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# **CNS-Targeted Antiretroviral Strategies: When Are They Needed and What to Choose**

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## **Abstract**

*Purpose of Review* Neurocognitive disorders are not uncommon in HIV-positive patients but their pathogenesis is multifactorial and incompletely understood. After excluding contributing comorbidities, several factors may impair neurocognition including severe immune suppression, incomplete antiviral efficacy, persistent immune activation, vascular abnormalities and neurotoxicity of drugs. The effectiveness of targeted antiretroviral strategies on these risk factors is unknown.

*Recent findings* Recent studies support the idea that residual cerebrospinal fluid HIV RNA in the setting of plasma viral suppression is associated with compartmental immune activation but the link to neuronal damage is debated. Some authors have reported an incomplete antiviral efficacy in macrophage-derived cells but targeted antiretroviral regimen switches have not been performed. Additionally, improvements in neurocognition using drugs with better central nervous system penetration or maraviroc (associated with favorable immunological properties) have been observed in pilot studies. Trials evaluating specific interventions for cardiovascular health (including brain white matter abnormalities) and neurotoxicity of antiretrovirals are warranted.

*Summary* Central nervous system targeted antiretroviral strategies are needed in patients with uncontrolled cerebrospinal HIV replication and they may be suggested in subjects with low CD4 nadir, individuals carrying drug-resistant viruses and those with compartmental immune activation.

**Keywords:** HIV-associated neurocognitive disorders; cerebrospinal fluid; antiretrovirals; pharmacokinetics; pharmacodynamics; compartmentalization.

## **Introduction**

Central Nervous System (CNS) manifestations in HIV-positive patients radically changed after the introduction of highly active antiretroviral treatment (HAART). In the current era, CNS opportunistic infections and HIV-associated dementia are mostly observed in late-presenting subjects with severe immune depression. Despite such favorable outcomes the health-adjusted life expectancy of people living with HIV is lower than their negative counterparts.(1) Besides an increasing incidence of cerebrovascular accidents, HIV-positive individuals often complain of neurocognitive impairment (NCI): the prevalence varies according to age, geographical setting and diagnostic methods. A recent study in five different centers in Europe found a prevalence of HIV-associated neurocognitive disorders (HAND) of 35%; the vast majority of the affected subjects (83%) did not show any impairment in everyday life thus falling in the asymptomatic HAND category (ANI).(2)

The pathogenesis of such disturbances is unclear and a recent manuscript reviewed the controversies around this issue.(3) After excluding contributing factors such psychoactive drug use, mood disorders, education, HCV-coinfection and host genetic factors, some patient-related (age, cardiovascular risk factors, persistent inflammation), virus-related (persistent viral replication, microglia/perivascular macrophage infection, neurotoxicity or HIV proteins) and treatment-related (neurotoxicity) factors have been identified. Antiretroviral treatment may directly modify some of these factors (such as viral replication, certain cell type infection, drug neurotoxicity and, to a certain extent, immune activation), indirectly some others (such as cardiovascular risk profile) but has no effect on the toxicity of HIV proteins nor on the so called “legacy effect”.(4) The latter is the consequence of long term chronic HIV infection, where neuronal damage that was triggered before the initiation of HAART may not be completely reversible.(5)

Relying on cerebrospinal fluid (CSF) biomarkers for assessing brain parenchyma further enhances the degree of uncertainty regarding HAND features and pathogenesis. While CSF is partially produced from brain interstitial fluid it also originates from plasma filtration: discrepancies between

CSF and brain parenchyma HIV viral load as well as concentrations of drugs have been reported.(6)

A recent observation in macaques highlighted phylogenetic distances (by Simian Immunodeficiency Virus *env* gene deep sequencing) between viruses collected in brain tissue and CSF.(7)

Additionally the association among these surrogate markers is ambiguous. While lower immune activation markers have been found in subjects with ultrasensitive undetectable CSF HIV RNA, the correlation between either CSF viral load and neopterin (highlighting macrophage activation) with NCI is uncertain.(8)(9)

### **Risk factors for CNS involvement and the role of antiretroviral treatment**

In order to define the specificity of CNS-targeted antiretroviral strategies, we will now briefly summarize data regarding the risk factors for CNS involvement in HIV-positive subjects as well as the role of antiretrovirals and the strategies that have shown some benefits. In Table 1 we also include the effect of low CD4 nadir, high HIV DNA (either in peripheral blood mononuclear cells, PBMCs, or in monocytes) and plasma HIV RNA. While these three factors have been consistently associated with HAND, only plasma viral control may be impacted through treatment changes and adherence support strategies.

<b>Variable</b>	<b>Degree of association with HAND</b>	<b>Degree of association with ARVs' choice</b>	<b>Other Relevant Factors</b>	<b>Beneficial interventions</b>
<b>Low CD4 nadir</b>	High	-	-	None
<b>High HIV DNA</b>	Moderate	Low	Duration of viral suppression	None

<b>Plasma HIV RNA</b>	High	High	Several	Genotype-based, adherence
<b>CSF HIV RNA</b>	Moderate	High	Several	Unclear
<b>Symptomatic CSF escape</b>	High (neurological symptoms)	High	Low nadir CD4, resistant-associated mutations	Genotype-based, CNS-targeted
<b>Asymptomatic CSF escape</b>	Low	Moderate	Plasma HIV RNA	Unclear
<b>Residual CSF HIV RNA</b>	Unclear	Unclear	Duration of viral suppression	None
<b>Macrophage derived cells infection</b>	Low	Moderate	Viral tropism	None
<b>Compartmental immune activation/inflammation</b>	Moderate	Unclear	Low nadir CD4	None
<b>Neurotoxicity</b>	Moderate	High	Host genetics	Unclear
<b>Cardiovascular risk profile</b>	High	Moderate	Several	None

**Table 1. Risk Factors (and the degree of association) for HIV-associate neurocognitive disorders and published beneficial interventions.** “ARV”, antiretrovirals; “CSF”, cerebrospinal fluid.

CSF HIV RNA is usually 1 Log<sub>10</sub> lower than plasma HIV RNA; although its correlation to brain parenchyma viral replication is still uncertain, subjects affected by HIV-associated dementia often present higher CSF viral loads.(10) The CSF response to treatment usually parallels plasma viral load decay although compartmentalized viruses may show a different trajectory. Despite systemic control of HIV replication, the detection of CSF viral RNA varies according to the used methods: it is approximately 10% using commercially available kits and up to 60% using ultra-sensitive assays.(11) The exact clinical relevance of a detectable CSF HIV RNA under suppressive treatment is uncertain. In longitudinal studies it has been associated with compartmental immune activation with no case of overt neurological symptoms and no increase in CSF biomarkers of neuronal damage.(12)(13) The management of this condition is also debated: a single study reported no effect of an intensified HAART containing CSF penetrating or non-penetrating antiretrovirals.(14) The detection of resistance-associated mutations (RAMs) supports antiretroviral regimens’ incomplete antiviral efficacy and suggests a potential benefit of genotype-based treatment optimization. The presence of

CSF RAMs is a feature reported in almost all cases of neurologically symptomatic disease: the clinical, radiological and virological response to optimized antiretroviral combinations supports the effect of adequate viral suppression in controlling this HIV-associated encephalitis.(15)(16)

Since microglia and perivascular macrophages are myeloid lineage cells that can be infected by HIV, viral control in the CNS needs to be optimized to these specific cells: *in vivo* functional imaging techniques support the persistence of microglial cells activation despite HIV RNA undetectability in plasma and CSF.(17) *In vitro* data suggest that the antiviral effect in activated and resting macrophages may be different according to certain drugs.(18) The evidence supporting the association with this specific activity and neurocognition resides in a single unreplicated study.(19) However, incomplete efficacy in macrophage-derived cells may be extrapolated from an *ex vivo* study: higher rilpivirine and lopinavir concentrations in patients' withdrawn CSF were associated with a higher antiviral effect in PBMCs, astrocytoma and glioblastoma-derived cells.(20)

The effect of intrathecal immune activation on cognition is unclear. While several studies correlated CSF biomarkers (including soluble CD14 and CD163) or immune phenotypes (CD8 IFN gamma) with HAND, others found no association.(21) (22) Compartmentalized inflammation has however been linked to blood-brain barrier damage and the persistence of IgG production: the latter is not entirely specific to HIV and it has been shown to decline in patients treated during primary infections.(23) In this context maraviroc has a unique activity beyond its antiviral effect: it is associated with CCR5 blockade and with anti-inflammatory properties that are under evaluation in certain CNS inflammatory conditions.(24) In pilot studies maraviroc intensification has been associated with the improvement of brain magnetic resonance spectroscopy markers of neuronal integrity, and reductions in CSF inflammation (CXCL 10) and in activated CD16+ monocytes. The new CCR2/CCR5 antagonist cenicriviroc, still in development, has the potential to further reduce residual immune activation.(25)

Direct or indirect toxicity of antiretrovirals has been suggested following several *in vitro* and *in vivo* pieces of evidence.(26) The largest experience is with efavirenz, which has been linked to neuropsychological and sleep disturbances: these symptoms are affected by plasma concentrations and patients' genetic variation in drug metabolizing enzymes. Three studies reported lower neurocognitive performance associated with efavirenz use and the drug is now avoided in patients with psychiatric comorbidities (for the risk of suicidality) and with HAND. As for the other drugs, several mechanisms have been proposed including interference with amyloid metabolism, reactive oxygen species production and mitochondrial dysfunction: almost all drugs had some degree of *in vitro* neuronal toxicity, but some were relatively less harmful (tenofovir, emtricitabine, darunavir and maraviroc).(27) Indirect toxicity may link certain ARVs to vascular abnormalities such as cerebral small vessel disease. An autopsy study reported vascular wall abnormalities in 72.2% of decedents studies. This finding was associated with protease inhibitor use and with a history of HAND during life.(28) This piece of evidence along with reports of lower cognition in patients with traditional cardiovascular risk factors and with vascular white matter abnormalities (on brain magnetic resonance imaging) highlights the relevance of this issue.(29) The effect of risk factor control on cognition in HIV-positive patients with HAND is currently unknown and needs to be assessed: nevertheless it is plausible that improving vascular health may be beneficial.

### **CNS-targeted antiretrovirals: what to choose?**

Attempts to target treatment of the CNS in HIV-positive individuals may theoretically consider all the above-mentioned risk factors. The large majority of the studies included the use of the CNS penetration/effectiveness score (CPE) developed and validated from the CHARTER study group in the United States. The CPE ranks ARVs according to their properties, CSF penetration and, in a few cases, antiretroviral activity: the score of an antiretroviral regimen is calculated by adding the values of individual drugs. (30) Higher CPE score regimens have been associated with lower CSF HIV RNA

while the association with asymptomatic CSF escape and with neurocognition is less certain. The explanation for the latter potentially lies in the multifactorial pathogenesis of HAND as well as in the presence of potentially irreversible damage (either from severe immune depression or from vascular abnormalities). Interestingly, in patients with persistently undetectable CSF HIV RNA a better mood over time was observed, potentially linking depression with residual viral replication and inflammation.(31) A well designed randomized clinical trial of CNS-targeted HAART in patients with HAND was prematurely interrupted for slow accrual and imbalance among study arms. In patients with plasma HIV RNA <50 copies/mL the use of a CNS-targeted treatment was associated with an improvement in global cognition (with very small sample size, 7 vs. 6 participants).(32) Importantly, patients in the CNS-targeted arm were receiving more “complex” regimens and showed worse control of plasma HIV replication.(32)

Other longitudinal studies supported a trend to better cognition over time if patients were treated with regimens with lower CPE scores (along with the decrease in CD4+/CD8+ T lymphocyte ratio in one of them). (33)(34) Another interesting but still unpublished single-arm study described the effect of increasing the CPE in 31 patients with HAND: new combinations with higher CPE score [ $\geq 3$  points (and total CPE  $\geq 9$ ) were associated with improved cognition (in 51% of the included subjects) and mood.(35)

Two other “rankings” have been tested in cross sectional studies. The first measured CSF concentrations in patients and compared those values to 95% inhibitory concentrations: subjects with higher ratios (95% inhibitory quotients) had a lower risk of CSF escape with controlled plasma HIV RNA.(36) The second one reflects the differential activity (and intracellular concentrations) ARVs reach in resident macrophages: it has been shown that some drugs have a limited entry or activity in such cells, while others (such as NRTIs) may be more active given the lower natural nucleotides’ intracellular pool.(37) A single study found out that drugs with higher monocyte efficacy score were associated with better cognition.(19)



Three studies reported potential concern of neurotoxicity: two of them were conducted in naïve patients randomized to different regimens. The first showed poorer cognition and worse spectroscopic markers of neuronal integrity after three years of treatment (with 10/22 patients receiving efavirenz).(38) The second randomized and controlled trial (comparing tenofovir/emtricitabine/efavirenz to zidovudine/lamivudine plus nevirapine) reported worse cognition in the efavirenz arm (119 patients) after 2 years of treatment.(39) This was observed after an initial improvement in cognition that follows the inhibition of viral replication and fits in the model of neurotoxicity proposed by Underwood and colleagues.(40) The third study measured neurocognitive performance in patients stopping their antiretroviral regimens: surprisingly, cognition improved in all subjects (although more in those interrupting efavirenz-based regimens).(41)

In Table 2 we review the rankings of commercially available drugs (with the exception of tenofovir alafenamide, lacking substantial data). Some drugs are not included in the scores because they have not been updated; nevertheless recent data may suggest a high CPE score and 95% inhibitory quotient for dolutegravir and moderate/high values for elvitegravir and rilpivirine.(42) The macrophage activity score has not been published for newer drugs, but several data point out a similar (or even greater) *in vitro* antiviral activity of integrase strand transfer inhibitors in macrophages (as compared to lymphocytes).(43) As for neurotoxicity, most of the data are derived from *in vitro* assessments (with the exception of efavirenz-associated toxicity); data on dolutegravir are conflicting. Two yet unpublished studies reported different findings: one observed neurite growth, while the other no effect of dolutegravir on *in vitro* cultures of rat neurons.(44)(45) There is also a relevant debate on dolutegravir neuropsychological effects: some but not all cohorts reported an increase in discontinuation due to sleep disturbances and mood changes.(46)(47) These effects seem to be mild and reversible and their clinical relevance and risk factors (female gender, older age and abacavir coadministration) need to be fully understood.

Table 2 also shows that the selection of the best CNS-targeting drug may be challenging: no guideline-recommended compound is at the same time effective in the CNS/CSF, active on macrophage-derived cells, lacking *in vitro* or *in vivo* neurotoxicity and neutral/beneficial on patients' cardiovascular risk. Maraviroc has been used as an intensification strategy in a pilot study: a significant improvement in cognition was observed in 9 patients when compared to 5 subjects with standard antiretroviral treatment.(48)

		CPE score	95% Inhibitory Quotients	Monocyte efficacy score	<i>in vitro</i> neurotoxicity	Cardiovascular risk
NRTIs	Abacavir	3	na	3	+	++
	Emtricitabine	3	na	12.5	-	0
	Lamivudine	2	na	50	+	0
	Tenofovir DF	1	na	50	0	-
	Zidovudine	4	na	50	+	0
NNRTIs	Nevirapine	4	na	20	+	0
	Efavirenz	3	6.4	100	++	0
	Etravirine	2	5.1	na	+	0
	Rilpivirine	na	na	na	+	0
PIs	Atazanavir	2	0.4	na	+	-
	Atazanavir/r	2	2.8	na	+	-
	Darunavir/r	3	8.2-18.5	na	0	+
	Lopinavir/r	3	1.5	na	+	++
INSTIs	Raltegravir	3	0.7	na	+	-
	Elvitegravir/r	na	na	na	+	0
	Dolutegravir	na	na	na	?	0
EIs	Maraviroc	3	na	na	-	0
	Enfuvirtide	1	na	50	na	0

**Table 2. The ranking of commonly prescribed antiretroviral drugs as for Concentration Penetration/Effectiveness (CPE) scores, 95% inhibitory quotients, monocyte efficacy scores, *in vitro* neurotoxicity and impact on cardiovascular risk.** “NRTIs”, nucleoside reverse transcriptase inhibitors; “NNRTIs”, non-nucleoside reverse transcriptase inhibitors; “PIs”, protease inhibitors; “INSTIs”, integrase strand transfer inhibitors; “EIs”, entry inhibitors. “na”, not available. “/r”, boosted by ritonavir.

### CNS-targeted antiretrovirals: when to consider their use?

After acknowledging the relevance of systemic HIV RNA suppression, there are some conditions where CNS-targeted antiretrovirals may be recommended. Unfortunately the evidence is inconclusive for most of the clinical scenarios.

In patients presenting with HIV-associated dementia or symptomatic CSF escape there is enough evidence to suggest the use of drugs with high CPE score. In the two published series of subjects with progressive neurological symptoms due to HIV escape, HAART optimization (using CPE scores and genotype resistance testing) was associated with clinical, radiological and virological improvements.(15)(16) In patients with asymptomatic CSF escape or residual CSF replication there is no evidence of beneficial interventions. Nevertheless, the finding of a higher prevalence of CSF escape in patients with recent low level plasma viremia supports the need for optimizing antiretroviral treatments and patient adherence to medications.(49)

There is no definitive recommendation in patients with HAND with undetectable plasma and CSF HIV RNA. Some longitudinal data seem to point out that higher CPE may be beneficial in improving cognition in the setting of optimal viral control: the supporting evidence is however insufficient for generating clear statements. CSF viral compartmentalization would be another case in which targeting the CNS compartment may be beneficial: however this definition relies upon the use of advanced virological techniques (including sequencing of *env* or other viral genes) and specific treatments have not been evaluated.(50) The available pieces of evidence may however suggest several interventions that need to be evaluated in prospective studies: we will now discuss them as if occurring in patients with HAND who have controlled viral replication in both compartments.

a. *CD4+ T lymphocytes nadir <200/uL:*

Late presenters have a higher risk of developing CNS-affecting conditions including HAND. In these patients less drug regimens (HAART combinations with 2 or fewer antiretroviral drugs) have been associated with incomplete antiviral response, viral rebound and HIV-associated encephalitis;

the latter event has been reported primarily in individuals simplified to protease inhibitor monotherapy.(51) Although the evidence is limited, using three drug regimens and including at least one drug with high CPE score is what we do in our patients.

*b. Being infected with HIV carrying resistance-associated mutations:*

Inhibitory quotients are lower in the CSF given the scarce amount of drug reaching the compartment: resistance associated mutations may therefore be more relevant for intrathecal HIV replication. An interesting study corrected the CPE score with the global sensitivity score (GSS, a measure of activity of every drug in the regimen): this correction improved the association between neuropenetration/efficacy and cognition.(52) We usually include two fully active drugs in these patients' antiretroviral regimens.

*c. Previous HIV-associated dementia or symptomatic CSF escape*

These conditions are usually associated with long durations of untreated HIV infection, severe immune depression and the potential for relapsing over time. In a cohort of HIV-positive patients cared for privately in India the prevalence of symptomatic CSF escape approximated 1%. Patients had low CD4+ nadir, CNS-affecting conditions at baseline and were treated with suboptimal antiretroviral regimens (with a very high prevalence of resistance associated mutations after virological failures).(53) We usually maintain three drugs with high CPE scores in patients with such worrisome previous conditions.

*d. On efavirenz-containing regimens:*

The evidence linking efavirenz to neurocognition is significant; we usually prefer not to use the drug in patients with HAND.(54) The use of less "neurotoxic" regimens needs to be studied and a pilot clinical trial is ongoing (NCT03163277).

*e. High CSF neopterin:*

This biomarker is produced by activated macrophages and, after an initial decline in response to initiation of HAART, remains elevated in the CSF in a proportion HIV-positive patients (55% in a recent report including patients with undetectable HIV RNA since 10 or more years).(55) In subjects with elevated CSF neopterin despite viral control, immune modulating strategies may be considered: maraviroc intensification (limited evidence) or its inclusion in the regimen (yet to be studied, neutral results have been reported with a three drug regimen containing maraviroc in HIV-positive naïve patients) warrant further studies in these individuals.

*f. High cardiovascular risk with or without white matter abnormalities:*

Age and a high cardiovascular risk have been associated with HAND and with T2 hyperintense abnormalities in brain white matter (WMA). The latter is a well known condition in geriatric medicine and it has recently been associated with cognition and incomplete CSF viral control in people living with HIV.(56) In HIV-negative individuals strict control of cardiovascular factors (blood pressure, LDL cholesterol and fasting glucose) is suggested although the reversibility of such a condition is unknown. In our patients with WMA we try to reduce cardiovascular risk by promoting healthy lifestyle (diet, exercise, smoking cessation), reducing LDL cholesterol and including cardiovascular neutral antiretroviral drugs.

## **Conclusions**

Standard HAART regimens are highly effective in HIV-positive patients and their beneficial effects on the central nervous system are outstanding. In those patients with neurocognitive deficits several factors need to be taken into account when contributing comorbidities have been excluded. In patients with HAND we usually reinforce adherence to medications, we optimize HAART according to historical genotype resistance testing, we replace efavirenz and we reduce cardiovascular risk as much as possible (by controlling risk factors and by using neutral drugs). In patients with low nadir CD4+

count, previous HIV-associated dementia or symptomatic CSF escape we suggest to use fully active drugs and to include at least one compound with high CPE score. In subjects with persistent compartmental immune activation, maraviroc-containing regimens and novel strategies need to be studied.

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## References

Papers of particular interest, published recently, have been highlighted as:

•• Of major importance

1. Hogg RS, Eyawo O, Collins AB, Zhang W, Jabbari S, Hull MW, et al. Health-adjusted life expectancy in HIV-positive and HIV-negative men and women in British Columbia, Canada: a population-based observational cohort study. *Lancet HIV*. 2017 Mar 2;
2. Haddow LJ, Laverick R, Daskalopoulou M, McDonnell J, Lampe FC, Gilson R, et al. Multicenter European Prevalence Study of Neurocognitive Impairment and Associated Factors in HIV Positive Patients. *AIDS Behav*. 2017 Jan 31;
3. Nightingale S, Winston A, Letendre S, Michael BD, McArthur JC, Khoo S, et al. Controversies in HIV-associated neurocognitive disorders. *Lancet Neurol*. 2014 Nov;13(11):1139–51. ••
4. Johnson TP, Patel K, Johnson KR, Maric D, Calabresi PA, Hasbun R, et al. Induction of IL-17 and nonclassical T-cell activation by HIV-Tat protein. *Proc Natl Acad Sci U S A*. 2013 Aug 13;110(33):13588–93.
5. Heaton RK, Clifford DB, Franklin DR, Woods SP, Ake C, Vaida F, et al. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology*. 2010 Dec 7;75(23):2087–96.
6. Curley P, Rajoli RKR, Moss DM, Liptrott NJ, Letendre S, Owen A, et al. Efavirenz Is Predicted To Accumulate in Brain Tissue: an In Silico, In Vitro, and In Vivo Investigation. *Antimicrob Agents Chemother*. 2017 Jan;61(1).

7. Jaclyn Mallard, Brittany Rife, Emily Papazian, Arianna Noggle, David J. Nolan, Marco Salemi, Kenneth C. Williams. CNS PARENCHYMA AND CHOROID PLEXUS, NOT CSF, ARE VIRAL RESERVOIRS IN MONKEYS WITH AIDS. In the Abstract book of the Conference on Retroviruses and Opportunistic Infections, Seattle, WA, USA; 2017.
8. Motta I, Allice T, Romito A, Ferrara M, Ecclesia S, Imperiale D, et al. Cerebrospinal fluid viral load and neopterin in HIV-positive patients with undetectable viraemia. *Antivir Ther.* 2017 Feb 15;
9. Edén A, Marcotte TD, Heaton RK, Nilsson S, Zetterberg H, Fuchs D, et al. Increased Intrathecal Immune Activation in Virologically Suppressed HIV-1 Infected Patients with Neurocognitive Impairment. *PloS One.* 2016;11(6):e0157160.
10. Bai F, Iannuzzi F, Merlini E, Borghi L, Tincati C, Trunfio M, et al. Clinical and viro-immunological correlates of HIV associated neurocognitive disorders (HAND) in a cohort of antiretroviral-naïve HIV-infected patients. *AIDS Lond Engl.* 2017 Jan 14;31(2):311–4.
11. Ferretti F, Gisslen M, Cinque P, Price RW. Cerebrospinal Fluid HIV Escape from Antiretroviral Therapy. *Curr HIV/AIDS Rep.* 2015 Jun;12(2):280–8.
12. Edén A, Nilsson S, Hagberg L, Fuchs D, Zetterberg H, Svennerholm B, et al. Asymptomatic Cerebrospinal Fluid HIV-1 Viral Blips and Viral Escape During Antiretroviral Therapy: A Longitudinal Study. *J Infect Dis.* 2016 Sep 28; ••
13. Anderson AM, Muñoz-Moreno JA, McClernon DR, Ellis RJ, Cookson D, Clifford DB, et al. Prevalence and Correlates of Persistent HIV-1 RNA in Cerebrospinal Fluid During Antiretroviral Therapy. *J Infect Dis.* 2017 Jan 1;215(1):105–13.
14. Yilmaz A, Verhofstede C, D'Avolio A, Watson V, Hagberg L, Fuchs D, et al. Treatment intensification has no effect on the HIV-1 central nervous system infection in patients on suppressive antiretroviral therapy. *J Acquir Immune Defic Syndr 1999.* 2010 Dec 15;55(5):590–6.
15. Canestri A, Lescure F-X, Jaureguiberry S, Moulignier A, Amiel C, Marcelin AG, et al. Discordance between cerebral spinal fluid and plasma HIV replication in patients with neurological symptoms who are receiving suppressive antiretroviral therapy. *Clin Infect Dis Off Publ Infect Dis Soc Am.* 2010 Mar 1;50(5):773–8.
16. Peluso MJ, Ferretti F, Peterson J, Lee E, Fuchs D, Boschini A, et al. Cerebrospinal fluid HIV escape associated with progressive neurologic dysfunction in patients on antiretroviral therapy with well controlled plasma viral load. *AIDS Lond Engl.* 2012 Sep 10;26(14):1765–74.
17. Garvey LJ, Pavese N, Politis M, Ramlackhansingh A, Brooks DJ, Taylor-Robinson SD, et al. Increased microglia activation in neurologically asymptomatic HIV-infected patients receiving effective ART. *AIDS Lond Engl.* 2014 Jan 2;28(1):67–72.
18. Gavegnano C, Schinazi RF. Antiretroviral therapy in macrophages: implication for HIV eradication. *Antivir Chem Chemother.* 2009;20(2):63–78.
19. Shikuma CM, Nakamoto B, Shiramizu B, Liang C-Y, DeGruttola V, Bennett K, et al. Antiretroviral monocyte efficacy score linked to cognitive impairment in HIV. *Antivir Ther.* 2012;17(7):1233–42.

20. Mora-Peris B, Winston A, Garvey L, Else LJ, Shattock RJ, Herrera C. HIV-1 CNS in vitro infectivity models based on clinical CSF samples. *J Antimicrob Chemother.* 2016 Jan;71(1):235–43.
21. Bryant AK, Moore DJ, Burdo TH, Lakritz JR, Gouaux B, Soontornniyomkij V, et al. Plasma soluble CD163 is associated with postmortem brain pathology in human immunodeficiency virus infection. *AIDS Lond Engl.* 2017 Apr 24;31(7):973–9.
22. Schrier RD, Hong S, Crescini M, Ellis R, Pérez-Santiago J, Spina C, et al. Cerebrospinal fluid (CSF) CD8+ T-cells that express interferon-gamma contribute to HIV associated neurocognitive disorders (HAND). *PloS One.* 2015;10(2):e0116526.
23. Calcagno A, Romito A, Atzori C, Ghisetti V, Cardellino C, Audagnotto S, et al. Blood Brain Barrier Impairment in HIV-Positive Naïve and Effectively Treated Patients: Immune Activation Versus Astrocytosis. *J Neuroimmune Pharmacol Off J Soc NeuroImmune Pharmacol.* 2017 Mar;12(1):187–93.
24. Martin-Blondel G, Brassat D, Bauer J, Lassmann H, Liblau RS. CCR5 blockade for neuroinflammatory diseases--beyond control of HIV. *Nat Rev Neurol.* 2016 Feb;12(2):95–105.
25. Thompson M, Saag M, DeJesus E, Gathe J, Lalezari J, Landay AL, et al. A 48-week randomized phase 2b study evaluating cenicriviroc versus efavirenz in treatment-naïve HIV-infected adults with C-C chemokine receptor type 5-tropic virus. *AIDS Lond Engl.* 2016 Mar 27;30(6):869–78.
26. Underwood J, Robertson KR, Winston A. Could antiretroviral neurotoxicity play a role in the pathogenesis of cognitive impairment in treated HIV disease? *AIDS Lond Engl.* 2015 Jan 28;29(3):253–61.
27. Robertson K, Liner J, Meeker RB. Antiretroviral neurotoxicity. *J Neurovirol.* 2012 Oct;18(5):388–99.
28. Soontornniyomkij V, Umlauf A, Chung SA, Cochran ML, Soontornniyomkij B, Gouaux B, et al. HIV protease inhibitor exposure predicts cerebral small vessel disease. *AIDS Lond Engl.* 2014 Jun 1;28(9):1297–306.
29. Su T, Wit FWNM, Caan MWA, Schouten J, Prins M, Geurtsen GJ, et al. White matter hyperintensities in relation to cognition in HIV-infected men with sustained suppressed viral load on combination antiretroviral therapy. *AIDS Lond Engl.* 2016 Sep 24;30(15):2329–39.
30. Hammond ER, Crum RM, Treisman GJ, Mehta SH, Marra CM, Clifford DB, et al. The cerebrospinal fluid HIV risk score for assessing central nervous system activity in persons with HIV. *Am J Epidemiol.* 2014 Aug 1;180(3):297–307.
31. Hammond ER, Crum RM, Treisman GJ, Mehta SH, Clifford DB, Ellis RJ, et al. Persistent CSF but not plasma HIV RNA is associated with increased risk of new-onset moderate-to-severe depressive symptoms; a prospective cohort study. *J Neurovirol.* 2016 Aug;22(4):479–87.
32. Ellis RJ, Letendre S, Vaida F, Haubrich R, Heaton RK, Sacktor N, et al. Randomized trial of central nervous system-targeted antiretrovirals for HIV-associated neurocognitive disorder. *Clin Infect Dis Off Publ Infect Dis Soc Am.* 2014 Apr;58(7):1015–22.



33. Vassallo M, Durant J, Biscay V, Lebrun-Frenay C, Dunais B, Laffon M, et al. Can high central nervous system penetrating antiretroviral regimens protect against the onset of HIV-associated neurocognitive disorders? *AIDS Lond Engl*. 2014 Feb 20;28(4):493–501.
34. Cross HM, Combrinck MI, Joska JA. HIV-associated neurocognitive disorders: antiretroviral regimen, central nervous system penetration effectiveness, and cognitive outcomes. *South Afr Med J Suid-Afr Tydskr Vir Geneesk*. 2013 Sep 18;103(10):758–62.
35. Gilles Force; Valerie Hahn; Helene Defferriere; Natacha Darchy; Jacques Ropers; Philippe Aegerter; Constance Delaugerre; Gilles Peytavin; Pierre De Truchis. Week48 Cognitive Improvement in HAND After Switch to HAART Based on CHARTER Score +3. In the Abstract book of the Conference on Retroviruses and Opportunistic Infections, Boston, MA, USA; 2016.
36. Calcagno A, Simiele M, Alberione MC, Bracchi M, Marinaro L, Ecclesia S, et al. Cerebrospinal fluid inhibitory quotients of antiretroviral drugs in HIV-infected patients are associated with compartmental viral control. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2015 Jan 15;60(2):311–7.
37. Aquaro S, Svicher V, Schols D, Pollicita M, Antinori A, Balzarini J, et al. Mechanisms underlying activity of antiretroviral drugs in HIV-1-infected macrophages: new therapeutic strategies. *J Leukoc Biol*. 2006 Nov;80(5):1103–10.
38. Winston A, Puls R, Kerr SJ, Duncombe C, Li P, Gill JM, et al. Differences in the direction of change of cerebral function parameters are evident over three years in HIV-infected individuals electively commencing initial cART. *PloS One*. 2015;10(2):e0118608.
39. Zhang F, Heaton R, Wu H, Jin H, Zhao H, Yu X, Franklin D, Mu W, Vaida F, Letendre S. Randomized Clinical Trial of Antiretroviral Therapy for Prevention of HAND. In the Abstract book of the Conference on Retroviruses and Opportunistic Infections, Seattle, WA, USA; 2015.
40. Underwood J, Robertson KR, Winston A. Could antiretroviral neurotoxicity play a role in the pathogenesis of cognitive impairment in treated HIV disease? *AIDS Lond Engl*. 2015 Jan 28;29(3):253–61.
41. Robertson KR, Su Z, Margolis DM, Krambrink A, Havlir DV, Evans S, et al. Neurocognitive effects of treatment interruption in stable HIV-positive patients in an observational cohort. *Neurology*. 2010 Apr 20;74(16):1260–6.
42. Letendre SL, Mills AM, Tashima KT, Thomas DA, Min SS, Chen S, et al. ING116070: a study of the pharmacokinetics and antiviral activity of dolutegravir in cerebrospinal fluid in HIV-1-infected, antiretroviral therapy-naïve subjects. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2014 Oct;59(7):1032–7.
43. Canducci F, Ceresola ER, Saita D, Castagna A, Gianotti N, Underwood M, et al. In vitro phenotypes to elvitegravir and dolutegravir in primary macrophages and lymphocytes of clonal recombinant viral variants selected in patients failing raltegravir. *J Antimicrob Chemother*. 2013 Nov;68(11):2525–32.
44. Sandy Hinckley; Sean Sherman; Brookie M. Best; Jeremiah Momper; Qing Ma; Scott R. Letendre; Ron Ellis; Anne Bang. Neurotoxicity Screening of Antiretroviral Drugs With

Human iPSC-Derived Neurons. In the Abstract book of the Conference on Retroviruses and Opportunistic Infections, Boston, MA, USA; 2016.

45. Cagla Akay-Espinoza, Anna L. Stern, Rebecca I. Nara, Nina Panvelker, Jean Li, Kelly L. Jordan-Sciutto. DIFFERENTIAL IN VITRO NEUROTOXICITY OF ANTIRETROVIRAL DRUGS. In the Abstract book of the Conference on Retroviruses and Opportunistic Infections, Seattle, WA, USA; 2017.
46. Hoffmann C, Welz T, Sabranski M, Kolb M, Wolf E, Stellbrink H-J, et al. Higher rates of neuropsychiatric adverse events leading to dolutegravir discontinuation in women and older patients. *HIV Med.* 2017 Jan;18(1):56–63.
47. Fettiplace A, Stainsby C, Winston A, Givens N, Puccini S, Vannappagari V, et al. Psychiatric Symptoms in Patients Receiving Dolutegravir. *J Acquir Immune Defic Syndr* 1999. 2017 Apr 1;74(4):423–31.
48. Gates TM, Cysique LA, Siefried KJ, Chaganti J, Moffat KJ, Brew BJ. Maraviroc-intensified combined antiretroviral therapy improves cognition in virally suppressed HIV-associated neurocognitive disorder. *AIDS Lond Engl.* 2016 Feb 20;30(4):591–600.
49. Nightingale S, Geretti AM, Beloukas A, Fisher M, Winston A, Else L, et al. Discordant CSF/plasma HIV-1 RNA in patients with unexplained low-level viraemia. *J Neurovirol.* 2016 May 18; •
50. Ritola K, Robertson K, Fiscus SA, Hall C, Swanstrom R. Increased human immunodeficiency virus type 1 (HIV-1) env compartmentalization in the presence of HIV-1-associated dementia. *J Virol.* 2005 Aug;79(16):10830–4.
51. Clarke A, Johanssen V, Gerstoft J, Clotet B, Ripamonti D, Murungi A, et al. Analysis of neurocognitive function and CNS endpoints in the PROTEA trial: darunavir/ritonavir with or without nucleoside analogues. *J Int AIDS Soc.* 2014;17(4 Suppl 3):19526.
52. Fabbiani M, Grima P, Milanini B, Mondì A, Baldonero E, Ciccarelli N, et al. Antiretroviral neuropenetration scores better correlate with cognitive performance of HIV-infected patients after accounting for drug susceptibility. *Antivir Ther.* 2015;20(4):441–7.
53. Dravid Ameet, et al. Cerebrospinal Fluid (CSF) HIV Escape is Associated with Progressive Neurologic Deterioration in Patients on Virologically Suppressive Antiretroviral Therapy (ART) in Western India. In the Abstract book of the European AIDS Conference, Barcelona, Spain; 2015.
54. Ciccarelli N, Fabbiani M, Di Giambenedetto S, Fanti I, Baldonero E, Bracciale L, et al. Efavirenz associated with cognitive disorders in otherwise asymptomatic HIV-infected patients. *Neurology.* 2011 Apr 19;76(16):1403–9.
55. Gustaf O. Hammarlund, Arvid Edén, Åsa Mellgren, Dietmar Fuchs, Henrik Zetterberg, Lars Hagberg, Staffan Nilsson, Aylin Yilmaz, Magnus Gisslén. CNS INFLAMMATION STILL PRESENT AFTER >10 YEARS OF EFFECTIVE ANTIRETROVIRAL THERAPY. In the Abstract book of the Conference on Retroviruses and Opportunistic Infections, Seattle, WA, USA; 2017.
56. Kugathasan R, Collier DA, Haddow LJ, El Bouzidi K, Edwards SG, Cartledge JD, et al. Diffuse White Matter Signal Abnormalities on Magnetic Resonance Imaging Are Associated

With Human Immunodeficiency Virus Type 1 Viral Escape in the Central Nervous System Among Patients With Neurological Symptoms. *Clin Infect Dis Off Publ Infect Dis Soc Am.* 2017 Apr 15;64(8):1059–65. ••