of quantification of the assay. Among protease inhibitors (PIs), median (IQR) concentrations in ng/mL across all brain regions were 208.3 (116.5-360.3) for saquinavir (SQV), 54.7 (25.0-168.2) for nelfinavir (NFV), and 25.0 (25.0-25.0) for LPV. All ATV concentrations were below the lower limit of quantification of the assay. While LPV concentrations were below the lower limit of quantification of the assay in GP and CGM, high concentrations were present in WM [250.5 (25.0; 956.3)]. Among non-nucleoside reverse transcriptase inhibitors (NNRTIs), median (IQR) concentrations in ng/mL were 35.9 (29.3-40.8) for EFV and 25.0 (25.0-73.2) for nevirapine (NVP). Figure 1 graphically depicts the range of concentrations of each drug in brain tissue as well as the ratio of the measured concentration of each drug in all brain regions to its published concentration in CSF.

Compared with published drug concentrations in CSF, the measured concentrations in brain tissue were statistically significantly higher for TFV ($p<0.001$), LPV ($p<0.001$), and EFV ($p=0.031$) with a

trend toward higher concentrations of SQV (p=0.06). Brain tissue concentrations were lower than published drug concentrations in CSF for NVP (p=0.001) and possibly ABC (p=0.10). Brain tissue drug concentrations did not statistically differ from published drug concentrations in CSF for NFV (p=0.25), FTC (p=0.43), and 3TC (p=0.74). Comparisons were not performed for drugs that were uniformly below the lower limit of quantification of the assay (ATV, D4T).

Drug concentrations did not differ between brain regions for individual drugs (all p values > 0.20). The largest difference was for LPV (p=0.26), particularly comparing WM to other brain regions (mean 175.4 vs. 25.0, p=0.10). Since the power of these comparisons was limited by the small sample sizes, we combined all regional brain concentrations of all drugs (n=89). Concentrations most strongly correlated between CGM and either GP (rho=0.70, p<0.0001, Figure 2) or WM (rho=0.66, p<0.0001). The correlation between drug concentrations in GP and WM was weaker but still statistically significant (rho=0.43, p=0.018). As shown in Figure 2, most drug concentrations in WM fell above the line of identity compared with drug concentrations in CGM but this difference was not statistically significant (p=0.16).

We next combined drug concentration data from all participants and brain regions and performed exploratory modeling by multivariate regression (N=89 drug concentrations for all participants in all brain regions). These analyses identified that higher ART drug concentrations in brain tissue were associated with longer estimated duration of HIV infection (rho=0.38, p=0.0007), longer duration of ART (rho=0.23, p=0.042), lower HIV RNA in plasma (rho=-0.40, p=0.0006, Figure 3), worse GDS (rho=0.40, p=0.0015), and possibly lower nadir CD4+ T-cell count (rho=-0.21, p=0.085). Analyses that adjusted for ART drug and brain region confirmed the associations with estimated duration of HIV infection (β=0.797, p=0.015), HIV RNA in plasma (β=-49.3, p=0.0001), and GDS (β=42.3, p=0.017) with weaker associations with nadir CD4+ T-cell count (β=-0.35, p=0.053) and duration of ART

(β=1.03, p=0.101). Including all of these covariates in a single model identified that higher ART drug concentrations were most strongly associated with lower HIV RNA in plasma (β=-75.6, p=0.031) and worse GDS (β=51.6, p=0.016). ART drug concentrations in brain tissue were not associated with age, sex, race/ethnicity, or current CD4+ T-cell count. The relationship between ART drug concentrations in brain tissue and HIV RNA in CSF followed a quadratic pattern (R^2=0.137, p=0.0036, Figure 3).

Discussion

In this project, ART drug concentrations were measured in brain tissue that was collected at autopsy from adults who died with HIV disease. Brain tissue drug concentrations were higher than published drug concentrations in CSF for at least one drug in every ART class tested, which included NRTIs (TFV), NNRTIs (EFV), and PIs (LPV and possibly SQV). Brain tissue concentrations exceeded published concentrations in CSF to the greatest extent for TFV and SQV (and in WM for LPV). ART drug concentrations in gray matter (GM) (either GP or CGM) appeared to be more similar to each other than to ART drug concentrations in WM, although these differences did not reach statistical significance in this small study. If ART drug concentrations in WM do differ from those in GM, this may be due to a different vascular pattern and lipid content in WM that could influence drug delivery and accumulation.[16]

In the current ART prescribing environment, the discordance between published TFV concentrations in CSF and those measured here in brain tissue may be the most impactful finding. TFV has low plasma protein binding (<1%), which favors distribution into the CNS, but is not highly lipophilic (LogP: -1.6) and has highly positive polarity that may require active transport across the BBB, characteristics which do not favor distribution into the CNS. Transporter enzymes that contribute to TFV distribution through the BBB and BCB include organic anion transporters 1 and 3, and multidrug resistance associated

proteins (MRP) 2 and 4 but not the permeability glycoprotein (P-glycoprotein).[17-19] The different distribution of the active transporters in these interfaces could influence TFV distribution into the CNS.

One animal study reported good distribution of TFV into CSF without reaching high concentrations in deep brain tissue[20] and a non-human primate study found comparable zidovudine concentrations in CSF and brain tissue.[21] More recent analyses in non-human primates found that TFV concentrations in brain tissue are higher than concentrations in CSF.[11] A potential explanation for high TFV concentrations in brain tissue despite poor CSF distribution[7] could be related to different expression of efflux transporters at the blood-brain and blood-CSF barriers.[22] Furthermore, TFV is administered as a prodrug, which may reach higher intracellular concentrations, which could theoretically result in lower extracellular drug concentrations, e.g., in CSF.

In addition to TFV, the other drugs that diverged most from reported concentrations in CSF were PIs, namely LPV, SQV, and NFV. While SQV and NFV are no longer used in clinical settings, LPV is still sometimes prescribed in low- and middle-income countries. LPV is highly lipophilic (LogP: 5.9), is a smaller molecule than ATV, and is substantially bound to drug-binding proteins (98-99%). LPV and other PIs are substrates for molecular transporters that can influence CNS exposure, such as the active efflux transporter P-glycoprotein.[23] Although LPV concentrations in CSF are much lower than those in plasma, they appear to be in the therapeutic range[24] even as a monotherapy regimen.[25] Our findings indicate a potentially more complex situation with LPV concentrations present principally in WM – and at much higher concentrations than published concentrations in CSF – and below 25 ng/mL in cortical and deep GM. Since the myelin in WM is lipid-rich, an explanation for the observed substantial LPV regional variation may its high lipophilicity.

We also identified associations between drug concentrations in brain tissue and plasma HIV RNA and neurocognitive performance. The direction of the relationship with plasma HIV RNA suggests that

drug concentrations in brain may reflect those in blood since lower plasma HIV RNA was associated with higher drug concentrations in brain. The direction of the relationship with neurocognitive performance, however, indicates that this may come at a cost: higher drug concentrations in brain were associated with worse neurocognitive performance. This is consistent with recent concerns about ART neurotoxicity.[26, 27] The quadratic relationship between drug concentrations in brain and CSF HIV RNA could be artifactual but might also be due to the influence of two factors that were not measured in this analysis, ART drug resistance and neuroinflammation. Both of these conditions would be more common in persons with advanced HIV disease or AIDS, such as our participants. When HIV is susceptible to the administered ART, HIV RNA would be suppressed by high ART drug levels and neuroinflammation would be relatively low. When ART drug resistant HIV is present in the CNS, high ART drug concentrations may not suppress HIV RNA in CSF (and the brain), leaving only the toxicity of the drugs without the benefit of viral suppression. In this scenario, neuroinflammation would be relatively worse, which could also increase BBB permeability and result in high drug concentrations in the brain. Either scenario would result in high drug concentrations in the brain but only one would result in HIV suppression, consistent with our observations.

Our study has multiple limitations. The sample size is very small. This project was intended, however, to be a pilot project and it fulfilled its primary aim, which was to describe ART drug concentrations in brain tissue from adults who died with HIV. Prior published studies have focused on animals and have provided valuable insights into ART drug concentrations in the brain, but ultimately human data are needed to determine how well the animal studies generalize to humans. Another limitation is the lack of dosing data immediately prior to death. While we attempted to limit the influence of this by qualitatively measuring drug concentrations in intracardiac aspirate from autopsy as a coarse indicator of recent dosing, the long half-life of several of the ART drugs means that they could

have been dosed days prior to death. The measured drug concentrations may provide useful information about the accumulation of ART drugs in the brain but their generalizability to regularly dosed, living persons is uncertain. Our study also lacked drug concentrations quantitatively measured in plasma and CSF since autopsies were not performed sufficiently quickly to collect them. While using published drug concentrations in CSF provided some insights, historical data remains an inferior standard compared with actual data from the same participants. Finally, the brain tissue used in these analyses were collected between 2000 and 2006. While the specimens were stored in monitored, -80^{o}C freezers, the length of storage could affect our results. Also, our study did not include drugs like tenofovir alafenamide or integrase inhibitors, which are commonly prescribed today. Research characterizing concentrations of these drugs in the brain are needed.

Conclusions

The manuscript reports ART drug concentrations from human brain tissue collected at autopsy, finding that they exceed published ART drug concentrations in CSF and that higher concentrations are associated with viral suppression in blood, longer duration of HIV infection and its treatment, and worse global neurocognitive performance. Larger studies are needed in which modern ART drugs are quantified in CSF, brain tissue, and blood to validate these pilot findings and to better understand the mechanisms of different ART distribution patterns into the CNS.

Disclosures

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All authors contributed to the writing and editing of the manuscript. RJE, VS, CLA, DJM, and SLL designed and implemented the study, obtained funding for it, and collected the data. NNB performed the assays of ART drug concentrations in brain tissue. MF, NNB, and SLL analyzed the data.

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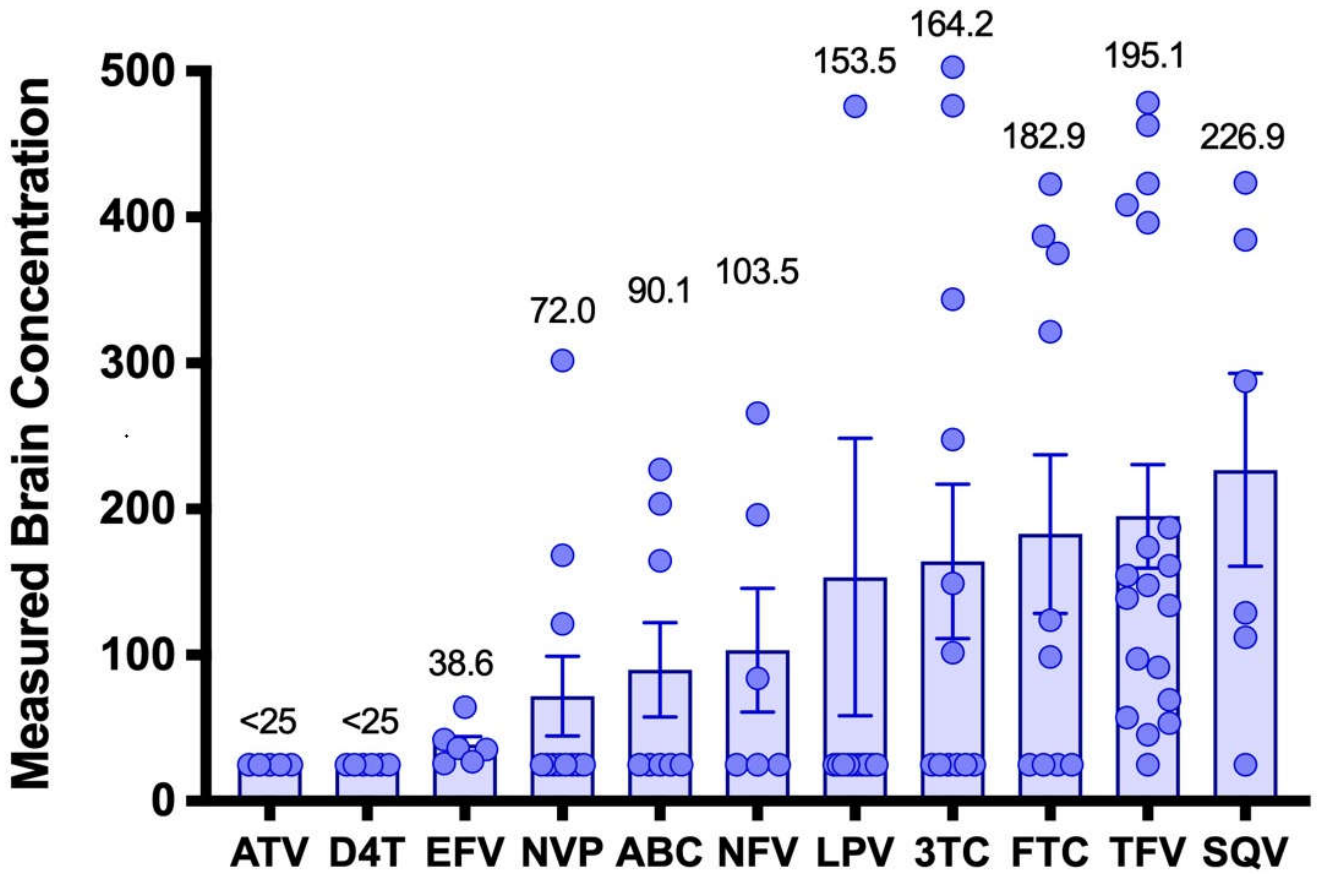
Figure Captions.

Figure 1. Drug Concentrations in Brain Tissue (left) and the Same Concentrations Divided by Published Drug Concentrations in CSF (right, see Table 2). The left panel graphically depicts the substantial variability in concentrations and the differences in concentrations between drugs and the right panel more clearly depicts that certain drugs appear to concentrate in brain tissue relative to published CSF concentrations (NFV, LPV, TFV, SQV). Bars are means and error bars are the standard error of the mean. Values over bars are means. One concentration data point is not shown to better display the patterns in the data (LPV, 1116 ng/mL). *No value is provided for ATV since both the measured values and the published CSF concentrations in CSF are below the assay sensitivity (25 ng/mL).



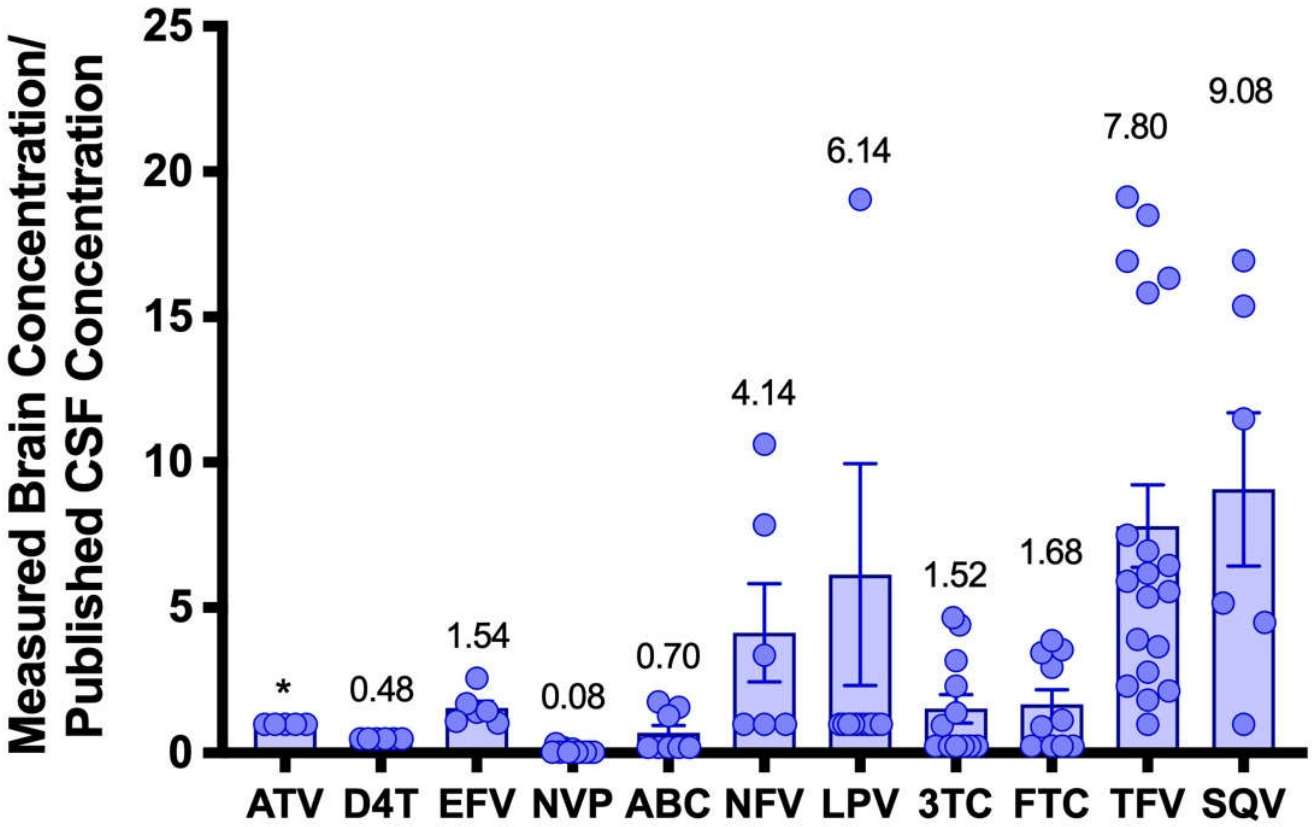
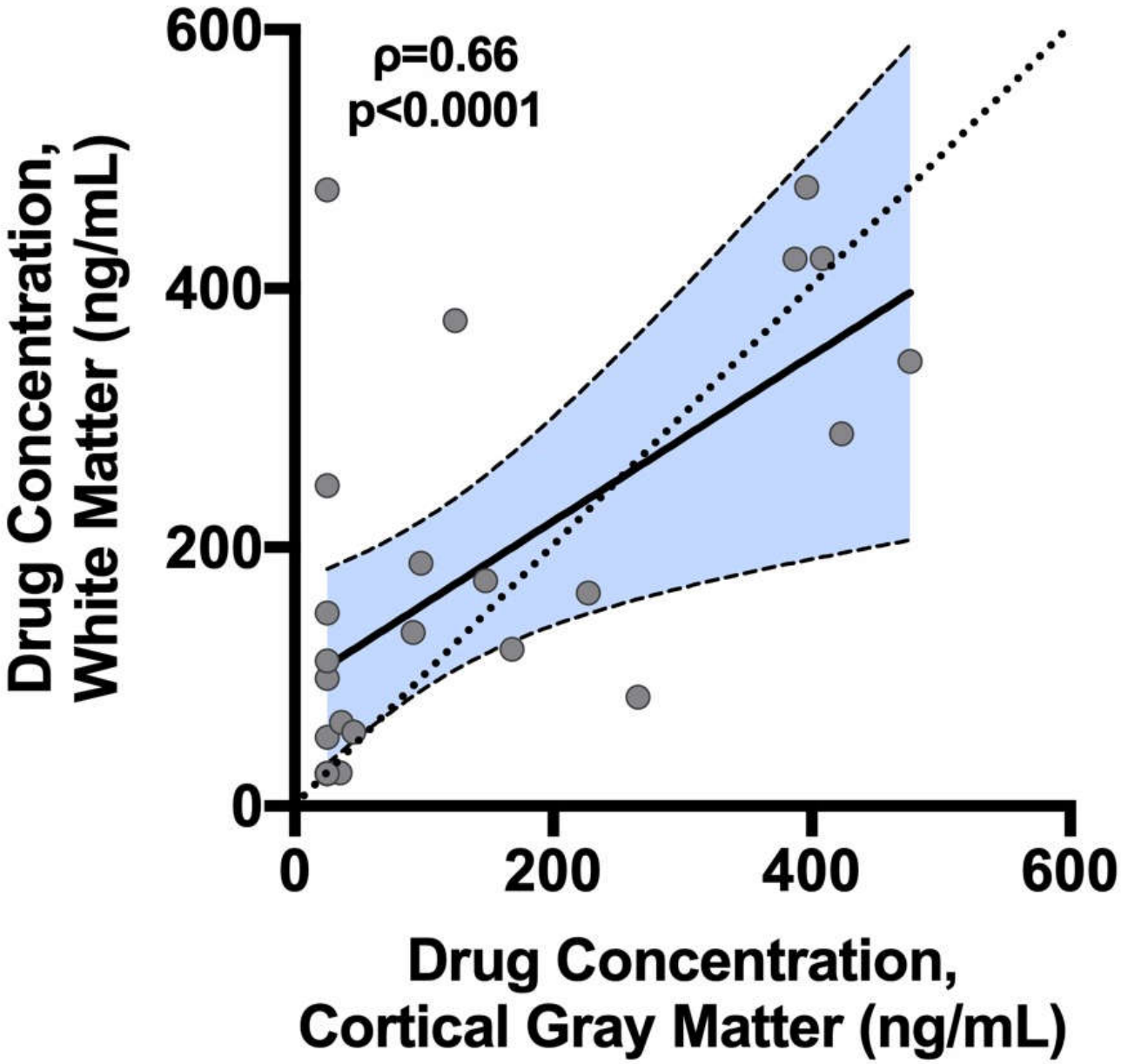
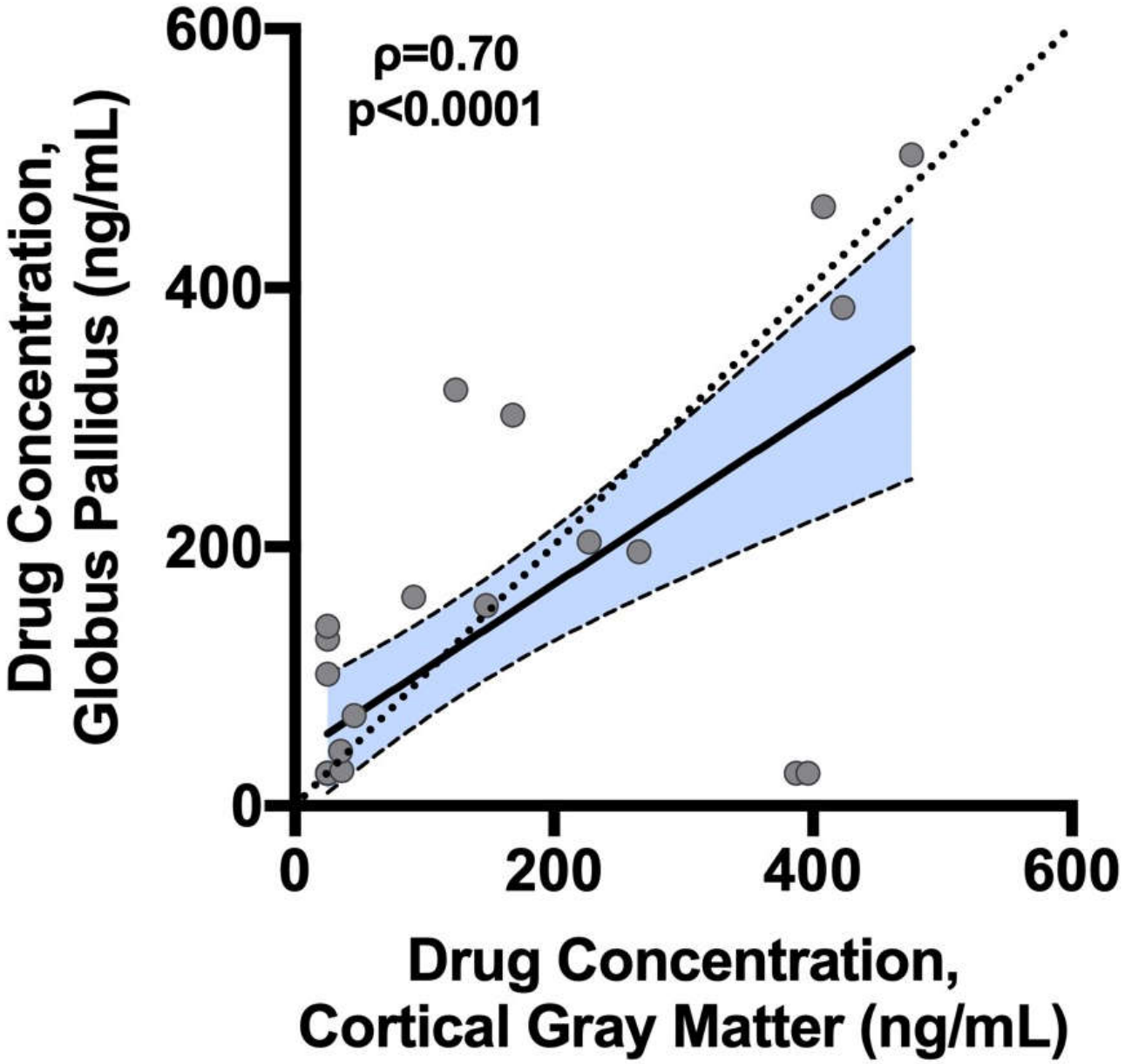


Figure 2. Correlation of ART Drug Concentrations Between Globus Pallidus, White Matter, and Cortical Gray Matter. The solid line is the regression line and the dashed lines demarcate the 95% confidence interval. The dotted line is the line of identity.





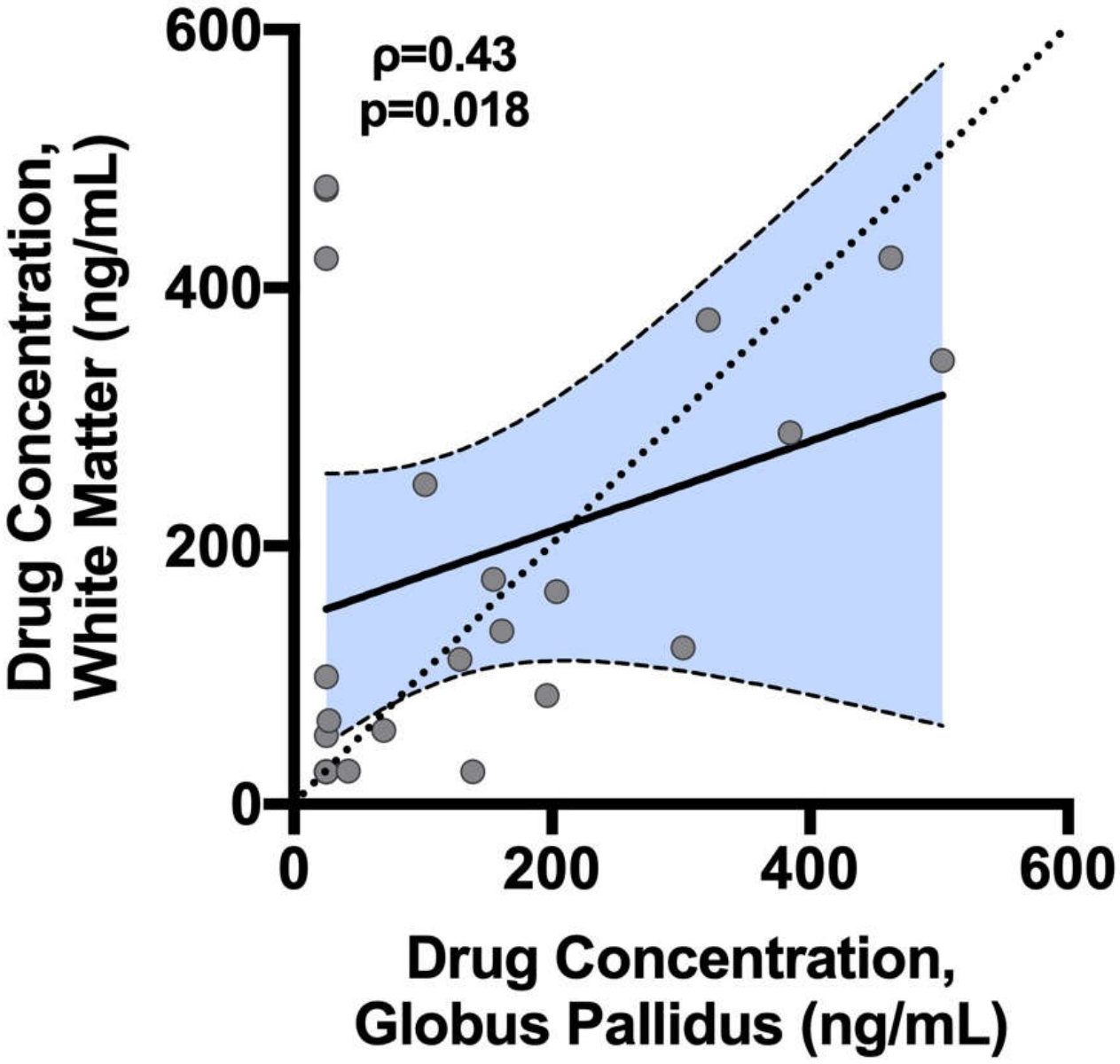
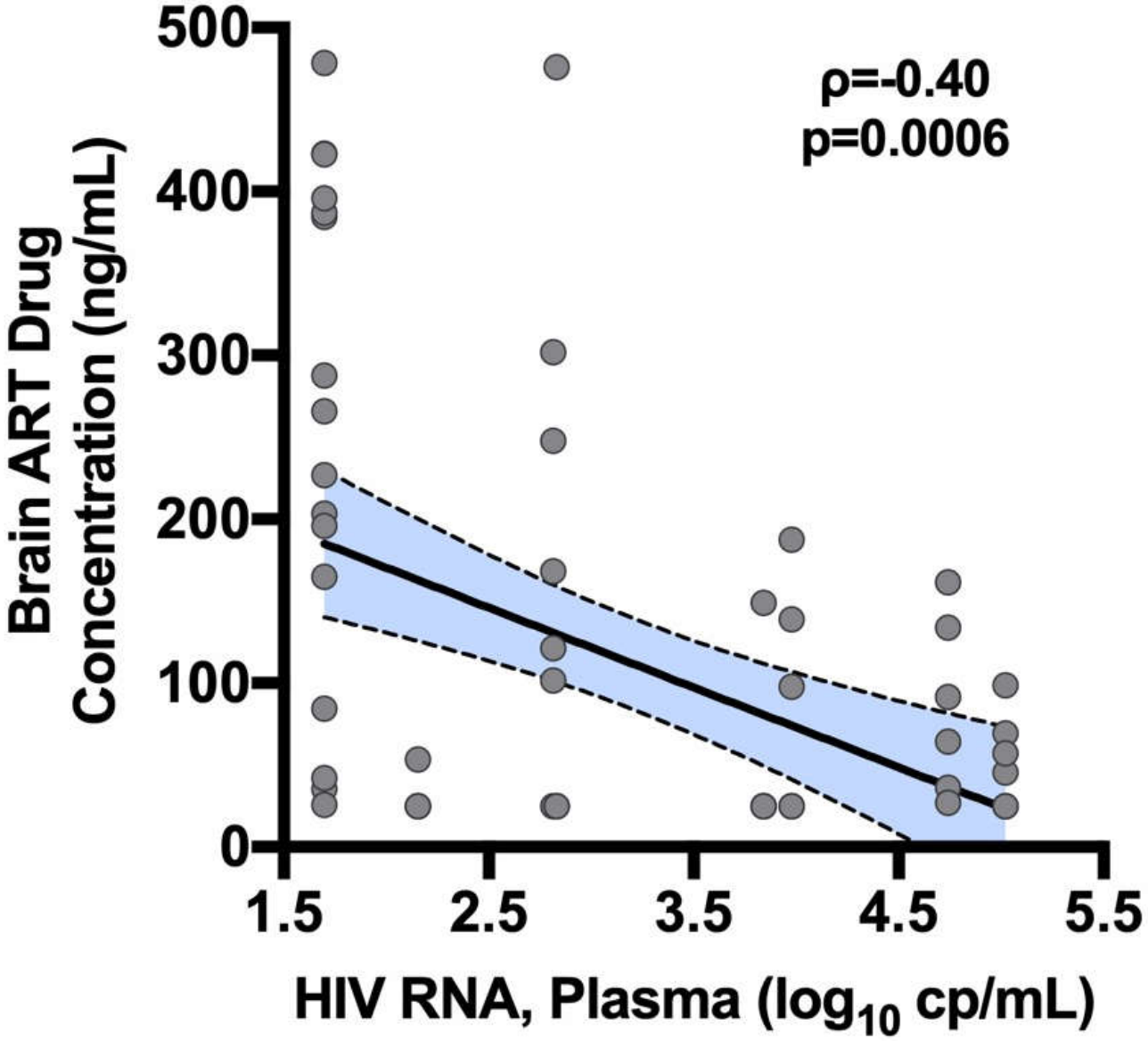


Figure 3. Relationships between ART Drug Concentrations and HIV RNA in Plasma (left) and CSF (right). The solid line is the best fit line and the dashed lines demarcate the 95% confidence interval.



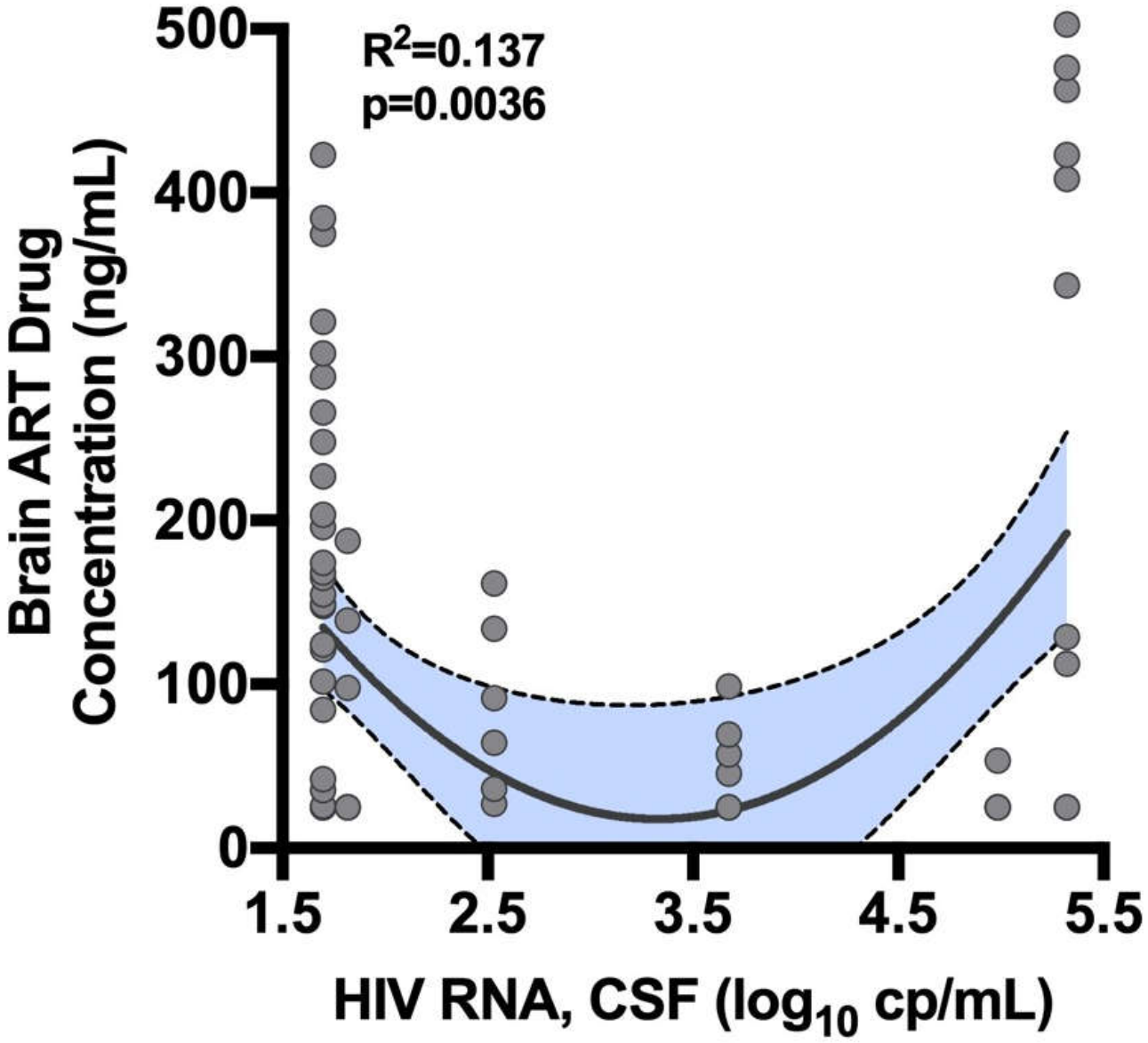


Table 1. Demographics characteristics of study population.

Case	Sex	Age at Death	Race/Ethnicity	Time from Death to Autopsy (Hours)	Duration of HIV (Months)	Last Antemortem ART Regimen	Regimen Duration at Last Visit (Months)	Plasma HIV RNA (log_{10})	Last Antemortem CD4+ T-Cell Count (/μL)	Nadir CD4+ T-Cell Count (/μL)
1	M	45	W	5	244.9	ABC/D4T/EFV/NFV/SQV	115	1.70	785	13
2	M	40	H	10	202.4	TDF/FTC/LPV-r	1.3	5.02	78	3
3	F	32	H	21	75.5	D4T/3TC/NVP	20.4	2.81	59	53
4	M	35	H	6	102.1	ZDV/3TC/LPV-r	19	2.83	39	39
5*	M	50	W	4	NR	TDF/FTC/LPV-r	6.7	NR	NR	NR
6	M	42	W	8	196.7	TDF/EFV/ATV-r	18.3	4.74	13	13
7*	M	38	W	30	220	TDF/3TC/LPV-r/SQV	NR	NR	NR	NR
8	M	40	H	7	NR	TDF/FTC/ATV-r	8.9	1.70	53	7
9	M	45	W	20	185.9	TDF/FTC/ABC/NVP/FPV-r	20.6	2.16	246	114
10	M	37	H	19	208.3	ABC/TDF/NVP	1.7	3.98	9	4
11	F	40	W	24	76.9	3TC/NFV/NVP	NR	1.70	NR	295
	9M,2F		6W,5H							
Median (IQR)		**40.0 (37.0-45.0)**		**10.0 (6.0-21.0)**	**196.7 (102.1-208.3)**		**18.3 (4.2-20.5)**	**2.82 (2.03-4.32)**	**56 (19.5-204)**	**13.0 (5.5-83.5)**
Mean (SD)		**40.4 (5.0)**		**14.0 (9.0)**	**168.1 (65.0)**		**23.5 (35.2)**	**2.96 (1.32)**	**160.2 (263.3)**	**60.1 (95.0)**

ABC=Abacavir, D4T=Stavudine, EFV=Efavirenz, NFV=Nelfinavir, SQV=Saquinavir, TDF=Tenofovir, FTC=Emtricitabine, LPV-r=Lopinavir-ritonavir, 3TC=Lamivudine, NVP=Nevirapine, ZDV=Zidovudine, ATV-r=Atazanavir-ritonavir, FPV-r=Fosamprenavir-ritonavir. NR=Not Recorded. M=Male, F=Female, W=White, H=Hispanic. IQR=Interquartile Range, SD=Standard Deviation. *=No Antemortem Assessment Performed

Table 2. ART drug concentrations among different brain tissue regions.

	n	Overall median (IQR)	WM median (IQR)	GP median (IQR)	CGM median (IQR)	CSF (ng/mL)
Protease Inhibitors						
Atazanavir (ATV)	2	**25.0 (25.0-25.0)**	25.0 (25.0-25.0)	25.0 (25.0-25.0)	25.0 (25.0-25.0)	**10.3**[28]
Lopinavir (LPV)	4	**25.0 (25.0-25.0)**	250.5 (25.0; 956.3)	25.0 (25.0; 25.0)	25.0 (25.0; 25.0)	**16.8**[24]
Saquinavir (SQV)	2	**208.3 (116.5-360.3)**	200.0 (112.4; 287.6)	256.7 (128.9; 384.5)	224.1 (25; 423.3)	**< 25**[29]
Nelfinavir (NFV)	4	**54.7 (25-168.2)**	54.6 (25; 84.3)	110.5 (25; 196.1)	145.4 (25; 265.8)	**< 25**[30]
Non-Nucleoside Reverse Transcriptase Inhibitors						
Efavirenz (EFV)	2	**35.9 (29.3-40.8)**	45.2 (25.9; 64.5)	34.7 (27.2; 42.3)	35.9 (35.4; 36.4)	**15.6**[9]
Nevirapine (NVP)	4	**25.0 (25.0-73.2)**	25.0 (25.0; 97.2)	25.0 (25.0; 301.9)	25.0 (25.0; 132.5)	**932.0**[31]
Nucleoside/Nucleotide Reverse Transcriptase Inhibitors						
Tenofovir (TFV)	7	**147.9 (80.6-291.8)**	174.0 (57.4; 423.0)	154.7 (104.2: 312.1)	97.7 (45.6; 396.1)	**5.5**[22]
Emtricitabine (FTC)	4	**111.4 (25.0-361.7)**	236.9 (43.4; 410.8)	173.2 (25.0; 321.4)	74.5 (25.0; 321.4)	**109.0**[32]
Lamivudine (3TC)	4	**63.4 (25.0-271.8)**	198.5 (56.0; 319.6)	63.3 (25.0; 402.3)	25.0 (25.0; 363.4)	**107.8**[33]
Abacavir (ABC)	3	**25.0 (25.0-174.5)**	25.0 (25.0; 164.8)	114.3 (25; 203.7)	25.0 (25.0; 227.1)	**128.0**[34]
Stavudine (D4T)	2	**25.0 (25.0-25.0)**	25.0 (25.0-25.0)	25.0 (25.0-25.0)	25.0 (25.0-25.0)	**51.6**[35]

WM: white matter; GP: globus pallidus; CGM: cortical gray matter) by ART drug class. CSF=Cerebrospinal Fluid, IQR=Interquartile Range. The lower limit of quantification was 25.0 ng/ml