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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 promotor 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-kB 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.

KEYWORDS
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 effectivity 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 permeable[34] (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 infection[41] 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

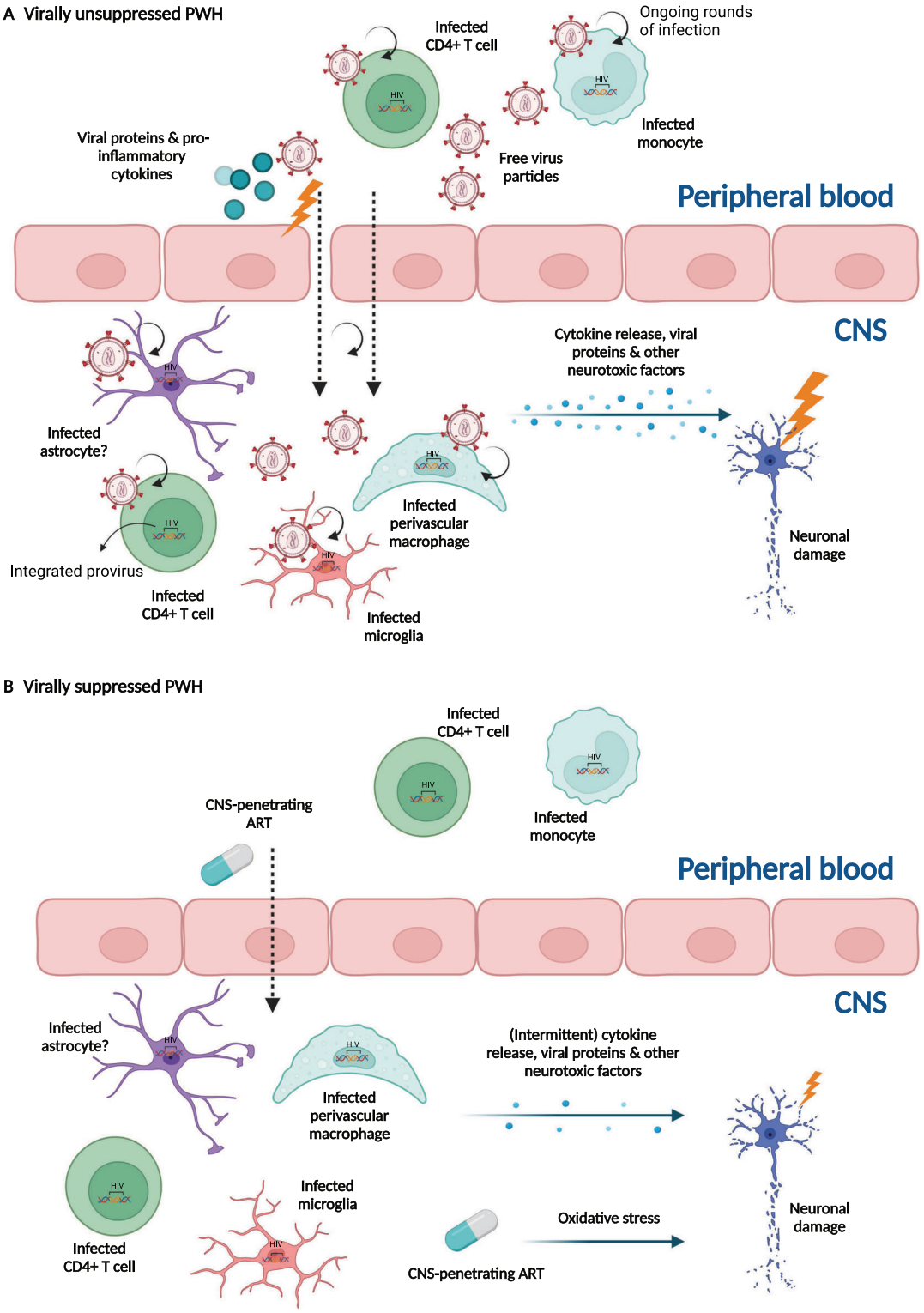

FIGURE 1 **Overview of neuropathogenesis in virally unsuppressed and suppressed PWH. (A) Virally unsuppressed PWH:** The exposure of viral proteins and inflammatory cytokines may cause increased permeability of the blood–brain barrier. This can contribute to the entrance of HIV within the CNS via free 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 morrow.[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 Hμglia.[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 hμglia 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-kB,[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/EBPγ, a truncated form of C/EBP, chicken ovalbumin upstream promotor 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 transcription[17] 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-kB,[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.

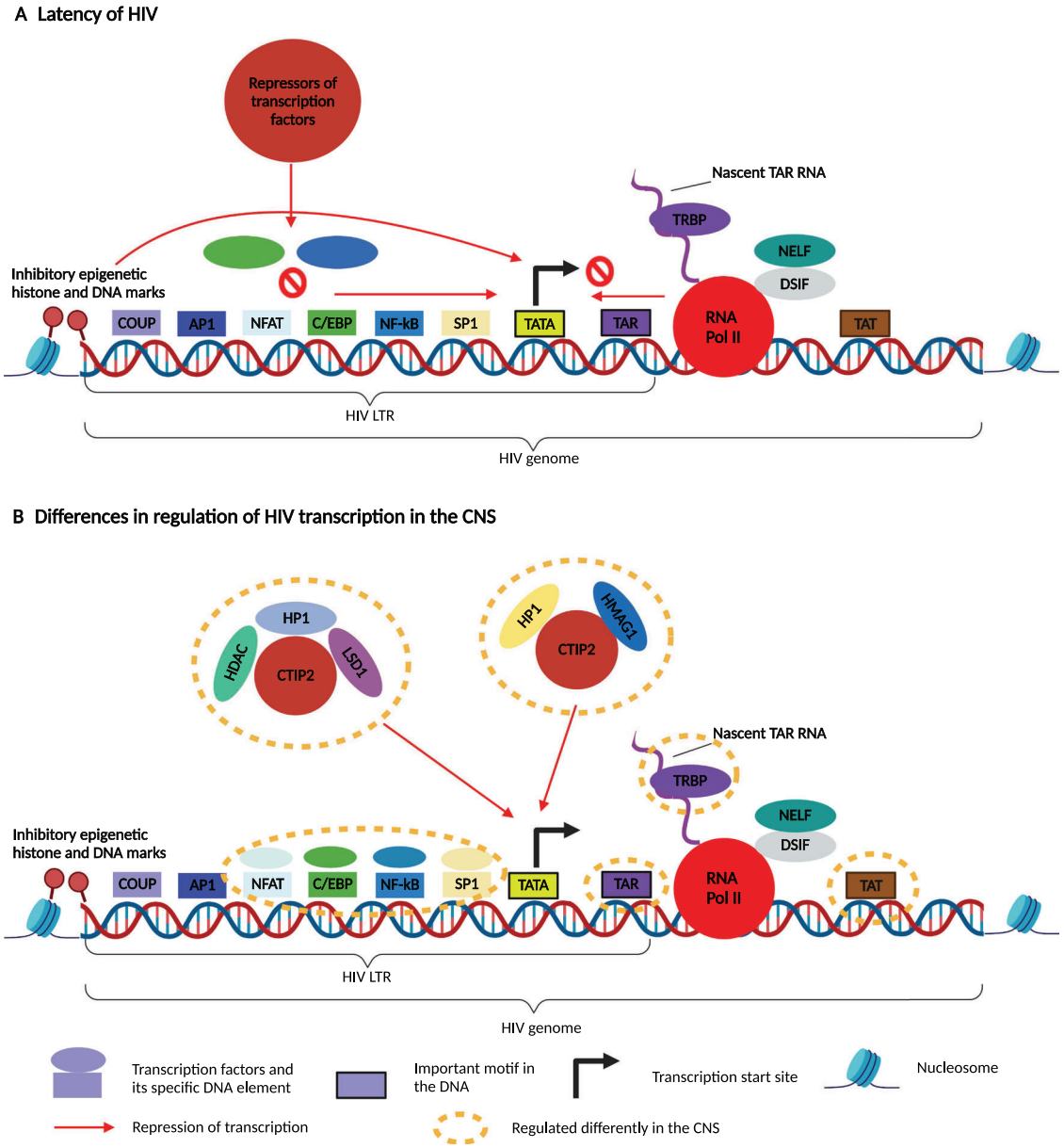

FIGURE 2 **HIV latency regulation in periphery and its differences within the CNS. (A) Latency of HIV:** The presence of transcription repression factors and inhibitory epigenetic around the HIV integration site and LTR prevent the transcription of HIV. Tat is not transcribed, which is needed for full-length 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; HMAG1, 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; TRPB, 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-kB	Altered binding of NF-$\kappa\beta$ 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
Unique transcription factors		
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
Epigenetic regulation		
Increased levels of COUP-TF interacting protein (CTIP2, histone deacetlyase (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 systems fails to detect the presence of transcriptional 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-kB inducing kinase (NIK), allowing for the accumulation of NF-kB.[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

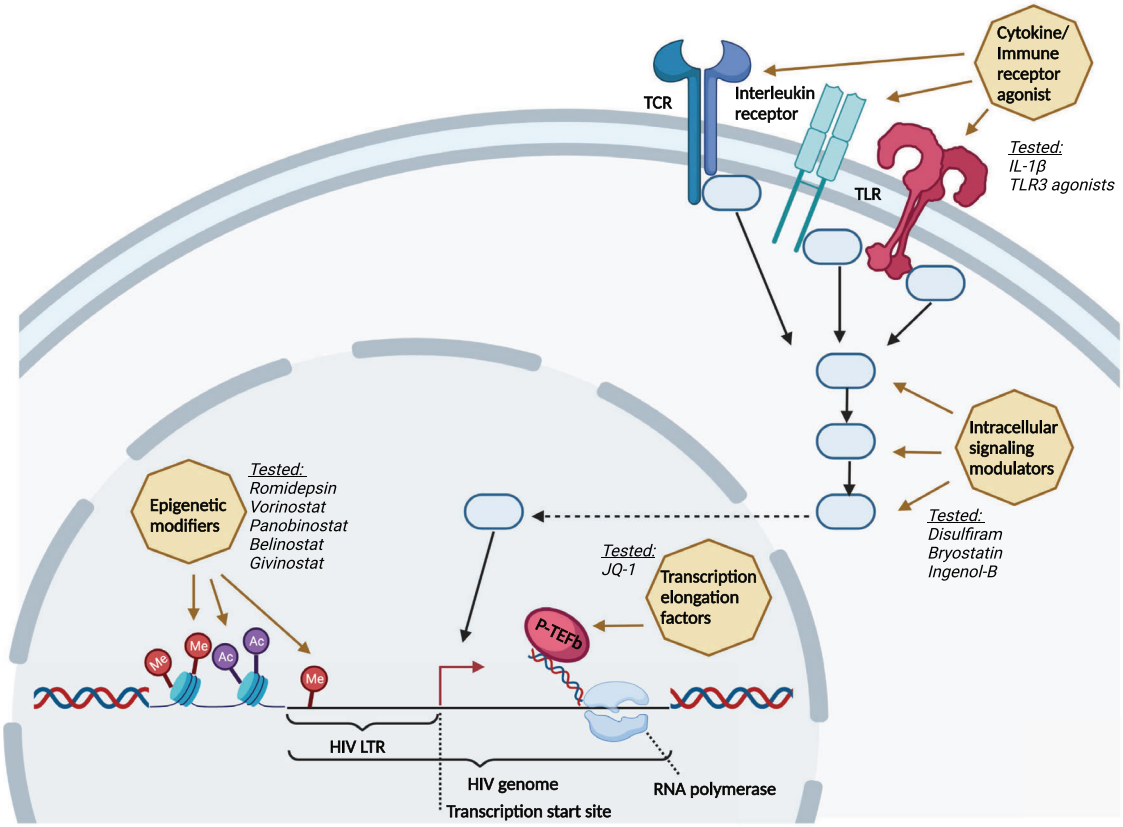

FIGURE 3 **The different classes of LRAs and compounds that are tested for their efficacy in culture models CNS cells or CNS quasispecies.** Latency reversal agents (LRAs) can be divided within 5 classes, based on their working mechanisms. Within each of these classes, subclasses are defined. Within the figure, the LRAs that have been tested on CNS culture models or CNS viral quasispecies 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, bryostatin, 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
Epigenetic modifiers	
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
Intracellular signaling modulators	
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
Cytokine or immune receptor agonists	
ILs and cytokines	IL-1β, 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
Transcription elongation factors	
Bromodomain and extraterminal domain (BET) inhibitors	JQ1, MMQO
Unclassified	
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-1β.[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
Microglial models			
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
Astrocyte models			
Disulfiram, romidepsin, panobinostat	Astrocytes	Viral transcription	22
IL-1β	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. Postmortem 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 markers[170] and accumulation of amyloid beta,[171] which may have detrimental consequences such as neuronal injury. At last, it has been shown that the LRAs prostratin and bryostatin 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 reviewedearlier.[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 traces, 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 evaluation 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.

REFERENCES

1. Rojas-Celis V, Valiente-Echeverría F, Soto-Rifo R, Toro-Ascuy D. New challenges of HIV-1 infection: how HIV-1 attacks and resides in the central nervous system. *Cells*. 2019;8: 1245.
2. Deeks SG, Lewin SR, Ross AL, et al. International AIDS Society global scientific strategy: towards an HIV cure 2016. *Nature Medicine*. 2016;22: 839-50.
3. Finzi D, Blankson J, Siliciano JD, et al. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nature Medicine*. 1999;5: 512-7.
4. Balcom EF, Roda WC, Cohen EA, Li MY, Power C. HIV-1 persistence in the central nervous system: viral and host determinants during antiretroviral therapy. *Current Opinion in Virology*. 2019;38: 54-62.
5. Bednar MM, Sturdevant CB, Tompkins LA, et al. Compartmentalization, viral evolution, and viral latency of HIV in the CNS. *Current HIV/AIDS Reports*. 2015;12: 262-71.
6. Enting RH, Prins JM, Jurriaans S, Brinkman K, Portegies P, Lange JMA. Concentrations of human immunodeficiency virus type 1 (HIV-1) RNA in cerebrospinal fluid after antiretroviral treatment inflated during primary HIV-1 infection. *Clinical Infectious Diseases*. 2001;32: 1095-9.
7. Davis LE, Hjelle BL, Miller VE, et al. Early viral brain invasion in iatrogenic human immunodeficiency virus infection. *Neurology*. 1992;42: 1736-9.
8. Schnell G, Price RW, Swanstrom R, Spudich S. Compartmentalization and clonal amplification of HIV-1 variants in the cerebrospinal fluid during primary infection. *Journal of Virology*. 2010;84: 2395-407.
9. Valcour V, Chalermchai T, Sailasuta N, et al. Central nervous system viral invasion and inflammation during acute HIV infection. *Journal of Infectious Diseases*. 2012;206: 275-82.
10. Sturdevant CB, Joseph SB, Schnell G, Price RW, Swanstrom R, Spudich S. Compartmentalized replication of R5 T cell-tropic HIV-1 in the central nervous system early in the course of infection. *PLoS Pathogens*. 2015;11: 1-24.
11. Joseph SB, Kincer LP, Bowman NM, et al. Human immunodeficiency virus type 1 RNA detected in the central nervous system (CNS) after years of suppressive antiretroviral therapy can originate from a replicating CNS reservoir or clonally expanded cells. *Clinical Infectious Diseases*. 2019;69: 1345-52.
12. Churchill MJ, Gorry PR, Cowley D, et al. Use of laser capture microdissection to detect integrated HIV-1 DNA in macrophages and astrocytes from autopsy brain tissues. *Journal of NeuroVirology*. 2006;12: 146-52.
13. Thompson KA, Cherry CL, Bell JE, McLean CA. Brain cell reservoirs of latent virus in presymptomatic HIV-infected individuals. *American Journal of Pathology*. 2011;179: 1623-9.
14. Churchill MJ, Wesselingh SL, Cowley D, et al. Extensive astrocyte infection is prominent in human immunodeficiency virus - associated dementia. *Annals of Neurology*. 2009;66: 253-8.
15. Ko A, Kang G, Hattler JB, et al. Macrophages but not astrocytes harbor HIV DNA in the brains of HIV-1-INFECTED AVIREMIC INDIVIDUALS ON SUPPRESSIVE ANTIRETROVIRAL THErapy. *Journal of Neuroimmune Pharmacology*. 2019;14: 110-9.
16. Zhang YL, Ouyang YB, Liu LG, Chen DX. Blood-brain barrier and neuro-AIDS. *European Review for Medical and Pharmacological Sciences*. 2015;19: 4927-39.
17. Churchill MJ, Cowley DJ, Wesselingh SL, Gorry PR, Gray LR. HIV-1 transcriptional regulation in the central nervous system and implications for HIV cure research. *Journal of NeuroVirology*. 2015;21: 290-300.
18. Brese RL, Gonzalez-Perez MP, Koch M, et al. Ultradeep single-molecule real-time sequencing of HIV envelope reveals complete compartmentalization of highly macrophage-tropic R5 proviral variants in brain and CXCR4- using variants in immune and peripheral tissues. *Journal of NeuroVirology*. 2018;24: 439-53.
19. Rose R, Paz Gonzalez-Perez M, Nolan DJ, Cross S, Lamers SL, Luzuriaga K. Ultradeep HIV-1 proviral envelope sequencing reveals complex population structure within and between brain and splenic tissues. *Journal of virology*. 2021;95.
20. Cao S, Woodrow KA. Nanotechnology approaches to eradicating HIV reservoirs. *European Journal of Pharmaceutics and Biopharmaceutics*. 2019;138: 48-63.
21. Kim Y, Anderson JL, Lewin SR. Getting the "Kill" into "Shock and Kill": strategies to eliminate latent HIV. *Cell Host and Microbe*. 2018;23: 14-26.
22. Gray LR, On H, Roberts E, et al. Toxicity and in vitro activity of HIV-1 latency-reversing agents in primary CNS cells. *Journal of NeuroVirology*. 2016: 455-63.
23. Gama L, Abreu CM, Shirk EN, et al. Reactivation of simian immunodeficiency virus reservoirs in the brain of virally suppressed macaques. *Aids*. 2017;31: 5-14.
24. Gray LR, Cowley D, Welsh C, et al. CNS-specific regulatory elements in brain-derived HIV-1 strains affect responses to latency-reversing agents with implications for cure strategies. *Molecular Psychiatry*. 2016;21: 574-84.
25. Swanstrom R, Coffin J. HIV-1 pathogenesis: the virus. Cold Spring Harbor Perspectives. *Medicine*. 2012;2.
26. McMichael AJ, Borrow P, Tomaras GD, Goonetilleke N, Haynes BF. The immune response during acute HIV-1 infection: clues for vaccine development. *Nature Reviews Immunology*. 2010;10: 11-23.
27. Stein J, Storcksdieck genannt Bonsmann M, Streeck H. Barriers to HIV cure. *HLA*. 2016;88: 155-63.
28. Mzingwane ML, Tiemessen CT. Mechanisms of HIV persistence in HIV reservoirs. *Reviews in Medical Virology*. 2017;27: 1-12.
29. Joseph SB, Arrildt KT, Sturdevant CB, Swanstrom R. HIV-1 target cells in the CNS. *Journal of NeuroVirology*. 2015;21: 276-89.
30. Xu R, Feng X, Xie X, Zhang J, Wu D, Xu L. HIV-1 Tat protein increases the permeability of brain endothelial cells by both inhibiting occludin expression and cleaving occludin via matrix metalloproteinase-9. *Brain Research*. 2012 Feb 3;1436: 13-9.
31. Banks WA, Freed EO, Wolf KM, Robinson SM, Franko M, Kumar VB. Transport of human immunodeficiency virus type 1 pseudoviruses across the blood-brain barrier: role of envelope proteins and adsorptive endocytosis. *Journal of Virology*. 2001 May 15;75: 4681-91.
32. Leibrand CR, Paris JJ, Ghandour MS, et al. HIV-1 Tat disrupts blood-brain barrier integrity and increases phagocytic perivascular macrophages and microglia in the dorsal striatum of transgenic mice. *Neuroscience Letters*. 2017;640: 136-43.
33. Cioni C, Annunziata P. Circulating gp120 alters the blood-brain barrier permeability in HIV-1 gp120 transgenic mice. *Neuroscience Letters*. 2002;330: 299-301.
34. Kaul M, Garden GA, Lipton SA. Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature*. 2001;410.
35. Machado Andrade V, Stevenson M. Host and Viral Factors Influencing Interplay between the macrophage and HIV-1. *Journal of Neuroimmune Pharmacology*. 2019;14: 33-43.
36. Sharma V, Creegan M, Tokarev A, et al. *PLOS Pathogens*. 2021 Dec 7;17: e1010105.
37. León-Rivera R, Veenstra M, Donoso M, et al. Central nervous system (CNS) viral seeding by mature monocytes and potential therapies to reduce CNS viral reservoirs in the cART era. *mBio*. 2021;12: 1-17.
38. Veenstra M, León-Rivera R, Li M, Gama L, Clements JE, Berman JW. Mechanisms of CNS viral seeding by HIV+ CD14+ CD16+ monocytes: establishment and reseeding of viral reservoirs contributing to HIV-associated neurocognitive disorders. *mBio*. 2017;8.
39. Ferretti F, Gisslen M, Cinque P, Price RW. Cerebrospinal fluid HIV escape from antiretroviral therapy. *Vol 12, Current HIV/AIDS Reports Current Medicine Group LLC 1*. 2015: 280-8.
40. Fox HS, Morsey BM, Niu M, Emanuel K, Lamberty BG, Trease AJ, SIV-infected CD4+ T cells carry virus to the brain. CROI 2022: poster Session-F01 CNS reservoirs, regulation, and escape. 2022

41. Maschke M, Kastrup O, Esser S, Ross B, Hengge U, Hufnagel A. Incidence and prevalence of neurological disorders associated with HIV since the introduction of highly active antiretroviral therapy (HAART). *Journal of Neurology Neurosurgery and Psychiatry*;69: 376-80.
42. González-Scarano F, Martín-García J. The neuropathogenesis of AIDS. *Vol 5*, *Nature Reviews Immunology*. 2005: 69-81.
43. Wang Y, Liu M, Lu Q, et al. Global prevalence and burden of HIV-associated neurocognitive disorder: a meta-analysis. *Neurology*. 2020;95: e2610-21.
44. Dewey J, Hana G, Russell T, et al. Reliability and validity of MRI-based automated volumetry software relative to auto-assisted manual measurement of subcortical structures in HIV-infected patients from a multisite study. *Neuroimage*. 2010;51: 1334-44.
45. Ances BM, Ortega M, Vaida F, Heaps J, Paul R. Independent effects of HIV, aging, and HAART on brain volumetric measures. *J Axquir Immune Defic Syndr*. 2012;59: 469-77.
46. Peluso MJ, Meyerhoff DJ, Price RW, et al. Cerebrospinal fluid and neuroimaging biomarker abnormalities suggest early neurological injury in a subset of individuals during primary HIV infection. *Journal of Infectious Diseases*. 2013;207: 1703-12.
47. Ghosh AK, Sarkar A, Mitsuya H. HIV-Associated Neurocognitive Disorder (HAND) and the prospect of brain-penetrating protease inhibitors for antiretroviral treatment. *Med Res Arch*. 2017;5.
48. Dickens AM, Yoo SW, Chin AC, et al. Chronic low-level expression of HIV-1 Tat promotes a neurodegenerative phenotype with aging. *Scientific Reports*. 2017;7: 1-11.
49. Hong S, Banks WA. Role of the immune system in HIV-associated neuroinflammation and neurocognitive implications Suzi. *Brain Behav Immun*. 2015: 1-12.
50. Zayyad Z, Spudich S. Neuropathogenesis of HIV: from initial neuroinvasion to HIV-associated neurocognitive disorder (HAND). *Current HIV/AIDS Reports*. 2015;12: 16-24.
51. Kraft-Terry SD, Buch SJ, Fox HS, Gendelman HE. A coat of many colors: neuroimmune crosstalk in human immunodeficiency virus infection. *Vol 64*, *Neuron*. 2009: 133-45.
52. Young AC, Yiannoutsos CT, Hedge M, et al. Cerebral metabolite changes prior to and after antiretroviral therapy in primary HIV infection. *Neurology*. 2014: 1592-600.
53. Sailasuta N, Ross W, Ananworanich J, et al. Change in brain magnetic resonance spectroscopy after treatment during acute HIV infection. *PLoS ONE*. 2012;7.
54. Ances BM, Letendre SL. CROI 2019: neurologic complications of HIV disease. *Topics in Antiviral Medicine*. 2019;27: 26-33.
55. Yilmaz A, Yiannoutsos CT, Fuchs D, et al. Cerebrospinal fluid neopterin decay characteristics after initiation of antiretroviral therapy. 2013.
56. Oliveira MF, Chaillon A, Nakazawa M, et al. Early antiretroviral therapy is associated with lower HIV DNA molecular diversity and lower inflammation in cerebrospinal fluid but does not prevent the establishment of compartmentalized HIV DNA populations. *PLoS Pathogens*. 2017;13.
57. Gisslen M, Keating SM, Spudich S, et al. Compartmentalization of cerebrospinal fluid inflammation across the spectrum of untreated HIV-1 infection, central nervous system injury and viral suppression. *PLoS ONE*. 2021;16.
58. Sanford R, Ances BM, Meyerhoff DJ, et al. Longitudinal trajectories of brain volume and cortical thickness in treated and untreated primary human immunodeficiency virus infection. *Clinical Infectious Diseases*. 2018 Nov 13;67: 1705-11.
59. Brew BJ, Barnes SL. The impact of HIV central nervous system persistence on pathogenesis. *Aids*. 2019;33: S113-21.
60. Spudich S, Robertson KR, Bosch RJ, et al. Persistent HIV-infected cells in cerebrospinal fluid are associated with poorer neurocognitive performance. *Journal of Clinical Investigation*. 2019;129: 3339-46.
61. Peluso MJ, Ferretti F, Peterson J, et al. Cerebrospinal fluid HIV escape associated with progressive neurologic dysfunction in patients on antiretroviral therapy with well controlled plasma viral load. *Aids*. 2012;26: 1765-74.
62. Perez-Valero I, Ellis R, Heaton R, et al. Cerebrospinal fluid viral escape in aviremic HIV-infected patients receiving antiretroviral therapy: prevalence, risk factors and neurocognitive effects. *AIDS*. 2019 Mar 1;33: 475-81.
63. Rubin LH, Sacktor N, Creighton J, et al. Microglial activation is inversely associated with cognition in individuals living with HIV on effective antiretroviral therapy. *AIDS*. 2018;32: 1661-7.
64. Wallet C, De Rovere M, Van Assche J, et al. Microglial cells: the main HIV-1 reservoir in the brain. *Frontiers in Cellular and Infection Microbiology*. 2019;9: 1-18.
65. Gray LR, Roche M, Flynn JK, Wesselingh SL, Gorry PR, Churchill MJ. Is the central nervous system a reservoir of HIV-1? *Current Opinion in HIV and AIDS*. 2014;9: 552-8.
66. Harrington PR, Schnell G, Letendre SL, et al. Cross-sectional characterization of HIV-1 env compartmentalization in cerebrospinal fluid over the full disease course. *Aids*. 2009;23: 907-15.
67. Evering TH. Molecular signatures of HIV-1 envelope associated with HIV-associated neurocognitive disorders. *Current HIV/AIDS Reports*. 2018;15: 72-83.
68. Schnell G, Joseph S, Spudich S, Price RW, Swanstrom R. HIV-1 replication in the central nervous system occurs in two distinct cell types. *PLoS Pathogens*. 2011;7.
69. Arrildt KT, LaBranche CC, Joseph SB, et al. Phenotypic correlates of HIV-1 macrophage tropism. *Journal of Virology*. 2015;89: 11294-311.
70. Gorry PR, Taylor J, Holm GH, et al. Increased CCR5 affinity and reduced CCR5/CD4 dependence of a neurovirulent primary human immunodeficiency virus type 1 isolate. *Journal of Virology*. 2002;76: 6277-92.
71. Lee B, Sharron M, Montaner LJ, Weissman D, Doms RW. Quantification of CD4, CCR5, and CXCR4 levels on lymphocyte subsets, dendritic cells, and differentially conditioned monocyte-derived macrophages. *Medical Sciences*. 1999;96: 5215-20.
72. Joseph SB, Arrildt KT, Swanstrom AE, et al. Quantification of entry phenotypes of macrophage-tropic HIV-1 across a wide range of CD4 densities. *Journal of Virology*. 2014;88: 1858-69.
73. Beauparlant D, Rusert P, Magnus C, et al. Delineating CD4 dependency of HIV-1: adaptation to infect low level CD4 expressing target cells widens cellular tropism but severely impacts on envelope functionality. *PLoS Pathogens*. 2017;13: 1-42.
74. Cunningham PH, Smith DG, Satchell C, Cooper DA, Brew B. Evidence for independent development of resistance to HIV-1 reverse transcriptase inhibitors in the cerebrospinal fluid. *AIDS*. 2000;14: 1949-54.
75. Venturi G, Catucci M, Romano L, et al. Antiretroviral resistance mutations in human immunodeficiency virus type 1 reverse transcriptase and protease from paired cerebrospinal fluid and plasma samples. *The Journal of Infectious Diseases*. 2000;181: 740-5.
76. Canestri A, Lescure FX, Jaureguiberry S, et al. Discordance between cerebral spinal fluid and plasma HIV replication in patients with neurological symptoms who are receiving suppressive antiretroviral therapy. *Clinical Infectious Diseases*. 2010;50: 773-8.
77. van Lelyveld SFL, Nijhuis M, Baatz F, et al. Therapy failure following selection of enfuvirtide-resistant HIV-1 in cerebrospinal fluid. *Clinical Infectious Diseases*. 2010;50: 387-90.
78. Henderson LJ, Johnson TP, Smith BR, et al. Presence of Tat and transactivation response element in spinal fluid despite antiretroviral therapy. *Aids*. 2019;33: S145-57. May 2018.
79. Joseph SB, Trunfio M, Kincer LP, Calcagno A, Price RW. What can characterization of cerebrospinal fluid escape populations teach us about viral reservoirs in the central nervous system? *Aids*. 2019;33: S171-9.

80. Edén A, Nilsson S, Hagberg L, et al. Asymptomatic cerebrospinal fluid HIV-1 viral blips and viral escape during antiretroviral therapy: a longitudinal study. *Journal of Infectious Diseases*. 2016;214: 1822-5.
81. Mukerji SS, Misra V, Lorenz DR, et al. Impact of antiretroviral regimens on cerebrospinal fluid viral escape in a prospective multicohort study of antiretroviral therapy-experienced human immunodeficiency virus-1 – infected adults in the United States. *Clinical Infecitous Diseases*. 2018;67: 1182-90.
82. Osborne O, Peyravian N, Nair M, Daunert S, Toborek M. The paradox of HIV blood–brain barrier penetrance and antiretroviral drug delivery deficiencies. *Trends in Neurosciences*. 2020;xx: 1-14.
83. Nightingale S, Geretti AM, Beloukas A, et al. Discordant CSF/plasma HIV-1 RNA in patients with unexplained low-level viraemia. *Journal of NeuroVirology*. 2016;22: 852-60.
84. Trunfio M, Joseph SB, Ghisetti V, et al. Symptomatic cerebrospinal fluid HIV-1 escape with no resistance-associated mutations following low-level plasma viremia. *Journal of NeuroVirology*. 2018;24: 132-6.
85. Lustig G, Cele S, Karim F, et al. T cell derived HIV-1 is present in the CSF in the face of suppressive antiretroviral therapy. *PLoS Pathogens*. 2021;17.
86. Tantale K, Garcia-Oliver E, Robert MC, et al. Stochastic pausing at latent HIV-1 promoters generates transcriptional bursting. *Nature Communications*. 2021;12.
87. Mishra M, Varghese RK, Verma A, et al. Genetic diversity and proviral DNA load in different neural compartments of HIV-1 subtype C infection. *Journal of NeuroVirology*. 2015;21: 399-414.
88. Gonzalez-Perez MP, Peters PJ, O'Connell O, et al. Identification of emerging macrophage-tropic HIV-1 R5 variants in brain tissue of AIDS patients without severe neurological complications. *Journal of Virology*. 2017;91: 1-21.
89. Caragounis EC, Gisslén M, Lindh M, et al. Comparison of HIV-1 pol and env sequences of blood, CSF, brain and spleen isolates collected ante-mortem and post-mortem. *Acta Neurologica Scandinavica*. 2008;117: 108-16.
90. Burkala EJ, He J, West JT, Wood C, Petito CK. Compartmentalization of HIV-1 in the central nervous system: role of the choroid plexus. *AIDS*. 2005;19: 675-84.
91. Tso FY, Kang G, Kwon EH, Julius P, Li Q, West JT, et al. Brain is a potential sanctuary for subtype C HIV-1 irrespective of ART treatment outcome. *PLoS ONE*. 2018;13.
92. Trillo-Pazos G, Diamanturos A, Rislove L, et al. Detection of HIV-1 DNA in microglia/macrophages, astrocytes and neurons isolated from brain tissue with HIV-1 encephalitis by LaserCapture Microdissection. *Brain Pathol*. 2003;13: 144-54.
93. Cochrane CR, Angelovich TA, Byrnes SJ, et al. Intact HIV proviruses persist in the brain despite viral suppression with ART. *Annals of Neurology*. 2022.
94. Desplats P, Dumaop W, Smith D, et al. Molecular and pathologic insights from latent HIV-1 infection in the human brain. *Neurology*. 2013;80: 1415-23.
95. Gianella S, Pond SLK, Oliveira MF, et al. Compartmentalized HIV rebound in the central nervous system after interruption of antiretroviral therapy. *Virus Evolution*. 2016;2: 1-12.
96. Kaalund SS, Johansen A, Fabricius K, Pakkenberg B. Untreated patients dying with AIDS have loss of neocortical neurons and glia cells. *Frontiers in Neuroscience*. 2020;13: 1-10.
97. Gallo V, Deneen B. Glial development: the crossroads of regeneration and repair in the CNS. *Neuron*. 2014;83: 283-308.
98. Barat C, Proust A, Deshiere A, Leboeuf M, Drouin J, Tremblay MJ. Astrocytes sustain long-term productive HIV-1 infection without establishment of reactivable viral latency. *Glia*. 2018;66: 1363-81.
99. Díaz L, Martínez-Bonet M, Sánchez J, et al. Bryostatin activates HIV-1 latent expression in human astrocytes through a PKC and NF-κB-dependent mechanism. *Scientific Reports*. 2015;5: 1-12.
100. Gorry PR, Ong C, Thorpe J, et al. Astrocyte infection by HIV-1: mechanisms of restricted virus replication, and role in the pathogenesis of HIV-1-associated dementia. *Current HIV Research*. 2003;1: 463.
101. Churchill M, Nath A. Where does HIV hide? A focus on the central nervous system. *Vol 8*, *Current Opinion in HIV and AIDS*. 2013: 165-9.
102. Russell RA, Chojnacki J, Jones DM, et al. Astrocytes resist HIV-1 fusion but engulf infected macrophage material. *Cell Reports*. 2017;18: 1473-83.
103. Al-Harti L, Joseph J, Nath A. Astrocytes as an HIV CNS reservoir: highlights and reflections of an NIMH-sponsored symposium. *Journal of NeuroVirology*. 2018;24: 665-9.
104. Valdebenito S, Castellano P, Ajasin D, Eugenin EA. Astrocytes are HIV reservoirs in the brain: a cell type with poor HIV infectivity and replication but efficient cell-to-cell viral transfer. *Journal of Neurochemistry*. 2021;158: 429-43.
105. Herz J, Filiano AJ, Smith A, Yogev N, Kipnis J. Myeloid cells in the central nervous system. *Immunity*. 2017;46: 943-56.
106. Ginhoux F, Prinz M. Origin of microglia: current concepts and past controversies. *Cold Spring Harbor Perspectives in Biology*. 2015;7: 1-15.
107. Garcia-Mesa Y, Jay TR, Checkley MA, et al. Immortalization of primary microglia: a new platform to study HIV regulation in the central nervous system. *Journal of NeuroVirology*. 2017;23: 47-66.
108. Rodrigues V, Ruffin N, San-Roman M, Benaroch P. Myeloid cell interaction with HIV: a complex relationship. *Vol 8*, *Frontiers in Immunology Frontiers Media SA*. 2017.
109. Gumbs SBH, Berdenis van Berlekom A, Kübler R, et al. Characterization of HIV-1 infection in microglia-containing human cerebral organoids. *Viruses*. 2022;14: 829.
110. Rai MA, Hammonds J, Pujato M, Mayhew C, Roskin K, Spearman P. Comparative analysis of human microglial models for studies of HIV replication and pathogenesis. *Retrovirology*. 2020;17.
111. Avalos CR, Abreu CM, Queen SE, et al. Brain macrophages in simian immunodeficiency virus-infected, antiretroviral-suppressed macaques: a functional latent reservoir. *mBio*. 2017;8: 1-16.
112. Abreu C, Shirk EN, Queen SE, et al. Brain macrophages harbor latent, infectious simian immunodeficiency virus. *Aids*. 2019;33: S181-8. June 2018.
113. Llewellyn GN, Alvarez-Carbonell D, Chateau M, Karn J, Cannon PM. HIV-1 infection of microglial cells in a reconstituted humanized mouse model and identification of compounds that selectively reverse HIV latency. *Journal of NeuroVirology*. 2018;24: 192-203.
114. Smolders J, Heutinck KM, Fransen NL, et al. Tissue-resident memory T cells populate the human brain. *Nature Communications*. 2018;9.
115. Wong ME, Johnson CJ, Hearps AC, Jaworowski A. Development of a novel in vitro primary human monocyte-derived macrophage model to study reactivation of HIV-1 transcription. *Journal of Virology*. 2021;95.
116. Gumbs SBH, Raphael K, Gharu L, et al. Human microglial models to study HIV infection and neuropathogenesis: a literature overview and comparative analyses. *Journal of NeuroVirology*. 2022;1: 3.
117. Castellano P, Prevedel L, Eugenin EA. HIV-infected macrophages and microglia that survive acute infection become viral reservoirs by a mechanism involving Bim. *Scientific Reports*. 2017;7: 1-16.
118. Cenker JJ, Stultz RD, McDonald D. Brain microglial cells are highly susceptible to HIV-1 infection and spread. *AIDS Research and Human Retroviruses*. 2017;33: 1155-65.
119. Janabi N, Peudenier S, Héron B, Ng KH, Tardieu M. Establishment of human microglial cell lines after transfection of primary cultures of embryonic microglial cells with the SV40 large T antigen. *Neuroscience Letters*. 1995;195: 105-8.
120. Melief J, Sneeboer MAM, Litjens M, et al. Characterizing primary human microglia: a comparative study with myeloid subsets and culture models. *GLIA*. 2016;64: 1857-68.
121. Alvarez-Carbonell D, Ye F, Ramanath N, Dobrowolski C, Karn J. The glucocorticoid receptor is a critical regulator of HIV latency in human

microglial cells. *Journal of Neuroimmune Pharmacology*. 2019;14: 94-109.
122. Alvarez-Carbonell D, Garcia-Mesa Y, Milne S, et al. Toll-like receptor 3 activation selectively reverses HIV latency in microglial cells. *Retrovirology*. 2017;14: 1-25.
123. Harschnitz O, Studer L. Human stem cell models to study host–virus interactions in the central nervous system. *Vol 21, Nature Reviews Immunology Nature Research*. 2021: 441-53.
124. Ryan SK, Gonzalez MV, Garifallou JP, et al. Neuroinflammation and EIF2 signaling persist despite antiretroviral treatment in an hiPSC Tri-culture model of HIV infection. *Stem Cell Reports*. 2020;14: 703-16.
125. dos Reis RS, Sant S, Keeney H, Wagner MCE, Ayyavoo V. Modeling HIV-1 neuropathogenesis using three-dimensional human brain organoids (hBORGs) with HIV-1 infected microglia. *Scientific Reports*. 2020;10.
126. Premeaux TA, Mediouni S, Leda A, et al. Next-generation human cerebral organoids as powerful tools to advance neurohiv research. *mBio*. 2021;12.
127. Mbonye U, Karn J. The molecular basis for human immunodeficiency virus latency. *Annual Review of Virology*. 2017;4: 261-85.
128. Lange UC, Verdikt R, Ait-Ammar A, Van Lint C. Epigenetic crosstalk in chronic infection with HIV-1. *Seminars in Immunopathology*. 2020: 187-200.
129. Gray LR, Cowley D, Crespan E, et al. Reduced basal transcriptional activity of central nervous system-derived HIV type 1 long terminal repeats. *AIDS Research and Human Retroviruses*. 2013;29: 365-70.
130. Burdo TH, Gartner S, Mauger D, Wigdahl B. Region-specific distribution of human immunodeficiency virus type 1 long terminal repeats containing specific configurations of CCAAT/enhancer-binding protein site II in brains derived from demented and nondemented patients. *Journal of NeuroVirology*. 2004;10: 7-14. SUPPL. 1.
131. Ross HL, Gartner S, McArthur JC, et al. HIV-1 LTR C/EBP binding site sequence configurations preferenstially encountered in brain lead to enhanced C/EBP factor binding and increased LTR-specific activity. *Journal of NeuroVirology*. 2001;7: 235-49.
132. Corboy JR, Garl PJ. HIV-1 LTR DNA sequence variation in brain-derived isolates. *Journal of NeuroVirology*. 1997;3: 331-41.
133. Cowley D, Gray LR, Wesselingh SL, Gorry PR, Churchill MJ. Genetic and functional heterogeneity of CNS-derived tat alleles from patients with HIV-associated dementia. *Journal of NeuroVirology*. 2011;17: 70-81.
134. Boven L, Noorbakhsh F, Bouma G, et al. Brain-derived human immunodeficiency virus-1 Tat exerts differential effects on LTR transactivation and neuroimmune activation. *Journal of NeuroVirology*. 2007;13: 173-84.
135. Spector C, Mele AR, Wigdahl B, Nonnemacher MR. Genetic variation and function of the HIV-1 Tat protein. *Medical Microbiology and Immunology*. 2019;208: 131-69.
136. Gosselin D, Skola D, Coufal NG, et al. An environment-dependent transcriptional network specifies human microglia identity. *Science (1979)*. 2017;356: 1248-59.
137. Zhang Y, Sloan SA, Clarke LE, et al. Purification and characterization of progenitor and mature human astrocytes reveals transcriptional and functional differences with mouse. *Neuron*. 2016;89: 37-53.
138. Hogan TH, Krebs FC, Wigdahl B. Regulation of human immunodeficiency virus type 1 gene expression and pathogenesis by CCAAT/enhancer binding proteins in cells of the monocyte/macrophage lineage. *Journal of NeuroVirology*. 2002;8: 21-6. SUPPL. 2.
139. Schwartz C, Catez P, Rohr O, Lecestre D, Aunis D, Schaeffer E. Functional Interactions between C/EBP, Sp1, and COUP-TF regulate human immunodeficiency virus type 1 gene transcription in human brain cells. *Journal of Virology*. 2000;74: 65-73.
140. Rohr O, Aunis D, Schaeffer E. COUP-TF and Sp1 interact and cooperate in the transcriptional activation of the human immunodeficiency virus type 1 long terminal repeat in human microglial cells. *Journal of Biological Chemistry*. 1997;272: 31149-55.
141. Ye F, Alvarez-Carbonell D, Nguyen K, et al. Recruitment of the CoREST transcription repressor complexes by Nerve Growth factor IB-like receptor (Nurr1/NR4A2) mediates silencing of HIV in microglial cells. Margottin-Goguet F, editor. *PLOS Pathogens*. 2022;18: e1010110.
142. Ong CL, Thorpe JC, Gorry PR, Bannwarth S, Jaworowski A, Howard JL, et al. Low TRBP levels support an innate human immunodeficiency virus type 1 resistance in astrocytes by enhancing the PKR antiviral response. *Journal of Virology*. 2005;79: 12763-72.
143. Taylor JP, Pomerantz1 R, Bagasra1 O, et al. TAR-independent transactivation by Tat in cells derived from the CNS: a novel mechanism of HIV-1 gene regulation. Vol. 1. *The EMBO Journal*. 1992.
144. Yang L, Morris GF, Lockyer JM, Lu M, Wang Z, Morris CB. Distinct transcriptional pathways of TAR-dependent and TAR-independent human immunodeficiency virus type-1 transactivation by Tat. *Vol 235, VIROLOGY*. 1997.
145. Taylor JP, Pomerantz RJ, Raj 'Gv, et al. Central nervous system-derived cells express a KB-binding activity that enhances human immunodeficiency virus type 1 transcription in vitro and facilitates TAR-independent transactivation by Tat. *JOURNAL OF VIROLOGY*. 1994.
146. Narasipura SD, Kim S, Al-Harthi L, Silvestri G. Epigenetic regulation of HIV-1 latency in astrocytes. *Journal of Virology*. 2014;88: 3031-8.
147. Marban C, Forouzanfar F, Ait-Ammar A, et al. Targeting the brain reservoirs: toward an HIV cure. *Frontiers in Immunology*. 2016;7: 1-13.
148. le Douce V, Herbein G, Rohr O, Schwartz C. Molecular mechanisms of HIV-1 persistence in the monocyte-macrophage lineage. *Retrovirology*. 2010;7:1-16.
149. Le Douce V, Colin L, Redel L, et al. LSD1 cooperates with CTIP2 to promote HIV-1 transcriptional silencing. *Nucleic Acids Research*. 2012;40: 1904-15.
150. Rohr O, Lecestre D, Chasserot-Golaz S, et al. Recruitment of Tat to heterochromatin protein HP1 via interaction with CTIP2 inhibits human immunodeficiency virus type 1 replication in microglial cells. *Journal of Virology*. 2003;77: 5415-27.
151. Le Douce V, Forouzanfar F, Eilebrecht S, et al. HIC1 controls cellular- and HIV-1- gene transcription via interactions with CTIP2 and HMGA1. *Scientific Reports*. 2016;6.
152. Forouzanfar F, Ali S, Wallet C, et al. HIV-1 Vpr mediates the depletion of the cellular repressor CTIP2 to counteract viral gene silencing. *Scientific Reports*. 2019;9: 1-10.
153. Sadowski I, Hashemi FB. Strategies to eradicate HIV from infected patients: elimination of latent provirus reservoirs. *Cellular and Molecular Life Sciences*. 2019;76: 3583-600.
154. Sarabia I, Bosque A. HIV-1 latency and latency reversal: does subtype matter? *Viruses*. 2019;11: 1-26.
155. Zerbato JM, Purves HV, Lewin SR, Rasmussen TA. Between a shock and a hard place: challenges and developments in HIV latency reversal. *Current Opinion in Virology*. 2019;38: 1-9.
156. Ait-Ammar A, Kula A, Darcis G, et al. Current status of latency reversing agents facing the heterogeneity of HIV-1 cellular and tissue reservoirs. *Frontiers in Microbiology*. 2020;10.
157. Abner E, Jordan A. HIV "shock and kill" therapy: in need of revision. *Antiviral Research*. 2019;166: 19-34.
158. Fidler S, Stöhr W, Pace M, et al. Antiretroviral therapy alone versus antiretroviral therapy with a kick and kill approach, on measures of the HIV reservoir in participants with recent HIV infection (the RIVER trial): a phase 2, randomised trial. *The Lancet*. 2020;395: 888-98.
159. Darcis G, Kula A, Bouchat S, et al. An in-depth comparison of latency-reversing agent combinations in various in vitro and ex vivo HIV-1

latency models identified bryostatin-1+JQ1 and ingenol-B+JQ1 to potently reactivate viral gene expression. *PLoS Pathogens*. 2015;11: 1-36.
160. Campbell GR, Bruckman RS, Chu YL, Spector SA. Autophagy induction by histone deacetylase inhibitors inhibits HIV type 1. *Journal of Biological Chemistry*. 2015;290: 5028-40.
161. Edara VV, Ghorpade A, Borgmann K. Insights into the gene expression profiles of active and restricted red/green-HIV + human astrocytes: implications for shock or lock therapies in the brain. *Journal of Virology*. 2020;94: 1-25.
162. Gisslén M, Rosengren L, Hagberg L, Deeks SG, Price RW. Cerebrospinal fluid signs of neuronal damage after antiretroviral treatment interruption in HIV-1 infection. *AIDS Research and Therapy*. 2005;2.
163. Alvarez-Carbonell D, Ye F, Ramanath N, et al. Cross-talk between microglia and neurons regulates HIV latency. *PLoS Pathog*. 2019;15: 1-43.
164. Silver J, Schwab ME, Popovich PG. Central nervous system regenerative failure: role of oligodendrocytes, astrocytes and microglia. *Cold Spring Harb Perspect Biol*. 2015;7: 1-22.
165. Garg H, Mohl J, Joshi A. HIV-1 induced bystander apoptosis. *Vol*. 2012;4: 3020-43.
166. Olivier IS, Cacabelos R, Naidoo V. Risk factors and pathogenesis of HIV-associated neurocognitive disorder: the role of host genetics. *International Journal of Molecular Sciences*. 2018;19.
167. Singh V, Dashti A, Mavigner M, Chahroudi A. Latency reversal 2.0: giving the immune system a seat at the table. *Current HIV/AIDS Reports*. 2021;18: 117-27.
168. Hashemi P, Sadowski I. Diversity of small molecule HIV-1 latency reversing agents identified in low- and high-throughput small molecule screens. *Medicinal Research Reviews*. 2020;40: 881-908.
169. Curty G, Iñiguez LP, Nixon DF, Soares MA, de Mulder Rougvie M. Hallmarks of retroelement expression in T-cells treated with HDAC inhibitors. *Frontiers in Virology*. 2021;1.
170. Proust A, Barat C, Leboeuf M, Drouin J, Tremblay MJ. Contrasting effect of the latency-reversing agents bryostatin-1 and JQ1 on astrocyte-mediated neuroinflammation and brain neutrophil invasion. *Journal of Neuroinflammation*. 2017;14: 1-16.
171. Proust A, Barat C, Leboeuf M, et al. HIV-1 infection and latency-reversing agents bryostatin-1 and JQ1 disrupt amyloid beta homeostasis in human astrocytes. *GLIA*. 2020;68: 2212-27.
172. Dental C, Proust A, Ouellet M, Barat C, Tremblay MJ. HIV-1 latency-reversing agents prostratin and bryostatin-1 induce blood–brain barrier disruption/inflammation and modulate leukocyte adhesion/transmigration. *The Journal of Immunology*. 2017;198: 1229-41.
173. Subramanian S, Bates SE, Wright JJ, Espinoza-Delgado I, Piekarz RL. Clinical toxicities of histone deacetylase inhibitors. *Pharmaceuticals*. 2010;3: 2751-67.
174. McMahon JH, Evans VA, Lau JSY, et al. Neurotoxicity with high dose disulfiram and vorinostat used for HIV latency reversal. *Aids*. 2022;36: 75-82.
175. Rasmussen TA, Tolstrup M, Møller HJ, et al. Activation of latent human immunodeficiency virus by the histone deacetylase inhibitor panobinostat: a pilot study to assess effects on the central nervous system. *Open Forum Infectious Diseases*. 2015.
176. Munoz-Moreno JA, Carrillo-Molina S, Martínez-Zalacaín I, et al. Preserved central nervous system functioning after use of romidepsin as a latency-reversing agent in an HIV cure strategy. *AIDS*. 2021: 1-19. Nov.
177. Chan P, Ananworanich J. Perspective on potential impact of HIV central nervous system latency on eradication. *Aids*. 2019;33: S123-33.
178. Clayton KL, Collins DR, Lengieza J, et al. Resistance of HIV-infected macrophages to CD8 + T lymphocyte-mediated killing drives activation of the immune system article. *Nature Immunology*. 2018;19: 475-86.
179. Cosenza MA, Zhao ML, Lee SC. HIV-1 expression protects macrophages and microglia from apoptotic death. *Neuropathology and Applied Neurobiology*. 2004;30: 478-90.
180. Giri MS, Nebozyhn M, Raymond A, et al. Circulating monocytes in HIV-1-infected viremic subjects exhibit an antiapoptosis gene signature and virus- and host-mediated apoptosis resistance. *J Immunol*. 2009;182: 4459-70.
181. Eugenin EA, Berman JW. Gap junctions mediate human immunodeficiency virus-bystander killing in astrocytes. *Journal of Neuroscience*. 2007;27: 12844-50.
182. Ousman SS, Kubes P. Immune surveillance in the central nervous system. *Nature Neuroscience*. 2012;15: 1096-101.
183. Ganesh A, Lemongello D, Lee E, et al. Immune activation and HIV-Specific CD8+ T cells in cerebrospinal fluid of HIV controllers and noncontrollers. *AIDS Research and Human Retroviruses*. 2016;32: 791-800.
184. Terry RL, Getts DR, Deffrasnes C, van Vreden C, Campbell IL, King NJC, Inflammatory monocytes and the pathogenesis of viral encephalitis. *Vol. 9*, *Journal of Neuroinflammation*. *BioMed Central*; 2012.
185. Matejuk A, Vandenbark AA, Offner H. Cross-talk of the CNS with immune cells and functions in health and disease. *Vol 12*, *Frontiers in Neurology Frontiers Media SA*. 2021.
186. Kruize Z, Kootstra NA. The Role of Macrophages in HIV-1 persistence and pathogenesis. *Frontiers in Microbiology*. 2019;10: 1-17.
187. Bruner KM, Wang Z, Simonetti FR, et al. A quantitative approach for measuring the reservoir of latent HIV-1 proviruses. *Nature*. 2019;566: 120-5.
188. Procopio FA, Fromentin R, Kulpa DA, et al. A novel assay to measure the magnitude of the inducible viral reservoir in HIV-infected individuals. *EBioMedicine*. 2015;2: 874-83.
189. Gisslén M, Price RW, Andreasson U, et al. Plasma concentration of the neurofilament light protein (NFL) is a biomarker of CNS injury in HIV infection: a cross-sectional study. *EBioMedicine*. 2016;3: 135-40.
190. Chaillon A, Gianella S, Dellicour S, et al. HIV persists throughout deep tissues with repopulation from multiple anatomical sources. *Journal of Clinical Investigation*. 2020;130: 1699-712.
191. Garrido C, Margolis DM. Translational Challenges in Targeting Latent HIV infection and the CNS reservoir problem. *J Neurovirol*. 2015;21: 222-6.
192. Sakai M, Higashi M, Fujiwara T, et al. MRI imaging features of HIV-related central nervous system diseases: diagnosis by pattern recognition in daily practice. *Vol 39*, *Japanese Journal of Radiology Springer Japan*;. 2021: 1023-38.
193. Henrich TJ, Hsue PY, VanBrocklin H. Seeing is believing: nuclear Imaging of HIV persistence. *Frontiers in Immunology*. 2019;10: 1-11.
194. Horti AG, Naik R, Foss CA, et al. PET imaging of microglia by targeting macrophage colony-stimulating factor 1 receptor (CSF1R). *Proc Natl Acad Sci U S A*. 2019;116: 1686-91.
195. Serrano ME, Kim E, Petrinovic MM, Turkheimer F, Cash D. Imaging synaptic density: the next holy grail of neuroscience? *Frontiers in Neuroscience*. 2022;16.
196. Chaganti J, Brew BJ. MR spectroscopy in HIV associated neurocognitive disorder in the era of cART: a review. *Vol 18*, *AIDS Research and Therapy BioMed Central Ltd*. 2021.

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