Published in final edited form as:

J Infect Dis. 2010 July 1; 202(1): 161–170. doi:10.1086/653213.

SIV-infected macaques treated with highly active antiretroviral therapy (HAART) have reduced CNS virus replication and inflammation but persistence of viral DNA

M. Christine Zink^{1,2,3}, Angela K. Brice¹, Kathleen M. Kelly, Suzanne E. Queen¹, Lucio Gama¹, Ming Li¹, Robert J. Adams¹, Christopher Bartizal¹, John Varrone¹, S. Alireza Rabi⁴, David R. Graham¹, Patrick M. Tarwater⁵, Joseph L. Mankowski^{1,2,6}, and Janice E. Clements^{1,2,6,7}

¹Department of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, Baltimore MD, 21205

²Department of Pathology, Johns Hopkins University School of Medicine, Baltimore MD, 21205

³Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore MD, 21205

⁴Department of Medicine, Johns Hopkins University School of Medicine, Baltimore MD, 21205

⁵Division of Biostatistics and Epidemiology, Paul L. Foster School of Medicine, El Paso, TX 79905

⁶Department of Neurology, Johns Hopkins University School of Medicine, Baltimore MD, 21205

⁷Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore MD, 21205

Abstract

Background—In the era of highly active antiretroviral therapy (HAART) the prevalence of HIV-associated CNS disease has increased despite suppression of plasma viremia.

Methods—Using an SIV model system where all animals develop AIDS and 90% develop CNS disease by three months postinoculation (p.i.), pigtailed macaques were treated with a regimen of tenofovir disoproxil fumarate, saquinavir, atazanavir, and an integrase inhibitor starting at 12 days and euthanized at ~175 days p.i.

Results—Plasma and CSF viral loads declined rapidly after initiating HAART. Brain viral RNA was undetectable at necropsy but viral DNA levels were not different from untreated SIV-infected macaques. CNS inflammation was significantly reduced, with decreased brain expression of MHC Class II and GFAP and reduced CSF CCL2 and IL-6. Brain from treated macaques had significantly lower levels of IFN β , the Type I IFN-inducible gene myxovirus (influenza) resistance A (MxA), and indolamine 2,3-dioxygenase (IDO) mRNA suggesting suppressed immune hyperactivation, and fewer CD4⁺ and CD8⁺ T cells, suggesting reduced trafficking of T cells from peripheral blood. Brain levels of CD68 protein and TNF α and IFN γ RNA, while reduced, were not significantly lower, indicating continued CNS inflammation.

Corresponding author: M. Christine Zink, 733 N. Broadway, BRB 819, Baltimore, MD 21205, Phone: 410-955-9770, FAX: 410-955-9823, mczink@jhmi.edu.

Disclosures: M.C.Z is named as an inventor on a patent pending for minocycline to treat HIV infection. The patent will be held by the Johns Hopkins University. No other authors reported conflict of interests.

Conclusions—These data, generated in a rigorous, high viral load, SIV/macaque model showed benefits of HAART therapy on CNS virus replication and inflammation but no change in the levels of viral DNA and continued CNS inflammation in some individuals.

Keywords

SIV; HIV; AIDS; HAART; CNS; IFN; TNF α ; immune activation; NeuroAIDS

Introduction

The advent of highly active antiretroviral therapy (HAART) significantly changed the quality of life for many HIV-infected individuals. Adherence to HAART results in sustained suppression of plasma HIV RNA, reduced HIV-related morbidity and mortality, and restoration of immune function [1,2]. HAART-treated HIV-infected individuals can live for decades with plasma viral loads that are below standard limits of detection, although low levels of virus sometimes can be detected using more sensitive methods [1,3]. During HAART, proviral DNA can be detected in resting memory CD4⁺ T cells [4]. Upon HAART cessation, peripheral virus replication resumes quickly [2].

Several studies showing stable or increasing prevalence of HIV-associated neurological disease in HIV-infected individuals on HAART suggest that current regimens are not effective in reducing CNS virus replication [5,6]. Some studies have shown continued HIV replication or the presence of replication-competent virus in brains of HIV-infected, HAART-treated individuals [7,8]. A recent study revealed that poor CNS penetration of antiretrovirals allowed continued HIV CNS replication as indicated by higher CSF HIV viral loads [9]. A number of studies have demonstrated a surprising degree of ongoing neuroinflammation in brains of HAART-treated patients [10,11]. Thus, the brain likely constitutes an important reservoir for latent or replicating HIV in HAART-treated individuals. One reason may be that many antiretrovirals do not reach effective brain concentrations, either because the blood-brain barrier prevents entry or because the drugs are pumped out of the CNS by ATP-binding cassette (ABC) membrane transporters [12,13].

SIV infection of macaques provides an excellent model of systemic and neurological HIV infection [14-16]. We have developed and thoroughly characterized a model of HIV-associated neurological disease by coinoculation of pigtailed macaques with a neurovirulent molecularly cloned virus, SIV/17E-Fr, and a viral swarm, SIV/DeltaB670, that consistently results in encephalitis by 84 days postinoculation (p.i.) [17-24]. This model has numerous parallels to HIV infection, including rapid transient acute depletion of CD4⁺ T lymphocytes, development of characteristic CNS inflammation correlating with high CSF and brain viral load, and cognitive and motor deficits typical of HIV-associated neurological disease [17,18,25-29]. This model provides an outstanding, rigorous platform to study HIV nervous system disease pathogenesis and to test potential CNS-specific therapeutics. Using this model we can measure several key markers of HIV neurological disease progression: 1) viral RNA, DNA and protein, 2) inflammatory processes as measured by glial fibrillary acidic protein (GFAP), MHCII, CD68, IL-6 and CCL-2 expression, and 3) immune activation as measured by IFN β , TNF α , IFN γ , indolamine 2,3-dioxygenase (IDO) and myxovirus (influenza) resistance A (MxA) mRNA. In addition, virus is present in peripheral memory CD4⁺ T cells in this model as is seen in HIV-infected individuals [19,30].

The purposes of this study were to determine the extent to which HAART that suppresses plasma viral load inhibits CNS virus replication and subsequent neuroinflammation and whether the brain is a stable latent virus reservoir when there is effective virus suppression in plasma. We used an SIV/macaque model of HAART in which macaques are treated with four

antiretrovirals including the nucleotide reverse transcriptase inhibitor (NRTI) tenofovir (Gilead), the protease inhibitors (PIs) saquinavir (Roche) and atazanavir (Bristol-Myers Squibb), and the integrase inhibitor L-870812 (Merck [31]) beginning on day 12 p.i. This therapeutic combination, composed of predominantly non-CNS penetrant drugs, resulted in reduction of plasma viral load to below detectable levels by 70 days p.i. with kinetics similar to that seen in HIV-infected individuals following HAART initiation [30]. The results of this study showed significant benefits of early HAART therapy on CNS virus replication and inflammation.

Materials and Methods

Viruses and animal studies

Eleven juvenile pigtailed macaques (*Macaca nemestrina*) were intravenously inoculated with SIV/DeltaB670 (50 AID₅₀) and SIV/17E-Fr (10,000 AID₅₀) as previously described [17]. Beginning at 12 days p.i., five animals were treated daily with a four drug combination, referred to hereafter as HAART, continuing until necropsy (range, day 161-175). All HAART-treated and untreated macaques received the same stock viruses. Animals in each group were inoculated and treated as a cohort but the two groups (treated and untreated) were inoculated and treated at different times. Treatment included the NRTI tenofovir (Gilead) at a dose of 30 mg/kg QD subcutaneously, the PIs saquinavir (Roche) and atazanavir (Bristol-Myers Squibb) at doses of 205 and 270 mg/kg orally BID, respectively, and the integrase inhibitor L-870812 (Merck [31]) at a dose of 10 mg/kg orally BID. With the exception of atazanavir, which is considered to have moderate CNS penetration, these drugs do not reach effective levels in the CNS, either because they do not cross the blood-brain barrier or because they are pumped out by ABC membrane transporters [9,12,13]. Tenofovir dose was determined from previous studies [32]. Atazanavir and saquinavir doses were determined by pharmacokinetic studies in pigtailed macaques. Antiretrovirals were administered alone or in combination orally to three macaques in a series of increasing doses. Plasma was sampled every 2 hours for 12 hours. Plasma drug levels were determined by HPLC [33]. Doses used in this study were those that resulted in the same area under the curve as seen in humans treated with atazanavir and saquinavir. The dose of L-870812 in rhesus macaques was determined in studies by Hazuda et al [31]; we used the same dose in pigtailed macaques.

Three of the HAART-treated, PTa2, PYd2 & POy1, had tenofovir doses reduced to 10 mg/kg from day 71 until necropsy, and POy1 was taken off tenofovir entirely at day 107. This was done in response to increased serum creatinine levels, an indication of nephropathy, which has been reported in tenofovir-receiving humans and macaques [34-36].

Positive controls for infection included six macaques inoculated with SIV but not treated. They were euthanized between day 83 to day 88 p.i., when all untreated macaques have AIDS and most have SIV encephalitis [17]. Macaques were euthanized in accordance with federal guidelines and institutional policies. At euthanasia, macaques were perfused with sterile saline to remove blood from the vasculature prior to freezing or fixing tissues. Three negative control animals were mock-inoculated, and samples were obtained at the same time points as for the inoculated, untreated macaques.

CSF and plasma samples were taken on days 7, 10, and 14 days p.i. then weekly for HAART-treated macaques, and biweekly thereafter for untreated macaques. Samples were used to measure viral RNA by quantitative RT-PCR and monocyte chemoattractant protein-1 (CCL2) and IL-6 by ELISA [17,23,24].

Quantitation of SIV virions in plasma and cerebrospinal fluid (CSF)

Viral RNA was isolated from 400 µl of plasma and CSF collected longitudinally, using the QIAamp MiniElute Virus Spin kit (Qiagen). Samples were eluted in 40 µl of TE buffer and analyzed in triplicate by real-time RT-PCR as previously described [17,21]. The limit of detection for the assay was established as 10 copies/reaction (100 copy eq./mL).

Quantitation of viral cDNA in brain tissue

Genomic DNA was isolated from 50 mg of brain tissue (basal ganglia) using the DNeasy kit (Qiagen). One microgram of DNA was analyzed in triplicate by real-time PCR using specific primers and probes for SIV gag and IFNβ [20,22]. Copy numbers of SIV DNA were normalized to copy numbers of a single-copy cellular gene (IFNβ gene).

Quantitation of viral RNA and cytokine mRNA in brain tissue

Total RNA was isolated from 50 mg of brain tissue (basal ganglia) by use of the RNeasy kit (Qiagen), and treated with DNase (Ambion). One microgram of purified RNA was analyzed by real-time RT-PCR using specific primers and probes for SIV gag [22], IFNβ [20], MxA [20], TNFα [5'-GGCTCAGGCAGTCAGATCATC, 3'-GCTTGAGGGTTTGCTACAACATG, probe-TCGAACCCCAAGTGACAAGCCTGTAGC] IFNγ [5'-GTGTGGAGACCATCAAGGAAGACA, 3'-CGACAGTTCAGCCATCACTTGGAT, probe-ACTGACTCGAATGTCCAACGCAAAGC], IDO [5'-TGCTTTGACGTCCTGCTGG, 3'-TTCCTGTGAGCTGGTGGCA, probe-ATGCTGCTCAGTCCCCCAGGGACA] and CCL2 [5'-TGTCCCAAAGAAGCTGTGATCT, 3'-GGAATCCTGAACCCACTTCTG, probe-CAAGACCATTGTGGCCAAGGAGATCTG]. PCR reactions were performed in a Chromo4 thermocycler (Biorad) using a Multiplex PCR Mix (Qiagen). Cellular mRNA levels were normalized by 18S ribosomal RNA levels. Quantitation of gene expression was performed using the $\Delta\Delta$ Ct method [37] and is expressed as fold-change over uninfected animals calculated using median values.

Pathological assessment

Sections of CNS were examined microscopically in a blinded fashion by two pathologists and lesion severity measured using a semiquantitative system as described [17].

Quantitative immunohistochemical analysis

To detect viral gp41 in brain, a monoclonal antibody (kk41; AIDS Reagent Program) to the transmembrane portion of the SIVmac239 envelope that cross-reacts with SIV/17E-Fr and SIV/DeltaB670 was used. Macrophages in the brain parenchyma were identified by the marker CD68 (KP1, DAKO). MHC Class II (HLA-DR; DAKO) was used as a marker of macrophage and endothelial activation. GFAP (DAKO) was used for astrocyte activation. CD4+ and CD8+ T cells were quantitated in the subcortical white matter by antibodies (Novocastra and Vector, respectively). For uniformity, all samples were stained by an Optimax Plus automated cell stainer (BioGenex) and quantitation of immunohistochemical staining was performed as described [17]. Briefly, stained slides were blinded and examined at 200× magnification. Twenty adjacent fields of white matter constituting approximately 3 mm² were imaged for each animal; images were analyzed using IP Lab imaging software (Scanalytics, BD Biosciences, Rockville, MD). Images were binarized (each pixel converted to a value of 1 for positive or 0 for negative) and the total positive pixel area was calculated, providing a quantitative measure of the total area occupied by positively stained cells or portions of cells in the area evaluated. This technique has proven useful to differentiate even subtle changes in

inflammatory and viral markers between groups of animals in a variety of previous studies [17,23].

Statistical Analysis

Wilcoxon rank-sum test and k-sample medians test were used for comparisons between treated and untreated groups of macaques. Expression of MHC II, CD68, GFAP and gp41 in the brain were quantitated using 20 repeated measures on each tissue sample using a linear regression model clustered by animal and using robust standard error estimates, a method analogous to the two sample t-test. Coefficient and standard error estimates were calculated using generalized estimating equations (GEE) [38]. For variables with measurements taken repeatedly over time (i.e., CSF IL-6 and CSF:plasma CCL2 ratios), we used GEE in a linear regression model. For analyses of biomarkers measured repeatedly over time, the regression model was fit to measurements observed from day 35 on, whereby the intercepts measured the average day 35 marker value for each group and the slopes measured average rate of change over time post-day 35. *P*-values for this statistical comparison were calculated using an interaction term between treatment group and time. All statistical analyses were performed as two-sided tests.

Mathematical modeling of the decay in CSF viral load with HAART

Plasma viral load decay following HAART initiation was modeled using a mathematical model similar to that used by Perelson et al [39]. Briefly, two distinct cellular compartments were assumed to contribute to the viremia: one with a short half-life, *T*, and another with a longer half-life, *M*. Assuming 100% efficacy for the antiretrovirals, we can describe the kinetics of viral load drop following therapy initiation with the following equation:

$$V(t)=V_0 \left[Ae^{-\delta t} + Be^{-\mu_M t} \right]$$

where δ and μ_M are the decay rates of *T* and *M*, respectively. In this simplified model, half-life of the free virus is assumed too short to consider. V_0 is the pre-therapy viral load; *A* and *B* are proportionality constants representing the contributions of the *T* and *M* cellular compartments to the viral load, respectively. Curve Fitting Toolbox (version 1.2) of Matlab (R2007b, 7.5) was used to estimate δ , μ_M , *A* and *B* using nonlinear least-squares regression. The same biphasic exponential equation was applied to the viral load decay in the CSF.

Results

HAART caused rapid declines in plasma and CSF viral load

SIV-infected, HAART-treated macaques experienced marked declines in plasma and CSF viral RNA, reaching ≤ 100 copy eq./mL by ~50 days after treatment initiation (Figure 1a). Plasma viral load decline was similar to that seen in HIV-infected individuals [40, 41]. These low levels continued until euthanasia, with occasional samples that had <1000 copy eq./mL in plasma or <300 copy eq./mL in CSF. SIV-infected, untreated macaques had continuing high levels of plasma and CSF viral RNA (10^6 - 10^8 copy eq./mL) throughout infection.

Median levels of viral RNA in plasma and CSF were fit using the viral decay equation (Figure 1b, c). CSF viral decay rate in HAART-treated macaques was biphasic and paralleled the pattern observed in plasma [30].

HAART-treated macaques had lower brain viral RNA and protein but not viral DNA

Low CSF viral RNA levels in HAART-treated macaques during the late stages of infection reflected significantly lower levels of virus replication in the brain, as indicated by lower brain SIV RNA ($p = 0.004$; Figure 2a) and protein (gp41; $p = 0.043$; Figure 2b) levels at necropsy. In contrast, there was no significant difference in the levels of viral DNA in the brains of HAART-treated macaques as compared to untreated macaques ($p = 1.0$; Figure 2a).

HAART reduced brain MHC Class II, GFAP and T cell infiltrates

Activation and inflammation markers in brain were quantitated from SIV-infected, HAART-treated and untreated macaques using quantitative digital image analysis. Compared to untreated macaques, HAART-treated macaques had significantly lower levels of MHC Class II and GFAP in subcortical white matter, where inflammation is most prominent in SIV encephalitis ($p = 0.043$ and 0.040 , respectively; Figure 3a, b). Levels were not statistically different from those found in uninfected control macaques. There were lower CD68 levels in HAART-treated animals as compared to untreated macaques, but the difference was not significant ($p = 0.123$; Figure 3c).

There was a significant reduction in the number of CD4+ and CD8+ T cells ($p = 0.006$; Figure 3d, e) in subcortical white matter, suggesting reduced cell trafficking from peripheral blood to CNS.

HAART reduced CCL2 and IL-6 protein in CSF

CCL2 CSF:plasma ratios rose as expected during acute infection in both HAART-treated and untreated animals, peaking at 10 days p.i. (4434.9 pg/mL:621.3 pg/mL and 3660.7 pg/mL:405.8 pg/mL, respectively) and declining to pre-infection levels by day 14 (288.6 pg/mL:291.5 pg/mL and 256.2 pg/mL:248.0 pg/mL, respectively; Figure 4a). IL-6 CSF also increased during this period (Figure 4b). CCL2 CSF:plasma ratios and CSF IL-6 increased again in untreated macaques, but remained significantly lower after day 35 p.i. in HAART-treated macaques ($p < 0.001$ and $p = 0.041$, respectively; day 44 CCL2 CSF treated vs. untreated = 363.5 pg/mL vs. 976.0 pg/mL). A larger CCL2 CSF:plasma differential would suggest that cells are trafficking to the CNS by a chemokine gradient; these data suggest that CSF CCL2 values alone do not call cells [23]. In contrast, levels of brain CCL2 and IL-6 mRNA were not significantly lower ($p = 0.201$ and $p = 0.136$, respectively) in HAART-treated macaques as compared to untreated animals (Figure 4c, d), suggesting continued induction of inflammatory cytokines in the brain.

HAART reduced levels of some markers of innate and adaptive immune responses

To determine whether the profile of hyperactivated innate immune responses typically seen in HIV/SIV infection were altered in the brains of SIV-infected, HAART-treated macaques, we measured IFN β , MxA and TNF α mRNA. IFN β and MxA mRNA levels were significantly lower in the brains of HAART-treated macaques ($p = 0.018$ and 0.006 , respectively; Figure 5). While TNF α mRNA was reduced in HAART-treated macaques as compared to untreated macaques, the difference was not significant ($p = 0.144$). As macrophages are major TNF α producers, this observation is consistent with our finding that HAART did not significantly reduce the number of CD68-positive macrophages in the brain. Levels of IDO RNA were significantly lower ($p = 0.045$) in HAART-treated animals, but levels of IFN γ were not significantly different ($p = 0.269$; Figure 6).

Discussion

Using a rigorous SIV/macaque model characterized by high viral loads and high rates of CNS disease, HAART treatment with predominantly non-CNS penetrant agents initiated early during infection resulted in significantly reduced viral replication in CSF and brain; however, there was no decline in viral DNA brain levels. These observations suggest that plasma viral load may be an important determinant of CSF viral load, as indicated by the parallel pattern of viral RNA decline in the two compartments. Decreasing the number of infected macrophages and lymphocytes in the periphery likely reduces the number of infected cells trafficking to the brain [42]. Furthermore, decreased inflammation in the brain would result in reduced chemotaxis, thus reducing virus carried into the brain by trafficking monocytes and lymphocytes. Without antiretroviral therapy, the majority of macaques in this model develop AIDS and encephalitis, reaching the criteria for humane euthanasia by 84 days p.i. [17,18]. In contrast, HAART-treated macaques remained healthy until 175 days p.i., when euthanasia was elected to enable study of tissue reservoirs of virus [30].

Viral RNA brain levels of HAART-treated macaques were below the level of detection; however, there was no change in viral DNA levels as compared to untreated macaques. This supports our previous observations that viral seeding of the brain occurs during acute infection (15), suggesting that ongoing seeding of viral DNA in brain is not necessary for viral DNA to persist in the brain lifelong, regardless of therapy. However, this study did not examine whether brain SIV DNA was replication-competent. It remains to be determined whether inclusion of CNS-penetrating drugs in the HAART regimen would be more beneficial in lowering residual viral DNA or preventing reactivation of virus in the brain.

Continued CNS inflammation has been noted among patients on successful long-term HAART [10,11]. Our studies showed significant decreases in inflammation during HAART treatment in the majority of animals using this rigorous model, even with non-CNS penetrant agents. Elevated MHC Class II, GFAP, CD68, IL-6, and CCL2 in macaques with SIV encephalitis are strong indicators of the severity of CNS inflammation. Significant reductions in MHC Class II and GFAP in these HAART-treated macaques indicate that there was a decrease in brain inflammation; we also found a significant reduction in the number of CD4+ and CD8+ T cells in the white matter in HAART-treated macaques compared to untreated controls, further suggesting that there was decreased trafficking of inflammatory cells from the periphery to the brain. Future studies including behavioral assessments will determine whether this degree of residual inflammation was sufficient to impact motor or cognitive function.

Nonetheless, in agreement with studies showing large numbers of activated macrophages in HAART-treated, HIV-infected individuals, we did not observe a statistically significant decrease in CD68 expression in HAART-treated macaques, although there was some decline compared to SIV-infected, untreated macaques [10]. TNF α also was not significantly reduced in the treated macaques. There was a significant decrease in CSF:plasma ratio of IL-6 and CCL2, but the mRNA expression in brain for these genes was not significantly lower, suggesting that there was continued induction of inflammatory responses.

Chronic activation of Type I IFNs has been proposed as a pathogenic mechanism in HIV disease since Type I IFNs upregulate immunosuppressive molecules including programmed death ligand-1 (PD-1) [43], PDL-1 and other B7 family member proteins [44,45]. IFN γ also is produced by cells of the innate (dendritic and NK cells) and adaptive immune system (effector T cells). In addition to antiviral and immunomodulatory effects, IFN γ is a strong stimulator of immunosuppressive enzyme IDO [46]. IDO is, in turn, down regulated by tristetraproline, which also is induced by IFN γ and other proinflammatory cytokines [47,48]. During SIV infection, brain macrophages upregulate expression of innate immune response genes, particularly Type

I IFNs. This is followed by CSF and brain viral RNA level declines. IFN β is the first Type I IFN to be produced in response to viral infections; it reduces SIV replication in vitro in primary macaque macrophages by a transcriptional mechanism [20,22]. In HAART-treated macaques we observed several changes in innate immune responses in the brain including significant reductions in brain IFN β and MxA RNA levels. Collectively, the reduction of functional impairments of the immune system induced by Type I and Type II interferon would be beneficial during SIV/HIV infection, allowing ongoing effector T cell responses in HAART-treated animals.

This SIV model of HAART demonstrates that, despite control of virus replication in the periphery and the CNS, there is ongoing CNS inflammation that likely contributes to CNS disease in HIV-infected individuals on long-term HAART. The cause of these ongoing responses in the brain are unclear but can be investigated in this new SIV HAART model.

HAART was initiated in these macaques at the beginning of the asymptomatic stage of infection. It is not certain whether the results would be as favorable in HIV infection, when treatment is usually initiated at CD4 counts <350 cells/ μ L. Recent studies have shown that earlier HAART initiation results in decreased morbidity and mortality [49,50]. The ongoing pro-inflammatory responses detected in this model suggest that non-CNS penetrating HAART does not completely prevent ongoing changes in brain. Possible benefits of early initiation of therapy need to be balanced with the potential treatment toxicity. Selection of antiretrovirals that reach effective levels in the CNS therefore may be an important factor in preventing the mild cognitive changes and encephalitis that are observed with increasing frequency in HAART-treated patients. Future studies should include a comparison between the CNS effects of early vs. late therapy both with CNS-penetrating and non-penetrating drugs in this model and in HIV.

Acknowledgments

Funding/Support: These studies were supported by grants MH070306, MH069116, RR07002 and NS055648 from the National Institutes of Health. Drs. Brice and Kelly were supported by RR007002.

References

- Willig JH, Abrams S, Westfall AO, et al. Increased regimen durability in the era of once-daily fixed-dose combination antiretroviral therapy. *AIDS* 2008;22:1951–60. [PubMed: 18784459]
- El-Sadr WM, Lundgren JD, Neaton JD, et al. CD4+ count-guided interruption of antiretroviral treatment. *N Engl J Med* 2006;355:2283–96. [PubMed: 17135583]
- Havlir DV, Bassett R, Levitan D, et al. Prevalence and predictive value of intermittent viremia with combination hiv therapy. *JAMA* 2001;286:171–9. [PubMed: 11448280]
- Chun TW, Carruth L, Finzi D, et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* 1997;387:183–8. [PubMed: 9144289]
- Sacktor N, Lyles RH, Skolasky R, et al. HIV-associated neurologic disease incidence changes:: Multicenter AIDS Cohort Study, 1990–1998. *Neurology* 2001;56:257–60. [PubMed: 11160967]
- Tozzi V, Balestra P, Galgani S, et al. Changes in neurocognitive performance in a cohort of patients treated with HAART for 3 years. *J Acquir Immune Defic Syndr* 2001;28:19–27. [PubMed: 11579273]
- Kumar AM, Borodowsky I, Fernandez B, Gonzalez L, Kumar M. Human immunodeficiency virus type 1 RNA Levels in different regions of human brain: quantification using real-time reverse transcriptase-polymerase chain reaction. *J Neurovirol* 2007;13:210–24. [PubMed: 17613711]
- Langford D, Marquie-Beck J, de Almeida S, et al. Relationship of antiretroviral treatment to postmortem brain tissue viral load in human immunodeficiency virus-infected patients. *J Neurovirol* 2006;12:100–7. [PubMed: 16798671]

9. Letendre S, Marquie-Beck J, Capparelli E, et al. Validation of the CNS Penetration-Effectiveness rank for quantifying antiretroviral penetration into the central nervous system. *Arch Neurol* 2008;65:65–70. [PubMed: 18195140]
10. Anthony IC, Ramage SN, Carnie FW, Simmonds P, Bell JE. Influence of HAART on HIV-related CNS disease and neuroinflammation. *J Neuropathol Exp Neurol* 2005;64:529–36. [PubMed: 15977645]
11. Eden A, Price RW, Spudich S, Fuchs D, Hagberg L, Gisslen M. Immune activation of the central nervous system is still present after >4 years of effective highly active antiretroviral therapy. *J Infect Dis* 2007;196:1779–83. [PubMed: 18190258]
12. Bousquet L, Roucairol C, Hembury A, et al. Comparison of ABC transporter modulation by atazanavir in lymphocytes and human brain endothelial cells: ABC transporters are involved in the atazanavir-limited passage across an in vitro human model of the blood-brain barrier. *AIDS Res Hum Retroviruses* 2008;24:1147–54. [PubMed: 18729774]
13. Ronaldson PT, Persidsky Y, Bendayan R. Regulation of ABC membrane transporters in glial cells: relevance to the pharmacotherapy of brain HIV-1 infection. *Glia* 2008;56:1711–35. [PubMed: 18649402]
14. Ringler DJ, Hunt RD, Desrosiers RC, Daniel MD, Chalifoux LV, King NW. Simian immunodeficiency virus-induced meningoencephalitis: natural history and retrospective study. *Ann Neurol* 1988;23(Suppl):S101–7. [PubMed: 2831796]
15. Murray EA, Rausch DM, Lendvay J, Sharer LR, Eiden LE. Cognitive and motor impairments associated with SIV infection in rhesus monkeys. *Science* 1992;255:1246–9. [PubMed: 1546323]
16. Letvin NL, Walker BD. Immunopathogenesis and immunotherapy in AIDS virus infections. *Nat Med* 2003;9:861–6. [PubMed: 12835706]
17. Zink MC, Suryanarayana K, Mankowski JL, et al. High viral load in the cerebrospinal fluid and brain correlates with severity of simian immunodeficiency virus encephalitis. *J Virol* 1999;73:10480–8. [PubMed: 10559366]
18. Zink MC, Clements JE. A novel simian immunodeficiency virus model that provides insight into mechanisms of human immunodeficiency virus central nervous system disease. *J Neurovirol* 2002;8:42–8. [PubMed: 12491150]
19. Clements JE, Babas T, Mankowski JL, et al. The central nervous system as a reservoir for simian immunodeficiency virus (SIV): steady-state levels of SIV DNA in brain from acute through asymptomatic infection. *J Infect Dis* 2002;186:905–13. [PubMed: 12232830]
20. Barber SA, Gama L, Dudaronek JM, Voelker T, Tarwater PM, Clements JE. Mechanism for the establishment of transcriptional HIV latency in the brain in a simian immunodeficiency virus-macaque model. *J Infect Dis* 2006;193:963–70. [PubMed: 16518758]
21. Barber SA, Gama L, Li M, et al. Longitudinal analysis of simian immunodeficiency virus (SIV) replication in the lungs: compartmentalized regulation of SIV. *J Infect Dis* 2006;194:931–8. [PubMed: 16960781]
22. Barber SA, Herbst DS, Bullock BT, Gama L, Clements JE. Innate immune responses and control of acute simian immunodeficiency virus replication in the central nervous system. *J Neurovirol* 2004;10:15–20. [PubMed: 14982734]
23. Zink MC, Coleman GD, Mankowski JL, et al. Increased macrophage chemoattractant protein-1 in cerebrospinal fluid precedes and predicts simian immunodeficiency virus encephalitis. *J Infect Dis* 2001;184:1015–21. [PubMed: 11574916]
24. Mankowski JL, Queen SE, Clements JE, Zink MC. Cerebrospinal fluid markers that predict SIV CNS disease. *J Neuroimmunol* 2004;157:66–70. [PubMed: 15579282]
25. McArthur JC, McClernon DR, Cronin MF, et al. Relationship between human immunodeficiency virus-associated dementia and viral load in cerebrospinal fluid and brain. *Ann Neurol* 1997;42:689–98. [PubMed: 9392567]
26. Ellis RJ, Deutsch R, Heaton RK, et al. Neurocognitive impairment is an independent risk factor for death in HIV infection. San Diego HIV Neurobehavioral Research Center Group. *Arch Neurol* 1997;54:416–24. [PubMed: 9109743]

27. Brew BJ, Pemberton L, Cunningham P, Law MG. Levels of human immunodeficiency virus type 1 RNA in cerebrospinal fluid correlate with AIDS dementia stage. *J Infect Dis* 1997;175:963–6. [PubMed: 9086160]
28. Weed MR, Gold LH, Polis I, Koob GF, Fox HS, Taffe MA. Impaired performance on a rhesus monkey neuropsychological testing battery following simian immunodeficiency virus infection. *AIDS Res Hum Retroviruses* 2004;20:77–89. [PubMed: 15000701]
29. Mankowski JL, Queen SE, Tarwater PM, Fox KJ, Perry VH. Accumulation of beta-amyloid precursor protein in axons correlates with CNS expression of SIV gp41. *J Neuropathol Exp Neurol* 2002;61:85–90. [PubMed: 11829347]
30. Dinoso JB, Rabi SA, Blankson JN, et al. A SIV-infected macaque model to study viral reservoirs that persist during highly active antiretroviral therapy. *J Virol*. 2009
31. Hazuda DJ, Young SD, Guare JP, et al. Integrase inhibitors and cellular immunity suppress retroviral replication in rhesus macaques. *Science* 2004;305:528–32. [PubMed: 15247437]
32. Tsai CC, Follis KE, Beck TW, Sabo A, Bischofberger N, Dailey PJ. Effects of (R)-9-(2-phosphonylmethoxypropyl)adenine monotherapy on chronic SIV infection in macaques. *AIDS Res Hum Retroviruses* 1997;13:707–12. [PubMed: 9168239]
33. Washington CB, Flexner C, Sheiner LB, et al. Effect of simultaneous versus staggered dosing on pharmacokinetic interactions of protease inhibitors. *Clin Pharmacol Ther* 2003;73:406–16. [PubMed: 12732841]
34. Rollot F, Nazal EM, Chauvelot-Moachon L, et al. Tenofovir-related Fanconi syndrome with nephrogenic diabetes insipidus in a patient with acquired immunodeficiency syndrome: the role of lopinavir-ritonavir-didanosine. *Clin Infect Dis* 2003;37:e174–6. [PubMed: 14689363]
35. Barrios A, Garcia-Benayas T, Gonzalez-Lahoz J, Soriano V. Tenofovir-related nephrotoxicity in HIV-infected patients. *AIDS* 2004;18:960–3. [PubMed: 15060449]
36. Peyriere H, Reynes J, Rouanet I, et al. Renal tubular dysfunction associated with tenofovir therapy: report of 7 cases. *J Acquir Immune Defic Syndr* 2004;35:269–73. [PubMed: 15076241]
37. Schefe JH, Lehmann KE, Buschmann IR, Unger T, Funke-Kaiser H. Quantitative real-time RT-PCR data analysis: current concepts and the novel “gene expression's CT difference” formula. *J Mol Med* 2006;84:901–10. [PubMed: 16972087]
38. Zeger SL, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. *Biometrics* 1988;44:1049–60. [PubMed: 3233245]
39. Perelson AS, Essunger P, Cao Y, et al. Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature* 1997;387:188–91. [PubMed: 9144290]
40. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 1995;373:123–6. [PubMed: 7816094]
41. Wei X, Ghosh SK, Taylor ME, et al. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* 1995;373:117–22. [PubMed: 7529365]
42. Price RW, Parham R, Kroll JL, et al. Enfuvirtide cerebrospinal fluid (CSF) pharmacokinetics and potential use in defining CSF HIV-1 origin. *Antivir Ther* 2008;13:369–74. [PubMed: 18572749]
43. Cho HY, Lee SW, Seo SK, Choi IW, Choi I. Interferon-sensitive response element (ISRE) is mainly responsible for IFN-alpha-induced upregulation of programmed death-1 (PD-1) in macrophages. *Biochim Biophys Acta* 2008;1779:811–9. [PubMed: 18771758]
44. Wiesemann E, Deb M, Trebst C, Hemmer B, Stangel M, Windhagen A. Effects of interferon-beta on co-signaling molecules: upregulation of CD40, CD86 and PD-L2 on monocytes in relation to clinical response to interferon-beta treatment in patients with multiple sclerosis. *Mult Scler* 2008;14:166–76. [PubMed: 17942524]
45. Boasso A, Hardy AW, Landay AL, et al. PDL-1 upregulation on monocytes and T cells by HIV via type I interferon: restricted expression of type I interferon receptor by CCR5-expressing leukocytes. *Clin Immunol* 2008;129:132–44. [PubMed: 18650129]
46. Schroecksnadel K, Zangerle R, Bellmann-Weiler R, Garimorth K, Weiss G, Fuchs D. Indoleamine-2, 3-dioxygenase and other interferon-gamma-mediated pathways in patients with human immunodeficiency virus infection. *Curr Drug Metab* 2007;8:225–36. [PubMed: 17430111]
47. King EM, Kaur M, Gong W, Rider CF, Holden NS, Newton R. Regulation of tristetraprolin expression by interleukin-1 beta and dexamethasone in human pulmonary epithelial cells: roles for nuclear factor-

- kappaB and p38 mitogen-activated protein kinase. *J Pharmacol Exp Ther* 2009;330:575–85. [PubMed: 19435930]
48. Emmons J, Townley-Tilson WH, Deleault KM, et al. Identification of TTP mRNA targets in human dendritic cells reveals TTP as a critical regulator of dendritic cell maturation. *Rna* 2008;14:888–902. [PubMed: 18367721]
49. Tabarsi P, Tehrani AS, Baghaei P, et al. Early initiation of antiretroviral therapy results in decreased morbidity and mortality among patients with TB and HIV. *J Int AIDS Soc* 2009;12:14. [PubMed: 19607726]
50. Pao D, Pillay D, Fisher M. Potential impact of early antiretroviral therapy on transmission. *Curr Opin HIV AIDS* 2009;4:215–21. [PubMed: 19532053]

Fig. 1.

a. Plasma and CSF viral loads in SIV-infected HAART-treated and untreated macaques. HAART-treated macaques experienced rapid declines in plasma and CSF viral loads. In untreated macaques, plasma and CSF viral loads increased rapidly after inoculation and remained at levels of 6 to 8 logs for the remainder of the infection period. Arrow indicates initiation of HAART. **b.** Decline in CSF and plasma viral RNA showed parallel two-phase declines when fitted to the viral decay equation shown in **c** where T is the shorter half-life, M is the longer half-life, δ and μ_M are the decay rates of T and M , respectively, V_0 is the pre-therapy viral load and A and B are proportionality constants representing the contributions of the T and M cellular compartments to the viral load.

Fig. 2.

a. There were significantly lower levels of viral RNA ($p = 0.004$) but not viral DNA ($p = 1.0$) in the brains of HAART-treated macaques as compared to untreated macaques as measured by quantitative RT-PCR (for RNA) or PCR (for DNA). **b.** There was significantly lower viral gp41 protein ($p = 0.043$) in the brains of HAART-treated macaques as compared to untreated macaques as measured by quantitative immunohistochemistry.

Fig. 3.

HAART-treated macaques had significantly lower levels of MHC Class II (**a**) and GFAP (**b**) in subcortical white matter as compared to untreated macaques ($p = 0.043$ and 0.040 , respectively). There were lower levels of CD68 in HAART-treated animals as compared to untreated macaques (**c**), but the difference was not significant ($p = 0.123$). There was a significant reduction in the number of CD4+ and CD8+ T cells (**d**, **e**; $p = 0.006$).

Fig. 4. HAART resulted in lower CCL2 and IL-6 levels in CSF after day 35 p.i. ($p < 0.001$ and $p = 0.041$, respectively) in HAART-treated macaques. However, brain CCL2 and IL-6 RNA levels were not significantly lower ($p = 0.201$ and $p = 0.136$, respectively) in HAART-treated macaques.

Fig. 5.

IFN β and MxA RNA were significantly downregulated in the brains of HAART-treated macaques ($p = 0.018$ and 0.006 , respectively). While levels of TNF α RNA were lower in HAART-treated macaques, this difference was not significant ($p = 0.144$).

Fig. 6.

Levels of IDO RNA were significantly lower (**a**; $p = 0.045$) in HAART-treated animals. Levels of IFN γ were lower, but the difference was not significant (**b**; $p = 0.269$). Levels of IFN γ were not expressed as fold change from uninfected animals because the latter did not have detectable IFN γ in the brain.