Shocking HIV out of hiding: where are we with clinical trials of latency reversing agents?

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Purpose of review
To provide an overview of the initial experiences with the use of latency-reversing agents (LRAs) in clinical trials in HIV and to discuss and contrast results arising from these studies.

Recent findings
Although the clinical administration of histone deacetylase inhibitors (HDACis) and disulfiram to HIV-infected individuals on antiretroviral therapy significantly increased cell-associated HIV RNA in $CD4^+$ T cells and in some cases plasma HIV RNA, this did not reduce the frequency of latently infected cells in blood. Potential reasons for this include insufficient potency in latency reversal, lack of virus or immune-mediated cytolysis of virus-expressing cells and/or a high frequency of immune escape mutations in the recently activated virus. Analyses of HIV-specific T-cell responses *in vivo* did not demonstrate that HDACis impair immune cell effector functions.

Summary
More effective latency-reversing interventions and additional strategies to eliminate virus-expressing cells are needed. Key challenges include testing combinations of LRAs and/or LRAs with immune modulation to optimize potency in the absence of adverse events. A better understanding of the mechanisms of action of LRAs as well as strategies to enhance potency and penetration in tissue are key challenges for future studies.

Keywords
histone deacetylase inhibitors, HIV cure, HIV latency, HIV reservoir, latency-reversing agents

INTRODUCTION

Combination antiretroviral therapy (ART) effectively suppresses HIV replication, but treatment is life long and there is no cure. This is due to the persistence of long-lived latently infected resting $CD4^+$ T cells [1]. Latency is established early following infection [2■] and although there is an initial decline in the frequency of latently infected cells following ART, these cells persist indefinitely [3]. Latency is defined as replication competent provirus integrated into the genome of $CD4^+$ T cells, which is silenced through a variety of mechanisms that inhibit transcription, translation and virion production [4]. Current research is focused at developing interventions with the capacity to increase HIV transcription and/or virion production as a first step toward elimination of latently infected cells through virus-mediated cytolysis or induction of an HIV-specific cytotoxic T-cell response, referred to as shock and kill [5]. Latency-reversing agents (LRAs) with potential application as shock agents in the clinical setting have been identified including histone deacetylase inhibitor (HDACi) [6,7] and the antialcoholism drug disulfiram [8]. Other compounds including ingenols [9], Toll-like receptor 7 agonists [10], proteasome inhibitors [11], histone methyltransferases inhibitors [12] and cytokines such as interleukin-15 [13] also show activity as LRAs *in vitro* or in animal models.

The purpose of this review is to provide an overview of the initial experiences with the use of LRAs in the clinical setting and to discuss and

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KEY POINTS

- The use of LRAs to induce HIV expression in latently infected cells is investigated in clinical trials with the aim of exposing virus-expressing cells to virus-immune or immune-mediated cytolysis.

- In single-arm clinical trials, short-term administration of disulfiram or a histone deacetylase inhibitor (vorinostat, panobinostat or romidepsin) increased cell-associated HIV RNA, and in some cases also plasma HIV RNA, in HIV-infected individuals on ART.

- Despite reversing latency, none of the interventions to date has had a demonstrable effect on the latent HIV reservoir.

- More effective latency-reversing interventions and additional strategies to eliminate virus-expressing cells are needed.

contrast results arising from these proof-of-concept studies. To date, while the clinical administration of HDACi and disulfiram has been shown to significantly increase HIV transcription, and in some interventions virion production, in infected individuals on suppressive ART, this has not led to a reduction in the frequency of latently infected cells in blood. There are several possible explanations for this, including insufficient host immune responses directed against virus-expressing cells and/or insufficient magnitude of latency reversal. One common characteristic for these LRAs is that they are all drugs developed for other therapeutic indications and, therefore, their mode of action not specific for HIV. Consequently, off-target effects in uninfected cells may result in dose-limiting toxicities. It is hoped that ongoing clinical development of LRAs specifically tailored toward reversing HIV latency could result in therapeutics of higher specificity and potency without increasing the risk of adverse effects.

EFFECTS OF LATENCY-REVERSING AGENTS ON HIV LATENCY IN CLINICAL TRIALS

Vorinostat, approved by the US Food and Drug Administration in 2006 for the treatment of cutaneous T-cell lymphoma [14], was the first potent HDACi to be tested in HIV-infected individuals on suppressive ART (Table 1). In this study, a single dose of 400 mg vorinostat led to an almost five-fold increase in HIV transcription as measured by cell-associated unspliced (CA-US) HIV RNA [15], thus providing the first demonstration of disruption of HIV latency in humans. In a multiple-dose study,

daily vorinostat (400 mg) for 14 days also significantly increased HIV transcription as measured by CA-US RNA in addition to a nonsignificant increase in CA-US RNA in rectal tissue but no change in plasma HIV RNA [16▪▪]. In contrast, when vorinostat was given 3 days a week for 8 weeks, levels of CA-US RNA were overall not increased above baseline after 11 and 22 doses [17▪]. However, changes in HIV transcription were not assayed in the early phases of vorinostat treatment and it is possible that initial effects were overlooked.

Oral treatment with the more potent HDACi panobinostat (20 mg thrice weekly every other week for 8 weeks) significantly increased levels of HIV transcription and also increased the detection rate of plasma HIV RNA using a semiquantitative assay [18▪▪]. Finally, in a recent pilot study, three infusions of romidepsin (5 mg/m^2), in addition to significantly increasing HIV transcription, led to increases in plasma HIV RNA which in five of six individuals on suppressive ART were readily quantifiable using a standard clinical assay (Cobas Taqman, limit of quantification 20 copies/ml) [19▪▪]. Interestingly, although romidepsin induced viremia to an extent not seen with other HDACis, changes in CA-US HIV RNA were of a similar magnitude. To facilitate the killing of virus-expressing cells, LRAs should ideally induce not only HIV transcription but also viral protein expression [5], and recent advances in super sensitive assays to detect nanomolar quantities of p24 will enhance the detection of protein production [20].

Disulfiram was initially discovered as a potential LRA in a drug library screen, along with its metabolites [21]. The molecular mechanism of activation of HIV transcription is secondary to depletion of the phosphatase and tensin homolog resulting in activation of the Akt signaling pathway [22]. In a pilot clinical trial of 14 days of standard-dose disulfiram (500 mg/day) in HIV-infected individuals on ART, there was no overall effect on plasma HIV RNA using a supersensitive single copy assay (lower limit of detection = 0.8/ml), but a transient increase in plasma HIV RNA was noted in a post-hoc analysis in patients with immediate postdose sampling available and in patients with higher concentrations of disulfiram in plasma [23]. These findings inspired a subsequent dose-escalation study, in which 30 HIV-infected individuals on ART were administered 3 days of disulfiram at doses 500 (licensed dose), 1000 or 2000 mg. Disulfiram significantly increased levels of CA-US RNA in all dose cohorts, both on-disulfiram and postdosing [24▪▪]. Furthermore, disulfiram at 2000 mg/day resulted in a significant increase in plasma HIV-RNA. The reason for this extended effect on HIV production remains unclear

Table 1. Clinical trials of latency-reversing agents

| Clinical trial intervention | Mechanism of latency reversal | Trial design and size | Reported safety data (# drug-related AEs) | Effects on HIV latency | | Effects on HIV reservoir and viral rebound | | | | Effects on T cell immunology | | Tissue analyses |
				HIV transcription (cell-associated HIV RNA)	Plasma HIV RNA	HIV DNA	qVOA	TILDA	ATI	HIV-specific T-cell responses	T-cell activation	
Vorinostat 400 mg	Chromatin relaxation through inhibition of HDACs; pan-HDACi	Single-arm study, single dose of vorinostat (n = 8)	No AEs attributable to vorinostat	4.8-fold increase[b]	No change	No change	ND	ND	ND	ND	ND	ND
Vorinostat 400 mg		Single-arm study; daily vorinostat for 14 consecutive days (n = 20)	70 AEs (59 grade 2, 11 grade 2, no SAEs); of these 12 laboratory events (10 grade 1, 2 grade 2)	2.7-fold increase[c]	No change	No change	ND	No change	ND	No change	No change	Rectal tissue: a trend toward an increase in CA-US RNA, no change in HIV DNA
Vorinostat 400 mg		Single-arm study; vorinostat 3 days/week for 8 weeks (n = 5)	One AE reported (thrombocytopenia); in addition, transient GI symptoms and headache below grade 1 severity	No overall change	No change	No change	No change	ND	ND	ND	ND	ND
Panobinostat 20 mg	Chromatin relaxation through inhibition of HDACs; pan-HDACi	Single-arm study; panobinostat 3 days/week every other week for 8 weeks (n = 15)	16 AEs (all grade 1, no SAEs)[a]	2.9-fold increase[c]	Significant increase in detection rate (nonquantitative)	No change	ND	ND	Median time to viral rebound 17 days	No change	Increase in CD69 and HLA-DR expression	Rectal/sigmoid tissue: no change in HIV DNA. CA HIV-RNA not detected using ICH. No change in CSF HIV RNA or neuroinflammatory biomarkers
Romidepsin 5 mg/m²	Chromatin relaxation through inhibition of HDACs; selectively inhibits HDACs 1 and 2	Single-arm study; romidepsin as iv infusion once weekly for 3 weeks (n = 6)	35 AEs (all grade 1, no SAEs)[a]	3.8-fold increase[c]	Significant increase following romidepsin infusion (ranging from 21 to 119 copies/ml)	No change	No change	No change	ND	No change	Increase in CD69 and HLA-DR/CD38 expression	ND
Disulfiram 500 mg	Depletion of the phosphatase and tensin homologue protein and activation of protein kinase B	Single-arm study; daily disulfiram for 14 consecutive days (n = 16)	Not quantitatively reported	ND	Significant increase postdosing	ND	No change	ND	ND	ND	ND	ND
Disulfiram 500–2000 mg		Dose escalation study with 3 dose arms of 500, 1000 and 2000 mg disulfiram daily for 3 days (n = 30)	76 AEs (64 grade 1, 12 grade 2, no SAEs); of these 14 laboratory events (7 grade 1, 7 grade 2)	1.7- to 2.1-fold increase across dose-arms[c]	Significant increase postdosing in the 2000 mg dose-arm	No change	ND	ND	ND	ND or no change?	ND or no change?	ND
Ipilumumab 3 mg/kg	Blockade of CTLA4 binding with its ligands; induces T-cell activation	Case report of HIV patient with melanoma; 3 infusions of ipilimumab at three weekly intervals (n = 1)	n/a	Cyclical increases of variable magnitude up to 19.6-fold	Cyclical decreases and an overall decline using SCA	No change	ND	ND	ND	ND	ND	ND

[a]Laboratory abnormalities only counted as AE if associated with clinical signs, symptoms or actions.
[b]Analyzed in resting CD4$^+$ T cells.
[c]Analyzed in total CD4$^+$ T cells.

AE, adverse event; ATI, analytical treatment interruption; CA-US RNA, cell-associated unspliced HIV-RNA; CSF, cerebrospinal fluid; CTLA4, cytotoxic T-lymphocyte associated protein-4; GI, gastrointestinal; HDAC, histone deacetylase; HDACi, histone deacetylase inhibitor; ICH, immune histochemistry; IL7, interleukin 7; n/a, nonapplicable; ND, not done; qVOA, quantitative viral outgrowth assay; SCA, single copy assay; SAE, serious adverse event; TILDA, tat/rev induced limiting dilution assay.

but, interestingly, similar postintervention changes were observed in trials of vorinostat and panobinostat [16[■■],18[■■]].

EFFECTS OF LATENCY-REVERSING AGENTS ON THE HIV-1 RESERVOIR

Collectively, these studies provided evidence that it is possible in the clinical setting to disrupt the state of HIV latency. Yet, none of the clinical trials to date, using HDACi or disulfiram, resulted in a decrease in the frequency of latently infected cells measured as either cell-associated HIV-1 DNA, infectious units per million using a quantitative viral outgrowth assay [25] or inducible multiply-spliced HIV-1 RNA using the *tat/rev* induced limiting dilution assay (TILDA) [26[■]]. Also, viral rebound occurred within an expected time frame during analytical ART interruption following panobinostat treatment [18[■■]].

Understanding why reversal of latency did not impact the latent HIV-reservoir is important for advancing HIV cure strategies and for developing effective shock and kill combination approaches. There are several possible explanations. First, the magnitude of latency reversal achieved so far may simply be insufficient to cause virus-mediated cell lysis, especially if virus-expressing cells remain in a resting state or return to a resting state following short-term virus production. Also, it is unclear what percentage of latently infected cells in blood activates transcription, as the PCR-based assays used to date are unable to determine the frequency of individual cells that may be responding. The development of assays that reliably provide such information is a key challenge in cure research. Second, it is well known that chronic HIV infection is characterized by an impaired cytolytic capacity of CD8^{+} T cells that is improved but not fully restored by ART [27,28]. In the absence of therapeutic enhancement of HIV-specific immune responses prior to latency reversal, there is likely insufficient immune-mediated killing of virus-expressing cells [29]. Third, the high frequency of immune escape mutations in individuals treated with ART during chronic compared with acute infection [30[■■]] means that reversal of latency may not result in immune recognition and killing even if viral epitopes are presented. Fourth, more studies are needed to understand the effect of LRAs in tissues. More than 90% of infected CD4^{+} T cells reside in lymphoid tissue and there is preferential persistence of HIV on ART in lymphoid tissues [31], largely in HIV-infected T follicular helper cells, which are potentially protected from cytotoxic T cells inside B-cell follicles [32[■■]]. Clinical trials of LRAs have primarily investigated their effects in peripheral blood T cells, albeit with a few exceptions [16[■■],33]. Hence, the penetration and effects of LRAs in lymphoid tissue, most notably lymph nodes, are largely unknown. It is possible that latently infected T-cell subsets as well as T cells in different anatomical locations respond differently to LRAs and to date this has not been examined in the clinical trial setting. Infections of CD4^{+} T cells from lymph node *ex vivo* are remarkably more sensitive to death by pyroptosis than peripheral blood CD4^{+} T cells [34], and recent work suggests that LRAs have superior activity in latency reversal using an in-vitro model of HIV latency with CD4^{+} T cells from tonsillar lymphoid tissue compared with blood [35]. Future clinical trials of LRAs should therefore aim to include lymphoid tissue and apply new techniques that can determine the frequency of RNA-producing cells such as unstimulated TILDA. Finally, shock and kill approaches rely on complete inhibition of virus replication by combination ART. However, antiretroviral intensification studies with raltegravir demonstrated an increase in two-long terminal repeat circles in the two studies that sampled blood within the first weeks of raltegravir intensification [36,37]. These data are consistent with residual virus replication in at least 30% of individuals, potentially more commonly in individuals receiving protease inhibitors [37]. Residual virus replication may occur in parts of tissue wherein there is suboptimal penetration of antiretrovirals. The tissue concentrations of five of the most commonly used antiretroviral drugs were shown to be much lower in lymph node tissue [38] and there was evidence of virus evolution, although in these studies the follow-up was short and individuals had only completed 6 months of ART [39[■■]]. Other studies have demonstrated no viral evolution on suppressive ART [40,41] and no effect of ART intensification on multiple markers of viral persistence, but it is possible that these techniques may not be sensitive enough to demonstrate residual viral replication. Further work is needed to exclude the possibility of residual virus replication and whether other potent integrase inhibitors play a role in this setting. Toward this aim, we are currently performing a study of dolutegravir to assess the impact of ART intensification on markers of residual replication (ClinicalTrials.gov NCT02500446).

EFFECTS OF LATENCY-REVERSING AGENTS ON THE IMMUNE SYSTEM

A recent in-vitro study raised the concern that HDACi may adversely affect T-cell effector function and CTL killing of HIV-1-infected target cells, which has obvious implications for shock and kill

approaches. Following the exposure of PBMCs from infected individuals to clinically relevant concentrations of vorinostat, panobinostat and romidepsin, the interferon (IFN)-γ secretion response to peptide stimulation was suppressed for all three HDACis but most notably for romidepsin. Furthermore, prestimulation with HDACis impaired proliferation of virus-specific T cells and CTL killing of HIV-1-infected target cells [42]. Hence, it has been speculated that HDACi, and romidepsin in particular, might also impair immune-mediated elimination of virus-expressing cells *in vivo*. However, quantification of HIV-1-specific CD8+ and CD4+ T-cell responses in samples from clinical trials of HDACi did not support this hypothesis even though these analyses did not include proliferative responses of HIV-specific T cells. Intracellular secretion of IFNγ, interleukin-2 or tumor necrosis factor (TNF)-α did not change during or following multiple vorinostat dosing [16■■]. In fact, an increase in staphylococcal enterotoxin B-specific IFNγ producing CD8+ T cells was noted. Similarly, no negative impact on HIV-1-specific CD4+ or CD8+ T-cell secretion of IFNγ, TNFα or interleukin-2, including memory subset-specific secretions, was seen during or following panobinostat or romidepsin administration [19■■,43].

LRAs and HDACi in particular may also affect immune pathways in a nonspecific manner. Interestingly, although panobinostat and romidepsin transiently increased the expression of activation markers CD69, CD38 and HL-DR by approximately two-fold without lowering CD4+ T cell counts post-HDACi [19■■,44], vorinostat appeared to have no effect on markers of T-cell activation. This is in line with a recent ex-vivo study showing that vorinostat induced a dose-dependent downregulation in expression of genes related to T-cell activation [45]. Still, it is not clear whether the observed increases in T-cell activation are mediated by HDACi-induced viral production or a direct pharmacological effect of panobinostat and romidepsin on T cells. The absence of increases in HIV-1-specific immune responses during HDACi administration and the previous finding that in-vitro exposure to panobinostat also increased CD69 expression in PBMCs from uninfected donors [7] strongly suggest the latter. Also, the relationship between upregulation of T-cell activation and effective reversal of latency is incompletely understood, but some data offer insight. Using microarray analysis during vorinostat multiple dosing, it was found that upregulation of pathways, which play a key role in upregulating T-cell activation and differentiation, was associated with an increase in HIV-1 transcription [16■■]. Many further questions remain including the effects of LRAs in women, in the setting of coinfections including hepatitis B virus (HBV), CMV and EBV and whether there is a different response with nonsubtype B genotype.

BEYOND DISULFIRAM AND HISTONE DEACETYLASE INHIBITOR: OPTIMIZING SHOCK INTERVENTIONS

Because numerous factors in addition to chromatin organization restrict HIV expression in resting memory CD4+ T cells, LRAs with different targets, which may also have synergistic effects when used in combination, are of interest. The potent protein C kinase agonists bryostatin-1, prostratin and ingenol derivatives produced an increase in HIV transcription and release of HIV-RNA in supernatant following ex-vivo stimulation of resting CD4+ T cells from HIV-infected individuals on suppressive ART [9,46,47]. A single dose of bryostatin-1 is currently tested in a clinical trial in Spain in HIV-infected patients on ART (ClinicalTrial.gov identifier NCT02269605). The bromodomain and extraterminal inhibitor (BETi) JQ1 and other BETi compounds, which activate HIV from latency by inhibiting sequestration of positive transcription elongation factor b, are also potent inducers of HIV transcription, including the release of extracellular HIV-RNA in ex-vivo studies [46,48]. Moreover, several combinations of these potent LRAs, including bryostatin with HDACi, induced HIV production *ex vivo* at a magnitude approaching that of maximal T-cell activation using CD3/CD28 antibodies or phorbol myristate acetate/ionomycin [46,49■]. These data suggest that a two-step approach with an agent that activates HIV-1 transcription through increased accessibility of transcription factors added on to HDACi-induced chromatin relaxation might significantly increase the magnitude of latency reversal. However, neither of the compounds activated latent virus in an HIV-specific manner, and it is uncertain whether their associated toxicities will allow for coadministration in a clinical setting. Ongoing clinical trials of LRA are shown in Table 2.

Another concept is to exploit cellular apoptosis pathways to facilitate killing of virus-expressing cells [50]. Inhibitors of phosphoinositide 3-kinases (PI3Ks) are now undergoing clinical development in oncology. Because basal activity of the PI3K pathway promotes cell survival and inactivation results in apoptosis, PI3K inhibitors may induce killing of HIV-expressing cells if used in combination with LRAs. Moreover, recent data suggest that compounds affecting apoptosis pathways may also reverse latency. Using small molecule antagonists known as second mitochondrial-derived activator of

Table 2. Ongoing clinical trials of latency-reversing agents in HIV

Intervention	LRA drug class	Study title	Estimated enrollment	Status	Identifier
Romidepsin	HDACi	Evaluating the Safety and Efficacy of Single-Dose Romidepsin in Combination With Antiretroviral Therapy in HIV-Infected Adults With Suppressed Viral Load	45	Recruiting	NCT01933594
Chidamide	HDACi	Chidamide in Combination With Antiretroviral Therapy for Eradication of the Latent HIV-1 Reservoir (CHARTER)	12	Recruiting	NCT02513901
Romidepsin + Vacc4x vaccine	HDACi	Safety and Efficacy of the Histone Deacetylase Inhibitor Romidepsin and the Therapeutic Vaccine Vacc-4x for Reduction of the Latent HIV-1 Reservoir (REDUC)	20	Completed	NCT02092116
Romidepsin + MVA.HIVconsv vaccine	HDACi	Study to Evaluate the Safety and Effect of HIVconsv Vaccines in Combination With Histone Deacetylase Inhibitor Romidepsin on the Viral Rebound Kinetic After Treatment Interruption in Early Treated HIV-1 Infected Individuals	24	Not yet recruiting	NCT02616874
Panobinostat + PEG-IFNα-2a	HDACi	Reducing the Residual Reservoir of HIV-1 Infected Cells in Patients Receiving Antiretroviral Therapy (ACTIVATE)	30	Not yet recruiting	NCT02471430
Vorinostat + ChAdV63.HIVconsv (ChAd) prime and MVA.HIVconsv (MVA) boost vaccines	HDACi	Research In Viral Eradication of HIV Reservoirs (RIVER)	52	Not yet recruiting	NCT02336074
Vorinostat + hydroxychloroquine + maraviroc	HDACi	Efficacy of VHM After Treatment Interruption in Subjects Initiating ART During Acute HIV Infection	15	Ongoing, not recruiting	NCT02475915
Bryostatin-1	PKC agonist	Bryostatin-1 Effect on HIV-1 Latency and Reservoir in HIV-1 Infected Patients Receiving Antiretroviral Treatment (BRYOLAT)	12	Completed	NCT02269605
MGN1703	TLR9 agonist	Toll-like Receptor 9 Agonist Treatment in Chronic HIV-1 Infection (TEACH)	16	Ongoing, recruitment completed	NCT02443935
GS-9620	TLR7 agonist	Multiple ascending dose study of the TLR7 agonist GS-9620 in HIV+ participants on cART	24	Enrolling	Not registered
Poly-ICLC	TLR3 agonist	Enhancement by Poly-ICLC During HIV-1 Infection	15	Ongoing, not recruiting	NCT02071095
ALT-803	IL-15 super agonist complex	Proof of Principle Study of Pulse Dosing of IL-15 to Deplete the Reservoir in HIV Infected People (ALT-803)	10	Not yet recruiting	NCT02191098
Kansui	Herbal supplement containing ingenol	Immunologic Response to Kansui in Treated HIV+ Individuals: a Dose Escalation Study	9	Not yet recruiting	NCT02531295

HDACi, histone deacetylase inhibitor; IL, interleukin; PKC, protein kinase C; TLR, Toll-like receptor.

caspases (SMAC) mimetics to enhance apoptosis, an increase in HIV transcription and release of extracellular HIV-1 RNA in supernatant was seen in ex-vivo stimulation of resting CD4$^+$ T cells obtained from HIV-infected individuals on suppressive ART [51]. Interestingly, the SMAC mimetic birinipant was recently shown to reduce serum HBV DNA, serum HBV surface antigen and promote the elimination of HBV core antigen-containing hepatocytes in a mouse model of chronic HBV infection.

It is possible that similar effects could be seen in HIV.

Finally, as previous studies have shown HIV-enrichment in cells that express receptors involved in immune regulation, usually referred to as immune checkpoints [52,53], blockade of these receptors by monoclonal antibodies is now being explored in cure research. Immune checkpoint blockade may reverse latency as indicated by the recent case report of anti-cytotoxic T-lymphocyte associated protein-4 (CTLA-4) treatment (ipilimumab) in an ART-suppressed HIV-infected individual with melanoma [54▪▪]. In addition, immune checkpoint blockade could have the added advantage of enhancing the function of HIV-1-specific T cells as shown by studies of blocking the programmed death-1 receptor *in vitro* [55] and in macaque models [56].

CONCLUSION

In conclusion, a limited number of single-arm clinical trials have explored the effects of short-term administration of LRAs in HIV-infected individuals on suppressive ART. Although these studies provide evidence that HIV transcription can be increased in CD4$^+$ T cells, there has been no demonstrable effect on the frequency of latently infected cells. A reasonable interpretation is that more potent activators of HIV transcription are needed, potentially combined with immune enhancement. Enhanced protein or virion production may also be possible through combining LRAs that target different latency mechanism. These studies will require a substantial collaborative effort and investment from industry and academia.

Several important challenges must be met to accelerate scientific progress in latency reversal and cure research. The development of robust in-vitro models for high-throughput drug screening will be key to effectively identifying promising LRAs and selecting compounds for further ex-vivo and in-vivo testing. Also, the effect of LRA combinations or compounds that target multiple latency pathways must be explored in animal models or in clinical trials. An important consideration in this process is how to identify safe, effective and feasible combinations among the rapidly expanding candidate compounds for latency reversal and immune enhancement/modulation. Finally, we still need assays with improved reproducibility and sensitivity to quantify the HIV reservoir in blood and tissue, including single cell assays, better visualization of infected cells and assessment of replication competence.

Acknowledgements

Financial support and sponsorship

Conflicts of interest

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- ▪ of special interest
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