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REVIEW

Characteristics and mechanisms of latency-reversing agents in the activation of the human immunodeficiency virus 1 reservoir

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Abstract

The "Shock and Kill" method is being considered as a potential treatment for eradicating HIV-1 and achieving a functional cure for acquired immunodeficiency syndrome (AIDS). This approach involves using latency-reversing agents (LRAs) to activate human immunodeficiency virus (HIV-1) transcription in latent cells, followed by treatment with antiviral drugs to kill these cells. Although LRAs have shown promise in HIV-1 patient research, their widespread clinical use is hindered by side effects and limitations. In this review, we categorize and explain the mechanisms of these agonists in activating HIV-1 in vivo and discuss their advantages and disadvantages. In the future, combining different HIV-1 LRAs may overcome their respective shortcomings and facilitate a functional cure for HIV-1.

Introduction

Acquired immunodeficiency syndrome (AIDS), was first identified in 1981 and is now one of the three major infectious diseases in the world [1]. Human immunodeficiency virus 1 (HIV-1), which causes AIDS, is a retrovirus that contains two copies of the viral single-stranded RNA genome within the core of the virus particle. There are long terminal repeats (LTRs) at each end of the HIV-1 genome, and the genes between the LTRs encode the viral structural proteins Gag, Pol, and Env, as well as regulatory proteins required for viral replication, which include the HIV-1 transcriptional transactivator (Tat), viral structural protein expression regulator (Rev), helper protein negative factor (Nef), viral infection factor (Vif), viral protein U (Vpu), and viral protein R (Vpr).

HIV-1 is a single-stranded RNA virus whose genomic RNA is reverse transcribed to DNA, which then integrates into the genome of $CD4^+$ T cells [2]. During this process, some $CD4^+$ T cells carrying HIV-1 DNA become quiescent. The level of viral transcription in the latent HIV-1 reservoir is extremely low, and almost no virus particles are produced. In the absence of viral proteins, infected cells remain in a stable dormant state for a long time, and their presence is not recognized by the immune system. However, the integrated proviruses still have the ability to replicate and can be activated to produce infectious virions when highly active antiretroviral therapy (HAART) is interrupted. AIDS is very difficult to cure due to the latency reservoir established during the infection. The majority of latent proviruses are believed to be present in resting memory $CD4^+$ T cells, but some proviruses can persist in macrophages [3, 4], dendritic cells [5–7], astrocytes, and hematopoietic stem cells [8, 9].

Achieving a functional cure for AIDS has required solutions that can eliminate the latent virus. However, the most commonly used treatment, HAART, is not able to achieve this goal. Although it has been shown to significantly reduce

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the viral load in the peripheral blood to an undetectable level [10], it cannot completely eradicate latent HIV-1. As a result, patients receiving HAART may have to treat HIV-1 as a chronic disease, and those who stop taking the drugs may experience a resurgence of HIV-1 [11].

To address the problem of the latent HIV-1 viral reservoir, a "Shock and Kill" strategy has been proposed. In this approach, gene expression of the HIV-1 provirus is induced using latency reversing agents (LRAs), and HIV-1-bearing cells are subsequently eliminated by HAART [12]. In this review, we classify and summarize the mechanisms and roles of LRAs for a better understanding of the functions of these molecules in the process of AIDS treatment.

In general, LRAs can be divided into four categories: (1) small-molecule inhibitors that affect histone modifications, (2) DNA methylation inhibitors, (3) small molecules targeting transcriptional regulatory complexes, and (4) small-molecule inhibitors of the NF-κB pathway. Each of these is discussed separately below.

Small-molecule inhibitors that affect histone modifications

Histone deacetylase inhibitors

The function of histone deacetylase (HDAC) is to remove acetylation from lysine residues in histones. There are four types of HDAC (I-IV) that differ in their size, number of active centers, cellular localization, and homology to yeast HDAC proteins [13]. It has been reported that the regulation of chromatin function is associated with HIV-1 proliferation ability, and histone acetyltransferase and deacetylase are two chromatin-modifying enzymes that activate latent HIV-1 and are considered targets for treating HIV-1 infection [14]. HDACs can inhibit gene expression through deacetylation [15]. HDAC inhibitors typically interfere with the deacetylase activity of HDAC by blocking its catalytic domain [15, 16], resulting in an increase in histone acetylation levels, overcoming the inhibitory effect of HDACs and stimulating viral transcription in latently infected cells [17, 18] (Fig. 1). HDAC inhibitors can activate latent HIV-1 in vitro, induce the expression of transcripts and antigens, and reduce the reservoir of latent HIV-1 [19, 20]. Acetylation of the nucleosome 1 (NUC-1) protein has been recognized as a critical step in transcription initiation and subsequent activation of the virus [21]. For example, a reversible HDAC inhibitor, trichostatin A (TSA), has been shown to activate latent viruses in HIV-1-infected cell lines [22]. When cells are exposed to TSA, HDAC1 is translocated from NUC-1, increasing histone H4 acetylation, and activating the expression of latent virus [23]. CC-4a (Fig. 1) is another HDAC inhibitor that has anti-deacetylase ability and can activate HIV-1 transcription.

HDAC inhibitors can potentially be used in HIV-1 treatment to clear the latent reservoir [24, 25]. Clinical trials have demonstrated the efficacy of HDAC inhibitors [26] such as vorinostat, panobinostat, romidepsin (RMD), and valproic acid. Of these, RMD shows the highest potency and specificity against class I HDACs [27], and resistance experiments in monocytes have demonstrated the possibility of reducing the HIV-1 reservoir [17]. In a study using an in vitro T-cell model of HIV-1 latency, a sixfold increase in the amount of cellular HIV-1 RNA was observed after exposure to 40 nM RMD for 4 hours [28]. In preclinical models, the HDAC inhibitor entinostat (MS275) has proven successful

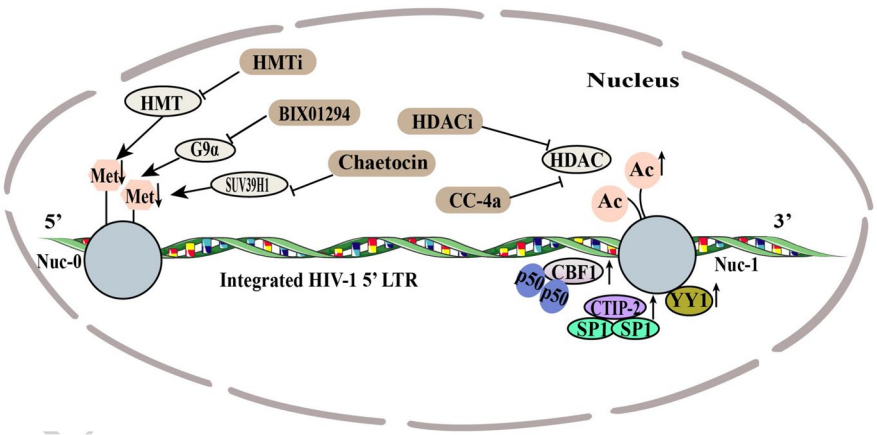

Fig. 1 Small-molecule compounds that modify histone methylation and acetylation activate latent HIV transcription. Histone methyltransferase inhibitors (HMT) reduce the level of histone methylation, which significantly activates latent HIV. BIX01294, chaetocin, and HMT inhibitors (HMTi) inhibit G9α, SUV39H1, and HMT, respectively, to facilitate the transcription of pre-viruses. Histone deacetylase inhibitors (HDACi) can inhibit the deacetylation of histones. With the accumulation of histone acetylation, the chromatin structure becomes loose, thus increasing the recruitment of transcription factors such as YY1, CTIP-2, p50-p50 homodimer, and CBF-1 to the HIV-1 5' LTR. CC-4a is a novel selective inhibitor of histone deacetylase I that shows promise in reactivating latent HIV-1 and has low cytotoxicity

in causing alterations in HDAC activity and pro-inflammatory cytokine expression levels in mice [29]. However, clinical trials have associated RMD with numerous serious adverse cardiac events [30]. Analysis of patient electrocardiograms revealed that the severity of the adverse effects was primarily influenced by the drug dosage and duration of administration. However, it was also observed that long-term oral administration of low doses of the drug might mitigate this toxicity [31]. In addition, HDAC inhibitors have many other drawbacks, including toxicity to normal cells, resulting in thrombocytopenia and neutropenia, as well as causing nausea, diarrhea, and fatigue [32–34]. Furthermore, the effectiveness of HDAC inhibitors in HIV-1 treatment is influenced by various factors, including pharmacokinetics, concentration, and exposure time [19].

Histone methylation inhibitors

Histone methylation not only participates in the structure of chromatin but also plays a critical role in regulating gene expression (Fig. 1). The histone methyltransferase (HMT) Suv39H1 mainly participates in the trimethylation of Lys9 of histone H3 (H3K9me3), leading to the silencing of HIV-1 transcription [35, 36]. An inhibitor of SUV39H1, chaetocin, has shown promise as a drug for reducing the HIV-1 viral reservoir and has been shown to produce a 25-fold increase in latent HIV-1 expression [37, 38] (Fig. 1). The combination of chaetocin and the HDAC inhibitor TSA has been shown to have a strong synergistic effect on HIV-1 expression [37]. Chaetocin does not activate T cells and can therefore be used without causing inflammation and related cytotoxic effects [37]. However, precise control of the concentration of chaetocin is crucial, because if used improperly, it can have deleterious effects on non-target cells [39]. H3K9me3 chromatin immunoprecipitation analysis showed that when SUV39H1 was incubated with HIV-1-infected cells, chaetocin could alter the HIV-1 LTR and significantly reduce trimethylation at H3K9 [40] (Fig. 1). Chaetocin can also promote the recombination of LTR chromatin, leading to reactivation of HIV-1 [37]. G9α is an important enzyme that is responsible for H3K9 dimethylation (H3K9me2). BIX01294, a small-molecule inhibitor of G9α (Fig. 1), when administered peripherally to mice, causes a reduction in H3K9 methylation [41] and activation of viral transcription. Histone methylation inhibitors, especially chaetocin, have shown positive effects in the treatment of HIV-1/AIDS [38].

DNA methylation inhibitors

DNA methylation is a critical epigenetic process that is involved in chromatin structure and gene regulation. Changes in methylation modifications are one of the epigenetic alterations caused by the integration of HIV-1 DNA into the host genome [42]. DNA methyltransferase inhibitors show promise in reversing abnormal DNA methylation processes, which makes them a potential target for reversing HIV-1 latency. By inhibiting DNA methyltransferases, these inhibitors can reactivate HIV-1 and potentially eliminate latent HIV-1 reservoirs. DNA methylation usually occurs at CpG sites, especially around transcription start sites [43] (Fig. 2). Studies have shown that the HIV-1 promoter is often affected by DNA methylation [44], and modification of DNA methylation proteins can affect the interaction between the virus and key transcription factors in the local epigenome, which may inhibit HIV-1 gene expression [45]. Therefore, DNA methyltransferase inhibitors can partially reactivate HIV-1 gene expression [46]. One inhibitor, 5-azacytidine (5-AzaC), a nucleoside analogue of cytosine, is phosphorylated by deoxycytidine kinase (Fig. 2). Administration of low doses of 5-AzaC to clinical patients that result in only minimal levels of DNA methylation [47] nevertheless leads to reactivation of HIV-1 [48]. DNA methyltransferase inhibitors may have significantly different effects on the activation of latent HIV-1, depending on the chromosomal location of the provirus and the epigenetic and transcriptional environment in the cell. MG98 is a specific

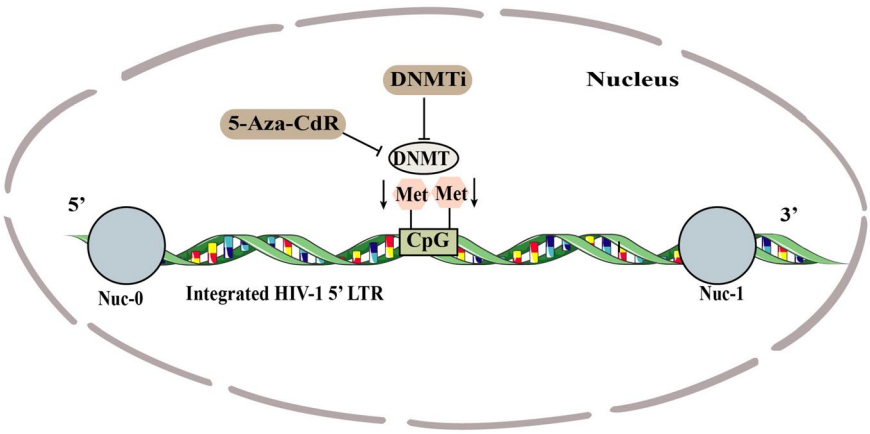

Fig. 2 DNA methyltransferase (DNMT) inhibitors (DNMTi) affect the transcription of HIV-1 by acting on DNA methylation. They can inhibit the aggregation of DNMT on the HIV-1 LTR, preventing the methylation of two CpG islands near the HIV-1 transcription initiation site, thus relieving the transcriptional suppression caused by the high methylation in the promoter region of HIV-1. 5-aza-2'-deoxycytidine (5-aza-CdR) is also an inhibitor of DNMT

inhibitor of human DNA methyltransferase 1 (DNMT1) that is generally well tolerated and easy to administer by intermittent intravenous infusion. However, in clinical trials, MG98 was found to cause side effects, including fever, chills, fatigue, and weakness [49–51].

Small molecules targeting transcriptional regulatory complexes

The 7SK snRNP signaling pathway is an important target of current HIV treatment strategies.

The transcription elongation factor P-TEFb was first identified as a regulator of HIV transcription, but subsequently, many other viral and host proteins have been found to interact with P-TEFb [52, 53], mainly by regulating its activity. For example, hexamethylene bisacetamide (HMBA) was originally developed as a treatment for leukemia, but subsequent research has shown that it may also act as a P-TEFb agonist in the treatment of HIV-1 infection. This drug activates the Akt signaling pathway, promoting the release of P-TEFb due to the phosphorylation of HEXIM. Subsequently, Tat recruits active P-TEFb to the vicinity of the trans-activation response (TAR) element, which phosphorylates the carboxy-terminal domain (CTD) of RNA polymerase II and promotes transcriptional elongation of HIV genes (Fig. 3) [54, 55].

Upregulation of P-TEFb activity can have negative effects on cells [56, 57]. For example, abnormally upregulated P-TEFb may participate in other signaling pathways [58, 59], affecting the cell's response to DNA damage [58, 60]. This suggests that P-TEFb may play a role in maintaining genome stability.

Bromodomain and extraterminal domain (BET) inhibitors are used to treat HIV-1 infection because they block the binding of bromodomain-containing proteins to P-TEFb. JQ1 is a well-known BET inhibitor that has been shown to reactivate latent HIV-1 provirus [61, 62] (Fig. 3). The mechanism of action of JQ1 involves inhibiting the binding of Brd4 to P-TEFb, thereby initiating gene transcription of the HIV-1 provirus.

BET inhibitors have also shown potential in regulating immune responses, preventing inflammation, and controlling cytokine synthesis [63–65]. Several BET inhibitors have been observed to exhibit HIV-1 reactivation properties and are currently undergoing clinical trials. For instance, RVX-208 and PFI-1 are considered potential candidates for anti-HIV-latency therapy. In a study investigating the ability of these two BET inhibitors to activate latent HIV-1 in latently infected Jurkat T cells in vitro as well as in patient-derived resting $CD4^+$ T cells in vivo [66], neither RVX-208 nor PFI-1 elicited widespread and robust T cell activation. At present, I-BET-151 remains the only BET inhibitor being tested for HIV-1 activation in vivo [67]. In general, due to

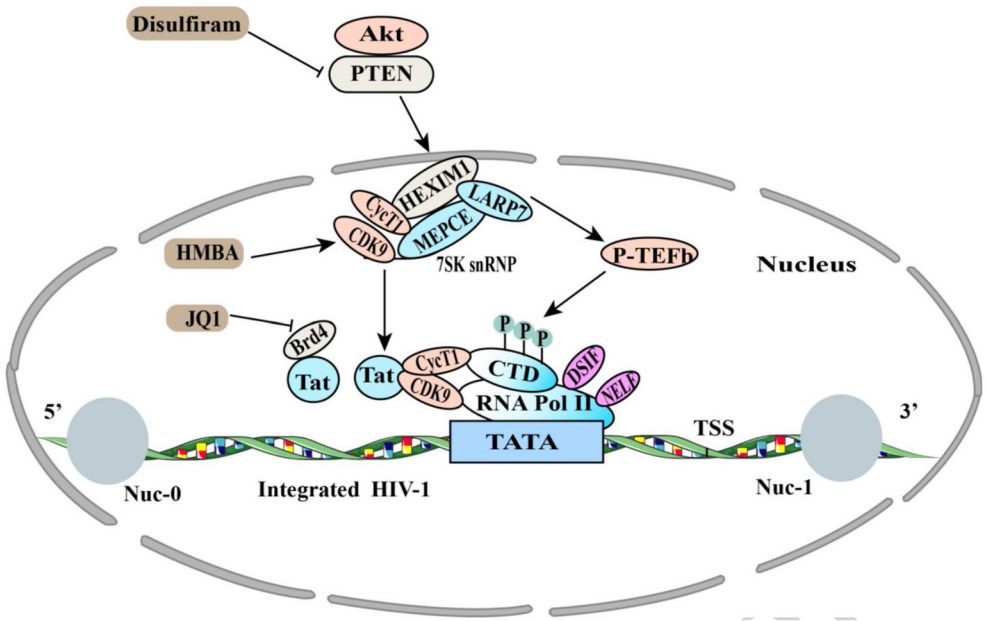

Fig. 3 Small-molecule compounds affect the activation of HIV-1 by acting on transcriptional regulatory complexes. Dithiothreitol not only causes the degradation of PTEN but also increases the phosphorylation of Akt, causing HEXIM1 to dissociate from the 7SK SNP complex, releasing P-TEFb. HMBA activates Akt through the PI3K pathway, and the phosphorylation of HEXIM-1 can also release P-TEFb. Tat recruits active P-TEFb to the vicinity of the trans-activation response (TAR) element and promotes phosphorylation of the RNA polymerase II (RNA Pol-II) carboxy-terminal domain (CTD), the dissociation of NELF, and activation of DSIF, thereby stimulating transcription elongation. Since JQ1 inhibits the competition between Brd4 and Tat for P-TEFb, it also stimulates transcription

their potency and low toxicity, BET inhibitors hold significant promise as potential candidates for future therapy against reactivated latent HIV-1.

The PTEN inhibitor disulfiram releases HEXIM1 from the 7sk snRNP and recruits P-TEFb from its transcriptionally inactive form to the HIV-1 promoter through the Akt signaling pathway [68, 69] (Fig. 3), stimulating transcriptional elongation and virus production. Disulfiram is a safe and well-tolerated drug that can maintain a low viral load [70, 71]. However, due to significant inter-individual differences in the pharmacokinetics and pharmacodynamics of disulfiram, it is difficult to determine the appropriate dose. In addition, there are significant inter-individual differences in the plasma levels of disulfiram and its metabolites. Participants in a clinical study were able to sustain low levels of virus within two months after disulfiram treatment [70], indicating that higher levels of drug exposure in vivo may have long-term effects on HIV-1 production.

Small-molecule inhibitors of the NF-κB pathway

In the classical NF-κB signaling pathway, when IκB is phosphorylated and degraded [72], most of the released p65/p50 is recruited to the nucleus, promoting gene transcription [73, 74].

Protein kinase C (PKC) agonists are commonly used as drugs to reverse HIV-1 latency by stimulating the classical NF-κB signaling pathway [75, 76] (Fig. 4). Drugs such as prostaglandins and bryostatins induce transcription from the dormant HIV-1 provirus by releasing active NF-κB and promoting its entry into the nucleus to bind to the HIV promoter region. For example, bryostatin-170 isolated from marine invertebrates activates IκBα kinase [77], leading to the phosphorylation and degradation of IκBα. PKC can stimulate P-TEFb activity, thereby reactivating latent HIV-1 with minimal cell toxicity [75]. Recent data from clinical and preclinical trials are highly encouraging and provide strong support for further investigation of PKC agonists as safe and effective LRAs in patients. In a non-human primate model of AIDS, the administration of certain PKC agonists resulted in the reactivation of latent simian immunodeficiency virus (SIV) without any apparent toxicity observed in vivo [78]. Likewise, in a humanized mouse model of AIDS, a synthetic bryostatin analogue not only demonstrated the ability to reactivate latent HIV-1 but also exhibited a potential killing effect against the virus [79]. Although PKC agonists have proven to be highly effective in reversing HIV-1 latency, their clinical application has been hindered by their side effects and cytotoxicity [76, 80, 81], which would have to be controlled before they can be used in killing strategies against HIV-1 (see Table 1).

Tumor necrosis factor (TNF) appears to be a major driver of HIV-1 transcription early in the disease [82]. While TNF is recognized by its receptor protein (Fig. 4), the TNFR-associated death domain (TRADD) interacts with the cytoplasmic death domain of TNFR1 through its own death domain to affect the secretion of growth factors and cell proliferation. Then, TNFR2 interacts with the TRAF protein, and receptor interaction proteins (RIPs) are recruited, activating several signaling cascades and leading to the activation of transcription factors to favor the binding of p65/p50 NF-κB complexes to binding sites present in the HIV-1

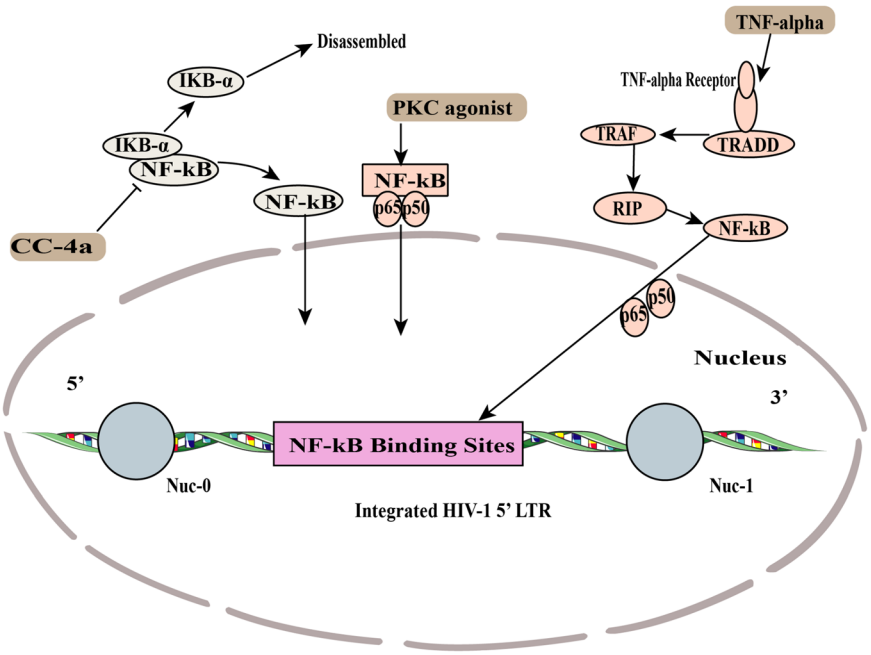

Fig. 4 Small-molecule inhibitors affect the latency of HIV-1 by acting on the NF-κB signaling pathway. TNF-α is recognized by TNF-α receptor and gradually recruits TRADD, TNF receptor-associated factor TRAF, and receptor-interacting protein (RIP), leading to activation of the NF-κB signaling pathway. NF-κB binds to the NF-κB binding site in the LTR promoter, thereby stimulating transcription. In addition, PKC agonists can also promote the entry of NF-κB into the nucleus. CC-4a as a novel HDAC inhibitor that can release IKB-α from the IKB-α/NF-κB complex and activate proviruses through the NF-κB signaling pathway

Table 1 Small molecules that affect HIV latency activation

Pathway	Category	Full name	Abbreviation
Histone modifications	Histone deacetylase inhibitors	Trichostatin A	TSA
		CC-4a	
		Vorinostat	
		Panobinostat	
		Romidepsin	RMD
		Valproic acid	
		Entinostat	
		Chidamide	
	Histone methylation inhibitors	Chaetocin	
		BIX01294	
DNA modifications	DNA methylation inhibitors	5-azacytidine	5-AzaC
		MG98	
The 7SK snRNP signaling pathway	P-TEFb agonist	Hexamethylene Bisacetamide	HMBA
	BET inhibitors	JQ1	
		RVX-208	
		PFI-1	
		I-BET-151	
	PTEN inhibitor	Disulfiram	
The NF-κB pathway	PKC agonists	Prostaglandins	
		Bryostatins	

LTR promoter [83]. Moreover, the close interaction between TNF and HIV-1 Nef persists throughout the course of the disease. Studies have shown that Nef interacts directly with proteins of the TRAF family (TRAF2, TRAF5, and TRAF6) to stimulate HIV-1 replication in individual cells and primary macrophages [84, 85]. Although TNF has been used to reduce the viral repertoire, it has limited ability to reactivate viruses and is virulent [86, 87].

The high degree variability of HIV-1 virus makes achieving a functional cure through a single treatment plan difficult. However, combining HAART with LRAs is a promising approach to help HIV-1 patients overcome the disease.

To overcome the challenges of curing HIV-1 infections, researchers are exploring various strategies to target viral reservoirs in T cells, adipose tissue, bone marrow, the central nervous system, and gut-associated lymphoid tissue [88]. One approach is the use of small molecules that can reactivate latent HIV-1 infection in different anatomical locations [89–91]. However, much research is still needed to optimize the use of these drugs and to evaluate their safety and efficacy.

The "Shock and Kill" strategy involves using a combination of small-molecule LARs, which can enhance treatment specificity and reduce cellular toxicity, and HAART. Although no single LRA is perfect, using different agents in combination can help to mitigate the drawbacks of each drug.

Activation of proviruses activates the immune system, which contributes to the "Shock and Kill" strategy. Several LRAs have been documented to cause widespread activation of proviruses. For instance, chidamide, a benzamide-based HDAC inhibitor, has been demonstrated to successfully reactivate latent HIV-1 in cellular models and in primary $CD4^+$ T cells from HIV-1-infected individuals [92, 93]. Such findings highlight the potential of LRAs in aiding HIV-1 eradication strategies by activating latent viral reservoirs and rendering them vulnerable to immune recognition and elimination.

Through oral administration of chidamide and using HIV-1 DNA and HIV-1 RNA levels in patients for reference, researchers have observed a notable enhancement in the HIV-1-specific cellular immune response accompanied by a modest 37.7% reduction in cell-associated HIV-1 DNA levels [94]. Initially, at the initial dose, the researchers did not observe a significant increase in cell-associated HIV-1 RNA. However, as the dose was increased, changes became apparent. It is important to emphasize that individual LRAs have a tendency to over-activate the immune system, which can lead to various side effects. Combining multiple LRAs might help to minimize these side effects.

In summary, the combination of HAART and LRAs represents a promising approach to HIV-1 treatment. However, given the significant challenges posed by the variability of the virus, it is crucial to continue developing new and more-effective therapies.

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Declarations

Conflict of interest The authors declare that they have no competing interests.

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