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Targeting HIV latency: pharmacologic strategies toward eradication

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Abstract

The latent reservoir for HIV-1 in resting CD4⁺ T cells remains a major barrier to HIV-1 eradication, even though highly active antiretroviral therapy (HAART) can successfully reduce plasma HIV-1 levels to below the detection limit of clinical assays and reverse disease progression. Proposed eradication strategies involve reactivation of this latent reservoir. Multiple mechanisms are believed to be involved in maintaining HIV-1 latency, mostly through suppression of transcription. These include cytoplasmic sequestration of host transcription factors and epigenetic modifications such as histone deacetylation, histone methylation and DNA methylation. Therefore, strategies targeting these mechanisms have been explored for reactivation of the latent reservoir. In this review, we discuss current pharmacological approaches toward eradication, focusing on small molecule latency-reversing agents, their mechanisms, advantages and limitations.

Keywords

HIV-1; latency; reactivation; eradication; small molecules

Introduction

Highly active antiretroviral therapy (HAART) can reduce plasma HIV-1 levels in adherent patients to below the detection limit of clinical assays (<50 copies/ml) [1]. However, a latent reservoir of integrated HIV-1 proviruses in resting CD4⁺ T cells remains a barrier to eradication [2,3,4]. HIV-1 replicates in activated CD4⁺ T cells; the resting state of CD4⁺ T cells is non-permissive for viral gene expression. Latency can be established when activated CD4⁺ T cells become infected as they are transitioning back to a resting memory state, or when resting CD4⁺ T cells are directly infected. The absence of viral gene expression in latently infected cells enables evasion from immune surveillance. HAART cannot eradicate

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Conflicts of interest

The authors have no conflict of interest to declare.

latent HIV-1 because it only targets replicating virus. However, following cellular activation, these latent HIV-1 genomes can be transcribed, and viruses can be produced, leading to rapid rebound of viremia upon the discontinuation of HAART. The extreme stability of this latent reservoir makes life-long HAART necessary [5,6]. Given the toxicity, expense and potential for resistance during life-long HAART, elimination of the latent reservoir is an important goal.

The most widely discussed approach to eliminating this reservoir involves reactivating latent HIV-1 in patients on HAART. The antiretroviral drugs will prevent the released virus from infecting new cells, and it is hoped that the infected cells will die from viral cytopathic effects or be killed by the host cytolytic effector mechanisms. Reversing latency through T cell activation proved too toxic [7]. To reactivate the latent virus without inducing global T cell activation requires an understanding of the mechanisms that maintain latency (Figure 1). In latently infected cells, HIV-1 genomes are found within actively transcribed genes [8,9]. Transcriptional interference from the host gene can contribute to latency [8,9,10]. In addition, HIV-1 transcription can be silenced by epigenetic modifications and cytoplasmic sequestration of crucial host transcription factors.

To explore potential reactivation strategies, various assays and *in vitro* models have been developed. A viral outgrowth assay that detects release of replication-competent virus from resting CD4⁺ T cells isolated from patients on HAART is the standard measure of the latent reservoir [3,5]. However, this assay is difficult, costly and cannot be adapted for screening. Earlier studies employed chronically infected cell lines such as the ACH-2 T cell line [11] and the U1 promonocytic cell line [12]. These cells show minimal constitutive expression of HIV-1 genes but a marked upregulation following treatment with cytokines or mitogens. More recently, Jurkat T cell lines carrying HIV-1 constructs have been widely used. These include J-Lat, E4 and J89 [13,14]. However, these cell lines continuously proliferate and thus do not accurately represent latency *in vivo*. Recently, models that reflect HIV-1 latency more accurately have been developed using primary cells [15,16,17,18]. Among these is a *bcl-2*-transduced CD4⁺ T cell model that had been adapted for drug screening [19].

Using these models, strategies for reversing latency have been explored. Active gene transcription requires that the physical barriers imposed by nucleosome structure be overcome. Because latent HIV-1 is integrated into the host genome it is subject to the same epigenetic mechanisms that regulate expression of host genes, including DNA methylation and histone methylation and acetylation.

Generally, histones that have been maintained in a deacetylated form by histone deacetylases (HDACs) pack densely and are associated with transcriptional repression. By contrast, acetylation of specific lysine residues within nucleosomal histones by histone acetyltransferases (HATs) neutralizes positive charges on lysine residues and disrupts histone–DNA binding, enabling recruitment of transcriptional activators and transcription complexes. In the case of HIV-1 two nucleosomes, Nuc-0 and Nuc-1, are positioned precisely in relation to *cis*-acting regulatory elements on the promoter of the integrated HIV-1 provirus. This is true regardless of the nature of the integration site. Nuc-0 is positioned upstream of the modulatory region, whereas Nuc-1 is immediately downstream of the HIV-1 core promoter and *cis*-regulatory elements (Figure 1). Nuc-1 restricts HIV-1 transcription in models of latent HIV-1 infection and must be displaced during transcriptional activation [20]. During latency, HDACs are recruited to HIV-1 LTR by the host transcription factors, and hypoacetylated Nuc-1 prevents proviral transcription by impeding the binding of factors crucial for initiation of transcription. Disrupting of the recruitment of HDACs results in HIV-1 outgrowth [21], and some HDAC inhibitors induce HIV-1 transcription in cell models of HIV-1 latency and in resting CD4⁺ T cells isolated

from patients on HAART [22]. Moreover, in a recent clinical study in patients on HAART, a single dose of the HDAC inhibitor vorinostat (suberoylanilide hydroxamic acid, SAHA) increased HIV-1 gene expression in resting CD4⁺ T cells *in vivo* [23].

In addition to acetylation, histone H3 methylation at lysine 9 (H3K9) by histone methyltransferases (HMTs) is associated with a restrictive chromatin environment at the HIV-1 LTR. Binding of the heterochromatin-associated factor heterochromatin protein 1 γ (HP1 γ) to methylated H3K9 imposes further restrictions on the local chromatin environment [24]. Therefore, inhibitors of HMTs could be novel pharmacologic candidates for reactivating latent HIV-1 [25].

The role of DNA methylation in HIV-1 latency remains controversial. Two CpG islands flank the HIV-1 transcription start site (Figure 1) and, when hypermethylated, the transcriptional repressor methyl-CpG binding domain protein 2 (MBD2) can be recruited to HIV-1 LTR [26]. The HIV-1 LTR becomes hypermethylated when it is durably quiescent in J-Lat cell lines and CD4⁺ T cells infected *in vitro* [26]. However, reactivation of HIV-1 gene expression is not accompanied by significant CpG demethylation in the 5'HIV-1 LTR [27]. Even though a negative correlation was found between the CpG density in the 5'LTR and the level of reactivation by tumor necrosis factor α (TNF- α) and phorbol 12-myristate 13-acetate (PMA) in a cell line model of HIV-1 latency, HDAC inhibitors such as vorinostat efficiently reactivate the densely methylated HIV-1 promoter [27]. Therefore, it seems that the removal of DNA methylation might be beneficial but not required for reactivation of latent HIV-1. In addition, a recent study has failed to detect a high level of DNA methylation at the HIV-1 LTR in cells from patients on HAART [28].

To overcome cytoplasmic sequestration of host transcriptional factors, cellular signaling pathways inducing nuclear translocation of transcription factors can be targeted. For instance, agents activating protein kinase C (PKC) could induce nuclear translocation of nuclear factor κ B (NF- κ B) and reactivate latent HIV-1 (Figure 1). The positive transcription elongation factor b (P-TEFb) also plays an important part in the regulation of transcription. It is a complex of cyclin T1 (cycT1) and cyclin-dependent kinase 9 (CDK9). When recruited to promoters, active P-TEFb can phosphorylate the C-terminal domain of RNA polymerase II (RNA Pol II) and stimulate transcriptional elongation. HIV-1 Tat efficiently recruits active P-TEFb to the stem-loop structure of the *trans*-activation-responsive (TAR) region encoded by the initial ~50 nucleotides of the HIV-1 transcript and removes the block at transcriptional elongation [29] (Figure 1). However, under most circumstances in resting cells, P-TEFb is restricted in a transcriptionally inactive complex with HMBA-induced protein 1 (HEXIM1) and 7SK small nuclear RNA (snRNA) [30,31,32]. Therefore, agents that activate and release P-TEFb could reactivate latent HIV-1. However, such agents are likely to affect the transcription of many genes.

Ideally, agents or combinations of agents used to eradicate latent HIV-1 should have high efficacy in reactivating latent proviruses without inducing global T cell activation, and also acceptable pharmacologic and toxicologic properties. Agents that have been considered to date are discussed below.

Histone deacetylase inhibitors

HDACs are divided into groups based on homology. Class I HDACs (HDAC 1, 2, 3 and 8), class II HDACs (HDAC 4, 5, 6, 7, 9 and 10) and the class IV HDAC (HDAC11) are all zinc-dependent, whereas class III deacetylases (SIRT1-7) are NAD-dependent. Among these groups, only three class I HDACs (HDAC 1, HDAC 2, HDAC 3) are localized at the integrated HIV-1 LTR, as demonstrated by chromatin immunoprecipitation (ChIP) assays [33].

Known inhibitors of zinc-dependent HDACs can be classified into four structural categories: hydroxamates, carboxylates, benzamides and cyclic peptides. Compounds from all groups except the benzamides have been reported to reactivate latent HIV-1 in various model systems. The hydroxamic acids are efficient zinc chelators. This class includes vorinostat [34], suberoyl bis-hydroxamic acid (SBHA) [35], trichostatin A (TsA) [36], scriptaid [37], oxamflatin [38], givinostat (ITF2357) [39], belinostat (PXD101) [22], droxinostat [35] and the recently reported CG05/CG06 [40]. Carboxylates include valproic acid (VPA) [41] and sodium butyrate [42]. A cyclic peptide HDAC inhibitor apicidin, which was studied as an anticancer and antiprotozoal agent, was recently reported to reactivate latent HIV [43].

Compared with other latency-reversing agents, HDAC inhibitors have several advantages. These compounds have been intensively investigated especially as anticancer therapies; therefore a large amount is known about their pharmacologic and toxicologic properties. At least 13 HDAC inhibitors have entered clinical trials for various purposes [44], and VPA and vorinostat have been approved by the FDA for the treatment of neuropsychiatric conditions and cutaneous T cell lymphoma (CTCL), respectively. Clinical studies of VPA were prompted by the finding that VPA could induce outgrowth of HIV-1 from the resting CD4⁺ T cells of HIV-1-positive individuals receiving HAART [41]. One study, in which patients were also receiving intensified HAART, reported a significant decline in the level of latently infected resting CD4⁺ T cells in three out of four patients [45]. However, later studies found no evidence for VPA-induced decay of the latent reservoir [46,47], even in combination with intensified HAART [48].

VPA is a relatively weak pan-HDAC inhibitor. Therefore, a lack of potency might explain the failure of VPA to reduce the latent reservoir. Vorinostat is more potent in inhibiting class I HDACs, and effectively induces virus outgrowth from resting CD4⁺ T cells of HIV-1-infected individuals on suppressive HAART [49]. Phase I and II clinical trials of vorinostat in patients receiving suppressive HAART are ongoing. Recent results from a single-dose trial of vorinostat showed that, upon administration of the drug, acetylation of histone H3 and transcription of HIV-1 RNA in resting CD4⁺ T cells were induced simultaneously [23]. This proof-of-concept study demonstrated that it is possible to disrupt HIV-1 latency by an achievable and tolerable exposure to an HDAC inhibitor.

Givinostat (ITF2357) and belinostat (PXD101) have entered clinical trials, givinostat as an anti-inflammatory agent for juvenile idiopathic arthritis and belinostat as a treatment for various cancers. The safety profile of givinostat is promising [39]. However, the HIV-1 latency-reversing activities of givinostat and belinostat have only been tested in a limited number of cell lines, and more information on these compounds should be obtained from primary cell models or CD4⁺ T cells from patients on suppressive HAART. In addition, there has been increased interest in the latency-reversing activity of romidepsin (Istodax®), which is an FDA-approved treatment for CTCL.

Generally, HDAC inhibitors do not induce T cell activation or cytokine secretion, and do not upregulate CD4, CXCR4 or CCR5. A potential concern about these agents is that the modification of histone acetylation levels could affect expression of large numbers of genes. In addition, HDACs might not be the sole targets of these compounds. For instance, an alternative mechanism has been proposed for the action of vorinostat, involving the activation of PI3K/Akt pathways [34]. Generally, given the potency, relatively low toxicity and pharmacologic advantages of HDAC inhibitors, this is a group of promising candidates for future therapy against reactive latent HIV-1.

Histone methyltransferase inhibitors

Several HMTs, such as EZH2, SUV39H1 and G9a, have recently been reported to be involved in repression of latent HIV-1 gene transcription by inducing histone H3K9 methylation [24,50,51]. BIX01294, a specific G9a inhibitor, was the first HMT inhibitor reported to reactivate latent HIV-1 [51]. Chaetocin inhibits Suv39H1, and induces luciferase expression from a Jurkat T cell line bearing integrated HIV-1 provirus in which luciferase is produced from the 5'LTR as an in-frame fusion with p24^{gag} [52]. BIX01294 and chaetocin induce virus outgrowth from resting CD4⁺ T cells isolated from HIV-1-infected individuals on suppressive HAART [25]. Moreover, Friedman *et al.* reported that 3-deazaneplanocin A (DZNep), a broad-spectrum HMT inhibitor, is more potent in inducing latent HIV-1 than BIX01294 or chaetocin in a latently infected Jurkat T cell line called E4 [50].

Interestingly, all HMT inhibitors discussed above can enhance proviral reactivation by HDAC inhibitors such as vorinostat when they are used in combination [50,51,52]. Compared with the HDAC inhibitors, the research on HMT inhibitors for reactivation of latent HIV-1 is still at a preliminary stage. Their pharmacologic properties remain unknown, and further studies evaluating their effects on T cell activation still need to be done in primary cells. Nevertheless, HMT inhibitors are an interesting group of potential latency-reversing agents, especially given the synergistic effects with HDAC inhibitors.

DNA methyltransferase inhibitors

Although controversy remains about the degree of methylation of the HIV-1 LTR *in vivo*, there is interest in DNA methyltransferase (DNMT) inhibitors as reactivating agents. Decitabine (5-aza-2' deoxycytidine, aza-CdR) and its analog azacitidine (5-azacytidine, Vidaza®) are small molecule inhibitors of DNMTs and are approved by the FDA for the treatment of myelodysplastic syndrome [53]. Decitabine itself is a fairly weak inducer of latent HIV-1, yet it synergizes with TNF- α and prostratin to promote a significant increase in viral gene expression in most J-Lat cell lines [26,54]. This encourages further studies of DNA methylation inhibitors in combination with other classes of HIV-1 latency-reversing compounds in more model systems.

Protein kinase C activators

PKC plays an important part in transcriptional activation in T cells. PKC activation induces latent HIV-1 by targeting multiple regulatory elements on the HIV-1 LTR, through the NF- κ B and AP-1 signaling pathways.

A large group of PKC activators belong to a family of diterpenes called phorbol 13-esters. The most well-known among them are PMA and 12-deoxyphorbol-13-acetate (prostratin) [55]. PMA and prostratin are natural products [56]. In addition, another natural phorbol ester, 12-deoxyphorbol 13-phenylacetate (DPP), is reported to be 20–40-fold more potent than prostratin in ACH-2 cells [57]. A series of chemically synthesized phorbol 13-monoesters also showed activity in reversing HIV-1 latency [58].

PMA reactivates latent HIV-1 by activating T cells. In addition to inducing the release of cytokines, PMA also causes mitogenesis and is tumor-promoting. Hence, it is not suitable for clinical use. Prostratin induces transcription of latent HIV-1 in J-Lat T cells and peripheral blood mononuclear cells (PBMCs) from HIV-1-infected individuals on HAART [55,59]. In contrast to PMA, prostratin is not tumor-promoting [60], does not induce cell proliferation by itself [61,62] and inhibits PMA-induced tumor promotion in a mouse model [60]. However, prostratin induced strong upregulation of the activation markers CD69 and CD25, the α chain of the interleukin (IL)-2 receptor [61,62]. In addition, prostratin induces

marked upregulation of the transcription of several cytokines [59]. DPP, like prostratin, lacks tumor-promoting activity [60]. The ability of DPP to induce latent HIV-1 was also confirmed with PBMCs from HIV-1-infected individuals on HAART [63]. DPP regulates a very similar set of PKC-responsive genes to prostratin [57], and it induces secretion of the proinflammatory cytokine TNF- α [64]. Therefore, careful evaluation of cytokine-related toxicity should be undertaken when considering *in vivo* trials of these PKC activators.

All of these phorbol esters bind to the highly conserved cysteine-rich motif (C1 domain) in the regulatory region of the PKCs in the same site as the physiologic ligand diacylglycerol (DAG) [57]. The C1 domain displays a hydrophobic surface interrupted by a hydrophilic cleft. Upon binding, phorbol esters or DAG provide a hydrophobic cap on the hydrophilic cleft, masking its polarity and facilitating the association of the C1 domain with the lipid bilayer or other hydrophobic surfaces, translocating PKCs from the cytoplasm to the membranes. Therefore, phorbol derivatives with higher lipophilicities on the side chain exhibit higher biological activities and potencies as PKC activators [57,58]. The induction of NF- κ B by these phorbol esters through PKC activation is associated with inhibitor of NF- κ B (I κ B) kinase (IKK) activation, which leads to the degradation of I κ B α [55,58].

In addition to phorbol 13-esters, other diterpenes have been shown to reverse HIV-1 latency. Most are natural products extracted from plants in the *Euphorbia* family. For instance, the diterpene ester ingenol-3-angelate (I3A, PEP005) reactivates latent HIV-1 expression in U1 cells at concentrations of 2.5–25.0 nM. However, it induces the secretion of TNF- α and other cytokines in various cell types at similar doses [65,66]. SJ23B, a jatrophone diterpene, was reported to induce HIV-1 expression in J-Lat cells with an EC₅₀ of ~50 nM [67]. Four other latency-reversing compounds that share similar structures with SJ23B were isolated and have effective dose ranges from 10 to 50 μ M [68,69]. In addition, Huang *et al.* recently reported that an antitumor agent gnidimacrin, which is a daphnane diterpene isolated from plants in the *Thymeleaceae* family, activates HIV-1 production from the U1 and ACH-2 cell lines at picomolar concentrations [70].

A recently reported latency-reversing agent bryostatin-1 has a structure that is distinct from the PKC activators discussed above. As an antineoplastic agent, bryostatin-1 has been evaluated in several clinical trials for various cancers. Bryostatin-mediated HIV-1 reactivation involves activation of PKCs via the AMP-activated protein kinase (AMPK) pathway [71]. It does not induce expression of CD69 or CD25 in primary human CD4⁺ T cells [45].

Although PKC activators stimulate expression of integrated latent HIV-1, they also inhibit *de novo* HIV-1 infection in similar dose ranges [61,65,70,71]. This phenomenon is consistent with the observed downregulation of cell surface receptors CD4, CXCR4 and CCR5 in PBMCs treated with PKC activators, and inhibition of PKCs will abrogate this anti-HIV-1 effect [61,70]. Thus, in combination with HAART, PKC activators should not cause infection of additional cells.

The major concern with PKC activators is the non-specific induction of many genes following activation of PKC-related pathways, and the toxicity caused by the systematic release of cytokines. Most of the PKC activators discussed above induce PKC isozymes from the classical (α , β , γ) and the novel (δ , ϵ , η , θ) PKC isotypes. This lack of specificity probably contributes to toxicity. Trushin *et al.* reported that a single isotype of active PKC (PKC α or PKC θ) is capable of inducing HIV-1 gene expression. Transfection of vectors expressing constitutively active PKC α or PKC θ together with an HIV-1-LTR-driven luciferase reporter results in HIV-1 gene expression in Jurkat cells and primary human CD4⁺ T cells [72]. Therefore, toxicity might be limited by targeting certain PKC isozymes. With a

group of rationally designed DAG analogs, DAG lactones, it is possible to separate latency-reversing activity from the toxicity resulting from TNF- α secretion [64]. These results suggest that, even though it might be impossible to eliminate the toxicities of PKC activators, it might be feasible to minimize them to a clinically acceptable level.

Positive transcription elongation factor b (P-TEFb) activator

Hexamethylene bisacetamide (HMBA) can activate HIV-1 transcription via its effects on P-TEFb independent of NF- κ B [73,74,75]. HMBA transiently activates the PI3K/Akt pathway, which leads to the phosphorylation of hexamethylene bisacetamide-inducible protein (Hexim)-1 and the subsequent release of active P-TEFb [74]. The active P-TEFb can then be recruited to the HIV-1 promoter in an Sp-1-dependent manner in the absence of HIV-1 Tat, to stimulate viral transcriptional elongation. Interestingly, HMBA greatly increases the expression of Hexim-1, which can form a negative feedback loop upon the induction. Thus, suppression of cell proliferation is observed in the continuous presence of HMBA, despite the fact that the PI3K/Akt pathway is associated with cell growth and proliferation [74]. The efficacy of HMBA in reversing HIV-1 latency is relatively low in the *bcl-2*-transduced latently infected primary CD4⁺ T cell model compared with co-stimulation or PKC activators [19]. In clinical trials of HMBA for cancers, dose-related thrombocytopenia was prominently observed [76,77].

A novel approach to recruit activate P-TEFb to HIV-1 LTR involves agents that inhibit Brd4 [78]. Brd4 is a bromodomain protein that recruits P-TEFb to generic cellular promoters, and HIV-1 Tat competes with Brd4 for binding to P-TEFb when recruiting P-TEFb to HIV-1 LTR [79]. JQ1(S), originally developed as an anticancer molecule, binds to bromodomains and inhibits Brd4 [80]. JQ1(S) was recently found to reactivate latent HIV-1 proviruses in ACH-2 and J-Lat cell lines, and induced virus outgrowth from cultured resting CD4⁺ T cells from one of three patients on HAART [78]. Interestingly, JQ1(S) downregulates T cell activation genes and suppresses T cell proliferation [78]. This preferential induction of HIV-1 LTR would be advantageous when developing into a therapeutic.

Unclassified HIV-1 latency-reversing agents

With the improvement in assays and technologies for high-throughput screening, additional compounds that reactivate latent HIV-1 have been discovered. Because most cellular models used in screening assays are phenotypic instead of target-based, the mechanisms and targets of some of these compounds remain to be identified.

Through screening the Johns Hopkins drug library, which consists of clinical compounds and drugs in development, Yang *et al.* discovered that juglone (5HN, 5-hydroxynaphthalene-1,4-dione) potently reactivates latent HIV-1 in the *bcl-2*-transduced latently infected primary CD4⁺ T cell model without inducing global T cell activation [19]. 5HN induces the production of intracellular reactive oxygen species, and reactivates latent HIV-1 through NF- κ B signaling pathways. Yet the effect of 5HN on latent HIV-1 cannot be abolished by the pan-PKC inhibitor Gö6983, suggesting 5HN is not a PKC activator [19].

The FDA-approved drug disulfiram was identified as an HIV-1 latency-reversing agent using the same *bcl-2*-transduced model [81]. Disulfiram does not induce T cell activation or cytokine release. This result was expected because disulfiram has been clinically used to treat alcoholism for decades, and little toxicity was observed in the absence of alcohol or cocaine [81]. A clinical trial is currently evaluating whether short-term administration of disulfiram will accelerate the decay of the HIV-1 reservoir in patients on HAART. It is also possible that disulfiram can be used in combination with other latency-reversing agents.

Micheva-Viteva *et al.* reported that AV6, a 4-3',4'-dichloroanilino-6-methoxyquinoline compound derived from high-throughput screening, activates latent HIV-1 in a cell-line model [82]. AV6 requires nuclear factor of activated T cells (NFAT) for inducing latent HIV-1 expression, and cooperates with the HDAC inhibitor VPA for enhanced induction of viral gene expression [82]. We recently identified two structurally related classes of quinolin-8-ol derivatives that reactivate latent HIV-1 without inducing global T cell activation [83]. The two classes are Mannich adducts of 5-chloroquinolin-8-ol and quinolin-8-yl carbamates, and SAR was explored. These studies expand the number of known latency-reversing agents and provide new scaffolds for drug development.

Concluding remarks

The latent reservoir of HIV-1 remains a major barrier to its eradication. Classes of agents targeting different mechanisms involved in maintaining latency have been discovered, but several obstacles still remain. First, reactivation of the entire latent reservoir needs to be achieved. Incomplete reactivation could be caused by either suboptimal efficacy of the latency-reversing agent or the multifactorial nature of latency. Both problems might be overcome by using latency-reversing agents from different classes in combinations. In various systems, increased reactivation of latent HIV-1 has been obtained by using combinations of HMT inhibitors and HDAC inhibitors, HDAC inhibitors with the PKC activator prostratin and TNF- α or prostratin with DNA methylation inhibitors [50,53,84,85]. Second, toxicity and potential risks related to T cell activation still need to be closely monitored, especially when PKC activators are involved. Modifying the agents to target specific subtypes of PKCs, or exploring agents targeting downstream effectors in the signaling pathways, might help to reduce side-effects. In addition, when latency-reversing agents are used in combination, lower concentrations could be effective. This could help eliminate toxicity. Finally and most importantly, it is highly possible that the latently infected cells will not die from either viral cytopathic effects or host cytolytic mechanisms following reactivation [86]. In this case reactivation strategies must be coupled with strategies that kill productively infected cells. In summary, promising progress in the development of HIV-1 latency-reversing agents has been made, but important questions still need to be solved before we can finally reach the goal of eradication.

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Research Highlights

- Eradication strategies for HIV-1 involve reactivation of latent reservoir in resting CD4⁺ T cells.
- The maintenance and reactivation of latent HIV-1 is multi-factorial in mechanism.
- Pharmacological strategies targeting different reactivation mechanisms are discussed in this review.

Figure 1. The maintenance of HIV-1 latency and reactivation of latent HIV-1 provirus
 The HIV-1 DNA is depicted as a green ribbon, with the two nucleosomes, Nuc-0 and Nuc-1, depicted as blue spheres. Parts of the HIV-1 5'LTR that contain binding sites for major transcription factors (boxes in purple, blue and pink) and the transcriptional start site (TSS, yellow triangle) are highlighted in light yellow. **(a)** The maintenance of HIV-1 latency and potential targets for reactivation. In latent infection, HDACs and HMTs are recruited to HIV-1 LTR, resulting in histone deacetylation and methylation on Nuc-0 and Nuc-1, which leads to a restrictive environment for transcription initiation. DNA methyltransferases could introduce DNA methylation on the CpG islands, which could further silence transcription.

Most host transcriptional factors are sequestered in cytoplasm in resting cells. The inactive NF- κ B p50 homodimer binds to the NF- κ B site at HIV-1 LTR, whereas the active form, a p65/p50 heterodimer, is bound by I κ B in the cytoplasm. NFAT is in its phosphorylated inactive form. pTEFb is restricted in a transcriptionally inactive complex with Hexim-1 and 7SK snRNA. To overcome these obstacles to transcription of the HIV-1 provirus agents targeting these restrictive steps have been explored for the reactivation of latent HIV-1 (red arrows). **(b)** The active transcription of HIV-1 provirus. Upon cellular activation, I κ B is phosphorylated and degraded, releasing p65/p50 which translocates into nucleus and binds to NF- κ B sites on HIV-1 LTR. HATs are recruited to LTR, and acetylation disrupts histone–DNA binding, facilitating the recruitment of transcription factors and complexes. Cellular activation also results in the release of active pTEFb which phosphorylates the C-terminal domain of RNA polymerase II (RNA Pol II), stimulating transcriptional elongation. After the stem-loop structure of TAR is transcribed, HIV-1 Tat efficiently recruits active pTEFb to TAR, further stimulating the elongation of HIV-1 transcripts.

Table 1

Summary of small molecules reversing HIV-1 latency

	Agent	Structure	Major assays tested
HDAC inhibitors			
Hydroxamic acids	Vorinostat (SAHA)		U1, ACH-2 [34], J89 [35,49], RCT [34,49]
	SBHA		J89 [35]
	Trichostatin A (TsA)		U1, ACH-2, J49 [36], J89 [35], bcl-2 [19]
	Scriptaid		A7 [37], J89 [35]
	Oxamflatin		A7 [38], J89 [35], ACH-2, J-Lat [87]
	Givinostat (ITF2357)		U1, ACH-2 [39], J89 [35]
	Belinostat (PXD101)		U1 [22] ACH-2 [40], J89 [35]
	Droxinostat		J89 [35]

	Agent	Structure	Major assays tested
	CG05/CG06		ACH-2 [40]
Carboxylates	Valproic acid (VPA)		RCT [41], J89 [35], bcl-2 [19]
	Sodium butyrate		U1, ACH-2 [42], J89 [35]
Cyclic peptide	Apicidin		A10.6 [43], J89 [35]
HMT inhibitors			
	BIX01294		ACH-2, OM10.1 [51], E4 [50], RCT [78]
	Chaetocin		Jurkat-tat [52], E4 [50], RCT [78]
	3-Deazaneplanocin A (DZNep)		E4 [50]
DNA methylation inhibitor			

	Agent	Structure	Major assays tested
	Decitabine (5-aza-2' deoxycytidine, aza-CdR)		J-Lat [26,54], ACH-2, U1 [54]
PKC activators			
Phorbol 13-esters	PMA		RCT [63], J-Lat [55], bcl-2 [19]
	Prostratin		RCT [59,63], J-Lat [55], SCID-hu [62], bcl-2 [19]
	DPP		RCT [63], ACH-2 [57], bcl-2 [19]
	Phorbol 13-monoesters		Jurkat-LAT-GFP [58]
Diterpenes	Ingenol-3-angelate		U1 [65]

	Agent	Structure	Major assays tested
	SJ23B		J-Lat [67]
	Gnidimacrin		U1, ACH-2 [70]
Macrocyclic lactone	Bryostatin-1		THP-p89, J1.1 [71]
DAG analogs	DAG lactones		ACH-2 [64]
P-TEFb activators			
	HMBA		RCT [75], U1 [74], ACH-2 [73,74]
	JQ1(S)		ACH-2, J-Lat, RCT [78]
Unclassified			
	Juglone (5-HN)		bcl-2 [19]

	Agent	Structure	Major assays tested
	Disulfiram		bcl-2 [81]
	AV6		STNL5G cell lines, ACH-2 [82]
	Quinolin-8-ol analogs		bcl-2, J-Lat [83]

RCT, resting CD4⁺ T cells isolated from HIV-1 infected individuals on suppressive HAART; PBMC, peripheral blood mononuclear cells isolated from HIV-1 infected individuals on suppressive HAART; SCID-hu, thymocytes and peripheral blood lymphocytes from the SCID-hu (Thy/Liv) mouse model.