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HIV-1 transcriptional regulation in the central nervous system and implications for HIV cure research

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Abstract

Human immunodeficiency virus type-1 (HIV-1) invades the central nervous system (CNS) during acute infection which can result in HIV-associated neurocognitive disorders (HAND) in up to 50% of patients, even in the presence of combination antiretroviral therapy (cART). Within the CNS, productive HIV-1 infection occurs in the perivascular macrophages and microglia. Astrocytes also become infected, although their infection is restricted and does not give rise to new viral particles. The major barrier to the elimination of HIV-1 is the establishment of viral reservoirs in different anatomical sites throughout the body and viral persistence during long-term treatment with cART. While the predominant viral reservoir is believed to be resting CD4⁺ T-cells in the blood, other anatomical compartments including the CNS, gut-associated lymphoid tissue, bone marrow, and genital tract can also harbor persistently infected cellular reservoirs of HIV-1. Viral latency is predominantly responsible for HIV-1 persistence, and is most likely governed at the transcriptional level. Current clinical trials are testing transcriptional activators, in the background of cART, in an attempt to purge these viral reservoirs and reverse viral latency. These strategies aim to activate viral transcription in cells constituting the viral reservoir, so they can be recognized and cleared by the immune system, while new rounds of infection are blocked by co-administration of cART. The CNS has several unique characteristics that may result in differences in viral transcription and in the way latency is established. These include CNS-specific cell types, different transcription factors, altered immune surveillance, and reduced antiretroviral drug bioavailability. A comprehensive understanding of viral transcription and latency in the CNS is

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required in order to determine treatment outcomes when using transcriptional activators within the CNS.

Keywords

HIV-1; CNS; reservoirs; latency; persistence; transcription

Introduction

Despite abundant research and numerous advances in the treatment of human immunodeficiency virus type 1 (HIV-1), the virus continues to be a major global health issue, claiming several million lives annually. The use of combination antiretroviral therapy (cART) has resulted in the prevention of disease progression and restoration of life expectancy approaching that of the general population (Obel *et al*, 2011). Similarly, the introduction of cART also led to a reduction in the occurrence of HIV-1 encephalitis (HIVE), which was a hallmark of significant viral replication within the CNS. However, the use of cART remains life-long, as cessation of therapy invariably results in viral rebound and disease progression (Ananworanich *et al*, 2006). Additionally, of the 29 million people globally who require access to cART, only 10 million are currently receiving this life-saving medication (WHO, 2013). Additionally, the cost of scaling up cART access to all those that need it is unlikely to be sustainable. Furthermore, the use of cART itself is not without its own shortcomings, because many of the drugs have side effects and the emergence of drug resistance is ever present, especially where drug adherence is less than optimal. Together, all these factors point to the need to develop new strategies that aim to eradicate HIV-1 from the body so that patients can cease cART without the fear of viral rebound and disease progression.

It has been well documented in the literature that the resurgence of virus following cessation of cART is derived from viral reservoirs in latently infected cells located in different anatomical sites throughout the body (Eisele and Siliciano, 2012; Siliciano *et al*, 2003; Trono *et al*, 2010). These sites include the brain, blood, gut-associated lymphoid tissue, bone marrow, and genital tract (Eisele and Siliciano, 2012). Several groups have shown that the duration of cART treatment does not appear to alter the cellular reservoir size over time, as they are highly stable and long-lived (Pierson *et al*, 2000; Rong and Perelson, 2009). This has sparked a new area of investigation, termed HIV cure research, which aims to specifically target these viral reservoirs to eliminate them from the body, and contribute to a cure for HIV (Deeks *et al*, 2012).

There is contention in the literature about whether the central nervous system (CNS) can act as a viral reservoir (Brew *et al*, 2013; Churchill and Nath, 2013; Churchill *et al*, 2009; Yilmaz *et al*, 2010). We have shown that HIV-1 integrates into astrocytes of the CNS, and in fact can be detected in up to 20% of astrocytes in autopsy brain tissues, suggesting that astrocytes may be a potentially significant reservoir of HIV-1 DNA (Churchill *et al*, 2006; Churchill *et al*, 2009; Wesselingh and Glass, 2000). Other studies have identified infected perivascular macrophages and microglia within the CNS, both of which have long lifespans

and may also act as viral reservoirs (Churchill *et al*, 2006; Watkins *et al*, 1990; Wiley *et al*, 1986). However, it remains to be determined if the virus present within these cells is capable of re-emerging and seeding the periphery upon cART cessation. Nevertheless, the high frequency of cells harbouring HIV-1, and the long-lived nature of these cells, suggests that the CNS at least has the potential to act as a viral reservoir.

The “shock and kill” approach has been proposed as a mechanism by which the viral reservoir could be eliminated progressively over successive rounds of treatment (Deeks, 2012). It involves the use of transcriptional activators that are designed to turn on HIV-1 synthesis in latently infected cells. It is believed that once these cells start producing viral proteins they will be recognised by the immune system and cleared, while any new virions produced will be blocked from infecting new target cells due to the continued use of cART. At present, the majority of transcriptional activators under investigation include histone deacetylase inhibitors (HDACi), histone methyltransferase inhibitors (HMTi), IL-7, disulfiram, and prostratin (Bernhard *et al*, 2011; Kulkosky *et al*, 2001; Van Lint *et al*, 1996; Wang *et al*, 2005; Xing *et al*, 2011). Most of these compounds function at the transcriptional level, modifying chromatin structure to better enable access for transcription factors and the transcriptional machinery.

All current clinical trials using the “shock and kill” approach are mainly focusing on the peripheral viral reservoir, contained within the resting CD4⁺ T-cells, with little or no investigation on outcomes involving the CNS viral reservoir (Kent *et al*, 2013). While it is important to concentrate efforts on the predominant and well characterized viral reservoir in the blood, a greater understanding of the other less well-characterized reservoirs is warranted to ensure we have a comprehensive strategy that will target all sites of latency. While the CNS viral reservoir remains controversial, and its investigation also poses unique difficulties (i.e. tissue sampling), the nature of the CNS suggests that treatment outcomes and responsiveness within this compartment will be distinct. The blood brain barrier (BBB) differentially affects cART penetration and effectiveness within the CNS and would likely present similar problems for the use of transcriptional activators (Gray *et al*, 2013b; Letendre *et al*, 2010; Letendre *et al*, 2008). Similarly, immune surveillance is altered within the CNS, and therefore clearance of re-activated latently infected cells may be suboptimal (Ousman and Kubes, 2012). Finally, studies by our own group and others have shown that the viral strains present in the CNS are distinct to those found in the periphery, and these differences extend to the viral promoter (long terminal repeat or LTR) (Ait-Khaled *et al*, 1995; Gray *et al*, 2013a). Furthermore, these tissue specific changes in the LTR will result in altered responsiveness to the proposed transcriptional activators. Taken together, all of these factors suggest that the use of transcriptional activators within the CNS will most likely result in different responsiveness and effectiveness.

This review will focus on highlighting the differences that exist at the LTR level and the regulatory mechanisms that govern transcription, between the CNS and peripheral compartments. Understanding these differences will provide important insights into the potential treatment outcomes during “shock and kill” approaches. This will permit the optimisation of regimens to ensure we use the most effective strategy to eliminate HIV-1 of all viral reservoirs.

CNS cells infected by HIV-1

Within the CNS HIV-1 can infect several different cell types, which include macrophages, microglia and astrocytes (Churchill *et al*, 2009; Cosenza *et al*, 2002; Thompson *et al*, 2004). Perivascular macrophages and microglia are the main targets for active viral replication within the brain, and they are likely to contribute to the neurodegeneration seen in patients with HIV-associated neurocognitive disorder (HAND) (Heaton *et al*, 2010). They constitute the resident immunocompetent cells of the brain and respond to all types of insult, ranging from vascular problems to protein accumulation that is associated with some neurodegenerative diseases, such as Alzheimer's disease (Gehrmann *et al*, 1995). Microglia are relatively long-lived cells but in comparison, perivascular macrophages have a faster turnover and are replenished by monocytes migrating into the CNS (Carson *et al*, 2006). Microglia appear as highly branched cells when in a resting state and more rounded cells when activated (Guillemin and Brew, 2004). Perivascular macrophages are flat and elongated and located adjacent to brain microvasculature. Both cell types express CD4 and the necessary coreceptors (CCR5 & CXCR4) for HIV-1 entry, rendering them susceptible to HIV-1 infection.

Astrocytes have also been shown to be susceptible to HIV-1 infection (Churchill *et al*, 2006; Churchill *et al*, 2009; Thompson *et al*, 2004). Astrocytes are the most abundant cell type within the brain, and are involved in homeostasis and regulation of the microenvironment within the CNS (Kimelberg and Norenberg, 1989). Astrocytes express both CCR5 and CXCR4 on their surface but lack expression of CD4. Despite this, astrocytes can become infected via a CD4-independent mechanism, which likely involves the use of vesicles (Gray *et al*, 2014; Sabri *et al*, 1999; Tornatore *et al*, 1994). Until recently astrocyte infection was thought to be relatively rare (1–3%) (Wesselingh and Glass, 2000), however recent studies by our laboratory using more sophisticated and highly sensitive techniques have demonstrated much higher infection frequencies of up to approximately 20% depending on the extent of neurological disease (Churchill *et al*, 2009). However, despite the major block at virus entry there appears to be post-entry blocks that prevent astrocytes from supporting a productive infection, as only early transcripts such as *nef* and *tat* are detectable during persistent infection (Gorry *et al*, 1999; Gorry *et al*, 2003; Kleinschmidt *et al*, 1994). A recent study demonstrated that by increasing expression of a cellular protein, TAR RNA binding protein (TRBP), the post-entry block is significantly alleviated and progeny virus can be produced (Ong *et al*, 2005). TRBP is the cellular antagonist of the dsRNA-activated protein kinase (PKR). PKR has strong antiviral effects against HIV-1, and these data suggests that PKR activity is heightened due to low TRBP levels within astrocytes.

The remaining cells that constitute the CNS, namely oligodendrocytes, neurons, and brain microvascular endothelial cells, are largely believed to be resistant to HIV-1 infection (Gonzalez-Scarano and Martin-Garcia, 2005). The unique nature of the CNS microenvironment and the cells which form it, suggests that viral replication and transcription is distinct to that in the periphery. The transcription factors available within the cells will impose a strong selective pressure to favour HIV-1 LTRs with specific configurations to allow for optimal replication within the CNS. Indeed, LTRs derived from

the CNS often compartmentalize compared to those found in the periphery (Gray *et al*, 2013a). Their unique features will be discussed in more detail within this review.

CNS-derived HIV-1 promoters

Numerous studies have analyzed the HIV-1 genome from different anatomical sites to determine their relatedness and similarity. Earlier research established that different tissue compartments favour different HIV-1 strains, each with their own unique properties, and that these differences extend to specific parts of the HIV-1 genome, including Env, Nef, Tat, and LTR (Ait-Khaled *et al*, 1995; Ohagen *et al*, 2003; Thomas *et al*, 2007; Thompson *et al*, 2004). The mechanisms governing the development of tissue compartmentalization are numerous and likely include different immune selection pressures, cell type-specific differences in replication or gene expression, local concentrations of antiviral drugs and/or drug resistance, and co-infections that alter the cellular microenvironment. The CNS presents several differences within this list that likely exacerbate HIV-1 compartmentalization within this compartment. These include reduced immune surveillance, CNS-specific cell types, altered antiviral penetration and effectiveness due to the BBB and cell types present, and the unique CNS cellular microenvironment.

Our research has demonstrated the compartmentalized nature of the HIV-1 LTR promoter between the CNS and other tissues including peripheral blood mononuclear cells (PBMC), spleen and lymph node of HIV-1 infected patients (Gray *et al*, 2013a). At the genetic level, this compartmentalization is associated with nucleotide changes within the LTR that result in a modification of the transcription factor binding sites present, or alterations in the efficiency of the sites to bind the various transcription factors. Highly efficient CCAAT enhance binding protein (C/EBP) transcription factor sites have been suggested to have an involvement in the compartmentalization of CNS LTRs and in disease progression, especially within monocyte/macrophage lineage cells (Hogan *et al*, 2003; Liu *et al*, 2009; Ross *et al*, 2001). Other studies have identified an involvement of HIV-1 Vpr with C/EBP sites, or nucleotide changes within the LTR, that are associated with neurocognitive impairment (Burdo *et al*, 2004a; Burdo *et al*, 2004b; Li *et al*, 2011). Our own studies did not find a strong association between presence of C/EBP sites and CNS LTRs, nor did we find other mutations that were always specific to CNS LTRs. Instead, we often saw a range of mutations and changes (particularly within or surrounding the binding sites for Ets-1, USF, C/EBP, AP-1 and in the core promoter region) that often resulted in a common phenotype or transcriptional activity that was able to segregate CNS versus non-CNS LTRs (Gray *et al*, 2013a). CNS LTRs can be characterized as having lower basal transcriptional activity (relative to matched non-CNS LTRs), whilst retaining their ability to be activated by HIV-1 Tat (Gray *et al*, 2013a). The basis for the reduced activity remains unclear.

The unique nature of CNS-derived LTRs highlights the need to use these LTRs in experimental systems to ensure we obtain an accurate interpretation of their responsiveness to various transcriptional activators. Testing CNS-derived LTRs in CNS cells is essential to ensure we generate valid results and accurately recreate the *in vivo* setting.

Altered HIV-1 transcriptional regulation within the CNS

Perivascular macrophages, microglia and astrocytes within the CNS all produce their own transcription factors, although there is some commonality that is shared between these cells compared to their peripheral counterparts. Within the CNS, the most important transcription factors relevant for HIV-1 transcription include Sp1-4, NF- κ B, C/EBP and AP-1, which are summarised in Table 1.

Sp factors

The HIV-1 promoter contains three binding sites for Sp factors, which are located within the basal region (Figure 1) (Jones *et al*, 1986). The Sp and NF- κ B factor binding sites in the core promoter play an important role in regulating transcription and replication in a cell type-specific manner. Sp family members include Sp1, Sp2, Sp3, and Sp4, and all contain zinc finger DNA binding domains. The Sp family members have a strong affinity and specificity for recognising GC-rich sequences (GGGGCGGGGC) (Hagen *et al*, 1992; Kadonaga *et al*, 1987). Sp1 and Sp4 are transcriptional activators, whereas Sp3 has been shown to be a repressor of HIV-1 transcription (Majello *et al*, 1994). The cellular expression of Sp2 remains largely unknown and its relevance to HIV-1 transcription is questionable. Sp1 and Sp3 are ubiquitously expressed in human cells, although their relative ratios vary depending on the status of the cell and the cell type (Discher *et al*, 1998; Hagen *et al*, 1992; Suske, 1999). Sp4 is predominantly expressed in the brain (Hagen *et al*, 1995) and therefore provides an additional activator within this compartment. However, unlike Sp1, Sp4 does not synergise in the presence of multiple Sp binding sites, and is therefore believed to be a less potent activator of HIV-1 transcription compared to Sp1 (Hagen *et al*, 1995). The relative ratio of Sp activators to Sp repressors largely governs HIV-1 transcription outcomes. We have observed an abundance of Sp3, relative to Sp1, in astrocytes and this finding could explain in some part, the restriction of transcription within these cells (M. Churchill, unpublished data). Genetic variation within the LTR Sp sites is likely to play a key role in HIV-1 transcription. Mutations in the Sp sites have been shown to result in poorer recruitment of Sp factors, altered transcriptional activity, and changes in disease progression (Nonnemacher *et al*, 2004). Sp factors bound to the HIV-1 core promoter cooperatively interact and recruit other transcription factors (Tat, NF- κ B, TATA binding protein (TBP), P-TEFb) as well as the transcriptional machinery (TFIID, RNA Pol II) (Chiang and Roeder, 1995; Emili *et al*, 1994; Yedavalli *et al*, 2003). Sp factors also play an important role in regulating transcription by remodelling chromatin either by the recruitment of histone acetyltransferases (HATs) to promote transcription or recruitment of histone deacetylases (HDACs) to inhibit transcription (Doetzlhofer *et al*, 1999; Widlak *et al*, 1997).

NF- κ B

The NF- κ B transcription factor is one of the main modulators of the HIV-1 LTR in all cell types, and its sites in the LTR are essential for viral replication (Mingyan *et al*, 2009). The recruitment of NF- κ B proteins to the two sites in the enhancer region (Nabel and Baltimore, 1987) is facilitated by Sp factors binding in the adjacent basal/core promoter. Sp-NF- κ B protein:protein interactions are able to further modulate LTR activity by binding the LTR cooperatively to activate transcription synergistically (Perkins *et al*, 1993). However, only

Sp1 is able to form protein:protein interactions with NF- κ B, whereas Sp3 and Sp4 failed to do so, due to an inability to associate with other proteins. NF- κ B is composed of heterodimers of five c-rel protein family members: p65/RelA, NF- κ B1/p50, c-Rel, RelB, and NF- κ B2/p52. Functional NF- κ B usually consists of a p65/p50 heterodimer in T-cells and RelB/p50 heterodimer in macrophages (Asin *et al*, 2001; Nabel and Baltimore, 1987). Inactive NF- κ B normally exists in the cytoplasm where it is kept inactive via an association with its inhibitor, I κ B (Baeuerle and Baltimore, 1988). Activation of NF- κ B involves the phosphorylation of I κ B (in response to specific stimuli), releasing NF- κ B and allowing its translocation to the nucleus where it activates host and viral genes through initial recruitment of P-TEFb (Barboric *et al*, 2001; Beg *et al*, 1993). NF- κ B has also been reported to function in a repressive role via the recruitment of HDACs (Ashburner *et al*, 2001). Mature macrophages contain constitutively active NF- κ B in the nucleus (Griffin *et al*, 1989), whereas microglia and astrocytes contain comparatively low/moderate levels, which can be elevated upon activation. The constitutive pool of NF- κ B in macrophages facilitates low basal levels of HIV-1 transcription in the absence of cellular stimuli (Griffin *et al*, 1989). Deletion or mutation of the NF- κ B sites abolishes LTR activity and results in reduced virion output (Asin *et al*, 2001).

C/EBP

C/EBPs play a critical role in HIV-1 replication. The HIV-1 LTR contains three C/EBP binding sites upstream of the transcription start site (Mondal *et al*, 1994). In monocyte lineage cells, the presence of at least one upstream C/EBP binding site and C/EBP proteins is necessary for replication (Henderson *et al*, 1995). C/EBP factors activate HIV-1 transcription through direct binding to the LTR and also interact with many other nuclear proteins. These include chromatin remodelling complexes such as SWI/SNF and p300/CREB (Kowenz-Leutz and Leutz, 1999; Mink *et al*, 1997), which remodel the chromatin structure to increase the transcriptional activity of the HIV-1 LTR. C/EBP can also act synergistically with Sp proteins to activate transcription of the HIV-1 LTR (Schwartz *et al*, 2000).

Wigdahl and colleagues have extensively studied the relationship between C/EBP factors and their sites within the subtype B HIV-1 LTR to tissue tropism and disease progression. They showed that a 6G configuration of C/EBP site I results in increased C/EBP binding and LTR activity, and was predominantly encountered in CNS-derived viruses (Ross *et al*, 2001). In addition, C/EBP site II was also conserved, and maintained a high affinity site in CNS-derived LTRs. Interestingly, these high affinity C/EBP sites also correlated with development of HIV-1-associated dementia (HAD) and regions of the brain which undergo high rates of viral replication (Burdo *et al*, 2004a). Conversely, the low affinity C/EBP sites were found preferentially in regions of the brain which undergo low rates of viral replication. However, our studies did not find a similar association between high affinity C/EBP sites and CNS-derived LTRs, suggesting these mutations may be cohort or patient specific (Gray *et al*, 2013a). Overall these findings suggest that C/EBP site affinity may play a role in the maintenance of HIV-1 pathogenesis, as well as the maintenance of latent reservoirs in the CNS, but further studies are required to demonstrate this more conclusively.

HIV-1 transcriptional latency

The establishment of HIV-1 viral reservoirs is associated with viral latency which is largely governed by transcriptional silencing of the viral promoter (Redel *et al*, 2010). Under latent conditions, the viral transactivator of transcription (Tat) is absent or the low levels present are excluded from interacting with the LTR because it exists in a tightly coiled chromatin structure. Under these conditions, the basal/core promoter is occupied by repressive factors including, the NF- κ B homodimer p50/p50, Sp3 and histone deacetylases (HDAC) (Figure 2a). HDAC actively deacetylate the neighboring histones in nuc-0 and nuc-1 to enable the DNA to be more tightly coiled and associated with the nucleosomes (Van Lint *et al*, 1996). The lack of transcriptional activators and the closed chromatin structure prevents the recruitment of RNA polymerase II and the associated transcriptional machinery, resulting in no viral transcripts or proteins being produced. However, upon specific stimuli or cell activation, the bound repressors are displaced with activators including the NF- κ B heterodimer p50/p65, Sp1, Tat and histone acetyltransferases (HATs) (Figure 2b). The active recruitment of NF- κ B and HATs by Sp1 results in acetylation of neighboring histones in nuc-0 and nuc-1, relaxation of chromatin structure and the exposure of transcription factor binding sites within the LTR for full promoter activity, resulting in viral transcripts and subsequent proteins being produced (Jones *et al*, 1986; Perkins *et al*, 1993).

In addition to histone modification with acetyl groups, histones can also become methylated by histone methyltransferases (HMT) (du Chene *et al*, 2007; Imai *et al*, 2010). DNA itself can also be directly methylated by DNA methyltransferases (DNMT), although their involvement in regulating HIV-1 transcription is yet to be determined (Kauder *et al*, 2009). Both of these forms of methylation result in restriction of transcription and further condition the LTR into a latent state.

There may be differences in the mechanisms of latency establishment between the periphery and cells in the CNS, however studies addressing this are yet to be done. An SIV study showed that IFN- β suppresses SIV LTR activity in the CNS by inducing expression of the dominant negative isoform of C/EBP- β , resulting in the establishment of latency (Barber *et al*, 2006). Another study of post-mortem brain tissue from HIV positive latent cases, observed an increase in CTIP2, HP1, MeCP2 and HDAC1 levels, suggesting they may play a role in the establishment of latency within the CNS (Desplats *et al*, 2013).

Potential therapeutics to reverse HIV-1 transcriptional latency

The identification of viral reservoirs and a greater understanding of the regulatory mechanisms that control silencing of HIV-1 transcription has fostered the development of potential therapeutic interventions to reverse HIV-1 latency. The majority of these approaches have concentrated on the use of HDAC inhibitors (HDACi) with varying specificities, but have now been expanded to include a broader range of compounds that target all aspects of transcriptional restriction (Bernhard *et al*, 2011; Chun *et al*, 1998; Wightman *et al*, 2013; Xing *et al*, 2011). These are summarised in Table 2, along with information regarding ongoing or planned clinical trials, known penetrance into the CNS, and approximate potency for inducing HIV-1 transcription. These drugs cover a spectrum of

poor (Romidepsin, Tubastatin) to very good (Vorinostat, Disulfiram) CNS penetration, which will greatly alter their effectiveness against the CNS latent reservoir. Furthermore, these compounds will potentially exhibit altered toxicity within the CNS, which remains largely unknown, but needs to be evaluated in CNS cells to ensure we minimise any side effects, while maximising their efficacy.

The majority of clinical trials using these compounds are predominantly analyzing reductions in the viral reservoir exclusively within the blood. This is often due to ease of sampling and especially because this compartment is believed to harbour the largest and most significant viral reservoir. However, the outcome of these treatments on other compartments such as the CNS is important, especially considering the role CNS infection plays in HAND, and it's rising prevalence. We want to avoid a situation where patients are undergoing HIV-1 cure strategies to the detriment of their neurocognitive capabilities, with potentially increased or uncontrolled CNS infection.

To date, the use of HDACi inhibitors has been promising but also less favourable than first anticipated. Some groups have observed a small but appreciable decline in the size of the viral reservoir, while others have observed no reduction (Archin *et al*, 2012; Rasmussen *et al*, 2013). Worryingly, in the trials where declines were observed, only a fraction of the patients appeared to respond, suggesting that single interventions may not work in all patients. The vast majority of these trials have employed the use of pan-HDACi (Vorinostat, Panobinostat) that lack specificity to particular classes of HDAC. However, with the arrival and use of class specific (Romidepsin, Entinostat, Givinostat, Belinostat) or HDAC specific (PCI-34051, Tubastatin) HDACi, there is the potential for improved specificity and increased activity on the LTR, as well as reduced the incidence of unfavourable side effects.

Other compounds in the pipeline include disulfiram, which is a modulator of Akt signalling, HMBA, which is a Tat mimetic and has roles in chromatin remodelling, JQ1, which is a bromodomain inhibitor, and chaetocin, which is a histone methyltransferase inhibitor. Interestingly, HIV-1 Vpr has also been shown to play a role in activating both cellular and viral promoters (Felzien *et al*, 1998; Wang *et al*, 1995). There is also some evidence that Vpr can reactivate latently infected cells (Levy *et al*, 1994; Levy *et al*, 1995), suggesting it may be an attractive target for therapeutic applications. It's now becoming clearer that like cART, which requires a combination of antiretrovirals to achieve sustained virological suppression, "shock and kill" approaches will likely need to use a combination of transcriptional activators to achieve a robust and significant decline in viral reservoirs in all patients. These strategies have to be conducted in an environment where side affects are continually minimised, while specific activity against the LTR is optimised.

Conclusions

This review has highlighted the unique characteristics of the CNS that may potentially influence the establishment and maintenance of the CNS HIV-1 viral reservoir. Despite ongoing contention regarding the relevance of the CNS viral reservoir to overall viral persistence, the differences to the T-cell reservoir cannot be ignored. Firstly, it is widely acknowledged that CNS-derived viral strains are distinct to those found in other anatomical

compartments, and this extends to mutations in the LTR. Secondly, the CNS-specific cell types that are infected with HIV-1 have markedly different expression of cellular transcription factors and this will ultimately affect the transcriptional activity of the infecting virus. Thirdly, the reduced immune surveillance in the CNS will greatly influence treatment outcomes when trialling the use of transcriptional activators to purge viral reservoirs. In addition, the altered antiretroviral drug bioavailability in the CNS may potentially hinder the blocking of new rounds of infection. In combination, these two factors could potentially worsen outcomes within the CNS when using HIV cure strategies, leading to an expansion of infected cells, rather than achieving a reduction in the reservoir size.

A greater understanding of all the factors which govern viral infection, replication and persistence, and cART bioavailability and immune surveillance in the CNS is required in order to identify ways to best optimise future HIV cure strategies. Despite these obstacles, several solutions already exist within the literature and they may provide the key to ensuring success not only against the T-cell reservoir but against the CNS and other anatomical reservoirs. In the case of the CNS, the advent of Neuro-cART (cART regimens with adequate CNS-penetration and effectiveness) or even optimised cART, has resulted in control of CNS infection and the alleviation of HAND-related symptoms (Brew and Chan, 2014; McManus *et al*, 2011). Neuro-cART will also play an important role in protecting the brain during eradication strategies to ensure patients don't develop progressive CNS infections. Immunotherapy to boost the immune response against HIV-1 has also been proposed as a way to enable more rapid clearance of latently infected cells during HIV-1 activation strategies, and this may have some flow on effects for CNS immune surveillance. Studies analyzing CNS-derived LTRs within CNS cells will enable the identification of the most potent modulators of transcriptional activity that could then be incorporated into HIV-1 purging strategies. It is highly likely that the unique characteristics of each anatomical reservoir may be the very aspect which allows the development of tailored eradication strategies to address viral purging specific to that compartment.

In summary, while previous and current clinical trials designed to eradicate HIV-1 have experienced some encouraging results, their overall effectiveness has been limited. Thus, like with the use of cART, a combined approach to activate HIV-1 viral transcription will most likely be required to ensure that all latently infected cells are targeted. These combined approaches could be tailored to specifically address different anatomical reservoirs, and achieve the ultimate goal of removing all latently infected cells from the body.

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Figure 1. Structure of the HIV-1 LTR promoter

The HIV-1 promoter is divided into the U3, R, and U5 regions. U3 is further divided into the Modulatory, Enhancer (E) and Basal/Core regions (top blue bars). The HIV-1 promoter contains a highly conserved TATA box (orange) and three Sp factor binding sites (red) within the basal/core region, as well as two NF-κB binding sites (blue) within the enhancer region. The R region contains a *trans*-acting responsive element (TAR) (mauve) that forms an RNA stem loop structure upon transactivation that binds the viral protein Tat (transactivator of transcription). The modulatory region contains a negative regulatory element (NRE, top blue bars) and binding sites for both activators and repressors including, NFAT (burgundy), C/EBP (green), Ets (mauve), GATA (yellow) USF (orange) and AP1 (purple). Numbering is relative to the start site of transcription (black arrow).

Figure 2. HIV-1 LTR latency and activation

(a) In the absence of cellular stimulation or HIV-1 Tat, histone deacetylases (HDAC) are recruited to the LTR, inducing histone deacetylation and repressive changes in the chromatin structure of the HIV-1 LTR. In this state RNA polymerase II binds weakly to the LTR and transcription is abortive, with only short transcripts generated. (b) In the presence of cellular stimulation the HDAC complexes disassociate from the LTR and are replaced by histone acetyltransferases (HAT) which acetylate the surrounding histones in nucleosomes nuc-0 and nuc-1, and subsequently cause the histones to disassociate from the HIV-1 proviral DNA. HIV-1 Tat can also recruit HAT to the LTR. Furthermore, Tat is acetylated by HAT and phosphorylated by CDK9 altering its biological activity. HIV-1 Tat interacts with the P-TEFb complex, composed of CDK9 and cyclinT1, to recruit to the TAR RNA in the transcribed 5' end of the HIV-1 LTR. The P-TEFb complex activates RNA polymerase II by phosphorylating the CTD. The activated polymerase has increased processivity and subsequently catalyses the synthesis of full-length HIV-1 transcripts. Repressive interactions are shown in red, and activating interactions are in green.

Table 1

Properties and functions of cellular transcription factors that act on the HIV-1 LTR

Transcription factor	Tissue distribution	Transcriptional Properties	Site present in CNS LTRs	Expression in CNS
Sp1	Ubiquitous, developmental variations	Activator, Synergistic, Protein-protein interactions	Present, mutations	Low in astrocytes
Sp2	Unknown	Unknown	Present, mutations	Unknown
Sp3	Ubiquitous	Repressor, Competes with Sp1	Present, mutations	High in astrocytes
Sp4	Predominantly neuronal cells	Activator	Present, mutations	High in neurons
NF- κ B	Ubiquitous	Activator, Repressor	Present, conserved	Depends on cell type, activation state
C/EBP	Differentially expressed, mainly found in liver, blood, adipose tissue, pancreas	Activator, Recruit co-activators	Largely conserved, Mutations to increase affinity, Duplications	Moderate
AP-1	Ubiquitous	Activator	Largely conserved, Duplications	Differential levels
USF	Ubiquitous	Activator	Largely conserved, Duplications	High in microglia, macrophages
GATA	Differentially expressed	Activator, Repressor	Largely conserved, Duplications	High in microglia, astrocytes

Table 2

Summary of currently proposed HIV-1 transcriptional activators

Name ^a	Type ^b	HIV-1 Clinical trials	CNS penetrance ^c	Potency ^d (fold increase HIV-1 transcription)
Vorinostat	HDACi (Pan)	Yes	+++	7-fold
Trichostatin-A	HDACi (Pan)	No	+++	50-fold
Romidepsin	HDACi (Class I)	Planned	-/+	9-fold
Panobinostat	HDACi (Pan)	Yes	Unknown	52-fold
Etinostat	HDACi (Class I)	No	-/+	53-fold
Givinostat	HDACi (Class I) HDACi (Class II)	No	+++	19-fold
Belinostat	HDACi (Class I) HDACi (Class II)	No	-/+	19-fold
Tubastatin	HDACi (HDAC6)	No	-/+	4-fold
PCI-34051	HDACi (HDAC8)	No	Unknown	ND
Disulfiram	Akt signalling	Yes	+++	4-fold*
HMBA	Tat mimetic	No	++	25-fold
JQ1	BRDi	No	++	7-fold*
Chaetocin	HMTi	No	Unknown	25-fold
IL-7	Cytokine	Yes	+++	ND
Bryostatins	PKC activator	Planned	++	13-fold*

^aHMBA, hexamethylbisacetamide;^bHDACi, histone deacetylase inhibitor; BRDi, bromodomain inhibitor; HMTi, histone methyltransferase inhibitor;^c-/+, poor/minimal CNS penetration; ++, good CNS penetration; +++, very good CNS penetration;^dFold increase in HIV-1 transcription determined in the ACH2 cell line; ND, not done;* evaluated in primary CD4⁺ T-cell latency model.