

REVIEW

Shock and kill within the CNS: A promising HIV eradication approach?

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Abstract

The most studied HIV eradication approach is the “shock and kill” strategy, which aims to reactivate the latent reservoir by latency reversing agents (LRAs) and allowing elimination of these cells by immune-mediated clearance or viral cytopathic effects. The CNS is an anatomic compartment in which (persistent) HIV plays an important role in HIV-associated neurocognitive disorder. Restriction of the CNS by the blood–brain barrier is important for maintenance of homeostasis of the CNS microenvironment, which includes CNS-specific cell types, expression of transcription factors, and altered immune surveillance. Within the CNS predominantly myeloid cells such as microglia and perivascular macrophages are thought to be a reservoir of persistent HIV infection. Nevertheless, infection of T cells and astrocytes might also impact HIV infection in the CNS. Genetic adaptation to this microenvironment results in genetically distinct, compartmentalized viral populations with differences in transcription profiles. Because of these differences in transcription profiles, LRAs might have different effects within the CNS as compared with the periphery. Moreover, reactivation of HIV in the brain and elimination of cells within the CNS might be complex and could have detrimental consequences. Finally, independent of activity on latent HIV, LRAs themselves can have adverse neurologic effects. We provide an extensive overview of the current knowledge on compartmentalized (persistent) HIV infection in the CNS and on the “shock and kill” strategy. Subsequently, we reflect on the impact and promise of the “shock and kill” strategy on the elimination of persistent HIV in the CNS.

Abbreviations: ART, antiretroviral therapy; BAF, BRG1/BRM-associated factor; BBB, blood–brain barrier; BET, bromodomain and extra terminal domain; C/EBP, CCAAT/enhancer binding protein; COUP, chicken ovalbumin upstream promoter transcription factor; CRF, circulating recombinant form; CSF, cerebrospinal fluid; CTIP2, COUP-TF interacting protein; DSIF, DRB-Sensitivity Inducing Factor; HAD, HIV-associated dementia; HAND, HIV-associated neurocognitive disorder; HDAC, histone deacetylase; HDACi, histone deacetylase inhibitor; HIC1, hypermethylated in cancer 1; HMAG1, high mobility group AT-hook 1; HMT, histone methyltransferase; HP1, heterochromatin protein 1; iPSC, induced pluripotent stem cell; LRA, latency reversing agent; LSD1, lysine specific demethylase; LTR, long terminal repeat; NELF, negative elongation factor; NFL, neurofilament light chain; NIK, NF- κ B inducing kinase; NR4A2, nerve growth factor IB-like nuclear receptor Nurr1; PET, positron emission tomography; PKC, protein kinase C; P-TEFb, positive transcription elongation factor; PWH, people with HIV; RNAPII, RNA polymerase II; SMAC, second mitochondria-derived activator of caspases; SP, SV40-promoter specific factor; TAR, transactivation response element; Tat, transactivator protein; TF, transcription factor; TRBP, TAR RNA binding proteins.

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astrocytes, CNS, compartmentalization, eradication, HAND, HIV, inflammation, latency, latency reversal, microglia, perivascular macrophages, persistence, reservoir, shock and kill

1 | INTRODUCTION

HIV-1, referred to as HIV from this point, is the major cause of AIDS. HIV is one of the major global health challenges, with approximately 38 million people infected.^{1,2} Despite the success of antiretroviral therapy (ART) at suppressing viral replication and reducing AIDS-related morbidity and mortality, HIV cure remains elusive due to the presence of latently infected cells and subsequent rebound viremia after ART interruption or cessation.³ Consequently, permanently eliminating the replication competent virus without the need for lifelong therapy is the ultimate goal for HIV cure.

HIV can reside in anatomic compartments including the CNS.^{4,5} HIV RNA and/or DNA have been detected in the cerebrospinal fluid (CSF)^{6–11} and postmortem CNS (myeloid)-resident cells, such as microglia and perivascular macrophages, in both untreated and virally suppressed individuals.^{12–15} The CNS is an immune-restricted anatomic compartment shielded from the periphery by the blood–brain barrier (BBB) with a unique microenvironment consisting of CNS-specific cell types, transcription factors (TFs), and immune surveillance.^{5,16,17} The adaption and isolated replication of HIV in the CNS, along with the poor penetration of ART across the BBB, give rise to genetically distinct HIV CNS populations, called compartmentalization in both ART naïve and treated individuals.^{10,11,18,19}

It is likely that HIV can persist in the brain and has the potential to cause a rebound of viremia upon ART cessation. Therefore, to achieve cure, HIV also needs to be eliminated from the CNS. Multiple strategies are currently designed that aim to eradicate the persistent HIV reservoir.²⁰ The most studied approach is the “shock and kill” strategy, aimed to reactivate (“shock”) the latent reservoir by latency reversing agents (LRAs), which will be subsequently cleared (“kill”) by the immune system or by virus-induced cytopathogenicity.²¹ Because of the BBB and the presence of genetically diverse compartmentalized viral populations with differences in HIV transcription profiles, reactivation of HIV in the brain by LRAs might be complex, occur with different effectiveness or have detrimental consequences on brain functioning.^{22–24} In this review, we will provide an extensive overview of (persistent) HIV infection in the CNS and the current knowledge on the “shock and kill” strategy. Subsequently, we will reflect on the impact and promise of the “shock and kill” strategy on the elimination of persistent HIV in the CNS.

2 | HIV INFECTION AND PERSISTENCE IN THE CNS

2.1 | HIV entry in the CNS

HIV causes infection of predominantly CD4⁺ T cells in lymph nodes at site of transmission, as reviewed earlier.²⁵ A few days after initial

infection, these infected CD4⁺ T cells start circulating throughout the body. Particularly high numbers of HIV-infected cells are present in the GALT, contributing to a systemic peak viremia in the first weeks after initial infection.²⁶ During this systemic peak of viremia, HIV can spread to various other tissues and anatomic compartments,^{27,28} including the CNS.^{4,29}

Exposure of the BBB to the HIV envelope and/or the HIV extracellular transactivator protein (Tat) in the periphery may cause increased permeability of the endothelial cell layer, a down-regulation of the tight junction proteins, all contributing to the penetration of HIV into the CNS.^{30–33} Moreover, proinflammatory cytokines and chemokines are secreted in the periphery and in the CNS during HIV infection, making the BBB more permeable³⁴ (Figure 1A). The most widely accepted mechanisms for the entry of HIV in the CNS are migration of either circulating cell-free virus, or trafficking of HIV-infected CD4⁺ T cells, and to a lesser extent, infected monocytes crossing the BBB into the CNS^{35–38} (Figure 1A). HIV RNA and inflammatory markers can already be detected in the CSF within the first weeks after infection.^{6–10} Generally, the viruses in CSF during this early stage of infection are largely derived from blood, which is termed a *noncompartmentalized* or *equilibrated* CSF infection.³⁹ In line with this observation, it was shown that infected CD4⁺ T cells could be detected in the CNS of SIV-infected rhesus macaques already 12 days post infection, whereas the number of infected monocytes was limited.⁴⁰ These observations are suggestive for migration of infected CD4⁺ T cells to be the primary mechanism for HIV entry in the brain.

2.2 | HIV neuropathogenesis

HIV infection can lead to an impairment of neurocognitive function, resulting in HIV-associated neurocognitive disorders (HAND). Because of the large variations seen in clinical symptoms and their severity, HAND is categorized in: asymptomatic neurocognitive impairment, mild neurocognitive disorder, and HIV-associated dementia (HAD).¹ Before introduction of ART, HAND was commonly seen in up to 70% of all patients with AIDS or symptomatic HIV infection⁴¹ of whom 20–30% developed HAD.⁴² Within the ART era, HAND is still seen in up to 42% of individuals,⁴³ but luckily HAD has significantly decreased.⁴¹

The development of HAND is characterized by pathologic neuronal degradation. Neuroimaging via MRI and computed tomography show atrophy of cerebral regions.^{44,45} Moreover, already soon after infection elevated levels of the biomarker neurofilament light chain (NFL), associated with neuronal injury, can be measured in the CSF.⁴⁶ The presence of neuronal loss implies that HIV infection either directly or indirectly leads to neuronal death. Infected cells release viral proteins such as gp120 and Tat, which are neurotoxic and promote neurodegeneration,^{47–49} (Figure 1A). Furthermore, CNS immune

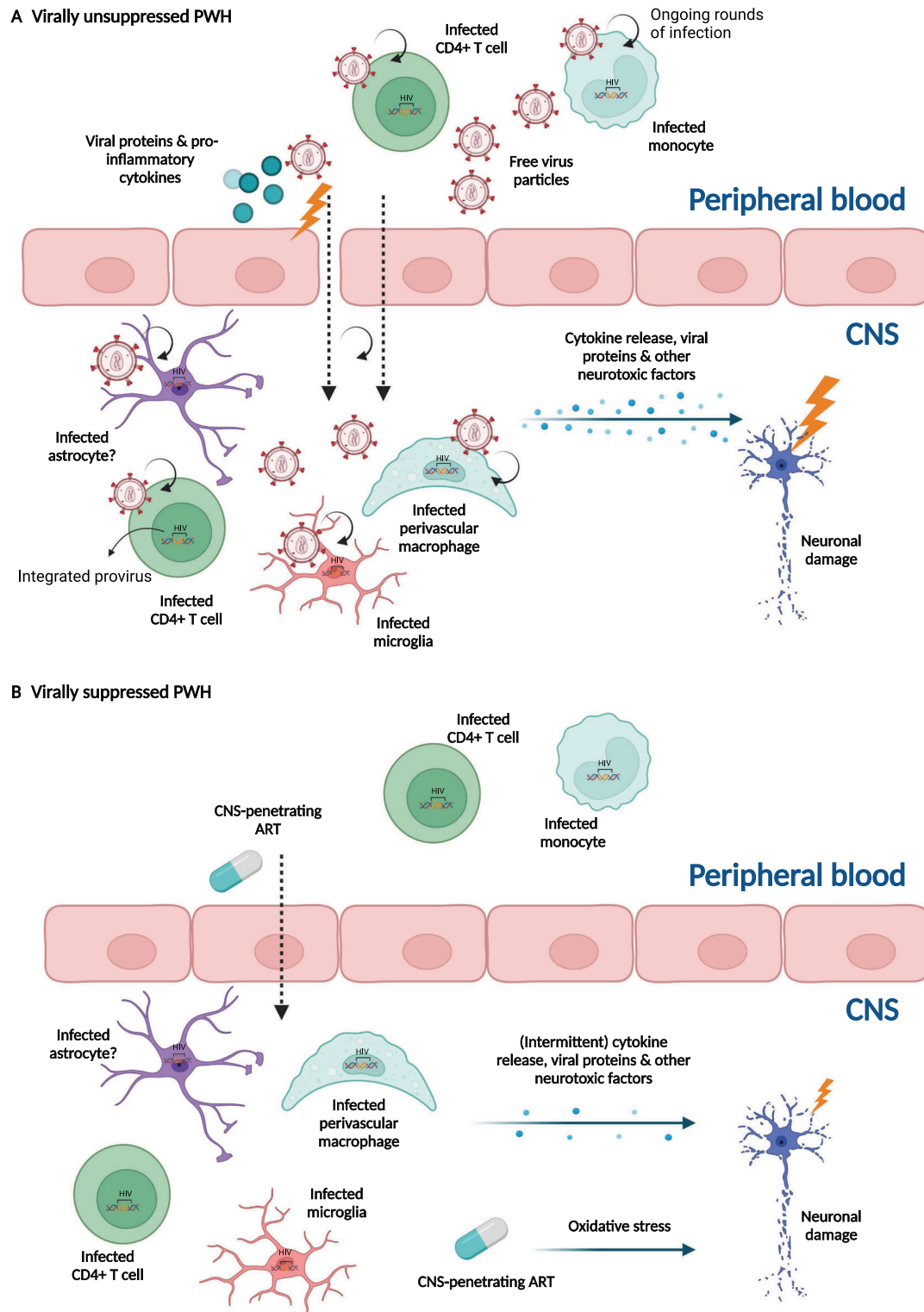


FIGURE 1 Overview of neuropathogenesis in virally unsuppressed and suppressed PWH. **(A) Virally unsuppressed PWH:** The exposure of viral proteins and inflammatory cytokines may cause increased permeability of the blood–brain barrier. This can contribute to the entrance of HIV within the CNS via free virus particles, infected CD4⁺ T cells, or infected monocytes. Subsequently, cells in the CNS can be infected. Ongoing rounds of viral infection occur, within the periphery and the CNS. Consequently, there can be a continuous influx of peripheral virus into the CNS. The presence of virus and viral proteins, release of cytokines and neurotoxic factors might cause neuronal damage and contribute to the development of HIV-associated neurocognitive disorder (HAND). **(B) Virally suppressed PWH:** In suppressed PWH, CNS-penetrating ART can pass the blood–brain barrier. These might cause oxidative stress, which might contributing to neuronal damage in these individuals. Cells in the periphery and CNS can be infected from before the start of therapy, but there are no rounds of ongoing infection. Nevertheless, these infected cells can still produce virus, cytokines, or can be intermittently activated, possibly also contributing to neuronal injury. *Created with Biorender.com.*

activation will occur resulting in additional neurotoxicity via neuroinflammation and the release of proinflammatory cytokines, which are also neurotoxic,^{9,50–53} (Figure 1A). Apart from viral factors, HAND was also shown to be increased by the presence of systemic inflammation and the presence of common comorbid conditions such as cardiovascular disease, chronic lung disease and diabetes.⁵⁴

It has been shown that initiation of ART causes a reduction in inflammatory markers in the CSF and brain,^{52,55–57} and reduces but does not completely reverse neuronal injury.⁵⁸ Also the incidence of HAND in HIV-suppressed individuals remains high, albeit its severity decreases.⁴¹ This might be caused by ART-induced oxidative stress in neurons, as reviewed in Brew *et al.* and Ghosh *et al.*,^{47,59} (Figure 1B). Furthermore, it is interesting to note that presence of infected cells in the CSF is related to neurocognitive disorders.⁶⁰ Moreover, some levels of inflammation markers in CSF were correlated to neurocognitive performance and others not.^{55,60} Also low levels of HIV RNA in CSF most likely caused by viral production from activated cells may contribute to HAND,^{60–62} (Figure 1B). Interestingly, activation of cells in the CNS was observed in virally suppressed individuals who underwent a positron emission tomography (PET) scan, of which the activation did correlate to neurocognitive performance.⁶³ However, it was also shown that not in all individuals experiencing HAND, viral RNA could be detected in the CNS.⁶² This could be a timing or stochastic issue due to limitations in frequent, longitudinal sampling of CSF. In some cases, HAND may be caused by ongoing viral replication due to insufficient drug penetration in the CNS, (Figure 1B), as will be discussed in more detail in Section 2.3.2.

2.3 | The CNS as a persistent (latent) reservoir of HIV

In order to consider an anatomic compartment as a biologically relevant HIV reservoir, it must fulfill several criteria. First, HIV DNA needs to be present in cells with a long lifespan or which undergo (homeostatic) proliferation. Second, the infected cells should be able to produce new replication-competent viral particles. At last, cells should have mechanisms to suppress viral replication and enable latent infection.^{64,65} There is evidence that the CNS may serve as a (latent) anatomic reservoir of HIV,^{4,5} although the strength of these lines of evidence varies. In the coming paragraphs we will discuss HIV CNS infection, compartmentalization and reservoir formation.

2.3.1 | HIV infection and compartmentalization in virally unsuppressed individuals

As indicated earlier in this review, early stages of infection of the brain are characterized by the entry of CD4⁺ *T cell-tropic* viral variants. Once in the CNS, HIV is exposed to a completely different microenvironment, which also includes brain-specific HIV target cells.²⁹ As a result, compartmentalized HIV populations, genetically distinct from viral populations replicating in the periphery, can be detected in the

first months after infection in the CSF of untreated people with HIV (PWH).^{8,10} The compartmentalization of viral quasispecies in the CSF is preserved throughout infection.^{10,66} The early initiation of ART was shown to limit but not exclude compartmentalization in the CSF.⁵⁶ Genetic characterization of the viral quasispecies is largely based on phylogenetic analysis of the highly variable HIV envelope glycoprotein gp120. This viral envelope protein is required for cell entry and determines affinity for the main CD4 receptor and the coreceptors CCR5 and/or CXCR4.⁶⁷ Viruses found in the CSF can evolve from R5 *T cell-tropic* viruses,⁶⁸ which require expression of the CCR5 coreceptor and high surface density of the CD4 receptor, to Macrophage-tropic (*M-tropic*) viruses, which also require the CCR5 coreceptor, but a low surface CD4 receptor density. As both of these receptors are expressed on CD4⁺ T cells, both viruses can efficiently infect these cells. *M-tropic* viruses can also infect cells from the macrophage-monocyte lineage, which are also present in the CNS, since these cells express CCR5 but have low CD4 surface expression.^{69–72} In vitro adaptation to *M-Tropic* variants demonstrated a lower functionality of the viral envelope gene, with reduced particle infectivity and prolonged entry transitions compared with *T cell-tropic* variants,⁷³ whereas in vivo characterization of the *M-Tropic* variants only showed increased susceptibility to soluble CD4.⁶⁹ Adaptation to *M-Tropic* variants might only occur in immune-privileged sites, however future studies are needed to elucidate the differences between *M-Tropic* and *T cell-tropic* variants.

In the CSF of ART naïve PWH, mostly compartmentalized R5 *T cell-tropic* variants were detected during the first 2 years of infection, after which evolution toward *M-tropic* viruses was observed.¹⁰ In the CSF of unsuppressed individuals in more advanced stages of infection, a mixture of both R5 *T cell-tropic* viral variants and *M-tropic* compartmentalized viral variants could be detected.⁶⁸ Moreover, viral isolates obtained from brain autopsies of individuals with severe neurologic symptoms also represented a mixture of *M-tropic* and *T cell-tropic* viruses.⁷⁰ It was observed that the *M-tropic* variants in CSF display a higher genetic diversity and decay slower after start of ART than R5 *T cell-tropic* viral quasispecies.⁶⁸ Consequently, it is hypothesized that *M-tropic* quasispecies represent infection of the relatively long-lived CNS-resident cells, whereas the R5 *T cell-tropic* variants represent clonal expansion and possibly an influx from peripheral cells responding to immune activation.^{10,68} This suggests that the CSF is an intermediate compartment in which the periphery, as well as the CNS, contribute to the viral population.⁶⁶ Moreover, when comparing drug resistance mutations patterns of viral sequences from paired CSF and plasma samples, independent evolution of the viral quasispecies in the CSF was observed.^{74,75} Altogether, providing evidence that the CNS serves as an independent site of viral replication in CNS-specific target cells resulting in viral compartmentalization.

2.3.2 | HIV infection and compartmentalization in virologically plasma-suppressed individuals

In the large majority of HIV-infected individuals on ART, viral replication is suppressed and the levels of HIV RNA in the CSF are below the

level of detection. However, in some individuals with an undetectable plasma viral load, HIV RNA can be detected in the CSF, a phenomenon referred to as CSF escape.^{61,76–79} A minority of the PWH experiencing CSF escape show persistent levels of elevated CSF viremia with undetectable plasma levels in longitudinal studies, which does suggest that persistent viral production or independent replication occurs within the CSF.^{11,80,81} This might be due to poor drug penetration over the BBB resulting in a limited potential to inhibit viral replication in the CNS, as reviewed before.⁸² In line with this explanation, it has been shown that usage of better CNS-penetrating ART reduces CSF escape.⁸³

Analysis of the CSF viral escape populations showed genetically diverse viral quasispecies in some individuals.^{11,84} In 1 individual, also the selection of resistance mutations was observed.¹¹ It is highly unlikely, because of the suppression of HIV replication in the periphery in these individuals, and the association of CSF escape with selection of drug resistance, that these were derived from trafficking peripheral cells.⁷⁹ At last, analysis of the CSF viral escape populations showed that they largely consisted of *T cell-tropic* variants,^{11,84,85} but within 1 individual also *M-tropic* variants were observed at consecutive time points.¹¹ Given the fact that *M-tropic* HIV viral strains are hardly observed within the periphery, it is likely that these M-tropic viruses originate from the CNS.⁷⁹ Generally, this supports the idea that the viral escape as observed in the CSF of these PWH on ART is the result of ongoing viral replication in CNS-resident cells in ART-suppressed individuals.^{11,79}

Apart from persistence of HIV in the CSF as a result of ongoing viral replication due to poorly penetrating antiretroviral drugs, viral latency might also be a mechanism of HIV persistence in the CSF.^{64,65} Latently infected cells are classically defined as cells that carry integrated HIV DNA and are transcriptionally silent, but upon activation produce replication competent virus causing a rebound in viremia when ART is stopped.² Their persistence is controlled by the half-life of the infected cells, their ability to proliferate and the epigenetic context of the integrated proviral genome.² It is also important to note that the current arsenal of ART cannot prevent viral production from activated cells. It is known that latent cells in the periphery can be activated resulting in viral production, and subsequently can revert back to latency.⁸⁶ Interestingly, the viral Tat protein can also be observed in the CSF of ART-suppressed individuals, indicating viral production.⁷⁸ However, it is difficult to discriminate viral production from ongoing (low-level) viral replication. In the majority of cases, viral RNA in CSF was detected just once (viral blip) indicative of viral production rather than ongoing viral replication.^{11,80,81} Consequently, the presence of viral RNA and Tat might be the result of viral production from intermittently activated latent cells.

2.3.3 | HIV reservoir analysis in CNS tissue of unsuppressed and suppressed PWH

Apart from studying viral RNA in the CSF, CNS tissue of PWH can be studied to get insight in viral reservoir formation. Multiple

studies have found compartmentalized HIV DNA sequences in CNS tissue as compared with peripheral tissues in virally unsuppressed individuals.^{19,87–90} Moreover, HIV DNA has been detected within CNS-resident cells in postmortem brain material of HIV-unsuppressed individuals.^{12–14,91–93} One study reported that the proviral genome sequences isolated from myeloid cells in the CNS were intact, suggestive of replication-competent virus.⁹³ This provides evidence that HIV replication occurs within the brain-specific myeloid cells in unsuppressed PWH.

However, in order to gain more insight into latency of HIV in the CNS, tissue of suppressed individuals needs to be studied. Within a study using single-molecule real-time sequencing of HIV isolates in the brain and lymphoid tissues of a virally suppressed individual 7 months before death, compartmentalization of HIV in postmortem brain tissue has been observed.¹⁸ Interestingly, phylogenetic analysis also incidentally showed viral strain compartmentalization between different brain regions, indicating that different compartments exist in the CNS.^{18,87} Furthermore, also in postmortem brain tissue of suppressed individuals, HIV DNA was found in CNS-resident cells, and was reported to be intact.^{15,91,93} Interestingly, in some of these resident CNS cells, no viral RNA was detected^{15,91,93}, suggestive for viral latency. Another study showed that directly after cessation of ART, compartmentalized viral populations in the CSF were detected, which were highly distinct from the viral populations present in the paired blood samples.⁹⁵ All in all, these data are indicating that the CNS may serve as a viral reservoir. Due to the limited availability of HIV-infected human brain material and paired CSF and plasma samples, further evidence regarding HIV latency in CNS human brain material is still lacking.

2.4 | HIV cellular reservoirs in the CNS

The data above are highly suggestive for the CNS to be a persistent HIV reservoir according to the criteria discussed in Section 2.3.^{65,66} Of brain-specific cells, neurons and oligodendrocytes are presumed to be overall resistant to HIV infection and thus do not meet the criteria for an HIV reservoir.⁹⁶ Astrocytes are long lived resident innate immune cells of the CNS that also have the ability to proliferate.^{59,97} HIV DNA has been detected in astrocytes in virally unsuppressed PWH,^{12–14,92} but not in virally suppressed individuals.¹⁵ Astrocytes were shown to be susceptible to HIV infection *in vitro*,^{98,99} however HIV infection was nonproductive.^{14,100,101} Due to the lack of CD4 surface expression on astrocytes, viral infection or transmission may occur via cell-to-cell contact, receptor-mediated endocytosis or via engulfment of neuronal debris or infected cells.^{102–104,105} Therefore, astrocytes are not considered to be a true cellular reservoir of HIV (Figure 1). Nevertheless, it is suggested that they may contribute to HIV-related cell injury, via the production of astrogliosis.¹

Multiple myeloid cell populations are present in the CNS,¹⁰⁵ and HIV DNA has been detected in microglia and perivascular macrophages in postmortem brain material of both ART-treated and virally unsuppressed PWH.^{12,13,15,64,91,92} Microglia are tissue-resident macrophages and are part of the human innate immune system.

Microglia are able to repopulate themselves throughout life and are thought to have a lifespan of approximately 4 year, whereas perivascular macrophages have a lifespan of months and need replenishment from the bone marrow.^{64,106} Microglia and perivascular macrophages express low levels of CD4, CCR5, and CXCR4 as compared with CD4⁺ T cells.¹⁰⁷ Therefore, it is likely that HIV DNA detected in the postmortem brains is derived from infection with compartmentalized *M-tropic* viruses. However, HIV reverse transcription has been shown to be inhibited via the expression of the restriction factor SAM domain and HD domain-containing protein 1 in cells of the myeloid lineage.¹⁰⁸ Nevertheless, human primary microglia cultures and in vitro microglial culture models have shown productive HIV infection with *M-tropic* viruses via the CD4 and CCR5 receptor, but did not support infection with *T-tropic* viruses.^{107,109,110} Moreover, studies with SIV in rhesus macaques and HIV-infected humanized immunodeficient mice show productive infection and suspected latency of replication competent virus in microglia and perivascular macrophages during ART suppression as shown by increase in viral load upon cell stimulation.^{23,111–113} Altogether, based on the cellular characteristics of microglia, the mounting evidence of susceptibility to productive HIV infection, and the presence of HIV DNA, microglia and to a lesser extent perivascular macrophages are thought to be the main HIV reservoir in the brain (Figure 1).

The peripheral CD4⁺ T cells are the most studied biologic relevant HIV reservoir, in which productive infection and latency is observed.²⁵ Because of the presence of CD4⁺ T cells in CSF due to trafficking and tissue-resident CD4⁺ T cells in the human brain parenchyma,¹¹⁴ it is likely that CD4⁺ T cells provide a cellular reservoir for HIV, (Figure 1). However, direct identification of CD4⁺-infected T cells in the CNS is challenging because of their low frequency in the CSF and the brain.²⁹ Nevertheless, sequencing envelope genes of viral isolates from CSF and postmortem brain tissues showed compartmentalization of both *M-tropic* and *T-cell-tropic* viruses.^{11,18,68} Specifically, the presence of CD26 on virion surfaces derived from individuals with CSF escape was observed, which has been shown to be an indication of *T-cell-tropic* virus. Also virions with CD36 on their surface, as a marker of *M-tropic* virus, have been observed in these individuals.⁸⁵ Altogether, this supports the idea that infected CD4⁺ T cells are present in the CNS, which implies that also latency in CD4⁺ T cells might occur in the CNS reservoir.

2.5 | Culture models for HIV CNS persistence studies

In order to get more insight into the details of HIV persistence and latency in the CNS, in vitro model systems are being used. As microglia are thought to be the main cellular HIV reservoir, in vitro studies of HIV infection in the CNS have been performed on myeloid cell model systems, such as the infected monocyte-derived macrophages.^{22,115} However, the translation of these results toward the CNS is limited, because of the distinct phenotype of microglia compared with macrophages.¹¹⁵

Therefore, current in vitro studies of HIV infection in the CNS are mostly done on microglial culture models, as reviewed earlier.^{110,116} Human primary microglia cultures and monocyte-derived microglia are highly susceptible to infection, showing continuous viral production up to 1 month postinfection.^{110,116–118} However, this is not representative of the small population of focally distributed infected microglial cells (1–10%) in the brain,^{13,15,91,92} which suggests that important restriction factors might be down-regulated in culture or that infection is greatly affected by surrounding CNS cells and the CNS environment in vivo. In vitro studies on human primary astrocyte cultures consistently report a low (nonproductive) HIV infection through CD4-independent mechanisms.^{102–104} Although this low infectivity (1–3%) is reminiscent of the small infected population in the brain, it is still unclear whether and how astrocytes become infected in vivo in the absence of the CD4 receptor. Long-term culture (>120 days) of primary microglia and astrocytes have led to viral latency suggesting that these primary cells retained at least some of the genes and/or restriction factors required for the induction of latency in vivo,^{104,117} such as the proapoptotic protein Bim, which surprisingly was up-regulated in latently infected macrophages.¹¹⁷ However, the technical challenges of obtaining fresh brain tissue and the limited number of cells postisolation complicates the use of primary cells for down-stream HIV (latency) analysis. To circumvent this, primary microglia have been immortalized to generate microglial cell lines such as HMC3, SV40 and H μ glia.^{107,119} We do not recommend the use of these cell lines for HIV infection studies as there is no to limited support of HIV infection and large transcriptomic discrepancies with primary microglia.^{107,110,116,120} Alternatively, latently HIV-infected clones derived from the H μ glia cell line show great promise as a model for the initial assessment of HIV latency reversal in microglia, although future studies will need to improve on the high levels of spontaneous progressive HIV reactivation observed in order to better recapitulate the suspected in vivo scenario.^{121,122}

Recent technologic advancements in stem cell research have enabled the generation of a variety of CNS cell types including cerebral organoids and opened unique opportunities to study host-virus interactions in the CNS.¹²³ HIV infection of induced pluripotent stem cell (iPSC)-derived microglia and organoid-derived microglia, decreased after the 1st week of infection, resembling HIV infection in vivo.^{109,110,116} 2D coculture models incorporating iPSC-derived microglia with iPSC-derived astrocytes and/or iPSC-derived neurons and 3D cerebral organoids can be used to address the true cellular targets of HIV in the CNS and the role of surrounding CNS cells in supporting infection and the development of neural injury.^{109,124,125} Furthermore, as iPSC-derived CNS cells and cerebral organoids can be maintained in culture for long periods of time (months to years), it will be interesting to investigate whether these cells revert to a latent state postinfection making them suitable models to study HIV persistence and latency in the CNS within a CNS-like environment.^{109,126} To conclude, several promising in vitro model systems are available to study HIV persistence and latency. However, it should be kept in mind that HIV CNS in vitro models can only partially recapitulate HIV persistence and latency in vivo and are a simplification of the complexity of

the human brain. Therefore, caution should be used when translating findings to the *in vivo* scenario.

2.6 | HIV transcription and latency in the CNS

2.6.1 | Regulation of (general) HIV transcription and latency

Multiple mechanisms are known to regulate transcriptional silencing of integrated HIV, which have been extensively reviewed,^{127,128} (Figure 2A). During latency, repressors of TFs and repressive epigenetic marks around the HIV integration site and long terminal repeat (LTR) inhibit transcription. The negative elongation factor (NELF) and DRB-sensitivity inducing factor (DSIF) cause pausing of the RNA polymerase II (RNAPII) on the LTR. In addition, latent cells are characterized by low levels of Tat. If levels of Tat are low, only low-level basal transcription of viral genes occurs and RNAPII is interrupted after the synthesis of short transcripts (± 80 base pairs) that include the transactivation response element (TAR).^{127,128} However, for productive infection these factors inhibiting HIV transcription need to be removed. Upon activation of the latent cells, Tat levels will increase.

Tat binds to the TAR element and recruits the P-TEFb complex (positive transcription elongation factor) to the LTR. The RNAPII carboxyterminal domain and DSIF are phosphorylated, causing dissociation of NELF, which enables the efficient transcription of the HIV genome. Moreover, Tat recruits several factors that are needed for the inhibition of the repressive (epigenetic) transcription mechanisms and efficient elongation of HIV transcription.^{127,128}

2.6.2 | HIV transcription in the CNS

Polymorphisms in HIV LTR of CNS strains isolated from autopsy tissue of virally unsuppressed PWH are reported to have considerable consequences on the latency and transcription of HIV as shown during *in vitro* infection in astrocyte and T cell lines.^{17,24,129} Generally, based on sequence analyses these LTR polymorphisms were expected to result in an altered binding of multiple TFs such as CCAAT/enhancer binding protein (C/EBP),^{130,131} NFAT,¹³² NF- κ B,¹³² or SV40-promoter specific factor (SP).^{24,132} One study reported that these CNS-derived LTR sequences resulted in reduced basal transcription in T cells and astrocytes compared with HIV populations present in the periphery.¹²⁹ Binding of these TFs to the LTR is essential for initiation of LTR-dependent transcription and synthesis of Tat protein. Viral sequences obtained from the CNS of PWH without suppressed ART indicate also sequence variations in the Tat gene.^{133–135} Overall, these sequences did not change the ability of Tat to transactivate the HIV LTR in glial, astrocyte, monocyte, and T cell lines, although this was the case in material obtained from some individuals,¹³³ (Figure 2B) and (Table 1).

Additionally, it has been shown that cultured primary microglia and astrocytes produce unique TFs with a distinct working mechanism, compared with other cells.^{17,136,137} First, HIV transcription in the CNS is majorly dependent on C/EBP TFs in the cells of the myeloid cell-line

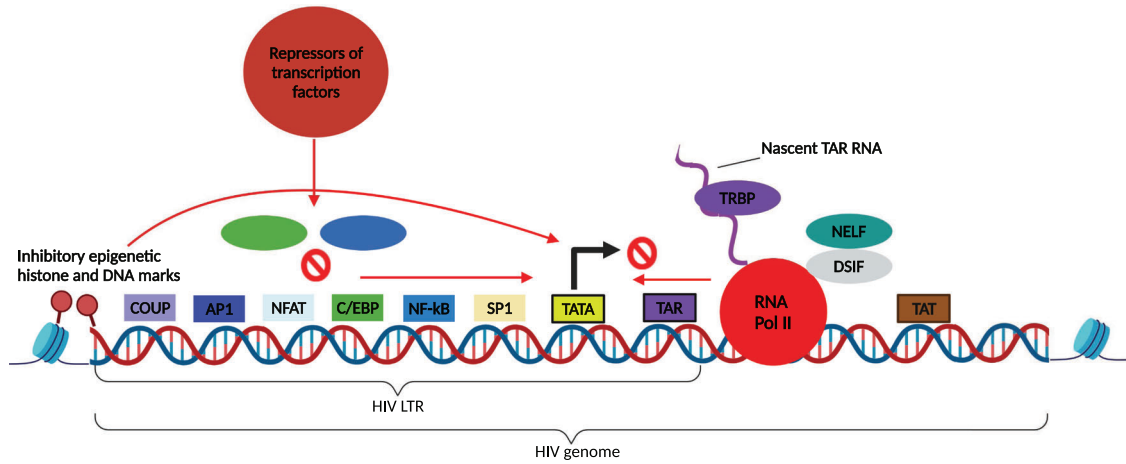
lineages.¹³⁸ Second, in *in vitro* glial cell-lines transcription inhibitors C/EBP γ , a truncated form of C/EBP, chicken ovalbumin upstream promoter TF (COUP), SP1 and SP3 show unique interactions with the LTR and can inhibit HIV transcription.^{139,140} Moreover, dexamethasone activates the glucocorticoid receptor and represses HIV transcription in *in vitro* immortalized microglia.¹²¹ Similarly, the Nerve Growth Factor IB-like nuclear receptor Nurr1(NR4A2) led to silencing of HIV by binding to the HIV LTR.¹⁴¹ Astrocyte cell lines express distinct levels of isoforms of SP resulting in restricted transcription¹⁷ and express low levels of TAR RNA binding proteins (TRBP),¹⁴² important for Tat-dependent HIV transcription. It has been suggested that in astrocyte and glial cell lines HIV transcription occurs in the absence of TAR^{143,144} and is more dependent on Tat binding to NF- κ B,¹⁴⁵ (Figure 2B) and (Table 1).

Transcription is also regulated via epigenetic regulation in multiple brain cell types, such as microglia and astrocytes.^{128,146} COUP-TF interacting protein (CTIP2), also known as BCL11b, is an important regulator in the epigenetics of HIV transcription and increased levels of CTIP2 are seen in microglial cells and contribute to differently regulated transcription.^{64,94} An extensive explanation of the role of CTIP2 within HIV latency has been previously reviewed.¹⁴⁷ At first, CTIP2 serves as a platform to anchor several protein complexes having different functions, which together disfavor viral reactivation.^{147,148} One complex of lysine specific demethylase (LSD1), histone deacetylases (HDACs) and heterochromatin protein 1 (HP1) induces the formation of heterochromatin.^{149,150} Another complex with hypermethylated in cancer 1 (HIC1) and high mobility group AT-hook 1 (HMAG1) with CTIP2 represses HIV transcription via the inhibition of TEFb,^{150,151} (Figure 2B). Interestingly, in infected cells obtained from postmortem brain tissues of PWH with and without ART therapy, an increase in CTIP2, HDAC, and HP1 levels was observed,⁹⁴ (Table 1). Second, CTIP2 regulates multiple cellular genes important in HIV transcription via TF p21 and thus indirectly inhibits transcription.¹⁴⁷ However, in microglia cell lines it is seen that this is counteracted via HIV viral protein R.¹⁵²

Altogether, these studies suggest that there might be a differentially regulated transcriptional activity and latency induction for HIV CNS viral populations as compared with those seen in peripheral blood. This can be caused by polymorphisms, especially in the LTR and its transcription bindings sites, and the expression of proteins, such as TFs, Tat and epigenetic modifiers. These differences might be the consequence of the selection pressure and different microenvironment in the CNS, leading to the compartmentalization of the CNS. These differences in transcription mechanisms may also contribute to differences in the establishment and maintenance of viral reservoirs observed in CNS and periphery (Figure 2).

Notably, these findings were obtained from studies performed mainly on *in vitro* cell lines and a few on cultured primary cells. As discussed before in Section 2.5, the translation of the results in these model systems should be done with caution, regarding their differences with the human *in vivo* situation. Moreover, most of the viruses isolated from human brain tissue to study these transcription mechanisms were derived from PWH without suppressive therapy, which might be not identical or comparable to the regulation of latency and transcription in PWH on successful therapy.

A Latency of HIV



B Differences in regulation of HIV transcription in the CNS

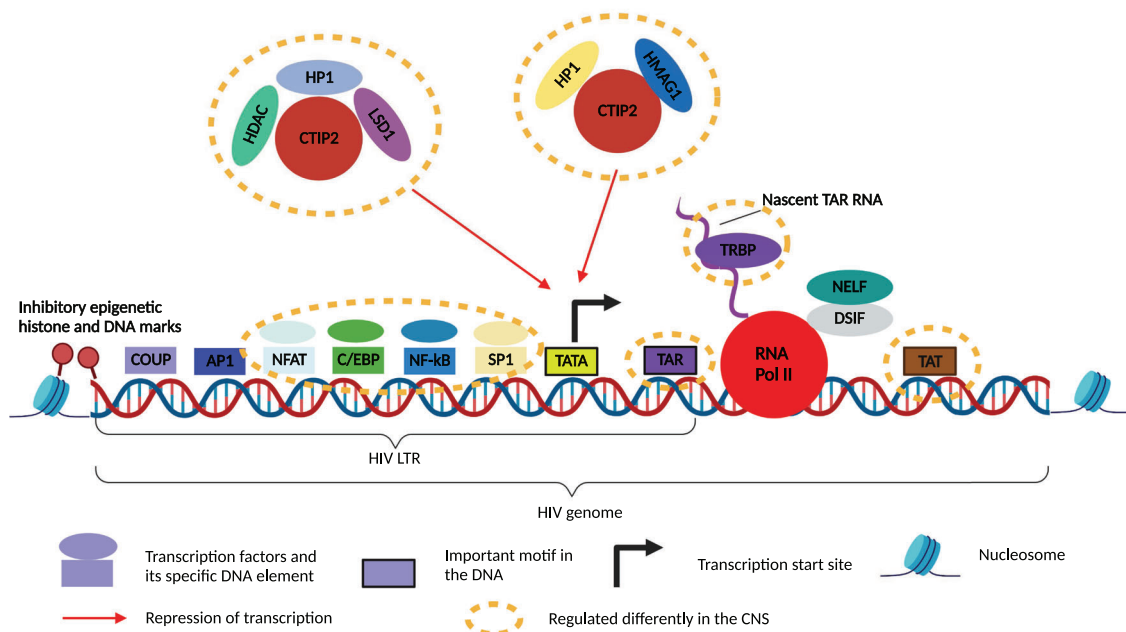


FIGURE 2 HIV latency regulation in periphery and its differences within the CNS. (A) **Latency of HIV:** The presence of transcription repression factors and inhibitory epigenetic around the HIV integration site and LTR prevent the transcription of HIV. Tat is not transcribed, which is needed for full-length HIV transcription. (B) **Differences in regulation of HIV transcription in the CNS:** Within the CNS multiple factors within transcription regulation are differently regulated compared with the general situation in the periphery. Factors of which it is reported that they are altered in the CNS are outlined with a dashed orange line. Polymorphisms in the LTR and the Tat gene cause an altered binding of transcription factors and a different function of Tat. Moreover, within brain target cells, unique isoforms and levels of C/EBP, SP1, SP3, COUP, and TRBP alter their transcriptional activity. At last, within brain target cells, increased levels of transcription repression factors are observed, which repress transcription by blocking TF binding sites or establishing epigenetic modifications. Created with Biorender.com. HDAC, histone deacetylase; CTIP2, COUP-TF interacting protein; HIC1, hypermethylated in Cancer 1; LSD1, lysine specific demethylase; HP1, heterochromatin protein 1; HMG1, high mobility group AT-hook 1; AP1, activator protein 1; C/EBP, CCAAT enhancer binding protein; COUP, chicken ovalbumin upstream promotor; SP1, specificity protein 1; LTR, long terminal repeat; RNA Pol II, RNA Polymerase II; TF, transcription factor; Tat, transactivator of transcription; TAR, transactivation response element; TRBP, TAR binding proteins; NELF, Negative Elongation Factor; DSIF, DRB-sensitivity inducing factor

TABLE 1 Differences of transcription regulation within the CNS compared with the periphery. Polymorphisms within the LTR of CNS viral populations, unique (expression of) transcription factors and a different epigenetic regulations within CNS cells might have an influence on the regulation of transcription and the induction of latency within the CNS. *LTR, long terminal repeat*

Change of transcription mechanism in the CNS	What effect?	Reference
CCAAT/enhancer binding protein (C/EBP)	Altered binding of C/EBP to LTR	130,131
NFAT	Altered binding of NFAT to LTR	132
NF- κ B	Altered binding of NF- κ B to LTR	132
SV40-promoter specific factor (SP)	Altered binding of SP to LTR	24,132
Transactivator protein (Tat gene)	No change on reactivation	133,134
<i>Unique transcription factors</i>		
Unique interactions of C/EBP, COUP, SP1 and SP3	Inhibition of HIV transcription in glial cells	139,140
Dexamethasone activates the glucocorticoid receptor	Repression of HIV transcription in microglia	121
Nerve growth factor IB-like nuclear receptor Nurr1 (NR4A2)	Silencing of HIV in microglia	141
Distinct levels of isoforms of SP	Restricted HIV transcription in astrocytes	17
Low levels of TAR RNA binding proteins (TRBP)	Restricted HIV transcription in astrocytes	142
<i>Epigenetic regulation</i>		
Increased levels of COUP-TF interacting protein (CTIP2, histone deacetylase (HDAC) and heterochromatin protein 1 (HP1)	Differently regulated transcription in microglia	94

3 | SHOCK AND KILL ERADICATION STRATEGY

3.1 | Theoretical approach

The persistence of latent reservoirs is in general the major obstacle to HIV cure. The immune system fails to detect the presence of transcriptionally silent latently infected cells, limiting recognition for elimination by immune-mediated clearance or direct viral cell-lysis by viral production. Theoretically, reactivation of HIV with LRAs by targeting the latency mechanisms ("shock") will lead to the synthesis of HIV RNA and viral protein production. Subsequently, these reactivated cells are ultimately recognized and killed ("kill") by the host immune defense mechanisms or viral cytolysis.^{21,153} This potential cure strategy is known as "shock and kill" and is performed in combination with ART.²¹

3.2 | Classes of LRAs

LRAs can be classified into different classes, based on their mechanism of action, shown in (Figure 3). First, epigenetic modifiers reverse the repressing epigenetic marks around the integrated provirus, which influence the transcription of HIV. The most studied ones are the histone methyltransferase (HMT) inhibitors and the HDAC inhibitors (HDACis). These agents reverse the repressing epigenetic acetyl and methyl marks in the integrated HIV-genome, its surrounding genome, and the associated histone tails in nucleosomes.^{21,153} The BRG1/BRM-associated factor (BAF) inhibitors modulate the histone position of the nucleosome of the integrated HIV DNA and facilitate thereby the

transcription of the HIV genome. Second, the intracellular signaling modulators include drugs that regulate the protein kinases in signaling pathways modulating the TFs binding to the LTR such as protein kinase C (PKC) agonists and compounds within the PI3K/Akt pathway or JAK/STAT pathway.^{21,153} Also second mitochondria-derived activator of caspases (SMAC) mimetics can be used, which inhibit the degradation of NF- κ B inducing kinase (NIK), allowing for the accumulation of NF- κ B.¹⁵⁴ Another class of LRAs are the cytokine or immune receptor agonists, which stimulate the immune cell by using ILs or cytokines, TCR, checkpoint inhibitors, or the TLR agonists. After transcription initiation, transcription elongation factors can be used to promote the activity of Tat, important for the elongation of the transcription.^{153,155} Important examples are the bromodomain and extra terminal domain (BET) inhibitors, which antagonize the inhibitor of P-TEFb and consequently activate the recruitment of P-TEFb to the LTR.^{21,153} Finally, a class of unclassified LRAs includes previously used drugs, which were found to reactivate HIV. However, their working mechanism for reactivating HIV is still unknown. Antioxidants and phosphatases are examples of these drugs that induced HIV latency reversal.¹⁵³ Examples of drugs of each class are listed in (Table 2) and in other reviews.^{153,156,157}

Many compounds in these classes have been studied in vitro, but only a few of them are studied in clinical trials. In most of these studies an increase in HIV transcription was observed, but without a reduction in HIV DNA.¹⁵⁵ Combinations of LRAs with different working mechanism may function in a synergistic manner to reactivate HIV and consequently increase the transcription of HIV.¹⁵⁷ Nevertheless, because of a decreased susceptibility of HIV-infected cells to apoptosis, direct inhibition of the immune cells by LRAs, immune

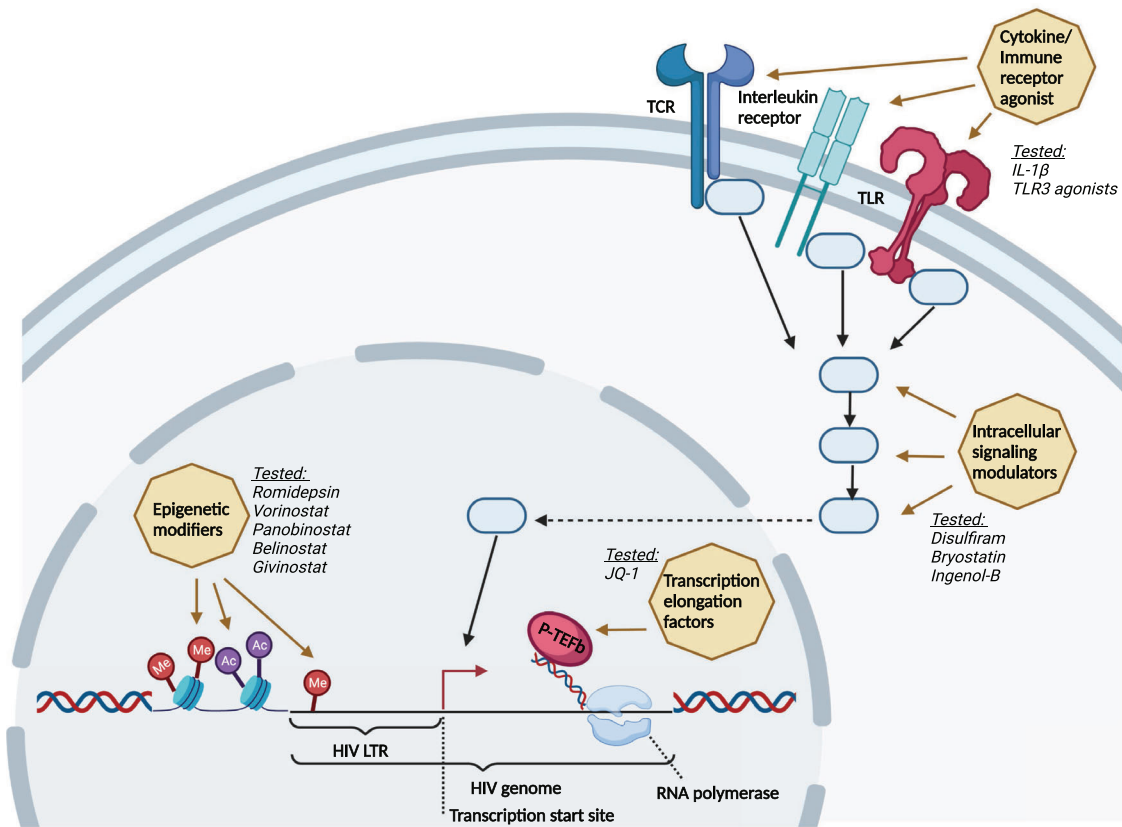


FIGURE 3 The different classes of LRAs and compounds that are tested for their efficacy in culture models CNS cells or CNS quaspecies. Latency reversal agents (LRAs) can be divided within 5 classes, based on their working mechanisms. Within each of these classes, subclasses are defined. Within the figure, the LRAs that have been tested on CNS culture models or CNS viral quaspecies are indicated. Created with Biorender.com.

escape mutations, issues with penetration of LRAs within tissues, decreased potency of some LRAs, and immune dysfunction in PWH, it is unlikely that reactivation alone is enough to completely eradicate latently infected cells.²¹ Therefore, stimulation of immune-based elimination and apoptotic pathways combined with LRAs will be needed to eradicate HIV-infected cells.¹⁵⁸

4 | SHOCK AND KILL IN THE CNS

The evidence for HIV persistence and potential latency in CNS-specific cells and resident CD4⁺ T cells emphasizes that also the CNS reservoir needs to be considered in HIV curative strategies. One of the most studied approaches is the “shock and kill” strategy (Section 3). In the coming paragraphs, issues regarding the effectivity and safety of “shock and kill” within the context of the CNS will be discussed.

4.1 | Penetration of LRA compounds to the CNS

Penetration of LRAs in the CNS could be a limiting factor for the success of the “shock and kill” strategy. Churchill *et al.*¹⁷ reviewed many of the currently known LRAs and showed that CNS penetration varied

from poor as in the case of romidepsin toward very good as seen with vorinostat and disulfiram. Although most LRAs show relatively good CNS penetration, multiple approaches are under development, such as nanoparticle delivery, to improve the passage of drugs/compounds across the BBB.²⁰

4.2 | Latency reversal in CNS cells

HIV transcription and latency can be differentially regulated in the CNS as compared with that in peripheral CD4⁺ T cells (Section 2.6.2). Although there is no direct proof of latency in the CNS, there are strong indications of latency as discussed in Section 2.3. It is likely that these differences have an impact on the responsiveness and efficacy of the LRAs.²⁴

Several *in vitro* studies have been performed with different LRAs that showed variable effects in the previously discussed culture model systems (Section 2.5) of microglia (Figure 3) and (Table 3).^{22,115,122,159,160} Contradictory data were shown regarding the induction of viral transcription of romidepsin, panobinostat, vorinostat, bryostatins, and the BET inhibitor JQ1 in monocyte-derived macrophages,^{22,115} (Table 3). A study testing HIV reactivation of HDACis (belinostat, givinostat, panobinostat, romidepsin, and vorino-

TABLE 2 Overview of the different classes of LRAs and examples of drugs. Latency reversal agents (LRAs) are classified within 5 different classes and multiple subclasses. Of each of these classes examples of drugs are listed.^{153,156,157}

(Sub)class of LRA	Examples of drugs
<i>Epigenetic modifiers</i>	
Histone methyltransferases (HMT) inhibitors	Chaetocin, AZ505, BIX01294
Histone deacetylase inhibitors (HDACis)	Vorinostat (SAHA), panobinostat, romidepsin, chidamide, valproic acid, belinostat, givinostat
BRG1/BRM-associated factor (BAF) inhibitors	Pyrimethamine
<i>Intracellular signaling modulators</i>	
Protein kinase C (PKC) agonists	Bryostatin, Ingenol-B, prostatin
Modulators in the PI3K/Akt pathway	Disulfiram
Modulators in the JAK/STAT pathway	Benzotriazole
Second mitochondria-derived activator of caspases (SMAC) mimetics	SBI-0637142, CAPE
<i>Cytokine or immune receptor agonists</i>	
ILs and cytokines	IL-1 β , IL-2, IL-7, IL-15 agonists
TCR activators	Maraviroc
Checkpoint inhibitors	Anti-CTLA4, anti-PD1
TLR agonists	TLR2,3,7,8, 9 (MGN1703) agonists
<i>Transcription elongation factors</i>	
Bromodomain and extraterminal domain (BET) inhibitors	JQ1, MMQO
<i>Unclassified</i>	
Antioxidants	Auranofin
Phosphatases	SMAPP1

stat) on in vitro-infected monocyte-derived macrophages showed that these HDACis reduced the amount of integrated HIV DNA, but without measurable reactivation because of the formation of autophagosomes.¹⁶⁰ This might explain the differences in measurable HIV reactivation between the studies,¹⁶⁰ but also donor variability has been suggested to cause these differences.¹¹⁵ Bryostatin/ingenol-B combined with JQ1 reactivated HIV in microglial cell lines.¹⁵⁹ Finally, it is reported that treatment with TLR3 agonists reactivated HIV transcription in immortalized primary microglia,¹²² (Table 3).

Although microglia are suggested to be the main reservoir of HIV in the brain (Section 2.4), multiple studies have tested the effects of LRAs on primary astrocytes or astrocyte cell lines (Figure 3) and (Table 3). Some LRAs did induce viral transcription in primary astrocytes, such as romidepsin, panobinostat, and disulfiram,²² whereas others did not such as IL-1 β .¹⁵⁹ However, in general, conflicting data were reported regarding the reactivation effects of LRAs in the astrocytes, for example for bryostatin and vorinostat.^{22,98,99,146,161}

Altogether, this indicates LRAs might have promising effects in the microglial or macrophage cells with respect to latency reversal and that conflicting data are seen in astrocytes, (Table 3). The discrepancy between microglia and astrocytes may be attributed to the fact that astrocytes have limited productive infection in vivo, ex vivo, and in vitro^{98,101,102,161} and consequently, have limited ability to increase their viral expression after reactivation. Moreover, it is important to

notice that many of these studies are performed on primary cells or even cell lines, which do not present the true HIV latency situation and are a simplification of the complex CNS reservoir, as discussed in Section 2.5. Last, some of these studies only report differences in viral transcription, whereas this is not directly result in an increase in viral production.

4.3 | Latency reversal of CNS viral populations

Some of the differences in HIV transcription and persistence in the CNS might be explained by compartmentalization of HIV and differences in LTR and Tat sequences, as discussed before in Section 2.6.2. A study on CNS-derived LTRs obtained from unsuppressed PWH, showed that viral reactivation by LRAs romidepsin, panobinostat, and JQ1 in combination with Tat was lower for the brain-derived viruses compared with the lymphoid-derived viruses in human fetal astrocytic cell lines (SVG),²⁴ (Figure 3). However, to the best of our knowledge, this is the only study in which these effects are investigated. Therefore, it is unknown whether these effects also occur for other LRAs and for viral strains obtained from suppressed individuals. This emphasizes the urgent need to screen the current LRAs for their efficacy toward CNS-derived viruses from both ART-suppressed and unsuppressed PWH.

TABLE 3 Overview of the effects of LRAs within culture models of different brain cells. The listed latency reversal agents (LRAs) are reported to have varying effects within astrocytes or (monocyte-derived) macrophages or microglia. PKC, protein kinase C

LRA	Cell type	What effect?	Reference
<i>Microglial models</i>			
Romidepsin, panobinostat, and JQ1	Monocyte-derived macrophages	Viral transcription	22
Vorinostat	Monocyte-derived macrophages	Little viral transcription	22
Bryostatin and borinostat	Monocyte-derived macrophages	HIV reactivation	115
Panobinostat	Monocyte-derived macrophages	No HIV reactivation	115
Belinostat, givinostat, panobinostat, romidepsin, and vorinostat	Monocyte-derived macrophages	Reduced amount of HIV DNA	160
Combination therapy bryostatin and Ingenol-B	Microglial cell lines	Reactivation of infected cells	159
TLR3 agonists	Immortalized primary microglia	Viral reactivation	122
<i>Astrocyte models</i>			
Disulfiram, romidepsin, panobinostat	Astrocytes	Viral transcription	22
IL-1 β	Astrocytes	No impact on viral transcription	161
Vorinostat	Astrocytes	Conflicting results on viral transcription	22,146,161
Bryostatin	Astrocytes	Conflicting results on viral transcription	98,99

4.4 | The impact of the “shock” in the CNS

It is important to keep in mind that *in vitro* and *ex vivo* experiments do not completely reflect the direct and indirect effects of LRAs within the complex human brain and its neurocognitive performance. LRAs themselves and the resulting viral reactivation might cause damage to the CNS. Therefore, it is needed to monitor potential toxic effects, preferably in 3D human cerebral organoid models, animal studies or clinical trials.

4.4.1 | Impact of viral reactivation in the CNS

To the best of our knowledge, only one *in vivo* study has been performed that investigated the potential of LRAs to reactivate HIV in the CNS.²³ This study showed that the administration of Ingenol-B and vorinostat in ART-suppressed SIV-infected macaques lead to a 10-fold higher viral load in CSF as compared with plasma in one macaque. Post-mortem *in situ* hybridization showed viral transcripts in myeloid cells (CD68⁺ cells) within the occipital cortex,²³ suggesting that perivascular macrophages or microglia represent an HIV reservoir that can be reactivated via the usage of these LRAs. However, in the latency reversed SIV-infected macaque, also increased levels of markers for neuronal degradation, inflammation, and SIV encephalitis were found in brain tissue and CSF compared with the macaque without latency reversal. This implies that although these LRAs might result in successful viral reactivation, the production of viral RNA and inflammatory markers of this HIV-cure strategy might also result in neuronal degradation and consequently HAND, as discussed previously in Section 2.2.

In line with this observation, ART interruption in PWH lead to increases in HIV RNA in CSF and plasma and increased levels of NFL

in the CSF,¹⁶² which is associated with neuronal degradation.^{46,162} Interestingly, it has been shown that neuronal damage enhances HIV expression in latently infected microglia,¹⁶³ suggesting a positive feedback loop after the first reactivation and its induced neuronal damage. Together, this indicates that reactivation of the CNS reservoir may impact neuronal degradation.

Moreover, it is important to be aware of the limited potential of CNS cells, predominantly neurons, to replenish as compared with peripheral cells.¹⁶⁴ Although the number of HIV-infected cells in the CNS is low, there could still be an effect of HIV-induced bystander apoptosis.¹⁶⁵ Therefore, LRA-induced activation of HIV-infected cells might lead to even more killing of surrounding (neuronal) cells with possible detrimental consequences on an individual's brain functioning. Altogether, viral reactivation induced by LRAs may result in a reduction of neuronal cells, which might lead to a decline in neurocognitive performance and the development of HAND.¹⁶⁶

4.4.2 | Neurotoxicity of LRAs

Besides the effects of viral reactivation in the CNS and its impact on neuronal functioning, LRAs might have direct neurotoxic effects as well. One important issue of latency reversal agents is that they may not only activate cells that harbor latent HIV, but instead cause activation of other resting cells.^{157,167} The first LRAs induced global (T cell) activation as bystander effect, leading to severe toxicity. Therefore, most contemporary LRAs are designed to more specifically activate HIV, for instance by preferential methylation of HIV DNA as discussed in section 3.2.¹⁶⁷ Nevertheless, current LRAs are not exclusively reactivating HIV-infected cells resulting in immune activation and toxicity in bystander cells, as discussed for CD4⁺ T cells.^{157,167-169} It is sugges-

tive that activation of noninfected cells in the CNS may lead to toxicity in these bystander cells as well. Moreover, it has been shown that the treatment of primary astrocytes with multiple combinations of LRAs resulted in secretion of several inflammation markers¹⁷⁰ and accumulation of amyloid beta,¹⁷¹ which may have detrimental consequences such as neuronal injury. At last, it has been shown that the LRAs prostatin and bryostatins damage the integrity of the BBB, allowing immune cells to cross this barrier and thus enhance neuroinflammation.¹⁷²

Neurotoxic effects of LRAs are reported in clinical trials as well. For example, HDACis and also disulfiram are associated with neurotoxic symptom.^{173,174} In a recent clinical trial investigating the combination of vorinostat and high dose disulfiram, the first 2 participants enrolled developed neurotoxicity grade 3, leading to cessation of the study.¹⁷⁴ In contrast, other clinical latency reversal studies reported that administration of the standard licensed doses of romidepsin and panobinostat was not associated with CNS side effects, as assessed by analysis of expression of CSF biomarkers and by performing cognitive tests.^{175,176} However, panobinostat could not be detected in the CSF, indicating that there was potentially no penetration of this LRA in the CNS.¹⁷⁵ Increased neurotoxicity as observed in some of these clinical trials might also be related to the usage of LRA combinations as opposed to LRA monotherapy. Together these results strongly suggest integration of CNS-specific monitoring in clinical studies to gain more insight in the efficacy of latency reversal and the consequences of LRA treatment and HIV reactivation in the CNS. In future, it may be a good idea to combine the “shock and kill” strategy with anti-inflammatory compounds, although these might reduce subsequent immune-mediated clearance.^{147,177}

4.5 | The “kill” in the CNS

The last important step of the “shock and kill” strategy is killing of the HIV-expressing cells, either via virus-mediated cytotoxicity or immune-mediated clearance. For both of these mechanisms, critical differences are to be expected in the CNS as compared with killing of CD4⁺ T cells in the periphery.

4.5.1 | Virus-mediated cell killing

It is reported that myeloid cells, including the perivascular macrophages and the microglial cells, are more resistant to cytopathic effects and apoptosis.^{147,148} The mechanisms behind the reduced cytopathic effects in myeloid cells are still largely unknown. However, recently it has been shown that CTL-mediated killing of infected macrophages compared with CD4⁺ T cells required longer cell–cell contact and that a higher concentration of secreted IFN- γ by CTLs was needed for cellular killing.¹⁷⁸

Moreover, studies have shown that HIV infection makes infected macrophages and microglia more resistant to apoptosis.¹⁷⁹ Gene expression in monocytes from PWH shows apoptosis-resistant expression patterns,¹⁸⁰ and Bim, a proapoptotic negative regulator of CTIP2,

is up-regulated in latently infected macrophages *in vitro*.¹¹⁷ These would allow microglia and perivascular macrophages to harbor latent HIV for months or years.^{64,147} The influence of HIV on apoptosis in astrocytes is currently unknown, although resistance of HIV-infected astrocytes to apoptosis has been described.¹⁸¹

Current *in vitro* LRA studies using primary CNS cells did not show a clear direct effect on reducing the number of infected cells despite viral reactivation.^{22,98,99,115,122,146,159,161} However, as discussed before in section 3.2, it is unlikely that administering LRAs alone will eliminate these cells and therefore LRAs need to be combined with compounds that stimulate cell killing. To our knowledge, there are no studies on the effect of this strategy in the CNS of PWH on ART.

4.5.2 | Immune-mediated clearance

The most prominent immune cells in the CNS are the resident perivascular macrophages and microglia, but other peripheral immune subsets such as macrophages, dendritic cells, and T cells are also present.¹⁸² The number of HIV-specific CD4⁺ and CD8⁺ T cells is limited in the CNS as compared with the periphery but they can be detected in the CSF of PWH.¹⁸³ This indicates that immune-mediated clearance of HIV can occur in the CNS and is suggested to be one of the mechanisms that may be involved in killing reactivated cells following latency reversal.

As discussed in section 2.2, the induction of HIV inflammatory responses in the brain, results in microglia activation, an up-regulation of cytokines and chemokines, and the influx of cells from the periphery, including monocytes and lymphocytes. A process called viral encephalitis.¹⁸⁴ This can lead to neuronal injury and degradation. To avoid these inflammatory processes, immune responses are regulated strictly in the CNS.¹⁸⁵ As a result, immune-mediated clearance is limited in the CNS, which might also limit the “kill” part of this eradication strategy. Together, the increased resistance of HIV-infected CNS cells to apoptosis and the reduced ability of immune-mediated killing suggests that the “kill” after latency reversal in the CNS will be suboptimal or even very limited. If viral production/reactivation and immune activation by LRAs is induced, but the removal of the activated cells is limited, this could have detrimental consequences on neuronal injury and performance. Therefore, it is questionable whether inducing structural mechanisms of immune activation in the CNS via LRAs with a possible risk on neuronal degradation is beneficial and/or preferable while treating (suppressed) PWH.

4.6 | Evaluation of LRA effects in CNS

Most techniques to evaluate the effects and safety of HIV eradication strategies in PWH are developed for the peripheral CD4⁺ T-cell reservoir and are hard to use for the CNS reservoir.¹⁸⁶ Current assays to study the size of the HIV reservoir are the quantitative viral outgrowth assay, Tat/Rev Induced Limiting Dilution Assay, and the intact proviral DNA assay.^{187,188} These assays are generally performed on blood-derived samples, which do not reflect the CNS reservoir. Some

HIV-induced markers of CNS injury can be found in the blood.¹⁸⁹ Ideally, the impact of LRAs on the viral reservoir in the CNS is investigated on human brain tissue on time points before and after an intervention. However, it is clear that these analyses cannot be performed in the setting of human clinical trials.¹⁹⁰ Therefore, longitudinal analyses will depend on the analyses of viral RNA and biomarkers for neuroinflammation and neuronal injury in CSF,^{9,46,55} which are of interest for the evaluation of the effects and safety of HIV eradication strategies. However, this option is also restricted because of the high invasiveness of every puncture.¹⁹¹

Other less invasive options to study the effects and safety of HIV eradication strategies on the CNS *in vivo* are imaging techniques. Although, MRI does not allow us to detect changes at the cellular level, such as neuronal death, as discussed before.¹⁹² More informative techniques to monitor HIV infection in the CNS are nuclear imaging approaches such as single photon emission computed tomography and PET scans. These techniques are used to observe immune activation, inflammation and neuronal injury related to HIV infection via radioactive tracers, as reviewed earlier.¹⁹³ For example PET imaging of CSF1R and TSPO were shown to track neuroinflammation in microglia^{63,194} and tracers are available to monitor synaptic density.¹⁹⁵ These techniques are interesting to utilize in an HIV-context.⁶³ However, to directly target HIV-infected cells in the CNS, there is an urgent need of HIV-specific tracers, which are able to penetrate the CNS.¹⁹³ Metabolic imaging using magnetic resonance spectroscopy is another promising imaging technique measuring chemical changes in (neuro)metabolites, which can be used to monitor neuroinflammation and its related neuronal injury. Unfortunately, just a limited number of brain regions can be studied and comorbidities have a great confounding effect.¹⁹⁶ Altogether, these imaging techniques are of great promise to monitor the effects of eradication strategies and its safety in the CNS, although it is hard to monitor the direct viral reactivation of HIV. The usage and further development of these novel imaging techniques is of great importance to evaluate the effects and safety of HIV eradication strategies in the CNS.

5 | CONCLUDING REMARKS

Eradication of the latent and persistent CNS reservoir is important because continued viral production during ART can contribute to the development of HAND. Cessation of ART can lead to systemic viral recrudescence contributing to HAND. HIV RNA in the CNS has been associated with severe neurologic manifestations. The CNS is shielded from the periphery by the BBB, which results in a unique microenvironment with CNS-specific cells that may be infected by HIV such as microglia, perivascular macrophages, and astrocytes. Localized HIV replication in these cells results in the generation and selection of compartmentalized CNS-specific viral quasispecies. Moreover, in these cells, HIV transcriptional activity and latency induction may be different, due to cellular characteristics and polymorphisms in the LTR of these CNS viral populations. Most HIV cure research efforts to date have focused on the “shock and kill” strategy. Unfortunately, the evalu-

ation of HIV cure strategies in the CNS, including the “shock and kill” strategy is challenging, and several limitations regarding the success and safety of reactivation of the viral reservoir with LRAs in the CNS exist.

It is therefore important to get more insight in the mechanisms associated with HIV latency in the CNS and the potential impact of latency reversal on neuropathogenesis. Improved delivery of LRAs is being explored such as the development of nanoparticles that can specifically target latently infected HIV cells in the CNS. Moreover, the differences in the viral LTR sequences could imply that CNS-specific LRAs may be needed to specifically reactivate brain-specific viral populations. The use of combinations of LRAs with apoptotic inducers may also improve the efficacy and safety of the “shock and kill” strategy, by sensitizing cells for apoptosis shortly after HIV is reactivated and thereby limiting continued viral production and bystander cell death. Ideally, these strategies will lead to a higher efficacy and specificity of the drug compounds toward HIV-infected cells, in order to increase eradication of HIV-infected cells and limit the eradication of noninfected brain cells.

Furthermore, better *in vitro* models such as human cerebral organoid model systems or *in vivo* animal studies are needed to investigate mechanisms of HIV persistence in the CNS including latency, latency reversal and its impact on cell activation, viral production, immune activation, and safety. Robust HIV reactivation via LRAs likely leads to encephalitis, neuronal damage, or neuronal loss due to the relative aspecific LRA stimulation, induced inflammation, viral toxicity causing bystander cell death. Considering the limited potential to replenish these neurons, the “shock and kill” strategy might have detrimental consequences on brain function. Therefore, it is of great importance to monitor HIV RNA in CSF and specific CSF parameters during clinical trials that may help us to assess the impact and safety of the “shock and kill” strategy. Moreover, further development of imaging techniques can provide a less invasive method to evaluate the effects and safety of the LRAs in treated individuals. As an alternative, at least CNS parameters in the blood need to be monitored. Anti-inflammatory compounds could be combined with the “shock and kill” strategy to diminish neuroinflammation seen in PWH. However, it is questionable whether the elimination of infected and reactivated cells will still occur if inflammation is inhibited.

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AUTHORSHIP

M. M. N. designed the review and performed literature research. M. M. N and S. G. and wrote the manuscript. S. G., N. B., L. G., L. W., A. W., S. L., M. N., and J. S. revised the manuscript for content. L. J. performed literature research.

DISCLOSURE

The authors declare no conflicts of interest.

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