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HIV-1 PERSISTENCE IN THE CNS: MECHANISMS OF LATENCY, PATHOGENESIS AND AN UPDATE ON ERADICATION STRATEGIES

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Abstract: Despite -four decades of research into the human immunodeficiency virus (HIV-1), a successful strategy to eradicate the virus post-infection is lacking. The major reason for this is the persistence of the virus in certain anatomical reservoirs where it can become latent and remain aquiescent for as long as the cellular reservoir is alive. The Central Nervous System (CNS), in particular, is an intriguing anatomical compartment that is tightly regulated by the blood-brain barrier. Targeting the CNS viral reservoir is a major challenge owing to the decreased permeability of drugs into the CNS and the cellular microenvironment that facilitates the compartmentalization and evolution of the virus. Therefore, despite effective antiretroviral (ARV) treatment, virus persists in the CNS, and leads to neurological and neurocognitive deficits. To date, viral eradication strategies fail to eliminate the virus from the CNS. To facilitate the improvement of the existing elimination strategies, as well as the development of potential therapeutic targets, the aim of this review is to provide an in-depth understanding of HIV latency in CNS and the onset of HIV-1 associated neurological disorders.

Keywords: HIV-1, Latency, Transcription, Neurocognitive disorder, epigenetics

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1. Introduction

AIDS (Acquired Immune Deficiency Syndrome) is one of the most debilitating human diseases ever known to mankind. The causative agent was identified as HIV-1 (Human Immunodeficiency Virus 1) in the year 1981. Since its discovery, research efforts have been dedicated to developing anti-HIV-1 drugs targeting its entry and key viral enzymes, such as reverse transcriptase, integrase, and protease; these efforts have led to the development of highly active antiretroviral therapy (HAART) for the treatment of HIV-1 infection (Lassen et al., 2004a). HAART or antiretroviral therapy (ART) successfully lowered plasma HIV-1 RNA levels below the detection thresholds and has significantly reduced AIDS-related mortality (Hakre et al., 2012). However, despite increased drug specificity and efficiency, treatment does not eliminate the virus and, upon interruption, viral rebound is seen even in patients with low or undetectable plasma viremia (Mata et al., 2005). This is because in certain cells, HIV-1 has the ability to remain quiescent and thus "hides" in these cells, even in the presence of antiretrovirals, and reactivate upon therapy interruption.

Therefore, once infected with HIV-1, the individuals are destined to take medication throughout their life to suppress the viral load in blood. While HAART and ART can improve immune function, it can be aberrant and incomplete often leading to immune reconstitution inflammatory syndrome (IRIS), most likely due to an imbalanced recovery of host innate and adaptive immune response. Initiating ART at an early stage of infection is probably the only chance, if any, for successful immune restoration (Wilson and Sereti, 2013). In most patients, owing to the ability of the virus to adapt to host immune response, and the evolution of viral variants, the medication becomes less effective, often resulting in drug replacement within the HAART regimen throughout infection (Alqatawni et al., 2020; Hokello et al., 2021b; Sharma et al., 2021). On the other hand, some of the medications are reported to have toxic side effects in patients, making the treatment less desirable and intolerable (Deeks et al., 2012). Moreover, these drugs are reported to have poor penetrability into certain anatomical compartments like the central nervous system (CNS) which hinders the effectiveness of the treatment.

The CNS is considered an "immune privileged" site and the brain a sanctuary, due to tight regulation of migration of cells and other materials including the antiretrovirals into the CNS by the blood-brain barrier (BBB) and cerebrospinal fluid (CSF), thus facilitating the sustenance of HIV-1 (Salemi and Rife, 2016). Several aspects of viral entry, transcription, and latency are controlled by unique mechanisms in the brain.

This review discusses the important concepts of HIV-1 transcription and latency in the CNS, describes the onset of HIV-1 associated neurological disorders, and provides an update on how this information is being utilized to design current eradication strategies.

2. HIV-1 reservoirs: where does HIV-1 hide?

Non-adherence or termination of ART results in a rebound of HIV-1 and this resurgence occurs either as a result of residual viral replication in infected cells that persisted due to suboptimal penetration of antiretrovirals, or as a result of the existence of a small population of cells harboring integrated and intact proviruses that do not actively produce infectious virions, but have the capacity to do so when conditions are favorible (no antiretrovirals)

(Dufour et al., 2020). This small population of cells are in a state of "quiescence" or "latency" and can exist within various compartments in the body including brain, blood, gut-associated lymphoid tissue, bone marrow, and genital tracts (Eisele and Siliciano, 2012; Trono et al., 2010). According to Blankson et. al, a viral reservoir is defined as "a cell type or anatomical site in association with which replication-competent forms of the virus persist with more stable kinetic properties than the main pool of actively replicating virus" (Blankson et al., 2002). For a cell type to be considered a true reservoir, it must satisfy the following criterion: (i) viral DNA must be integrated into the host cell genome, (ii) cell should be capable of harboring the virus in a dormant and non-infectious state for a long period and this may include possessing the mechanism to establish and maintain latent infection, and (iii) cell should possess the ability to produce fully active replication-competent viral particles upon activation (Eisele and Siliciano, 2012). While at least two out of the three criteria of a true latency reservoir: the presence of HIV-1 integrated DNA and the mechanisms allowing the virus to persist for long period have been described in many cell types (Blankson et al., 2002), it has been somewhat of a challenge to determine whether the cells can produce replication competent virus. This is particularly true in case of CNS cells such as microglia, which reside in deep tissues and are inaccessible in living subjects. However, *ex vivo* quantification of cellular reservoirs in the periphery from patient blood was possible through quantitative viral outgrowth assay (QVOA), however, this tool cannot be used to identify the cellular reservoirs in the CNS due to their inaccessibility (Machado Andrade and Stevenson, 2019).

2.1. Central Nervous System

It is still unknown whether CNS is a true viral reservoir. A review by Gray et al. (Gray et al., 2014a) addressed this issue in detail and highlighted that the CNS satisfies most of the requirements to be classified as a viral reservoir. Evidence from *in vitro* experimental models and autopsied brains indicate that HIV-1 can infect several different cell types in the CNS, including macrophages, microglia, and to some extent, astrocytes (Churchill et al., 2006; Churchill et al., 2009; Cosenza et al., 2002). Perivascular macrophages and microglia within the CNS are the resident immune cells of the brain and respond to any type of injury. These cells are also known to harbor integrated HIV-1 in their genomes (Churchill et al., 2006; Gehrmann et al., 1995; Wallet et al., 2019). Both cell types are susceptible to HIV-1 infection as they express CD4 and the coreceptors (CCR5 and CXCR4) required for HIV-1 entry (Vallat et al., 1998). Astrocytes express the coreceptors required for HIV-1 entry but lack expression of CD4 (Gray et al., 2014b; Sabri et al., 1999). Despite the lack of CD4, astrocytes can still become infected via a CD4-independent mechanism (Tornatore et al., 1994). Peripheral macrophages have a relatively short half-life, however, a continuous supply of these cells in the CNS is maintained by circulating monocytes. In comparison, astrocytes and microglia have long half-lives (Carson et al., 2006; Sofroniew and Vinters, 2010). Due to the high number of cells harboring latent HIV-1, and their long half-lives, it can be suggested that these cells in the CNS satisy atleast two of the three characterestics of a true reservoir.

Since it is challenging to determine whether these cells produce replication competent viral particles using *ex vivo* quantification methods, the amount of HIV RNA collected from CSF can be considered as an acceptable substitute (Gianella et al., 2016). Comprehensive sequence and phylogenetic analyses on 14 individuals infected with HIV-1 who had been serially sampled in CSF and blood plasma before and after interruption of ART revealed that HIV-1 emerged from the CSF upon interruption of ART indicating that viral escape

from the CNS is possible (Gianella et al., 2016). Genetic and phenotypic analyses of HIV-1 *env* gene in four individuals with persistent CNS escape (three as part of the THINC study in UCSC and YALE, and one enrolled in Torino, Italy) indicate that replication-competent HIV-1 can persist in the CNS even when the patient is on ART (Joseph et al., 2019).

2.2 Blood Brain Barrier (BBB)

The blood brain barrier (BBB) is a semi-permeable barrier that selectively prevents the entry of ions, neurotransmitters and macromolecules from the periphery into the extracellular compartment of the CNS. It comprises of brain microvascular endothelial cells, pericytes, perivascular macrophages and perivascular astrocytes, interconnected through tight junctions. The combined surface area of this barrier spans 12 - 18 m^2 in an average human adult making it the largest interface for blood-brain exchange (Abbott et al., 2010; Abbott et al., 2006). The presence of energy-dependent ABC efflux transporters (ATP-binding cassette transporters) and solute carrier transpoters selectively pump any of the endogenous metabolites, proteins or xenobiotics ingested through diet or otherwise acquired from the environment out of the brain, to prevent any damage to the neurons. Factors that govern the entry of antiviral drugs across the BBB are high polar surface area (PSA, >80 $Å^2$), high unsaturation (> 6 hydrogen bonds that increase the lipophilicity of the compound), presence of rotatable bonds and a molecular weight of > 450 Da (Abbott et al., 2010). While antivirals designed to target the brain are known to cross the BBB, the presence of various transporter and efflux mechanisms leads to minimal accumulation and low concentration of the drug in the CNS than in the periphery (Ene et al., 2011). This suboptimal concentration of the antiretroviral is insufficient to inhibit HIV-1 transcription and replication, as a result of which the virus is able to maintain a low level of replication in the CNS (Bertrand et al., 2016).

2.3 Viral entry into the CNS

Viral entry into the CNS can occur as early as within the first week of infection (Valcour et al., 2012). One of the popular theories that aim to explain the entry of HIV-1 into the CNS is the "Trojan horse theory" which proposes that the virus primarily enters the CNS through infected monocytes or CD4+T lymphocytes circulating in the plasma (Spudich and Gonzalez-Scarano, 2012). While the blood-brain barrier (BBB) tightly regulates the entry of foreign substances into the brain, many external and internal factors can alter its permeability, especially when physiological homeostasis is interrupted. The viral protein (transactivator of transcription) Tat is shown to alter the permeability of the BBB at least in part by decreasing the production of occludin in the endothelial tight junctions (Andras et al., 2003; Xu et al., 2012) (Fig 1). The viral envelope protein (gp120) mediates HIV-1 entry into the CNS via transcytosis across the BBB (Banks et al., 2001).

HIV-1 enters macrophages and microglia through the well-established CD4-mediated mechanism (Fig 1). Recently, a specific subset of infected monocytes that preferentially cross the BBB, the HIV+ CD14+ CD16+ monocytes, has been characterized (Veenstra et al., 2017). These cells express several proteins such as Junctional Adhesion Molecule-A (JAM-A), Activated Leukocyte Cell Adhesion Molecule (ALCAM), and chemokine receptors CCR2 that assist in crossing the BBB (Wallet et al., 2019). Although macrophages are CD4+ and express both CXCR4 and CCR5 coreceptors, HIV-1 entry occurs mostly through the coreceptor CCR5 (Berger et al., 1998). In contrast, astrocytes lack the expression of CD4, but

HIV-1 can still infect these cells by associating itself with intracellular vesicles containing the tetraspanin-family protein CD81 (Gray et al., 2014b; Vallat et al., 1998) (Fig 1). Infection occurs in microglial cells despite the high expression of cellular restriction factor SAMHD1 (SAM domain and HD domain 1) (Rodrigues et al., 2017), probably due to its phosphorylation by cyclin kinase 1 (CDK1), which is induced in cells that cycle between G0 to G1 state (Mlcochova et al., 2017).

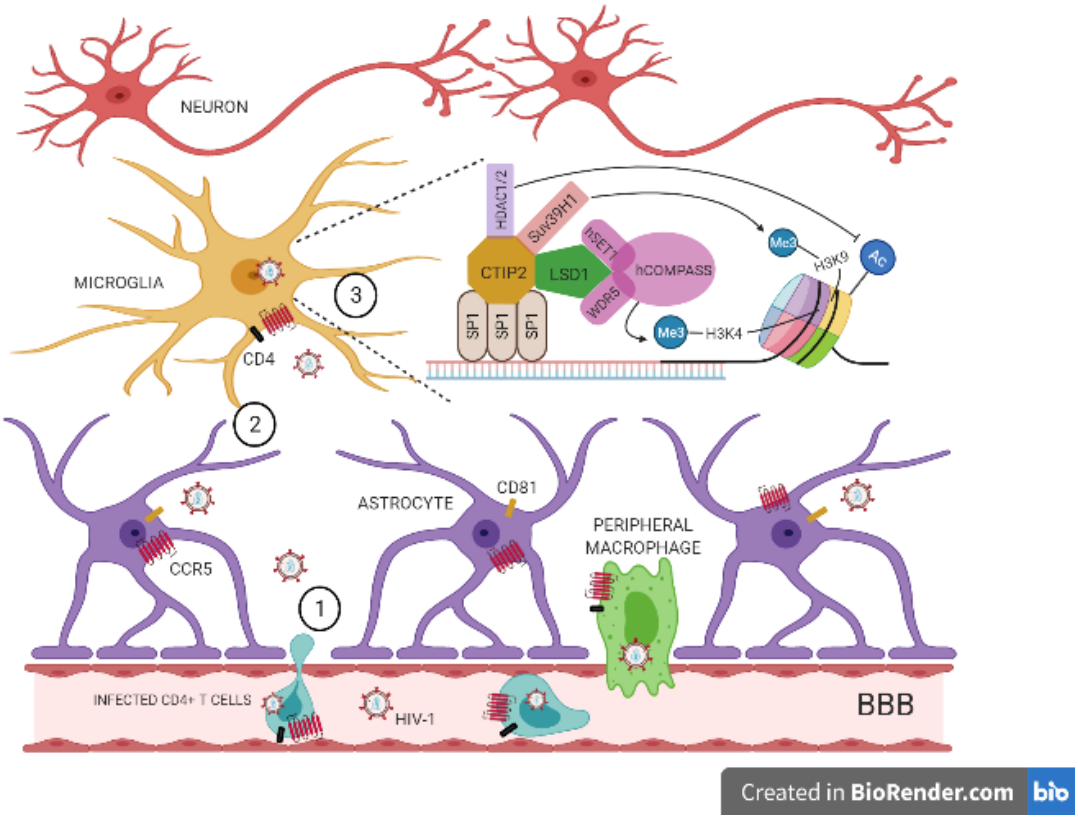

Fig 1. Viral entry into CNS cells and establishment of latency in microglial cells. 1. HIV-1 infection occurs primarily through infected CD4+T cells in the blood. Viral proteins can compromise the permeability of the BBB to facilitate the CNS entry of infected cells. 2. HIV-1 enters astrocytes mainly through the CD81 tetraspanin protein family, and enters microglia through the well-established CD4 mediated mechanism. 3. HIV-1 latency in microglia is established through the recruitment of histone deacetylases (HDAC1, HDAC2) and histone methyltransferase (Suv39H1) by CTIP-2 to the HIV-1 long terminal repeat (LTR) to induce repressive epigenetic marks on Lysine 9 of histone H3. CTIP2 acts in synergy with LSD1 which associates itself with two members of the hCOMPASS complex, hSet1, and WDR5 to bring about another repressive epigenetic mark on Lysine 4 of histone H3. The illustration was prepared using BioRender software.

Once inside the target cell, many factors influence viral replication. Many cells in the brain including macrophages and microglia express the proinflammatory cytokine, CXCL8 (IL-8), which plays a role in enhancing HIV-1 replication (Lane et al., 2001). CXCL8 mediated enhanced replication is dependent on nuclear factor-kappa beta (NF-κB) signaling (Mamik and Ghorpade, 2014). Besides, elevated IL8 levels are seen in the CSF of patients with HIV-1 associated dementia when compared with neurocognitively normal HIV-1-infected patients (Zheng et al., 2008). These findings suggest that HIV-1 develops specialized replication mechanisms in the CNS.

2.3.1 Compartmentalization

The presence of unfavorable environment that affects viral replication and a range of conditions limiting viral trafficking leads to the evolution of virus to that specific site resulting in viral compartmentalization (Salemi, 2013). HIV-1 compartmentalization in the CNS can occur either during primary or late infection, and the restricted entry into the CNS triggers viral genetic adaptation into a distinct HIV-1 metapopulation that can enter the protective barrier and contribute to latent viral reservoir (Lamers et al., 2011; Schnell et al., 2010). HIV-1 virus in the CNS possesses unique long terminal repeat (LTR) promoters, with mutations in the Sp1 motif directly adjacent to the two NF-κB binding sites, which render the virus more quiescent and may condition the virus into taking on a latent phenotype (Gray et al., 2016a). These mutations were absent from non-CNS-derived LTR sequences from the same patients demonstrating the distinct subpopulation of latent HIV-1 reservoir (Gray et al., 2016a). Major HIV-1 target cells within the CNS are perivascular macrophages, microglia and astrocytes (Burdo et al., 2013; Williams et al., 2001). They have long half-lives that allow the virus to persist and enable the maintenance of the viral reservoir within the CNS (Crowe et al., 2003; Koppensteiner et al., 2012; Sofroniew and Vinters, 2010).

Recent discovery of lymphatic vessels that drain from the brain dura matter to the deep cervical lymph nodes (Aspelund et al., 2015; Louveau et al., 2016) has particular relevance to HIV-1 infection as these vessels serve as physical conduits draining both CSF and brain interstitial fluid from CNS to periphery. HIV-1 infected cells in the CNS (latent or active), if mobile, could theoretically travel out of this compartment and 'reseed' the systemic reservoir (Spudich, 2016).

3. Establishment of latency in the CNS

Reverse transcription of retroviruses such as HIV-1 is essential for the integration and production of infectious virions (Sloan et al., 2011). Reverse transcription of viral RNA gives rise to at least two types of cDNA: linear and circular. Linear viral cDNA along with viral integrase, capsid proteins, and some viral cellular proteins form a pre-integration complex (PIC) that is responsible for carrying the proviral DNA into the nucleus (Hamid et al., 2017). The viral integrase then mediates the integration of viral DNA into the host cellular genome (Lusic and Siliciano, 2017). Host transcriptional factors such as NF-κB, nuclear factor of activated T-cells (NFAT), and activator protein 1 (AP-1) regulate HIV-1 long terminal repeats (LTR) transcription either individually or through functional synergy with one another (Hokello et al., 2021a; Hokello et al., 2020). Active transcription of the integrated provirus leads to the production of new viral progeny and this cycle is usually completed within days (Perelson et al., 1997). While the majority of infections are actively transcribed, some cells become latent (Dahabieh et al., 2015). This is post-integration latency and the mechanisms lead to this kind of latency are discussed below.

Circular viral cDNA, often containing either one or two copies of the long terminal repeat (LTR) region, is considered defective and is unable to integrate into the host genome. In the pre-integration state, viruses can produce viral transcripts such as Nef , Tat and Rev, but these transcripts are incompletely spliced and are unable to produce infectious virions (Hamid et al., 2017; Sloan et al., 2011). Hence, the presence of unintegrated, unproductive viral DNA characterizes pre-integration latency. Unintegrated viral DNA was first reported in brain and blood tissue of HIV-1 infected dementia patients, with considerably higher levels found in patients with HIV-1 encephalitis (Pang et al., 1990).

Historically, latent cells are thought to harbor transcriptionally silent HIV-1 provirus. However, recent evidence indicates that complete silencing of the HIV-1 promoter is a rare event and majority of latently infected cells express low levels of incomplete viral transcripts due to blocks at several stages (Hermankova et al., 2003; Lassen et al., 2004b; Lassen et al., 2006; Wilson and Sereti, 2013). However, in the presence of favorible conditions (no antiretrovirals, epigenetic modulation, presence of viral Tat), they can produce replication competent virus (Mohammadi et al., 2014; Razooky et al., 2015; Romani and Allahbakhshi, 2017). Recent evidence suggests that even unintegrated viral DNA can yield productive infections upon complementing/superinfection with other defective variants (Gelderblom et al., 2008; Quan et al., 2009). Activation of non-dividing cells such as resting $CD4^+$ T cells resulted in integration and subsequent production of active virions from unintegrated viral DNA maintained extrachromosomally for several weeks in a dormant state (Stevenson et al., 1990). Despite harboring non-productive provirus, latent cells are associated with markers of immune activation such as IFN (Stunnenberg et al., 2020), or increased CD4+ T cells expressing CD38, CCR5, and/or PD-1, even in the presence of antiretrovirals (Hatano et al., 2013).

Some common factors that drive susceptible cells into latency are briefly discussed below. Although the percentage of these cells is very small (approximately 1 in one million of resting CD4+T cells per infected individual), this latent pool prevents complete HIV-1 eradication in patients undergoing antiretroviral therapy (Siliciano et al., 2003; Tyagi and Bukrinsky, 2012). Using primary CD4+ T cells, for the first time we showed that levels of positive transcription elongation factor b (P-TEFb), which is involved in HIV-1 transcription elongation, are low in latently infected primary CD4+T cells confirming strong links between the defect in transcription and latency (Hokello et al., 2019; Tyagi et al., 2010).

3.1. General mechanisms of the establishment of latency

Mechanisms underlying HIV-1 latency are still under study. While several mechanisms acting at transcriptional and post-transcriptional level are proposed, it is well accepted that the establishment of latency is a multifactorial process (Dahabieh et al., 2015) (Fig 2).

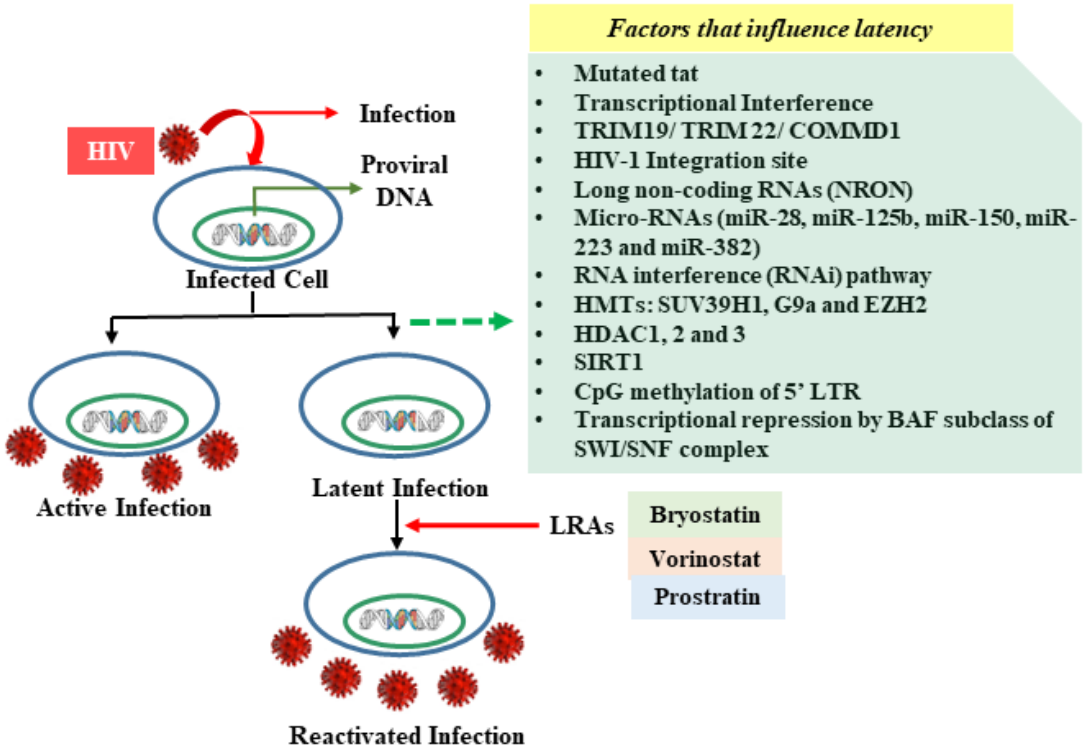

Fig 2: Schematic of the general factors that influence HIV-1 Latency

Several viral proteins influence the establishment of latency. HIV-1 Tat (transactivator of transcription) protein is critical for facilitating either active replication or reactivation of the latent virus (Jordan et al., 2001; Lin et al., 2003; Marzio et al., 1998; Tyagi et al., 2001). Several studies indicate that the attenuation of Tat may be involved in the establishment of latency: Natural variants of Tat harboring various mutations such as H13L (identified in latently infected U937 cells), WHA, WHB, WHC, and WHD (isolated from patient-derived HIV-1 strains) show reduced interaction with its cellular cofactor P-TEFb resulting in decreased trans-activation activity (Emiliani et al., 1998; Meyerhans et al., 1989; Reza et al., 2003). The force selecting defective Tats that can lead to latency favors Tat variants with revival activity sufficient to maintain a latent phenotype. Attenuation of Tat activity can thus serve as a mechanism of latency (Reza et al., 2003).

At the transcriptional level, proviral silencing can occur as a result of several factors: 1) Transcriptional interference that exists as a result of spatial occlusion or dislodgment of transcription initiation or elongation complexes from the provirus (Lenasi et al., 2008). 2) Integration of the provirus into a site that is or is susceptible to being repressive for transcription (Jordan et al., 2001). HIV-1 tends to avoid latency by preferentially integrating into actively transcribed genes. Once integrated, the provirus requires host transcriptional machinery for viral expression. Integration into sites that are susceptible to being repressive for transcription can lead to latency. 3) The absence of transcriptional factors required for HIV-1 expression in the host nucleus (Ganesh et al., 2003), and 4) the presence of cellular transcription repressors (Tyagi and Karn, 2007; Williams et al., 2006).

Transcriptional interference (TI) is defined as "the suppressive influence of one transcriptional process, directly and in *cis*, on a second transcriptional process." TI results

from the existence of two adjacent interfering promoters which may be convergent (transcribing in the same direction), divergent (transcribing in opposite directions), tandem (one upstream of the other but transcribing in the same direction), or overlapping (where promoter binding sites share a common DNA sequence), and when the stronger promoter reduces the expression of the weaker promoter (Shearwin et al., 2005). Han et al. demonstrated the presence of orientation-dependent TI using an experimental model with two systems in which HIV-1 proviruses are inserted in the exact same position within the host gene, but in different orientations with respect to the host gene (Han et al., 2008)

Cellular defense proteins (or restriction factors) are an integral part of the host's innate immune system. Several restriction factors are released in response to HIV-1 infection to decrease the progression of viral transcription and active replication. Some of these factors act during the early stages of the HIV-1 life cycle and induce latency: *TRIM22* acts as a transcriptional suppressor by decreasing the interaction between Sp1 and HIV-1 promoter (Turrini et al., 2015); *COMMD1*, inhibits HIV-1 replication by binding to κB-responsive promoters and decreasing the duration of NF-κB recruitment to chromatin (Maine et al., 2007); *PML (or TRIM19)*, restricts HIV-1 transcription by recruiting inhibitory cyclin T1 aggregation into PML nuclear bodies (Marcello et al., 2003).

Antisense transcription of the genome gives rise to different classes of RNAs such as small RNAs and non-coding RNAs (ncRNAs). These ncRNAs regulate chromatin structure by recruiting chromatin-modifying complexes through the formation of RNA scaffolds (Holoch and Moazed, 2015; Moazed, 2009). Several cellular lncRNAs either directly or indirectly contribute to HIV-1 latency. One such example is the lncRNA NRON that restricts HIV-1 gene expression by inducing Tat proteasomal degradation (Li et al., 2016). The inhibition of HIV-1 gene expression is also mediated by microRNAs and is evidenced in resting CD4+T cells. A cluster of cellular miRNAs including miR-28, miR-125b, miR-150, miR-223, and miR-382 target the 3' ends of HIV-1 messenger RNAs and inhibit gene transcription; inhibition of these miRNAs resulted in active transcription and translation of the HIV-1 provirus (Huang et al., 2007). Small RNAs employ RNA interference (RNAi) pathways to modify chromatin and target gene expression (Reinhart and Bartel, 2002; Volpe et al., 2002). RNAi pathways mediate transcriptional repressive events at the epigenetic level (Holoch and Moazed, 2015).

In addition to cellular and transcriptional factors, the post-translational modifications on histone proteins or epigenetic mechanisms also influence the establishment of latency. The N-terminus of histone proteins undergo post-translational modifications such as methylation, acetylation, phosphorylation, etc., and contribute to transcriptional activation or repression by transforming the chromatin conformation into an "open" or "closed" state respectively. The closed state of the chromatin is associated with a transcriptionally repressed or silent state which is characteristic of the integrated, but latent HIV-1 provirus. Of the several histone modifications that epigenetically influence HIV-1 latency, histone methylation and acetylation processes are well characterized. Depending on the site of modification, histone methylation could result either inactivation or suppression of gene expression and in contrast, DNA methylation results in gene suppression (Cedar and Bergman, 2009; Rose and Klose, 2014). Histone acetylation results in active gene transcription (Eberharter and Becker, 2002). Histone lysine crotonylation is a newly identified epigenetic modification, and it is a robust indicator of active promoters.

Lysine and arginine residues abundantly found on histones are prone to methylation by the enzymes histone methyltransferases (HMTs) (Migliori et al., 2010). HMTs such as SUV39H1, G9a, and EZH2 are closely associated with the latent provirus. Lysine residues of histone proteins can also be acetylated by histone acetyltransferases (HATs), while histone deacetylases (HDACs) mediate histone deacetylation (Yang and Seto, 2007). Promoters of actively expressed genes, as well as actively transcribed HIV-1, generally have acetylated histones whereas silent regions of the genome and silent LTRs of latent HIV-1 proviruses carry deacetylated histones (Eberharter and Becker, 2002; Van Lint et al., 1996). 18 HDACs are known in humans, among which HDAC1, 2 and 3, are the key players in silencing the HIV-1 promoter (Keedy et al., 2009). Numerous transcription factors such as AP4, c-Myc, and Sp1 (Imai and Okamoto, 2006; Jiang et al., 2007) YY1 (Yin Yang 1) and LSF (Late SV40 Factor) facilitate the recruitment of HDACs; and act as proviral transcription repressors. Our lab has identified a key player of the Notch signaling pathway, CBF-1, to recruit HDACs to the proviral LTR via polycomb group (PcG/PRC) corepressor complexes (PRC1 and PRC2) (Sharma et al., 2020; Tyagi and Karn, 2007). The HAT p300 mediates crotonylation at lysine 18 of Histone H3 when crotonoyl-CoA (which is formed from crotonate by the cytoplasmic/nuclear localized enzyme acyl-CoA synthetase 2 (ACSS2 or AceCS1)) is available (Luong et al., 2000; Sabari et al., 2015). It was recently reported that the latency reversal activity of the HDAC inhibitor, Vorinostat (SAHA) was augmented following ACSS2 induction and histone crotonylation (H3K4Cr) indicating that crotonylation of histone tails at the HIV-1 LTR plays a major role in regulating HIV-1 latency (Jiang et al., 2018).

Epigenetic modifications of several non-histone proteins also play an important role in HIV-1 transcriptional silencing (Siliciano and Greene, 2011). Members of HAT family: p300 and CBP acetyltransferase are known to acetylate Rel A/p65 subunit of NF-κB at lysine residues 218, 221, and 310 and consequently influence NF-κB functions including DNA binding and its assembly with IκBα and HIV-1 gene expression (Chen et al., 2001; Chen et al., 2002). HDAC3 and SIRT1 inhibit HIV-1 gene expression by deacetylating RelA/p65 subunit at lysine residues 221 and 310 respectively (Chen et al., 2001; Yeung et al., 2004). P300 acetylates HIV-1 Tat (a non-histone protein), a necessary step for the initiation of Tat-mediated transactivation; and SIRT1 deacetylates Tat both *in vitro* and *in vivo*. Tat regulates HIV-1 latency through the mechanism of reversible acetylation making it an extremely important player in the establishment of HIV-1 latency (Marcello et al., 2001; Pagans et al., 2005; Pearson et al., 2008).

The chromatin organization of the HIV-1 promoter is different in latent state and in a transcriptionally active state (Van Lint et al., 1996). Several reports indicate the importance of SWI/SNF complex, an ATP dependent chromatin remodeling complex that modulates chromatin remodeling of nuc-1 in HIV-1 infected cells, by remodeling the HIV-1 LTR and its contribution to the establishment and maintenance of HIV-1 latency (Treand et al., 2006). BAF and PBAF, distinct subclasses of the SWI/SNF complex, are recruited at different stages of the cell cycle and have opposing roles in HIV-1 transcription cycle. While PBAF potentiates HIV-1 transcription via acetylated Tat, BAF terminates transcription by positioning a repressive nuc-1 immediately downstream of the transcriptional start site (Agbottah et al., 2006).

3.2. HIV-1 latency in Microglia

Microglial cells are a part of the host's innate immune system and are the resident tissue macrophages of the CNS. Under normal physiological conditions, microglia support the development of CNS and synaptogenesis, participate in the immune response against infectious agents, and play a role in mitigating neuroinflammation. Microglia, therefore act as liaisons between the nervous and immune systems (Rojas-Celis et al., 2019).

It has been previously established that microglia serve as a CNS reservoir harboring latent HIV-1 provirus. The average lifespan of microglial cells is 4 years and their regeneration is slow but occurs throughout life. This nature of microglia allows the persistence of HIV-1 in the brain of the infected person, probably for the rest of their life. Besides, these cells are resistant to apoptosis, which makes it especially difficult to eliminate the infected population (Kumar et al., 2014). Several mechanisms have been proposed for establishing latency in microglia. Microglial cells express several proteins that act as transcriptional repressors, such as Sp1, Sp2, truncated form of liver-enriched transcriptional inhibitory protein (LIP), and/or C-EBPg (Schwartz et al., 2000). Tetherin, a host restriction factor is also implicated in developing proviral latency in microglia as experimental stimulation of HIV-1 infected human fetal microglial cells with interferon (IFN)-α did not revive viral RNA and DNA, probably due to the induction of tetherin (Geffin et al., 2013).

BCL11b, also known as COUP-TF interacting protein 2 (CTIP2) is an important factor for T-lymphocyte as well as spinal cord development and is highly expressed in microglia. Recently, CTIP2 has been identified as a key factor for establishing and/or maintaining viral latency in microglia by influencing cell microenvironment and favoring the formation of heterochromatin in the vicinity of the viral promoter. In the presence of CTIP2, histone deacetylases HDAC1 and HDAC2, and the histone methyltransferase (HMT), SUV39H1 are simultaneously recruited on the viral LTR, generating the repressive epigenetic mark, H3K9me3 (trimethylated lysine 9 of Histone H3) (Marban et al., 2007). Lysine specific demethylase 1 (LSD1) is discovered as a new factor working in synergy with CTIP2 towards the establishment of HIV-1 latency by recruiting two members of the hCOMPASS complex, hSet1 and WDR5 to the HIV-1 promoter, which induce another repressive epigenetic mark, H3K4me3 (trimethylated lysine 4 of Histone H3) (Le Douce et al., 2012) (Fig 1). Reports indicate that CTIP-2 also inhibits the P-TEFb by repressing its Cdk9 kinase activity (Cherrier et al., 2013). More recently, it was discovered that the repressive function of CTIP2 is linked to high mobility group AT-hook 1 (HMGA1) (Eilebrecht et al., 2014) and the recruitment of CTIP2 inactivated P-TEFb complex to the viral LTR by HMGA1 is a crucial step in inhibiting viral gene expression. Knockdown of CTIP2 in microglial cells resulted in the upregulation of cellular cyclin-dependent kinase inhibitor CDKN1A/p21^{waf} gene (Cherrier et al., 2013). In infected macrophages, the presence of HIV-1 Vpr activates p21 transcription stimulating subsequent viral expression. The recruitment of CTIP2 to p21 promoter counteracted with HIV-1 Vpr and led to repressed gene transcription (Vazquez et al., 2005). All these results strongly support the role of CTIP2 in establishing latency.

3.3. HIV-1 latency in Astrocytes

Astrocytes comprise the majority of glial cells in the brain and are essential for providing structural support for neurons and maintaining neuronal homeostasis. It is still unknown if astrocytes constitute a true cellular reservoir for HIV. Although HIV-1 enters astrocytes through a CD4-independent CD81 mediated manner, it is also known to enter the cells via endocytosis; however, particles entering via endocytosis do not integrate into the

host genome. In addition, astrocytes are shown to engulf fragments of HIV-1-infected macrophages, explaining the presence of viral DNA in the absence of infection, and some causes for restricted HIV-1 replication in astrocytes (Russell et al., 2017). One study demonstrated that HIV-1 production is decreased in proliferating astrocytes, but the infection of non-proliferating astrocytes leads to a robust and sustainable HIV-1 infection. Using a novel dual-color reporter virus (NL4.3 eGFP-IRES-Crimson) that encodes for all known viral proteins, researchers detected silent HIV-1 proviruses in a small fraction of astrocytes, and these could not be reactivated even in the presence of strong inducers such as tumor necrosis factor, indicating that the proviruses are either transcriptionally incompetent or have entered a state of deep latency (Barat et al., 2018). These results suggest that astrocytes may mediate pre-integration latency, and the small population that produces infection can contribute to the neurological disorders seen in infected patients.

One of the mechanisms that establish latency in astrocytes is through epigenetic regulation by class I HDACs and HMTs. SU(VAR)3–9, a well-known H3K9 trimethyltransferase, epigenetically silences the HIV-1 proviral DNA and causes latency in HIV-1-infected astrocytic cell models. To drive the HIV-1 out of latency, trimethylation of H3K9 is required in addition to anti-deacetylation, indicating the presence of a complex multi-layered latency structure in astrocytes and an additional step blocking latency reversal. Besides, DNA methylation, which is a well-established mechanism of latency employed in lymphocytes, does not mediate HIV-1 latency in astrocytes (Blazkova et al., 2009).

All these findings suggest that the cells of the CNS have developed unique mechanisms of latency that contribute to the persistence of HIV-1 in the CNS and to challenges encountered in eradicating it.

4. Latent HIV-1 and pathogenesis in the CNS

Normal neuronal function is disturbed by HIV-1 infection in the CNS. In the early stage of HIV-1 infection, complications in the CNS arise as a response to the detection of the virus in the form of multiple processes mediated by the immune system. In the intermediate stages, complications continue as an indirect consequence of the immune system dysfunction and the metabolic effects of the antiretroviral drugs. In later stages, the neurological complications exacerbate due to the development of opportunistic disorders in addition to the failing immune responses (Rojas-Celis et al., 2019).

HIV-1-infected cells cross the BBB during early infection and subsequently initiate a cascade of inflammatory mechanisms through the release of active virus or viral protein and/or cytokines/chemokines (Irish et al., 2009; Koenig et al., 1986). Migrating infected host cells express IL-1, IL-6, (TNFα), tumor growth factor–b, and prostaglandin E2, which bind glia receptors and activate additional inflammatory genes through a positive feedback mechanism leading to neuroinflammation (Roulston et al., 1995). In addition to neuroinflammation mediated by the physiologic response to HIV-1 infection, HIV-1 proteins such as Vpr, Tat, Nef, and gp120 expressed by infected cells activate interferon (IFN), apoptosis, and MAPK pathways in uninfected microglia and astrocytes and further exacerbate the inflammatory response (Yang et al., 2009a). While microglial activation and pro-inflammatory response is desirable under normal circumstances, excessive and persistent pro-inflammatory response surely leads to neurotoxicity.

The presence of persistent latent virus in the brain might lead to cognitive impairment and neurodegeneration by continuous release of proinflammatory responses and altering gene expression. A study by Desplats et al. reports that patients with latent HIV-1 display cognitive deficits, neurodegenerative alterations, and neuroinflammatory changes indicating that the presence of latent virus in the brain represents a distinct condition that manifests with pathologic features (Desplats et al., 2013). Indeed, infection of the CNS by either latent or active HIV-1 has been long associated with neurologic conditions, such as HIV-associated dementia (HAD), HIV-associated neurocognitive disorders (HAND), HIV encephalitis (HIVE), etc. (Clifford and Ances, 2013; Fauci, 1988).

4.1. HIV-1 Encephalitis (HIVE)

HIVE is characterized by the presence of infected macrophages in CNS, microgliosis, astrogliosis, and myelin loss (Everall et al., 2009). Although latent HIV-1 and HIVE cases displayed similar clinical and neurodegenerative traits, the extent of the cognitive and pathologic alterations was greater in the HIVE group (Desplats et al., 2013). At the molecular level, patients with HIVE showed increased levels of the epigenetic modulator of HIV-1, CTIP2 (Desplats et al., 2013). CTIP2 is a common regulator of gene transcription in the brain, implicated in the negative regulation of BDNF signaling, which is altered in several neurodegenerative disorders (Desplats et al., 2008; Tang et al., 2011). In microglial cells, CTIP2 assembles a multi enzymatic chromatin-modifying complex through the recruitment of SP1, HP1a, HDAC1, HDAC2, and SUV39H to the viral LTR region, and establishes a heterochromatic environment at the viral insertion site, thus silencing HIV-1 transcription (Marban et al., 2007). Recruitment of CTIP2 to the viral insertion sites during latency possibly alters the transcription of its target proinflammatory genes, triggering chronic inflammatory responses that ultimately lead to the development of HIVE (Desplats et al., 2013). Drugs that inhibit Janus Kinase (JAK) were shown to be effective in minimizing the HIVE symptoms in an HIV-1 infected SCID (severe combined immunodeficiency) mouse model (Haile et al., 2016) implicating the role of an important pathway in HIVE that can be targeted for developing therapeutic interventions in future.

4.2. HIV-1-associated neurocognitive disorders (HAND)

While the majority of cases of HIV-1 infection are asymptomatic, the presence of virus can be accompanied by immune activation in the CNS/CSF (Davis et al., 1992; Hecht et al., 2002; Taiwo and Hicks, 2002). Active replication of HIV-1 as discussed above can result in damage leading to neurocognitive disorders. HIV-associated neurocognitive disorder (HAND) is classified into three categories of disorders with increasing severity of dysfunction: i) asymptomatic neurocognitive impairment (ANI), ii) mild neurocognitive disorder (MND), and iii) HIV-associated dementia (HAD). Before the introduction of ART, the neurocognitive disorders were severe and often presented the severe immunosuppression stage of Acquired Immunodeficiency Syndrome (AIDS). The availability of ART has greatly ameliorated but did not completely eradicate the symptoms of HAND. Despite successful reduction of plasma viremia to undetectable levels, almost 50% of the patients on ART continue to suffer from less severe forms of HAND (Eggers et al., 2017). Normally, in HIV-1 infected patients, whether receiving stable ART or not, the CSF viral RNA load is typically lower than that in plasma. (Mellgren et al., 2005). However, in a subset of patients receiving stable ART for atleast 6 months, the CSF viral RNA load was found to be >200 copies/ml while the plasma viral load was <50 copies/ml (Eden et al.,

2010). These patients suffered neurological symptoms consistent with HAND indicating that despite successful suppression of plasma viremia with ART, HIV-1 persists in the CSF, presenting neurocognitive symptoms (Canestri et al., 2010). In these patients, HAND presents with mild symptoms such as disturbances in psychomotor function, processing, and memory, but it can swiftly take on its severe form, especially in those who interrupt treatment therapy or start treatment at an advanced disease stage (Heaton et al., 2010).

Many factors can contribute to the pathogenesis of HAND such as toxicity of the antiretrovirals, CNS inflammation in response to viral infection, release of HIV-1 transcripts from quiescent/latently infected cells, or even co-infection with other viruses such as hepatitis C virus can contribute to the pathogenesis of HAND (Sutherland and Brew, 2018). Two possibilities explain the existence of mild HAND symptoms despite antiretroviral therapy: i) Antiretrovirals cannot penetrate the BBB effectively and hence cannot completely eradicate HIV-1 in the infected cells. As a result, the damage initiated by primary HIV-1 infection is persistent as many cells of CNS are non-regenerating (Dahl et al., 2014; Koneru et al., 2014; McArthur et al., 2010). ii) The pro-inflammatory factors released by the infected cells in the periphery can "leak" into the CNS causing exacerbation of inflammatory responses in the CNS (Spudich and Gonzalez-Scarano, 2012). Moreover, viral factors such as the protein Tat, released by the infected cells in the periphery can freely pass the BBB and release more chemokines/cytokines and cause neuronal damage (Bagashev and Sawaya, 2013; Banks et al., 2005; Moran et al., 2014; Zayyad and Spudich, 2015). Drugs targeting the JAK/STAT pathway such as baricitinib, are shown to decrease the production of these pro-inflammatory factors and ameliorate the neurotoxic inflammatory response in an HIV-1 infected SCID (severe combined immunodeficiency) mouse model, showing the potential of this pathway in the treatment of HAND (Gavegnano et al., 2019)

Elevated levels of the macrophage activation marker, neopterin, as well as neurofilament light chain (NFL) which is associated with neuronal injury are elevated in the CSF of people suffering from HAND (Brew et al., 1996; Cinque et al., 2007; Peluso et al., 2013). Recently, systemic markers such as red blood cell count, mean red blood cell volume, mean cell hemoglobin, and iron transport deficiency in the brain have been suggested to be better indicators of neurologic dysfunction in HIV-1 infected patients. More recently, plasma markers such as soluble CD14 and lipopolysaccharide have also been considered as indicators of HAND (Ancuta et al., 2008; Spudich, 2014; Sun et al., 2010). Neuroimaging is an emerging tool owing to its noninvasiveness and superior detection sensitivity and is being increasingly used to monitor preclinical changes in subjects with HAND (Wang et al., 2011). Indeed, microglial activation was observed via PET in individuals undergoing ART (Vera et al., 2016).

4.2.1. Effect of ART on HAND

The introduction of ART has greatly improved the quality of life for people infected with HIV-1, by turning a fatal disease into a manageable chronic disease; although management of the disease is through lifelong therapy. However, it comes with its own set of challenges as even lifelong adherence to ART does not eliminate the latent reservoir. Several reports confirm the resurgence of HIV-1 derived from latent reservoirs or from persistently replicating cells (Eisele and Siliciano, 2012; Siliciano et al., 2003). Further, recent reports ruled out opportunistic infections as the reason behind emerging cases of

559 neurocognitive disorders in HIV-1 patients, and support the fact that HIV-1 infection itself
560 causes deficits in cognitive functioning (Christo et al., 2007).

561 Studies evaluating the effect of antiretroviral drugs on proper functioning of CNS are
562 ongoing. Few studies report that the use of antiretrovirals control the symptoms associated
563 with HAND, while others report exacerbation of symptoms upon withdrawal or therapy
564 interruption (Heaton et al., 2010; Underwood et al., 2015). Secondary effects of certain
565 antiretrovirals are indeed associated with neurological disturbances such as changes in
566 sleep quality, development of anxiety, and depression (Clifford et al., 2009). The onset of
567 these conditions affects the rigidity with which patients adhere to treatment.

568 **5. Current treatment strategies to eradicate HIV-1 from CNS reservoirs**

569 The complete eradication of HIV-1 virus in the Berlin patient and London patient raised
570 significant enthusiasm for developing a cure for HIV-1 infection (Gupta et al., 2019). Several
571 strategies are being explored and employed to control latently infected cells, namely, ART
572 or HAART, along with latency reversal agents (LRAs), and immune-based, cell-based, and
573 gene editing therapies (Table 1). To tailor an approach for viral eradication, a thorough
574 understanding of the specialized mechanisms adapted by the HIV-1 is essential to ensure
575 its replication in tightly regulated anatomical compartments such as the CNS. A cautionary
576 approach needs to be employed towards eradicating the virus from the CNS to minimize
577 neurotoxicity (neuroinflammation) and subsequent cell death of non-regenerating neuronal
578 population.

579 Table 1: List of Current strategies to eradicate HIV-1 from CNS reservoir

STRATEGY	INTERVENSION	REFERENCE
ANTIRETROVIRALS	EFAVIRENZ	163
	ZIDOVUDINE	90, 222
LATENCY REACTIVATING AGENTS	ROMIDEPSIN	152
	JQ-1	152
	PANOBINOSTAT	152
	BRYOSTATIN	152
	PROSTRATIN	152
	VORINOSTAT	43, 183, 226
	INGENOL B	43, 183
LATENCY PROMOTING AGENTS	DIDEHYDRO-CORTISTATIN A (dCA)*	28, 130
	ABX4641*	23

IMMUNOTHERAPEUTIC INTERVENTIONS	BRAIN DERIVED HIV-1-SPECIFIC CYTOTOXIC T CELLS	143
	ANTI-INFLAMMATORY DRUGS	6
	BROADLY NEUTRALIZING ANTIBODIES (BNABS) (RITUXIMAB)	111, 164, 187
	DUAL AND MULTI-AFFINITY ANTIBODIES	225
	CHIMERIC ANTIGEN RECEPTOR (CAR)T CELLS	92, 121, 144
GENE EDITING THERAPIES	CRISPR/CAS9	4, 10, 44, 52, 99, 131, 190, 192
THERAPEUTIC VACCINES	ALVAC-HIV + AIDSVAX B/E*	62, 151, 156
	VACC-4X*	197

Table 1. Strategies currently in use to eradicate the viral reservoir from CNS. * the efficacy of these interventions has not been validated in the CNS or in brain cells.

5.1. Antiretroviral therapy

Antiretroviral therapy is still the most effective therapy to curb HIV-1 early after infection. Relatively lower levels of microglial activation and neuronal damage markers are seen in the CSF when therapy is initiated at an early stage (Chan and Ananworanich, 2019). An antiretroviral drug with the best penetration into the brain and minimum neurotoxicity should be an obvious choice for viral suppression. As most antivirivals are administered orally, several factors contribute to their insufficient response in the CNS: First pass metabolism leading to decreased bioavailability, slow absorption and most importantly, the presence of BBB (Tatham et al., 2015). In order to increase the accessibility of the drug into the brain, several drug delivery approaches are being evaluated. Invasive methods include intracerebral injections and implants, and modulation of the BBB using ultrasound and osmosis. Non-invasive methods being explored to deliver drugs to the CNS include use of endogenous transporters, prodrugs, liposomes, nanoparticles, nanogels, dendrimers and monoclonal antibodies (Barnabas, 2019). Formulation of antiretrovirals into nanoparticles seems to be the best way to improve BBB permeability and subsequent site targeting. ART nanoparticles are envisioned to preserve the innate therapeutic and nontoxic properties of original drugs while increasing bioavailability in comparison with traditional pharmacokinetic properties (Osborne et al., 2020). To ensure effective migration across the BBB without compromising its structural integrity, the typical size of the antiretroviral nanoformulation should be less than 120 nm (Nair et al., 2016). In addition, transmigration of nanoparticles across the BBB increased 7.3-fold when utilizing a ferrous magnet-based liposome nanocarrier with synergistic support from transferrin receptors on the epithelium *in vitro* (Thomsen et al., 2019). Poloxamer-PLGA nanoparticles loaded with the integrase

inhibitor, elvitegravir, effectively crossed the BBB and suppressed HIV-1 replication in macrophages with low inflammatory response (Gong et al., 2020). Efavirenz, a non-nucleoside reverse transcriptase inhibitor, when administered through nanodiamonds, crossed the BBB and had a higher bioavailability in the brain with minimum side effects (Roy et al., 2018). Precise delivery of the antiretrovirals across to the specific site of interest across the BBB was possible with the discovery of magnetic nanoformulation (Nair et al., 2013). With the assistance of external magnetic field, magentic azidothymidine 5′-triphosphate (AZTTP) liposomes permeabilized across the BBB three times more efficiently than the free drug (Saiyed et al., 2010).

Many antiretrovirals that are approved by the FDA to target brain cross the BBB through an unknown mechanism. Some utilize transport proteins such as P-glycoprotein, MRP, and breast cancer resistance protein (BCRP) (Osborne et al., 2020). However, to date, even the most effective CNS penetrating drugs are associated with neurocognitive effects. Dolutegravir, a novel integrase inhibitor with excellent brain permeability was found to cause neuropsychiatric side effects (Letendre et al., 2014; Scheper et al., 2018). Infants born to women on dolutegravir showed severe neural tube defects (Zash et al., 2018). Similarly, although the nucleoside analog, Zidovudine, has been effective in treating HIV-1 Dementia (Hoogland and Portegies, 2014), a recent study has revealed that zidovudine upregulated several proinflammatory cytokines contributing to neuroinflammation in the CNS (Wu et al., 2017). Moreover, the effectiveness of these drugs is less in general in macrophages and their effect in astrocytes is not yet validated (Nath and Clements, 2011). Recently, limited off-target toxicity and improved macrophage uptake of hydrophobic lipophilic ART nanoparticles was successfully achieved through long-acting slow-effective release of antiretrovirals (LASER ART) in combination with CRISPR-Cas9 injections (Osborne et al., 2020). Improved macrophage uptake was also observed in a long-acting dolutegravir prodrug encapsulated in a poloxamer nanoformulation (Sillman et al., 2018).

5.2. Latency reactivating agents

Several agents were investigated for their potential to reactivate latent HIV-1, and many compounds have been successfully developed into LRAs. The main principle behind latency reversal is ‘shock and kill’, where the LRA ‘shocks’ the latent cells into expressing viral antigens, and ‘kills’ them by exposing the activated cells to HIV-1-specific cytotoxic T-lymphocytes (CTLs) (Margolis et al., 2016). The main disadvantage of using these agents is exacerbated cytotoxic response that can damage un-infected cells. Current LRAs are designed to reactivate the viral reservoir in CD4+T cells. Their efficacy in CNS cells is still under investigation. Some LRAs, including romidepsin, JQ-1, and panobinostat, can induce viral transcription in infected astrocytes *in vitro*, however, promising LRAs such as bryostatin and prostratin, when evaluated in astrocytes, have shown to contribute to neurocognitive impairment (Proust et al., 2020). Research efforts have been diverted to developing small molecule LRAs that do not induce excessive cytokine release and cytotoxicity via activated T-lymphocytes (Yang et al., 2009b). These include histone deacetylation inhibitors (HDACi) such as vorinostat; protein kinase C (PKC) agonists such as ingenols that induce NF-κB; and toll-like receptor (TLR) agonists (Spivak and Planelles, 2018). Studies carried out in macrophage/microglial cell lines demonstrated that a combination of LRAs, such as vorinostat and ingenol-B can reactivate latent virus with increased HIV-1 mRNA and protein levels (Darcis et al., 2015). The reactivation of latent virus in the brain (*in vivo*), even when on ART, can result in the synthesis of early viral

proteins that can trigger the release of proinflammatory mediators that can be neurotoxic when produced in excess. (Bruce-Keller et al., 2003). However, recent studies report that most LRAs are nontoxic to primary CNS cells at therapeutic concentrations and can be safely used for latency reversal in conjunction with ART (Gray et al., 2016b).

5.3. Latency Promoting Agents

Another strategy to incapacitate the ability of HIV-1 reservoir to reactivate is the "Block and Lock". Latency promoting agents (LPAs) possess the ability to inhibit HIV-1 transcription by inducing a deep latency state. An example of this approach is the potent inhibition of protein Tat from infected CD4+ T-lymphocytes by Didehydro-cortistatin A (dCA), an analog of the natural compound, cortistatin A. This inhibition, in combination with antiretroviral therapy and LRAs effectively inhibits viral reactivation (Chan and Ananworanich, 2019). dCA is shown to cross the BBB in microglia-like and astrocytic cell lines (Mediouni et al., 2015). While the potent inhibitory action of dCA is established in CD4+ T cells, its activity is yet unknown in the CNS (Mousseau et al., 2012). However, if a similar potency is seen in CNS cells, dCA will become a popular CNS intervention that can substantially mitigate Tat mediated neurotoxicity in addition to inhibiting latency reversal. Recent reports confirmed that levosimendan inhibits both the acute HIV-1 replication and the reactivation of latent HIV-1 proviruses in primary CD4+ T cells (Hayashi et al., 2017). This is a promising latency promoting candidate, which is already FDA approved. However, its efficacy and/ or toxicity needs to be evaluated in brain cells to determine its potential for eradicating the CNS reservoir. Another compound, ABX4641, targets HIV Rev, and blocks HIV-1 replication, but its efficacy is unknown in the CNS (Campos et al., 2015). Compounds targeting the viral proteins are expected to have fewer adverse effects on the host micro-environment. Hence, combining the 'Block and Lock' and 'Shock and Kill' strategies is an effective way to control the HIV-1 reservoir.

5.4. Immunotherapeutic interventions

Immunotherapeutic interventions are a wide range of treatment strategies that hold a lot of promise towards targeting HIV-1. Besides attempting to provide a functional cure, they also have potential to minimize morbidity associated with HIV-1 by decreasing inflammation, improving immune functioning, etc. However, the BBB poses a major barrier to the delivery of immunotherapeutics as well. The tight junctions between the endothelial cells of the BBB limit the entry of immune cells and mediators making the fight against HIV-1 inside the CNS more challenging (Muldoon et al., 2013). Recent research has focused on potentiating host humoral and cell-mediated response by inducing host inflammatory cascade to mitigate neurotoxicity associated with HIV-1. A combination of boosting the existing immune response, inducing additional immune responses to existing or novel HIV-1 immunogens as well as passive immunization can achieve this goal. To this effect, the generation of T cells that can recognize antigens expressed in the brain, derived from potent HIV-1-specific clones of cytotoxic T cells in the brain, is an attractive new strategy (Nath and Clements, 2011). However, the tradeoff is that the induction of the host immune response and providing additional boosts may tip the balance of the inflammatory casacade towards pro-inflammatory response, and thus, the release of excessive proinflammatory cytokines can exacerbate tissue cytotoxicity. To counter this cytotoxicity and support the insufficient immune responses in HIV-1-infected patients, the addition of anti-inflammatory drugs to immunosuppressive drugs has been an attractive approach to decrease the levels of

proinflammatory cytokines related to neurotoxicity (CCL2, CCL5, and CXCL10). This approach has shown positive results in a microglial cell model (Ambrosius et al., 2017).

A small subset of infected individuals generates antibodies against the highly conserved regions of the HIV env protein, which can neutralize a wide range of HIV strains, hence, these antibodies are aptly termed as broadly neutralizing antibodies (bnAB) (Stamatatos et al., 2009). However, CNS penetrance of anti–HIV-1 bnABs has yet to be evaluated in human studies. Low concentrations of the bnAB, rituximab was seen in the CSF of non-human primates infected with SIV, which translates into low CNS penetrance. This concentration increased with intrathecal administration, but its turnover was short with a low half-life (Rubenstein et al., 2003). Efforts are underway to develop recombinant antibodies, with longer half-lives and potential candidates are under evaluation in clinical trials (Lee et al., 2016).

Antibodies targeting surface markers B7-H1 are being developed to encourage cellular apoptosis of reactivated latent cells (Zhang et al., 2013). These antibodies have promise in eliminating infected latent cells that are resistant to apoptosis such as microglia. Development of multi-affinity antibodies is another attractive approach to combat viral infection. While bnABs can target the virus, they are not very effective in preventing the emergence of resistant mutants. To enhance the killing potential of the latent population, Dual affinity retargeting (DART) antibodies are being developed to target the CD3 receptor on activated effector CD8+ T cells and the HIV-1-specific gag or env antigens expressed on reactivated CD4+ T cells (Yang et al., 2018).

On a more technologically advanced front, designer immune responses are generated by constructing chimeric antigen receptors (CAR) by the fusion of CD4 epitope and CD3 chain signaling domain on effector T cells which facilitate the selection of HIV-1-infected CD4+ cells through the interaction between HIV-1 env and CD4 (Maldini et al., 2018). This strategy has not yet been optimized for specific eradication of latent population in the CNS. CAR-T cells designed against tumor cells have been demonstrated to cross the BBB showing successful outcomes in treating CNS tumors (O'Rourke et al., 2017), suggesting the utility of this therapy in overcoming CNS infection in the near future. CAR-T therapy, however, is associated with its own set of challenges: CAR-T cell-related encephalopathy syndrome (CRES) and cytokine-release syndrome (CRS) are among the most common side effects ranging from mild symptoms to more severe conditions leading to multi-organ failure (Hunter and Jacobson, 2019). Several neurotoxic effects are also known to associate with this therapy including confusion, delirium, aphasia, seizure, and loss of consciousness.

5.5. Gene editing based therapies

CRISPR/Cas9 is a novel gene-editing tool that has become increasingly popular to target and potentially repair faulty DNA sequences. In contrast to traditional gene-editing tools such as ZFNs and TALENs, CRISPR/Cas9 technology is a fast, more specific, and a cost-intensive approach and is being widely used to combat HIV-1. CRISPR/Cas9 uses a guided RNA and a Cas9 nuclease to excise target DNA sequences of cellular factors, and one of the first sequences that was targeted in the effort to eradicate HIV-1 infection is the NF-κB binding site located in the HIV-1 LTR (Ebina et al., 2013). Since then many studies have explored whether CRISPR/Cas9 could successfully excise fragments of integrated HIV-1

proviral DNA and whether it can be used with ART to eliminate HIV-1 from cellular reservoirs. To evaluate the combinatorial effect of ART and CRISPR/CAS9, humanized mice were subjected to sequential treatment of ART (LASER ART) followed by CRISPR/CAS9 targeted towards the HIV-1 LTR-Gag region. Complete elimination of HIV-1 was seen with no viral resurgence in the viral compartments of humanized mice even after two months following the termination of ART (Dash et al., 2019; Su et al., 2019). This is the first study to demonstrate that complete HIV-1 eradication is possible by employing multiple elimination strategies.

Traditionally, Cas9 and sgRNA are encoded within the plasmid DNA of the viral vectors that randomly integrate into the human genome, potentially giving rise to unintended off-target genetic effects. While formulating CAS9 and gRNA into ribonucleoproteins was an attractive alternative, delivering these ribonucleoprotein complexes remained a major challenge. The discovery of yarn-like DNA nanoclew (DNA NC) synthesized by rolling circle amplification (RCA) provided a novel method of polymeric nanoparticle delivery of CRISPR–Cas9 (Ali et al., 2014). Partial complementarity between the DNA NC and the sgRNA guide sequence greatly enhanced the extent of gene editing, and with the incorporation of cell-specific targeting ligands, the DNA NCs can be engineered to specifically target the cell types of interest (Sun et al., 2015). However, non-invasive delivery of Cas9/gRNA across the BBB is not fully explored yet. Kaushik et al. developed a novel, promising non-invasive mode of delivery that controls the release of Cas9/gRNA targeting HIV-1 LTR, on-demand, across the BBB by using magneto-electric nanoparticles (MENPs) as vehicles. These MENPs are small, ferromagnetic, non-toxic and are able to across the BBB under a static magnetic field. Treatment of latent HIV infected hμglia/HIV cells with MENPs reduced viral LTR expression levels confirming successful delivery across the BBB and targeting latent virus (Kaushik et al., 2019).

CRISPR/CAS9 technology is also being explored to redesign the gene expression of cells such as CTLs to target HIV-1 infected cells with enhanced specificity, thus increasing the efficiency of the host antiviral response to HIV-1 infected cells and activated reservoirs (Mehta et al., 2017). A major limitation of this technology is that it is mostly explored in CD4+T cells. Its efficacy is unknown in CNS cells. *Ex vivo* studies showed that edited and redirected CD4+T cells successfully targeted only a few infected cells and this approach has still largely been unsuccessful in eliminating all of the infected cells (Wang et al., 2018). Moreover, the incidences of off-target effects, undesirable gene mutations, and chromosomal translocations pose obstacles that need to be overcome.

However, gene therapy is still in its infancy but shows great promise in achieving the goal of eradicating total viral load from all the known HIV-1 reservoirs. CRISPR/Cas9 targets the root of the problem: integrated proviral DNA; thus, the capability to excise or inactivate the LTR, which is required for viral gene activation and expression, makes this strategy stand out. The potential for CRISPR/Cas9 in clinical therapy is still under investigation. Several issues will have to be resolved before CRISPR/CAS9 can be used clinically for HIV eradication. First, as a consequence of mutations in the virus in the reservoirs or in neighboring sites of the targeted cells, the gRNA sequence specific to the strain may be altered as a result of which recognition and cleavage by CRISPR/CAS9 may not occur (Badia et al., 2017). Second, the HIV-1 genome is about 10,000 bps and the gRNA targets a region of only 20 bps. This drastically increases non-specific targeting sites in the provirus in latently infected cells. Establishing a platform to evaluate gRNA candidates

against proviral DNA is especially important to improve tissue targeting and cleavage efficiency (Soriano, 2017). Finally, safe and effective mechanisms of delivery of CAS9 and gRNA is essential for successful therapy. While adenoviral vectors have been traditionally used in gene therapy, the packaging size of the vector is not ideal for CAS9/gRNA delivery. Substantial research is addressing these concerns and several promising modes of delivery such as DNA nanoclews and MENPs (discussed above) are being developed. Despite these roadblocks, CRISPR technology is evolving at a rapid pace and a promising pathway of complete HIV-1 eradication is not far away.

5.6. Therapeutic vaccines

There has been a lot of interest in developing a vaccine against HIV-1. Development of a vaccine against HIV-1 may prove effective for eliminating not only the plasma viral load but also for preventing future infections that may occur through the reactivation of latent reservoirs. The efficacy trial, RV144 study, has demonstrated a modest reduction in HIV-1 infection rates using a combination of ALVAC-HIV (canarypox vector) and AIDSVAX B/E (gp120 vaccine) (Gao et al., 2018; Rerks-Ngarm et al., 2009). However, efforts are underway to improve the efficacy of this candidate (Pitisuttithum et al., 2020). Another potential candidate under study is Vacc-4x developed from highly conserved regions of HIV-1 p24 viral core protein (Tapia et al., 2017). Vaccines targeted towards enhancing the cytotoxic response of T cells are of particular interest when it comes to targeting the CNS. However, the efficacy and adverse effects of enhancing the cytotoxic T cell responses in the CNS are not yet known. To date, there are no clinical studies targeted towards examining this effect in the CNS.

6. Future perspectives

The complete eradication of HIV-1 in two infected individuals under ART through allogenic transplantation of hematopoietic stem cells from donors expressing the naturally occurring CCR5Δ32 mutation has demonstrated that the cure for HIV-1 is possible through the transfusion of HIV-1 resistant stem cells. Besides the huge cost involved, it is unlikely that the majority of infected individuals can find compatible donors, making the search for an alternate effective strategy to eliminate the latent reservoir vital.

A major limitation of most current strategies is the identification of the latent reservoir. In theory, latently infected cells have completely repressed transcription and no viral proteins should be produced from them. However, there is evidence of sporadic viral transcript production latent cells (Symons et al., 2017). These findings indicate the possibility that latent HIV-1 provirus may exhibit a distinct molecular signature. There is considerable interest to identify "biomarkers" specific to the latently infected cell populations. Cell surface molecules that could distinguish latently infected cells from uninfected cells could function as potential biomarkers. Recent research has identified CD32a as a potential biomarker of latently infected CD4+ T cells, however only ~50% of the latent population was seen to express CD32a making it unlikely to be representative of the entire latent population (Descours et al., 2017; Garcia et al., 2018). The co-localization of CTIP2 and the microglial marker (Iba1) in human cortical glia, and the presence of repressive epigenetic marks in latently infected patients but not in HIV encephalitis (HIVE) patients indicates that CTIP2 can be considered a biomarker of brain HIV-1 latency (Desplats et al., 2013). Research

targeted towards the identification of a biomarker, especially in the CNS, will be useful for treating people on ART but who still suffer from HIVE and HAND.

Studies conducted on small molecule LRAs revealed that the "shock" caused by these small molecules is not sufficient to evoke significant latency reversal in the majority of the latent cell population (Chen et al., 2017). Future research should aim towards developing combinations of LRAs that target different areas of the genome and synergistically induce broad transcriptional responses (Hashemi et al., 2018). Development of strategies that improve the capacity of the cell to successfully "kill" may also enhance effectiveness when used in conjunction with the LRAs. Currently, there are no known small molecule compounds or drugs that lock HIV-1 provirus expression in the CNS by modulating the recruitment of HDACs, HMTs, DNA methyltransferases, etc. Identification of epigenetic modulators of transcription in the CNS represents an important focus for future research.

Lastly, while vaccines present an appealing option for HIV-1 prevention, but their effect on HIV-1 latency is unknown (Castro-Gonzalez et al., 2018). The inaccessibility of the viral genome in a latent state makes it difficult for vaccine-boosted CTL responses to target infected cells. The boosting of HIV-specific T cell responses in the peripheral tissues with vaccines may be effective, but if these immune cells are not able to effectively cross the BBB, this strategy would have limited efficacy in the CNS. Hence the future focus should be directed towards the design of vaccines that can effectively cross the BBB and elicit minimum amount of cytotoxic damage to uninfected cells.

7. Conclusion

Four decades of research on HIV-1 infection indicate that complete viral eradication is not possible without targeting latent viral reservoirs. The role of the CNS as a latent reservoir is still controversial. The cells of the CNS developed unique mechanisms to silence the integrated viral genome and facilitate viral persistence. The long lifespan of these cells is an added advantage as the silenced virus is harbored within them lasts for a long time. Viral infection of resident immune cells in the CNS such as macrophages and microglia is clinically significant, as a disruption of cellular functioning in these cells is attributed to the pathogenesis of HIV-1 associated neurodegeneration. Due to poor antiviral drug penetration, these anatomical compartments also turn into viral sanctuaries. This suggests that the brain harbors HIV-1 regardless of its latent state and that the effect of eradication strategies on the CNS has to be carefully considered before implementation. As discussed in this review, understanding mechanisms of HIV-1 latency in CNS reservoirs and the onset of HIV-1-associated neurological disorders is critical to designing strategies to eliminate HIV-1 from the CNS. Studies have aimed at eliminating the latent virus through several approaches and it can be suggested that a carefully tailored combination of two or more of these approaches can result in successful eradication of HIV-1.

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