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Pharmacokinetics and Pharmacodynamics of Antiretrovirals in the Central Nervous System

Calcagno A, Di Perri G and Bonora S.

Unit of Infectious Diseases, Department of Medical Sciences, University of Torino, Torino, Italy.

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Corresponding Author:
Andrea Calcagno,
Department of Infectious Diseases, University of Torino
c/o Ospedale Amedeo di Savoia,
C.so Svizzera 164
10159, Torino, Italy
+390114393884, fax +390114393818
andrea.calcagno@unito.it

Abstract

Background HIV-positive patients may be effectively treated with highly active antiretroviral treatment and such strategy is associated with striking immune recovery and viral load reduction to very low levels. Despite undeniable results central nervous system is commonly affected during the course of HIV infection with neurocognitive disorders being as prevalent as 20-50% of treated subjects.

Objective This review discusses the pathophysiology of central nervous system infection by HIV and the barriers to efficacious control of such mechanism including the available data on compartmental drug penetration and on pharmacokinetic/pharmacodynamic relationships.

Methods Articles pertaining to cerebrospinal fluid and central nervous system transfer of antiretrovirals, as well as neurocognitive disorders were identified from PubMed and from references of included articles. Articles including animal data or *in vitro* studies were included only when providing original data on drug penetration mechanisms.

Results In the reviewed articles a high variability in drug transfer to the central nervous system is highlighted with several mechanism as well as methodological issues potentially influencing the observed results. Nevirapine and zidovudine showed the highest cerebrospinal fluid to plasma ratios although target concentrations are currently unknown for the central nervous system. The use of the composite cerebrospinal fluid concentration effectiveness score has been associated with better virological outcomes (lower HIV RNA) but inconstantly with neurocognitive outcomes.

Conclusion These findings support the central nervous system effectiveness of commonly used highly antiretroviral therapies. The use of antiretroviral drugs with increased cerebrospinal fluid penetration and/or effectiveness in treating or preventing neurocognitive disorders needs to be addressed in well-designed prospective studies.

1.0 Introduction

HIV enters the central nervous system (CNS) early in the natural history of the disease with cerebrospinal fluid (CSF) HIV RNA recovered as early as 8 days after infection. [1] The presence of viral replication (in perivascular macrophages and microglia and, although restricted, in astrocytes) is

eventually associated with neuronal damage due to persistent immune activation and cytokines production: the clinical endpoint of untreated CNS HIV infection is the appearance of dementia (HAD). [2,3] With the introduction of highly active antiretroviral treatment (HAART) the incidence of dementia significantly declined; nevertheless cognitive impairment (asymptomatic and moderate according to the impact on everyday life and globally defined as HIV-associated neurocognitive disorders, HAND) remains highly prevalent. [4] Although several authors highlight the impact of traditional risk factors (age, drug and alcohol abuse, previous head injuries, cardiovascular risk abnormalities, opportunistic infections) [5] on neurocognitive impairment in HIV-positive subjects the role of neuro-effective HAART is crucial: it is significantly associated with CSF viral control but inconsistently with the prevention and treatment of HAND.

The purpose of this review is to analyse the pharmacokinetics and pharmacodynamics of antiretroviral drugs in the central nervous system considering the effect both on compartmental viral replication and on neurocognitive impairment.

2.0 Methods

After including studies and reviews on pathogenesis, diagnosis and treatment of neurocognitive disorders in HIV-positive patients we focused on pharmacokinetic and pharmacodynamic data. The aim was to include all studies containing pharmacokinetic data pertaining to and using the following search terms: [(HIV AND (central nervous system OR cerebrospinal fluid) AND (pharmacokinetics OR pharmacokinetic OR pharmacodynamic OR passage)]. For the pharmacodynamic chpater the following search terms were used: [(HIV AND (CPE OR central nervous system concentration effectiveness score OR HIV RNA)]. Review articles were included for references finding. Articles were not restricted based on year of publication or language. Articles identified by the PubMed search were further screened manually by review of the the full article text.

3.0 Pathophysiology of CNS injury by HIV

The neuropathogenesis of CNS damage is generally considered to be initiated and driven by HIV invasion and replication within the brain parenchyma; productive infection of brain perivascular macrophages and endogenous microglia and restricted infection of astrocytes have been demonstrated. [6,7] Consequently neuroinflammation and immune activation of resident glia (macrophages, microglia, astrocytes) have been associated with indirect neuronal injury. [2] With no antiretroviral treatment activated microglia, infiltrating macrophages, reduced synaptic and dendritic density and neuronal loss are the neuropathological correlates of HAD. [8,9] With the introduction of HAART lymphocytes infiltration was markedly reduced (and limited to immune-reconstitution inflammatory syndrome cases) while neuroinflammation was observed in different anatomical sites: while in pre-HAART specimens basal ganglia were involved, in post-HAART samples hippocampus and adjacent parts of entorhinal and temporal cortex were frequently involved. [10,11] Inflammatory cytokines and chemokines [Tumor Necrosis Factor alpha (TNF-alpha), Interleukin-6 (IL6), Interleukin-10 (10), chemokine C-C motif ligand 2 (CCL-2), C-X-C motif chemokine 10 (CXCL-10)] have been found to be abnormally elevated in HIV-positive patients and they have been linked to the alteration of blood brain barrier (BBB): viral factors (TAT, gp120) and lipopolysaccharide have been also implicated. [3] The impairment in BBB function has a crucial impact on the pathogenesis of HAD since it facilitates the penetration of HIV-infected monocytes thus increasing the viral biomass in the CNS. [12,13] BBB damage may persist despite effective antiretroviral treatment and a low nadir CD4+ T lymphocyte cell count has been recently identified as a predictor of such event. [14-16]

The immune cell trafficking from and toward the CNS has the potential to sustain the persistence of residual viremia; although the exact origin of the latter is still debated [17] it has been proven that drugs with lower diffusion into tissues (such as protease inhibitors) have been associated to

either higher residual viremia or replication in sanctuary sites (such as lymph nodes).[18] Furthermore CNS has been recognized as a site of compartmentalized viral replication with the possible divergent evolution of HIV quasispecies. [19,20] Approximately 10% of patients have detectable HIV RNA in the CSF despite plasma viral control; this "CSF-escape" is usually transient and it is not associated with neurological sequalae. [21] However different resistance-associated mutations may be selected in the CSF and cases of symptomatic (and severe) CSF-escape have been constantly reported in recent years. [22,23]

The compartmental pharmacokinetic and pharmacodynamic profile of antiretrovirals may be of relevant importance both for HIV control in the CNS and for the reduction of viral biomass in reservoir sites in sight seeking a functional cure.

4.0 HAND

A consensus research definition of HAND includes the sub-classifications asymptomatic neurocognitive impairment, mild neurocognitive disorder, and HIV-associated dementia. [24] This categorization relies upon the execution of a full battery of neurocognitive tests (assessing at least five domains, including attention–information processing, language, abstraction-executive, complex perceptual motor skills, memory, simple motor skills, or sensory perceptual skills) and upon the determination of functional status (usually self-reported). Patients presenting abnormalities in two cognitive domains (age-adjusted scores one standard deviation lower than the average) are diagnosed with asymptomatic neurocognitive impairment (ANI) or mild neurocognitive disorder (MND) (with no or mild impairment in daily living respectively); significant deficit in two cognitive domains (with scores lower than two standard deviations) and impairment in everyday living are the diagnostic criteria for HIV-associated dementia (HAD). Considerable uncertainty is still undeniable in the diagnosis, determinants, prognostic factors and treatment of HAND although HAART has been

associated with significant improve in symptoms and CSF markers of immune activation and neuronal damage in patients with HAD. [4] One of the key questions is whether the diagnosis of ANI has any relevance in the course of HIV-infection: recent data suggest that patients with ANI may progress to MND and that they have a significant impairment in performance-based tests (potentially affecting adherence to medications). [25,26] The uncertainty in this area is enhanced by the diagnostic criteria that, to some extent, may overestimate the prevalence of asymptomatic and mild forms on neurocognitive impairment.

Furthermore several authors highlight the high prevalence of other risk factor for neurocognitive decline such as the increasing age, the high cardio- and cerebrovascular risk, the often under-diagnosed presence of psychiatric illnesses, the use of psychotropic substances and the prevalence of chronic hepatitis (and specifically HCV). [5,27] The challenge of studying HAND is having an adequate well-matched control group in which all these confounding factors may be accounted for. [28] Nevertheless some HIV-associated (CD4 cell count at nadir below 200/mm^3, plasmatic or CSF HIV replication, cell-associated HIV DNA) and some other risk factors (age above 50 years, HCV infection, metabolic and glucose abnormalities, cardiovascular risk) have been identified and they may help selecting patients for accurate neurocognitive screening and follow up. Finally a therapeutic approach is not clearly defined since controlling HIV replication may be necessary but not sufficient: neither higher CNS-penetrating combined antiretroviral therapy nor adjuvant treatments have so far proven to be effective in preventing and reversing HAND. [29]

5.0 Mechanisms of drug passage to the CNS

To be efficacious drugs must reach adequate concentrations at the site of action: in the case of CNS infection by HIV the targets are macrophages, microglia and astrocytes within the brain parenchyma. After intestinal absorption orally administered antiretrovirals (ARVs) (the vast majority of

available drugs, with the exception of intravenous zidovudine and subcutaneous enfuvirtide) are transported by plasma proteins in the bloodstream and distributed to organs and tissues. The CNS is reached by a considerable blood flow (approximately 14% of cardiac output) but two anatomical barriers can be found that prevent the free passage of drugs into the brain: the BBB and the blood CSF barrier (BCB). The first one is characterized by endothelial cells connected by tight junctions and by the presence of astrocytes end feet: several substances are restricted from crossing the BBB. [30] Nevertheless tight junctions are absent in some areas of the brain (hypothalamus, area postrema, subfornical organ) and direct diffusion is possible. Several mechanisms have been identified for crossing the BBB and they affect each compound ability to reach the brain tissue: paracellular aqueous pathway, transcellular lipophilic pathway, transport proteins, receptor-mediated transcytosis and adsorptive transcytosis. Therefore both patients' and drugs characteristics influence ARVs passage into the CNS.

The study of antiretrovirals pharmacokinetics in the CNS has two key obstacles: the scarce data on tissue concentrations and the intracellular target of action. Obtaining brain tissue concentrations is limited in healthy patients (for obvious ethical reasons) and associated to potential bias in sick individuals (brain biopsies are usually performed in patients with severe CNS diseases and this may impact the results of measured concentrations): data on autoptic measurements are limited and they may be influenced by the time elapsed from death to the procedure. Furthermore brain parenchyma concentrations derive from different compartments (averaged as single measurement per gram of tissue) and they may be influenced by preparation and analysis procedures. [31] Microdyalisis is another option for directly measuring brain extracellular concentrations (through the use of intracranial catheters): it is however an invasive technique and the results may depend upon compounds characteristics. [32,33] CSF concentrations are easier to obtain but their reliability as marker of CNS exposure is still debated. Cerebrospinal fluid is believed to be produced by filtration from blood plasma (for 2/3rd) and from brain extracellular fluid (for 1/3rd) from which it is separated by one layer of ependymal cells;

nevertheless some difference in drugs concentration may be observed if CSF is withdrawn from cisterna magna or from lumbar space. [34] Several animal studies have suggested that cerebrospinal fluid is a surrogate reliable marker for most of the studied drugs; although the variability in predicting tissue concentrations was high it was considerably lower than plasma unbound concentrations and comparable to microdyalisis. [33, 35, 36] As an example animal data (non-human primates) confirmed the good correlation between zidovudine CSF and brain parenchyma concentrations; [32] data for other ARVs are more variable and they have been recently reviewed. [37] Additionally drug concentrations in brain tissue are not uniform; they may vary with the distance from the CSF, with the vascularity of brain regions, and between white and grey matter. [38] Since the perivascular areas are probably the main objective of antiretroviral therapy this may not be relevant in the delivery of drugs to target cells. [39]

The second pitfall in the evaluation of CNS exposure is the site of action: with the exception of enfuvirtide and maraviroc all antiretrovirals have intracellular targets. While non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs) and integrase strand transfer inhibitors (ISTIs) once inside the cells are ready for exerting their activity, NRTIS need to be phosphorylated (thrice or twice) to become active and compete with endogenous nucleosides. The direct relationship between plasma and intracellular concentrations support the measurement of the former; however no data is currently available on the concentrations reached inside CNS macrophages, microglia or astrocytes.

5.1 Patients' characteristics and blood brain barrier damage

Older age may affect the passage of several drugs into the CNS: reduced blood efflux, permissive BBB and altered CSF flow are some of the potential mechanisms. [40] Being atherosclerosis and cerebrovascular disease common in older HIV-positive patients this may be relevant.

[41,42] Furthermore as a consequence of declining renal function plasma concentrations of several ARVs have been shown to increase with increasing age. The only available data suggest that while plasma concentrations of efavirenz and tenofovir are increased in older subjects, efavirenz CSF concentration have a steep increase after 60 years of age. [43]

Meningeal inflammation (usually observed in acute infection, rebound encephalitis, CSF escape or with opportunistic infections) has the potential to modulate the penetration of ARVs: this is mediated by blood flow, BBB impairment and pH modifications. The latter mechanism has been identified in bacterial meningitis but it may be relevant for drugs very sensitive to pH, such as raltegravir. [44]

Finally BBB impairment has been considered as a key event in the pathogenesis of AIDS dementia complex and other HIV-related neurological complications. BBB alterations were found in 2 to 22% HIV-positive asymptomatic individuals, in about 50% of patients with AIDS and in 100% of patients with HAD. [45-47] Furthermore altered permeability may persist in a subset of patients (mostly those ones with low CD4+ T-lymphocytes nadir) despite antiretroviral treatment and it has been associated with a higher prevalence of HAND. [14-16] Theoretically a permissive barrier may allow the passage of both drugs and plasma proteins thus increasing the CSF total concentration but reducing the free drug concentrations: the net effect on antiviral efficacy is currently not known. [48] Tenofovir, emtricitabine and raltegravir CSF concentrations have been shown to be higher in presence of altered BBB and to be directly proportional to CSF to plasma albumin ratios (CSARs). [49-51]

5.2 Drugs characteristics

Four chemical characteristics that affect drug passage have been identified: molecular weight (the smaller the higher), lipophilicity (the higher the higher, measured as octanol water distribution coefficient, LogP), ionization (the higher the lower) and plasma protein binding (the lower the

higher). In Table 1 molecular size, LogP and unbound plasma fractions are shown for available ARVs. Nucleoside reverse transcriptase inhibitors (NRTIs) are small, poorly bound molecules with a generally high CSF to plasma ratio; tenofovir is an exception to this example since it is positively charged and thus it requires active transport to cross the BBB. Molecular size and lipophilicity can be graphically plotted and an area of optimal characteristics can be drawn as in a recent paper by Marzolini and coll.: drugs with a distribution coefficient (LogD, a measure of pH-dependant lipophilicity) between -1 and +5 and with cross sectional area between 20 and 70 ($Å^2$) showed the highest penetration into the CSF. [52]

Protein binding has been classically identified as one of the key characteristics affecting drug distribution into organs and tissues; highly protein bound molecules have less unbound (or free) drug available for exerting the effect or being transported outside the blood stream. The effect of proteins on antiviral effect has been studies *in vitro*: at higher levels intracellular ARVs concentrations are reduced as well as their antiviral effect. [53] This seems to be confirmed in the CSF since a direct relationship between plasma unbound fraction and CSF to plasma ratios has been shown for some ARVs. [54-56] Measuring unbound CSF concentrations has proven to be more challenging due to low drug and protein concentrations: CSF albumin is usually 7.8-40 mg/L in CSF and 35-55 g/L in plasma, with normal CSAR ranging from 6 to 9.5 according to age. [57] Data are available for few compounds: CSF drug concentrations were shown to be very close to plasma unbound ones. [56, 58, 59] Etravirine passage is unexpectedly peculiar: despite very low unbound plasma concentrations (approximately 0.1%) total etravirine CSF to plasma ratio was around 4%. [60] However etravirine was found to be highly protein bound in the cerebrospinal fluid although the authors were not able to understand target proteins. This unexpected finding may be explained by specific binding to other plasma/CSF proteins or to the effect of concomitantly administered drugs (since etravirine is often administered with boosted protease inhibitors).

5.3 Transporters and pharmacogenetics

Several transporting proteins have been found to be expressed at the BBB and at the BCB: p-glycoprotein (P-gp), Organic Anion Transporter 1, 2 and 3 (OAT1,2 and 3), Breast Cancer Resistance Protein (BCRP) and others. P-gp has been extensively studied as it mediates ATP-dependant efflux of several drugs towards the bloodstream thus potentially reducing the amount available for reaching the brain parenchyma; it has also being implicated in refractory epilepsy. [61] Positron emission tomography techniques were used to quantify (both in animal and in humans) the effect of functionally or pharmacologically inhibited P-gp: several substrates showed a huge increase in brain parenchyma diffusion. [62] Other transporters have been less extensively studies but they are expressed at the brain barriers and, for instance, protease inhibitors have been shown to be substrate of OAT1A2. [63,64]

The importance of understanding drug passage across BBB and BCB lies in the modulatory effects on transporters and on the possible influence of genetic polymorphisms affecting enzyme activity or expression. In human primates (using nelfinavir and zosiquidar, a P-gp inhibitor) P-gp blocking was associated with modest increases in CSF concentrations but extensive increments in brain concentrations. [65] Marzolini and coll. recently published their *in vitro* work on transporter kinetic measurements showing that large lipophilic drugs such as PIs have strong binding affinities to drug efflux transporters expressed at the BBB and thus are prevented from entering the brain. [52] When combined, ritonavir (having the highest affinity) will occupy a large proportion of the transporter binding sites and thus slow down the efflux rate of the co-administered PI thereby facilitating its brain entry. This was confirmed in a study comparing once-daily (800 mg with 100 mg ritonavir) to twice-daily darunavir (600 mg with 100 mg ritonavir twice-daily): CSF concentrations (as expected given the lower dose) but also CSF to plasma ratios were lower

possibly because of ritonavir reduced effect along the dosing interval. [66] Several other drugs are inhibitors or inducers of P-gp and the new pharmacoenhancer, cobicistat, has the same interacting potential on transporters (P-gp and BCRP) as ritonavir. [67,68]

Several genetic polymorphisms may affect metabolizing or transporting enzymes function or expression thus affecting drug exposure. While pharmacogenetic studies have extensively studied ARVs plasma pharmacokinetics, limited data are available on their effect on CNS exposure. Single nucleotide polymorphisms (SNPs) in *CYP2B6* have been associated with plasma efavirenz concentrations as well as to the occurrence of neuropsychiatric symptoms and withdrawal from treatment. [69,70] In a limited sample size study CYP2B6 slow metabolizing children had higher CSF nevirapine concentrations than fast metabolizers. [71] In the aforementioned study on darunavir CSF concentrations a borderline association was found between polymorphisms in the *SLCO1A2* gene (encoding for OAT1A2) and CSF concentrations. [66] Finally SNPs in the Hepatic Nuclear Factor 4 alpha (*HNFalpha4,* a nuclear factor implicated in the regulation of OATs) might explain some of the extreme variability observed in raltegravir CSF penetration. [51]

5.4 Plasma concentrations

A direct correlation between plasma and CSF concentrations has been demonstrated for the majority of ARVs (Table 1). Therefore factors affecting plasma concentrations may potentially affect CNS exposure; for instance unboosted atazanavir (400 mg without ritonavir) is associated with very low and often undetectable CSF concentrations, as expected from the low plasma exposure observed with such dosage. [72] Once-daily administered drug may therefore reach lower concentrations as it has been shown for darunavir/ritonavir (800/100 mg): even if no data are available it may also be relevant for abacavir (for which all data have been derived from the twice-daily dosage) and for maraviroc (studied at 150

mg once-daily with boosted protease inhibitors). [55, 73-76] Furthermore drug-to-drug interaction reducing plasma exposure of one ARV may significantly affect CNS exposure and efficacy.

6.0 ARVs' CNS penetration

Antiretrovirals CSF concentrations and pharmacokinetic parameters are summed up in Table 1. We briefly describe here some of the key pharmacological features of those compounds, according to drug classes.

6.1 NRTIs

NRTIs are small, hydrophilic molecules, poorly bound to plasma proteins reaching very variable CSF exposures. NRTIs are transported by Organic Anion Transporters (OATs) that have been showed to be present at the choroid plexus (OAT1 and OAT3); the modulation of their activity (either by other drugs such as probenecid or by genetic polymorphisms in the encoding genes) may be relevant for zidovudine, stavudine, lamivudine and tenofovir passage. [97] With the exception of didanosine (whose CSF exposure has been found to be undetectable or very low) the other NRTIs have been associated with therapeutic CSF concentrations. Tenofovir is ionized at physiological pH and this limits its uptake by membrane transporters. [50, 73, 77-96] CSF tenofovir concentrations have been described as very low (and with no sample above IC50, 201 ng/mL); previous animal data suggested a good CSF passage (through the blood CSF barrier and OATs-independent) but a poor penetration into deep brain tissue. [98]

6.2 NNRTIs

NNRTIs show different properties but they are small, lipophilic, highly protein bound (with the exception of nevirapine) compounds. [51,

58, 60, 71, 87, 99-103] The neuropsychiatric effects in efavirenz-recipients account for its passage into the CNS: nevertheless being the IC50 very low (0.5-1.3 ng/mL) and close to the limit of detection of the instruments, a few studies reported a poor passage into the CSF. While the data on rilpivirine (one single study) and on etravirine (two reports) are still limited, nevirapine high CSF to plasma ratios has been constantly confirmed: the compound properties as well as the *in vivo* data suggest that nevirapine is one of the ARVs with the highest CSF penetration.

6.3 PIs

PIs are large (with molecular weights above 500 Da), lipophilic, highly protein-bound (with the exception of indinavir) compounds with CSF concentrations approximately 1% of plasma concentrations; [54, 56, 59, 72, 104-121] they have been recognized as substrate of p-glycoprotein as well as OAT1A2 and this may limit the drug accumulation into the CNS (as well as into other key tissues such as lymph nodes). [64,116] While tipranavir has not been studied, the data in first-generation PIs were disappointing with nelfinavir, saquinavir and amprenavir being undetectable or below IC50s in most of the patients. Indinavir CSF exposure was somehow higher probably for the lower binding to plasma proteins: CSF concentrations were above the IC95 concentrations and it was mostly unbound (98.6%). The comparison among the three commonly prescribed protease inhibitors (atazanavir, lopinavir and darunavir) favours the last two since most of atazanavir concentrations were very low or undetectable. [117]

6.4 Entry inhibitors (Fusion inhibitors and CCR5 antagonist)

Enfuvirtide is a synthetic 36 amino acid oligopeptide (interacting with viral gp41) with a very large molecular weight: a single study

confirmed that CSF concentrations were below the limit of quantification (25 ng/mL) while a case report of emerging enfuvirtide-resistant CSF (and then plasma) viruses reported a CSF concentration of 55 ng/mL. [118, 119]

Maraviroc is a small, lipophilic, intermediately protein-bound compound that targets the human co-receptor CCR5 and that is effective in preventing R5-tropic HIV viruses entry into target cells. It is substrate of both cytochrome P450 3A4 and p-glycoprotein and drug-to-drug interactions, potentially affecting CSF penetration, have been reported. The available data have been obtained with twice-daily dosages (150 mg with PIs, 300 mg with NRTIs and nevirapine and 600 mg with efavirenz or etravirine): CSF concentrations were detectable, 2-3% of plasma concentrations and in the EC90 range (0.06-10.7 ng/mL). [55, 74-76]

6.5 Integrase Strand Transfer Inhibitors

Integrase inhibitors are the latest ARV drug class and they are somehow heterogeneous: while they are small, highly protein bound molecules, their lipophilicity varies considerably (raltegravir is hydrophilic while elvitegravir is lipophilic). So far no data has been released on elvitegravir CSF exposure while a single unpublished study reported dolutegravir low CSF to plasma ratios (0.4%) but CSF concentrations above IC50 in all samples [122]. Raltegravir pharmacokinetics has peculiar characteristics: very wide inter and intra-individual variability and an unclear pharmacokinetic/pharmacodynamic relationship. [49, 51, 123] Even if pH-dependant absorption may explain much plasma variability, raltegravir CSF to plasma ratios have been described as varying from 3 to 20%.

7.0 PHARMACOKINETIC/PHARMACODYNAMIC

7.1 Target concentrations

The study of the pharmacodynamic effect of ARVs in the CNS is complicated by the absence of a clear target. The optimal marker would be the inhibition of HIV tissue replication in the whole brain parenchyma: such marker is currently not feasible.

The use of CSF HIV RNA as a marker of antiviral activity is the most commonly used marker since it decreases with the introduction of HAART it parallels cognitive improvement in patients with HAD. [124-127] Nevertheless commercial kits for measuring HIV RNA have not been validated in the CSF and the threshold is currently unknown. Second generation methods can quantify as low as 20 copies/mL; very sensitive experimental techniques (quantifying 2 copies/mL) have been assessed and residual viremia (between 2 and 50 copies/mL) was associated with worse cognitive function. [128,129] The measurement of other CSF markers (such as neopterine or CCL2) may be useful for understanding the pathogenesis of neuronal damage and, potentially, for monitoring changes in immune activation or neuronal function but it is still not used but for research purposes. [3, 130]

The use of magnetic resonance spectroscopy (MRS) and positron emission tomography (PET) has the potential to describe neuronal integrity in different areas of the CNS and they have successfully been used to describe ARV effect: nevertheless these techniques are expensive, time-consuming and not-standardized. [131] A recent study using a selective ligand for the translocator protein expressed by activated microglial cells ([11C]-PK11195) showed that HIV-infected patients with longstanding virological suppression on cART and without comorbidities or drug and alcohol misuse, had focal areas of activated microglial cells, indicative of neuroinflammation, in several cortical regions. [132]

Finally one of the possibilities would be to monitor cognitive function after the introduction of ARVs: most of the studies reported an improvement after antiretroviral treatment initiation or modification (Table 2). [133] Nevertheless complete neurocognitive testing is time-consuming and it may be influenced by the choice of the control group and by learning effect (patients repeating slightly-modified tests may perform better). [28, 134]

Given the inaccessibility of *in vivo* brain tissue, CSF inhibitory concentrations (IC50, IC90 and IC95) have been used to compare the adequacy of ARVs exposure: this concentrations represent the level at which 50%, 90% or 95% of *in vitro* viral replication is inhibited (using wild-type viruses). However these *in vitro* protein-free concentrations have significantly variable values and the same drug has been judged to reach optimal or insufficient concentrations in different studies when compared to different thresholds. [49, 51, 123] A recent study have quantified both protein-free and protein-corrected ICs of several antiretrovirals using a standardized methodology; [135] our group recently reported better CSF viral control (as CSF HIV RNA below 50 copies/mL and a lower prevalence of CSF escape) when drugs showed higher 95% inhibitory quotients (as CSF exposure divided by IC95, derived by the aforementioned study). [136]

Following these observations there is no single PD marker in the CNS; the most reliable target would be the complete control of tissue viral replication.

7.2 CSF escape

In the majority of patients CSF HIV RNA is lower than plasma HIV RNA (approximately 1 Log_{10}): higher CSF viral loads have been associated with active neurological symptoms and with a shorter time to develop HAND. [137] In some patients despite plasma viral control

CSF HIV RNA may be detectable or 1 Log_{10} higher: this condition has been defined as “CSF escape”. The exact clinical relevance of CSF escape is currently unknown since it may occur in approximately 10% of patients on HAART and no neurological impairment was observed in a longitudinal study after 5 years of follow up: this event may therefore be similar to the emergence of plasma “blips”. [21, 138] However two case series and several case reports have clearly documented the concrete, though uncommon, possibility of symptomatic CSF escape: severe neurological syndromes and neuroradiological findings have been documented. [22,23, 139-142] In most of the subjects differential viral evolution (with resistance-associated mutation selected in the CSF compartment) was shown and it was explained by asymmetrical penetration of ARVs (with some cerebrospinal fluid concentrations below the limit of detection) but not confirmed by other reports. [143] In a large longitudinal study the factors associated with CSF escape were the presence of CSF pleocytosis, the use of a PI-containing HAART and ultrasensitive plasma HIV RNA level: [144] the poor CSF to plasma ratios observed with protease inhibitors (0 to 1.4% with currently used PIs) may possibly explain these results as well as persistent intrathecal immune activation and plasma residual viremia. In symptomatic patients, switching HAART using more neuro-effective drugs has been shown to improve symptoms and to reduce the CSF viral load, and it appears advisable.

7.3 Efficacy of monotherapy versus combination antiretroviral treatment

For a few compounds pharmacodynamic data are available: patients received monotherapy and CSF HIV RNA decay was monitored. While lopinavir/ritonavir and zidovudine had a significant effect on cerebrospinal fluid replication didanosine and saquinavir showed no significant effect. [145,146] Abacavir was tested as an adjunctive therapy in patients with HAD: neurocognitive performance and CSF HIV RNA

showed no significant change. [86] Protease inhibitors monotherapies have been tested given the need for reducing long-term toxicities and drug expenditure: this strategy is less effective than triple therapy but it is efficacious in the majority of patients. Concerns have been raised on the compartmental activity of low penetrating drugs such as PIs: several data on neurocognitive tests and a review of available data were reassuring on the effect of such strategies. [147-150] Nevertheless a few patients on darunavir/ritonavir (2 from the MONOI study), lopinavir/ritonavir and several subjects on atazanavir/ritonavir as single agents presented neurological symptoms and elevated CSF HIV RNA despite plasma viral control (3/20 in the ATARITMO study with atazanavir). [151,152] Furthermore even in patients with controlled CSF HIV RNA S100beta (a marker of astrocyte damage) rapidly increased after the interruption of NRTIs. [153]

Combination antiretroviral treatment is usually effective in the CNS compartment and a rapid decay in CSF HIV RNA is observed; however in some cases viral decay in the CSF and blood may differ. Slower decay of CSF HIV RNA has been noted in subjects with HAD and lower CD4 cell counts. [125,126, 154,155] Ninety percent of patients with undetectable plasma HIV RNA presented CSF HIV RNA below 50 copies/mL: nevertheless a compartmental residual viremia was measurable through sensitive methods. CSF low-level viremia was associated with neurocognitive impairment, with increased immune activation and it was unresponsive to intensification strategies (with maraviroc, enfuvirtide or raltegravir). [128,129, 156,157]

7.4 The CPE score

The CNS Concentration Effectiveness score (CPE score) has been proposed by a large collaborative study group in the USA (the CHARTER group): [158] in the revised 2010 version ARVs were scored 1 to 4 (where 4 is the most neuro-effective drug) according to drug

characteristics, pharmacokinetics and pharmacodynamic properties. [159] The composite CPE (obtained adding single drug scores to obtain a treatment score) has been used in several studies leading to conflicting results. Most of the studies found a lower CSF HIV RNA with higher CPE score while the effect on immune-activation, MRI cerebral metabolites concentrations and neurocognitive testing were less concordant among studies: the results are summed up in table 2. [160-172] Furthermore while several retrospective studies found an association between higher CPE scores and lower CSF viral loads [153, 154, 173-175] only one study (out of three) found a correlation with CSF escape. [21, 143, 176] Some reports tried to define a CPE cut off: respectively a value of 6 or 7 were found to be associated with heterogeneous CSF outcomes. [143,144, 173, 177]

Some limitations of the CPE score must be highlighted: the limited amount of evidence regarding PD data and regarding drugs standard dosages, the absence of a clear cut off, the validation in patients receiving triple therapies and with fully sensitive viruses. As an example a CPE corrected for plasma resistance associated mutations was a better predictor (compared to standard CPE) of HAND in a cross-sectional study. [178] For these reasons some authors (and the Italian guidelines) prefer not to use the aggregate CPE but they suggest that treatment optimization in patients with CNS diseases may include drugs with individual elevated neuro-effective score. [174, 179]

The CPE score is therefore a valuable and easy to use tool to implement the use of neuro-active drugs although with some limitations. Nevertheless a recent review using rigorous methods found that neuroHAART was effective in improving neurocognitive function and decreasing CSF viral load (although only two of those studies were adequately statistically powered): this confirms the possible optimization of CNS treatment and calls for prospective, randomized, adequately powered studies. [180] A very interesting study (randomized and controlled) was conducted by Ellis and coll. but unfortunately it was prematurely interrupted for slow accrual (326 patients screened and 59

enrolled): CNS-targeted HAART was not associated with either virological nor neurocognitive improvements although in patients with baseline suppressed viral load a trend for improved cognitive performances over time was observed. [171]

7.5 Efficacy in monocytes, macrophages and astrocytes

Given the peculiarity of infected cells in the CNS and several *in vitro* data, an increasing interest arouse on ARVs activity on monocyte, macrophages and astrocytes. *In vitro* data suggest that the endogenous nucleoside pool in resting macrophages is smaller than the one in activated lymphocytes and therefore that the effective phosphorylated NRTI concentrations required to inhibit HIV replication may be lower. [181] Shikuma and coll. used *in vitro* effective concentration in acutely infected macrophages (EC50) to calculate a "monocyte efficacy score" (ME score: 1/EC50*1000): surprising results were observed with tenofovir being 17 times more efficacious than abacavir (50 versus 3).[182] In 139 patients the composite score was nicely associated with neurocognitive performance and with presence of HAND or minor motor cognitive disorder.

Recent data challenging infected astrocytes with several NRTIs, NNRTIs and raltegravir reported that some drugs (zidovudine, lamivudine and stavudine) may have inadequate inhibitory activity in astrocytes, with 90% effective concentrations (EC90) exceeding those achievable in the CSF. [183]

These preliminary observation warrant further studies on the differential efficacy of ARVs according to target cells: the repeated association between HIV reservoir size (measured as PBMC- or monocyte-associated quantitative HIV DNA) and HAND support the implementation of

specific drug strategies in selected patients (those with low CD4+ cells nadir, high HIV RNA zenith and high cumulative viremia for instance). [184, 185]

7.6 Potential adjunctive effect of maraviroc in the CNS

Maraviroc is a CCR5 antagonist that bind the human co-receptor thus preventing the stable interaction between R5-tropic HIV and target cells: the mechanism of action is therefore peculiar since it blocks an endogenous receptor and it has an extracellular target. The compound has been associated with some immunological benefits such as a higher CD4 increase and, although less than expected, reduced immune activation in patients with poor immunological recovery. [186] The drug, used in combination with other ARVs, has been proven to be effective in blocking HIV entry both in naïve and in experienced patients. The CNS target cells are usually expressing the CCR5 and most of the viruses are R5 tropic in the CSF (even if patients harbour X4-tropic viruses); discordant tropism (X4 in CSF samples and R5 in plasma) has been rarely reported thus suggesting that maraviroc may be effective in treating CNS HIV infection in most of the patients. [187]

While being CNS protective as monotherapy in macaques model and suppressing CSF HIV RNA in patients with neurological symptoms, three studies evaluated the effects of maraviroc intensification. In one it was not associated with the control of CSF residual viremia despite good compartmental penetration. [156] After 14 days of treatment intensification small increases in cerebral metabolite markers of neuronal integrity (NAA/Cr ratios) were observed and they were associated with maraviroc plasma exposure; concomitantly higher plasma concentration were associated with lower CSF CXCL10 (IP-10) concentrations, an inflammatory chemokine. [188,189] Both for its activity

in CNS target cells and for the non antiviral properties maraviroc treatment (either as switch or as intensification) may be an option in neurologically impaired HIV-positive patients with suppressed plasma viral load.

8.0 ARV toxicity in the CNS:

It must be highlighted that most ARVs have a well-described toxicity in the peripheral nervous system while little is known on their toxicity profile in CNS neurons. Some *in vitro* data (immortalized cell lines and peripheral dorsal root ganglia neurons) showed the potential for ARVs to produce neuronal damage: using primary cultures of rat forebrain, Robertson and coll. showed that several antiretroviral achieved toxic concentrations in the CSF without any additive effect. [190-192] Recent data further explored this hypothesis and the production of oxygen reactive species was confirmed in pigtail macaques and rats *in vivo* (with the exposure to zidovudine, saquinavir and ritonavir). [193]

PIs and efavirenz have been associated with glucose and metabolic disturbances eventually leading to dyslipidaemia, glucose intolerance and to abnormal fat distribution (lipodystrophy); the cumulative exposure to PIs has further being implicated in the increasing cardiovascular event observed in HIV-positive patients. [194] Previous studies suggest that HIV-infected patients are at increased risk of ischemic cerebrovascular disease, potentially caused by infective vasculitis, brain opportunistic diseases, cardiac embolism, hypercoagulopathy, or HIV infection itself. [195,196] Among a variety of brain vessel diseases, cerebral small vessel disease (CVSD) has been associated with ischemic stroke during life and cerebral infarction at autopsy. Recently it was demonstrated that mild and moderate/severe small vessel diseases were associated with protease inhibitor-based HAART exposure and that HAND was associated with mild CSVD (after adjusting for vessel mineralization, HIV encephalitis, microglial nodular lesions, white matter lesions, or older age).[197] Further to this potentially relevant effect on cerebrovascular disease, PI-based

combination treatment has been associated with reduced amyloid phagocytosis and increased neuronal accumulation justifying some of the shared and clinical features with Alzheimer's dementia. [198,199]

Efavirenz effects in the CNS are well-characterized (abnormal dreams, dizziness) and associated with higher plasma concentrations and to single nucleotide polymorphisms in genes encoding for proteins involved in the drug metabolism or transport. Furthermore being on efavirenz was independently associated with the diagnosis of HAND in a cohort of stable HIV-positive patients. [200] One recent study reported that cognition improved for up to 96 weeks in a group of immunologically and virologically stable patients who elected to come off treatment; the improvement was significant in all participants but greater in efavirenz recipients. [133]

These results raise the possibility that ARVs concentrations to some extent may have some detrimental effects: this may be particularly relevant for individuals with specific genetic profiles but it must be compared to the clear beneficial effect of HAART on compartmentalized viral control.

9.0 Conclusions

Highly active antiretroviral treatment is very effective in controlling HIV replication and in increasing patients' immune system thus preventing opportunistic diseases. In the central nervous system the same rule applies, although persistent immune activation have been demonstrated despite antiviral efficacy. Antiretrovirals penetration into the CNS may depend on several drug and patient characteristics: the use of more neuro-effective drugs (high penetration and compartmental activity) has been associated with better cerebrospinal fluid viral control and in some, but not all studies, with better neurocognitive performances. ARV regimens based on neuro-effective drugs may be suggested in patients with increased pharmacological needs (CSF escape, CNS compartmentalized viruses, high intrathecal immune activation) and neurocognitive disorders. The use of

antiretroviral drugs with increased cerebrospinal fluid penetration and/or effectiveness in treating or preventing neurocognitive disorders needs to be addressed in well-designed prospective studies aiming also at understanding the exact impact of antiretrovirals neurotoxicity.

Conflict of Interest

A. Calcagno has received travel grants or speaker's honoraria from Abbott, Bristol-Myers Squibb (BMS), Merck Sharp & Dohme (MSD) and Janssen-Cilag. S. Bonora. has received grants, travel grants and consultancy fees from Abbott, Boehringer-Inghelheim, BMS, Gilead-Sciences, GSK, MSD, Pfizer and Janssen-Cilag. G. Di Perri has received grants, travel grants and consultancy fees from Abbott, Boehringer-Inghelheim, BMS, Gilead-Sciences, GSK, MSD, Pfizer, Roche and Tibotec (Johnson & Johnson).

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Drug	Molecular weight (Da)	LogP	Protein binding (%)	Protein-free IC50 (ng/mL)	Protein-free IC95	Median CSF	Max CSF	Min CSF	CPR (%)	Correl CSF/P	References
NRTIs											
Abacavir	286	1.20	50	457.6	n.a.	128	384	37	36	n.a.	73, 84-87, 101
Didanosine	236	-1.24	<5	1180.0	n.a.	0	n.a.	0	negligible	n.a.	88, 89
Emtricitabine	247	-1.40	<4	70	n.a.	109	386	39	43	no	50, 90
Lamivudine	229	-1.40	16-36	549.6	n.a.	95-134	300	12	12-22	n.a.	91, 92, 101
Stavudine	224	-0.72	negligible	112.0	n.a	51.6	110	0	27	n.a.	91, 93-95, 101
Tenofovir	287	1.25	<7	201.6	n.a	5	32	<0.9	4	no	50, 96-98

Zidovudine	267	0.05	30-38	5.3	n.a	45-50	283	0	2-674	n.a.	78-85, 89, 91, 101
NNRTIs											
Efavirenz	315	4.60	99.5-99.7	1.3	4.7	11.1-13.9	51.8	0.2	0.5	n.a.	58, 90, 100
Etravirine	435	3.67-5.54	99.9	0.9	3.5	9.5	38.9	2	1-4.3	yes	60
Nevirapine	266	2.50	60	32	253	932	1837	219	62.6	n.a.	87, 101, 102
Rilpivirine	366	3.80-5.47	>99	0.27	0.7-1.3[a]	0.8	1.6	0.5	1.4	no	103
PIs											
Amprenavir	505	1.85	90	5.3	31	n.a.	123	<10	1.6	n.a.	105, 106
Atazanavir	704	4.50	86	1.7	6.5	7.9-10.3	40	<5	0.9	yes	59, 72
Darunavir	547	1.80	95	0.4	1.9	30-55.8	212	<0.4	0.6-1.4	yes	56, 59, 66, 108
Fosamprenavir	585	0.84-1.92	90	5.3	31	26.1-23.4	>200	<0.4	1.2	yes	107
Indinavir	613	2.90	60	4.3	21	174	693	94	9.9	yes	120-121
Lopinavir	628	3.91-4.69	98-99	3.1	17	11.2-26.4	74	<5	0.2-0.5	yes	54, 109-112
Saquinavir	670	3.8	98	3.6	14	<1.4	6.7	<1.4	negligible	n.a.	104, 113-115
Tipranavir	602	6.9	>99.9	53	261	n.a.	n.a.	n.a.	n.a.	n.a.	
EI and R5I											
Enfuvirtide	4491	n.a.	92	18-1260	n.a.	<25	<25	<25	negligible	no	118, 119
Maraviroc	513	3.6-4.3	76	0.05-2.3[a]	10.7[a]	2.6-35	173	<0.5	2.2-29	no	55, 74-76
ISTI											
Elvitegravir	448	4.5	98-99	3.9	54[a]	n.a	n.a.	n.a.	n.a.	n.a.	
Dolutegravir	419	0.98-1.10	>98.9	0.2	n.a.	18.2	23.2	3.7	0.4	yes	122
Raltegravir	444	-0.39	83	3.6	44	14.5-31	187	<2	3-20	yes/no	49, 51, 123

Table 1. Antiretrovirals characteristics and published cerebrospinal fluid exposure. "CSF" cerebrospinal fluid, "IC50" 50% inhibitory concentration, "IC95" 95% inhibitory concentration, "CPR" CSF to plasma ratio, "Correl CSF/P" correlation between CSF and plasma levels, "n.a." not available. [a]Respectively EC50 and EC90 values.

Reference	n	Design	CPE version	Higher CPE → CSF VL	Higher CPE → NC testing	Areas NC testing	CPE cut off
Cysique *et al.* [160]	37	prospective single arm	2008	**lower CSF VL**	**better NC tests**	6	≥2
Tozzi *et al.* [161]	185	prospective single arm	2008	not done	**better NC tests**	4 and 8	no
Marra *et al.* [162]	26	prospective single arm	2008	**lower CSF VL**	**worse NC tests**	8	≥2
Winston *et al.* [163]	30	prospective randomized	2008	not done	**better NC tests**	Cogstate	no
Smurzynski *et al.* [164]	2636	prospective single arm	2008	not done	**better NC tests with >3 drugs**	3	no
Arendt *et al.* [165]	3883	prospective single arm	2010	**lower CSF VL** n=68	**better NC tests**	2	no
Garvey *et al.* [166]	101	retrospective single arm	2008 & 2010	not done	**no effect**	Cogstate	no
Rourke *et al.* [167]	545	prospective single arm	2008 & 2010	not done	**better NC tests**	4	≥1.5 (2008)
Robertson *et al.* [168]	860	prospective randomized	2010	not done	**no effect**	4	no
Ciccarelli *et al.* [169]	101	prospective single arm	2010	not done	**better NC tests**	8	≥6
Kahouadji *et al.* [170]	54	prospective single arm	2008	not done	**worse NC tests**	2	no
Ellis *et al.* [171]	49	prospective randomized	2008	**no effect**	**no effect**	8	no (2.5 vs. 1)
Vassallo *et al.* [172]	246	prospective controlled	2010	not done	**stable or better NC tests**	8	no (8.1 vs. 6.9)

Table 2. Studies investigating the relationship between CNS concentration effectiveness score (CPE) and cerebrospinal fluid HIV RNA and/or neurocognitive performance. CPE version 2008 ans 2010 are respectively referenced as [155] and [156]. "VL" viral load, "NC" Neuro Cognitive.