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Opinion

Reversal of Latency as Part of a Cure for HIV-1

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Here, the use of pharmacological agents to reverse HIV-1 latency will be explored as a therapeutic strategy towards a cure. However, while clinical trials of latency-reversing agents LRAs) have demonstrated their ability to increase production of latent HIV-1, such interventions have not had an effect on the size of the latent HIV-1 reservoir. Plausible explanations for this include insufficient host immune responses against virus-expressing cells, the presence of escape mutations in archived virus, or an insufficient scale of latency reversal. Importantly, these early studies of LRAs were primarily designed to investigate their ability to perturb the state of HIV-1 latency; using the absence of an impact on the size of the HIV-1 reservoir to discard their potential inclusion in curative strategies would be erroneous and premature.

Trends

Pharmacologically induced expression of latent virus is investigated as part of a cure for HIV-1 infection.

Recent data from clinical trials show that short-term administration of a latency-reversing agent (LRA) may increase HIV-1 transcription and plasma HIV-1 RNA.

So far, reversal of HIV-1 latency by histone deacetylase inhibitors has not been associated with a reduction in the size of the latent reservoir.

Possible explanations for the lack of an effect on the size of the latent HIV-1 reservoir include insufficient immune response against virus-expressing cells, the presence of cytotoxic T lymphocyte (CTL) escape variants, and/or an insufficient degree of latency reversal achieved with current approaches.

Shock and Kill

The introduction of combination antiretroviral therapy (cART) to treat human immunodeficiency virus-1 (HIV-1) infection in the mid-1990s provided clinicians with a therapeutic opportunity to suppress viral replication and restore the immune function of infected individuals. Initially, the potency of cART even raised hopes that this treatment might be able to eradicate HIV-1 infection after few years of therapy [1]. However, with the demonstration that a minute fraction of resting memory $CD4^+$ T cells carries quiescent but replication-competent provirus, it became clear that this would not be the case [2,3]. The nonproductive infection in long-lived memory T cells most likely occurs as a consequence of normal immunological physiology of the $CD4^+$ T cell. Usually, an infected cell dies rapidly due to viral expansion within the cell or owing to killing by the immune system. However, when, in rare cases, a $CD4^+$ T cell is infected as it is transitioning to a resting memory state, this sets the stage for latent infection in a long-lived cell [4]. Alternatively, $CD4^+$ T cells may become infected directly in the resting state [5]. In the silent resting state such cells do not produce any HIV proteins and, therefore, their infected status remains unrecognised by the immune system and unresponsive to antiretroviral therapy (ART). This reversibly nonproductive state of infection, denoted HIV-1 latency [6], constitutes a hiding mechanism by which HIV-1 may persist for decades evading host immune responses and potent cART. Therefore, the development of therapies capable of exhausting this latent viral reservoir, primarily residing within long-lived $CD4^+$ T cells, has become a highly prioritized goal in HIV-1 research.

One approach towards this aim, often referred to as 'shock and kill' [7], is characterized by the use of pharmacological agents to reverse HIV-1 latency and turn on production of viral proteins in latently infected cells, as this would theoretically expose such cells to killing by immune-mediated mechanisms or viral cytopathic effects. A wide range of LRAs has been investigated *in vitro* and *ex vivo* [8,9] with a few candidates being advanced to testing in experimental clinical trials [10–20]. The focus of this article is to summarize and consider the results arising from recent clinical trials in HIV-1 using LRAs. Specifically, we will discuss why these interventions have still not shown any durable effect on the size of the latent HIV-1 reservoir, but we also argue that this

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should not deter us from further pursuing the shock and kill approach. Studies with LRAs were primarily designed to investigate the effect of these drugs on the state of HIV-1 latency, that is, their ability to deliver the shock, and should be evaluated accordingly. Multiple other barriers to curing HIV-1 infection, including the presence of cytotoxic T lymphocyte (CTL) escape mutations in archived virus, and waning CTL responses during chronic infection, require other or additional interventions and must be addressed in separate studies.

Clinical Experiences with LRAs

The concept of eliminating latently infected $CD4^+$ T cells through activating HIV-1 from latency was initially tested using interleukin (IL)-2 and T cell activators such as anti-CD3 antibodies (OKT3). However, IL-2 treatment did not consistently impact the latent HIV-1 reservoir, and although the combined use of IL-2 and OKT3 caused a marked activation of the T cells, there were unacceptable toxicities and also irreversible decreases in $CD4^+$ T cells [21–23]. Rooted in these experiments began a search for compounds capable of inducing HIV-1 expression without causing global T cell activation; indeed, the absence of increases in T cell activation marker expression became part of the drug screen investigations. Histone deacetylase inhibitors (HDACi) appeared to match that profile, but, as discussed below, absence of T cell activation is not characteristic for all HDACi.

By virtue of its capacity to inhibit histone deacetylases, though requiring very high concentrations for *in vitro* efficacy [24,25], valproic acid (VPA) was initially used to test this hypothesis in clinical trials but showed no consistent effect on the latent HIV-1 reservoir [10–13]. Subsequently, vorinostat, an HDACi approved by the FDA for the treatment of cutaneous T cell lymphoma [26], became the first potent HDACi to be tested in a clinical HIV-1 trial. In this study, administration of a single dose of vorinostat to HIV-1 infected patients on suppressive cART led to an almost fivefold increase in HIV-1 transcription as measured by cell-associated HIV-1 RNA in resting $CD4^+$ T cells [14]. Similar results were seen with daily vorinostat dosing for 14 consecutive days, although changes in HIV-1 transcription were measured in total rather than resting $CD4^+$ T cells [18]. By contrast, when vorinostat was given 3 days per week for 8 weeks, this resulted in only modest increases in HIV-1 expression [15]. The anti-alcoholism drug, disulfiram, initially discovered as a potential LRA in a drug library screen [27] and recently tested for its effect on HIV-1 latency, also appeared to modestly increase HIV-1 transcription [16,20]. In addition, based on promising *in vitro* data [28], our group conducted a clinical trial with the highly potent HDACi panobinostat, which was approved by the FDA in 2015 for the treatment of multiple myeloma [29]. Panobinostat was added to suppressive cART thrice weekly every other week for 8 weeks in 15 HIV-1 infected patients, which resulted in a significant increase in levels of cell-associated unspliced HIV-1 RNA in $CD4^+$ T cells. Moreover, in contrast to the vorinostat studies, a significant increase in plasma HIV-1 detection rate, as assessed by a nonquantitative assay, was seen during panobinostat treatment [17]. Even more compelling were the results of a recent pilot study in which romidepsin infusions (5 mg/m^2 weekly for 3 weeks) led to increases in plasma HIV-1, which, in five of six study participants, were readily quantifiable using a standard clinical assay (Cobas Taqman®; detection limit of 19 copies/mL) [19]. Collectively, these results demonstrate that we presently have access to pharmaceuticals that are capable of inducing production of latent HIV-1 without causing significant toxicities. Still, none of the studies conducted to date using HDACi or disulfiram has demonstrated a significant effect on the size of the latent HIV-1 reservoir, measured as total HIV-1 DNA, integrated HIV-1 DNA, or quantitative viral outgrowth. Also, plasma HIV-1 RNA rebound occurred within an expected time frame in all patients undergoing analytical cART interruption following panobinostat treatment [17]. However, while a direct effect of LRAs on the viral reservoir would clearly have been a desirable result, using the absence of such an impact to discard the shock-and-kill approach entirely would be erroneous and premature. As discussed below, there are several possible potential explanations for the lack of an effect on the reservoir; future studies need to

address these mechanisms to advance the field and to further explore whether reversal of HIV-1 latency might form part of a cure.

Why Successful Latency-Reversing Interventions Have Not Reduced the Latent HIV-1 Reservoir

Understanding why reversing HIV-1 latency in clinical trials did not reduce the frequency of latently infected cells will be key to developing new and more effective interventions. Several barriers need consideration. First, activating HIV-1 expression in latently infected cells may not in itself lead to the death of these cells if they remain in a resting state; boosting of CTLs may be required for the elimination of virus-expressing cells [30]. By contrast, increases in the expression of T cell activation markers, which is seen following treatment with some HDACi but not all [28,31], may not be causally associated with HIV-1 expression but may be a direct pharmacological effect of HDACi on T cells. Second, CTLs play an important role in controlling HIV-1 replication during acute infection, but chronic infection is characterized by an impaired cytolytic capacity of $CD8^+$ T cells, which is not restored by cART [32]. Moreover, owing to the selection pressure induced by CTLs and the high frequency of virus sequence evolution during uncontrolled replication, mutations that confer CTL resistance are quickly acquired and archived in long-lived $CD4^+$ T cells [33]. Indeed, it was recently shown that, in patients initiating cART more than 3 months after HIV-1 infection, CTL escape variants completely dominated the latent viral reservoir [34]. Third, it is possible that the extent of latency reversal currently achieved in clinical trials is insufficient to mobilize significant proportions of the latent reservoir. At present we do not have a good understanding of the correlation between inductions in cell-associated HIV-1 RNA or plasma HIV-1 RNA during treatment with LRAs and the proportion of the reservoir that is targeted by these interventions. For example, when plasma HIV-1 RNA is detected in the 100 copies/mL range following romidepsin infusion [19], it is unclear whether this corresponds to having mobilized very little, say 1%, or a significant proportion, say 25%, of the inducible latent reservoir. Additionally, it is unknown whether the shock provided by LRAs *in vivo* activates previously transcriptionally silent proviruses or merely increases already ongoing low-level proviral transcription. Finally, it must also be kept in mind that the great majority of HIV-1-infected cells are located in the lymphoid tissues, primarily gut-associated lymphoid tissue and lymph nodes, although other anatomical compartments such as the central nervous system (CNS) may also constitute relevant reservoirs for HIV-1 [35–37]. Yet, clinical studies of LRAs have primarily analyzed effects on HIV-1 production and reservoir size in circulating $CD4^+$ T cells with only few investigations directed towards tissue reservoirs [18,38]. Thus, there is limited knowledge of the penetration of LRAs into lymphoid tissue and anatomical compartments potentially harboring HIV-1, and of their virological effects. Future studies need to address these important questions, the resolution of which may further illuminate why LRA interventions to date have failed to reduce the size of the latent HIV-1 reservoir (see Outstanding Questions).

Latency Mechanisms Targeted with Current Approaches

The detailed mechanisms of HDACi effects on chromatin remodeling and HIV promoter accessibility have been extensively reviewed elsewhere [39]. Results arising from clinical trials using HDACi to reverse HIV-1 latency suggest that not all HDACi are equal. As illustrated in Table 1, one of the most apparent differences between vorinostat on one side and panobinostat and romidepsin on the other side is the clear effect on markers of T lymphocyte activation during treatment with panobinostat and romidepsin. This effect on T lymphocyte activation was also observed following *ex vivo* stimulation of both HIV-infected and healthy donor peripheral blood mononuclear cells (PBMCs) [28,40] and, thus, is likely a direct pharmacological effect on T cells rather than an effect secondary to increases in HIV-1 production. Disulfiram is mechanistically distinct from the HDACi in that it depletes the phosphatase tension homolog (PTEN), resulting in activation of the Akt signaling pathway [41]. Activation of Akt signaling results in nuclear factor-κB (NF-κB) translocation to the nucleus creating a favorable milieu important for HIV reactivation

Table 1. Clinical Trials of Latency-Reversing Agents

	Drug Dosing (doses)	HIV-1 Transcription (fold > baseline)	Plasma HIV-1	Post Dosing Viral Effect	T cell Activation	Reservoir Size	Refs
Vorinostat							
Archin *et al.* (2012)	400 mg (1)	4.8	No change	ND[c]	ND	ND	[14]
Elliott *et al.* (2014)	400 mg daily (14)	2.7	No change	Yes	No change	No change	[18]
Archin *et al.* (2014)	400 mg TIW[c] (22)	1.3	No change	ND	ND	No change	[15]
Panobinostat							
Rasmussen *et al.* (2014)	20 mg TIW (12)	2.9	Increased[a]	Yes	Increased	No change	[17]
Romidepsin							
Sogaard *et al.* (2015)	5 mg/m^2 (3)	3.8	Increased	No	Increased	No change	[19]
Disulfiram							
Spivak *et al.* (2014)	500 mg daily (14)	ND	Increased[b]	Yes	ND	No change	[16]
Elliot *et al.* (2015)	Dose escalation (3)	~2	Increased	Yes	ND	ND	[20]

[a]Determined nonquantitatively by nucleic acid testing (NAT) using a transcription-mediated amplification (TMA)-based assay (Procleix Ultrio Plus®, Novartis).
[b]The subgroup of patients with a measurable metabolite had an increase in low-level viremia.
[c]Abbreviations: ND, not determined; TIW, three times a week.

[42] as well as in T lymphocyte activation and differentiation [43]. The impact of disulfiram on T cell activation has not been investigated *in vivo* in the setting of HIV-1 infection, but no effect was reported in primary T cell models of HIV latency [27]. An unexpected finding in HDACi clinical trials in HIV-1 is a distinctive feature of post-dosing increases in virus production beyond the dosing window and past the point where all direct measures of drug pharmacodynamics have returned to baseline levels. Thus, one could speculate that LRA treatment impacts global T cell homeostasis, elevating the dynamic transcriptional pattern of the proviruses. These observations further emphasize the need to dissect the transcriptional activity of the individual provirus in order to study not only the steady state equilibrium, but also the direct LRA shock effect and sustained long-term effects.

Optimizing Latency-Reversal Strategies

Until now, only well characterized drugs 'borrowed' from other medical fields have been tested as LRAs in clinical trials and the potential for optimizing latency-reversal strategies is enormous (Figure 1, Key Figure). Ideally, the proportion of latently infected cells induced to express HIV-1 proteins by LRAs *in vivo* should approach that seen with maximal T cell activation *ex vivo*. This was indeed used as a benchmark in a recent *ex vivo* analysis in which levels of HIV-1 production following stimulation with combinations of LRA were normalized to that seen with maximal T cell activation using phorbol myristate acetate (PMA)/ionomycin. Interestingly, in contrast to stimulation with any single LRA, combinations of certain HDACi and the protein C kinase (PKC) agonist bryostatin-1 robustly induced HIV-1 transcription up to around 50% of that achieved with maximal T cell activation [44]. However, whether it will be possible in the clinical setting to achieve the same pharmacological pressure for a comparable duration without

Key Figure

Optimization of Shock-and-Kill Therapies

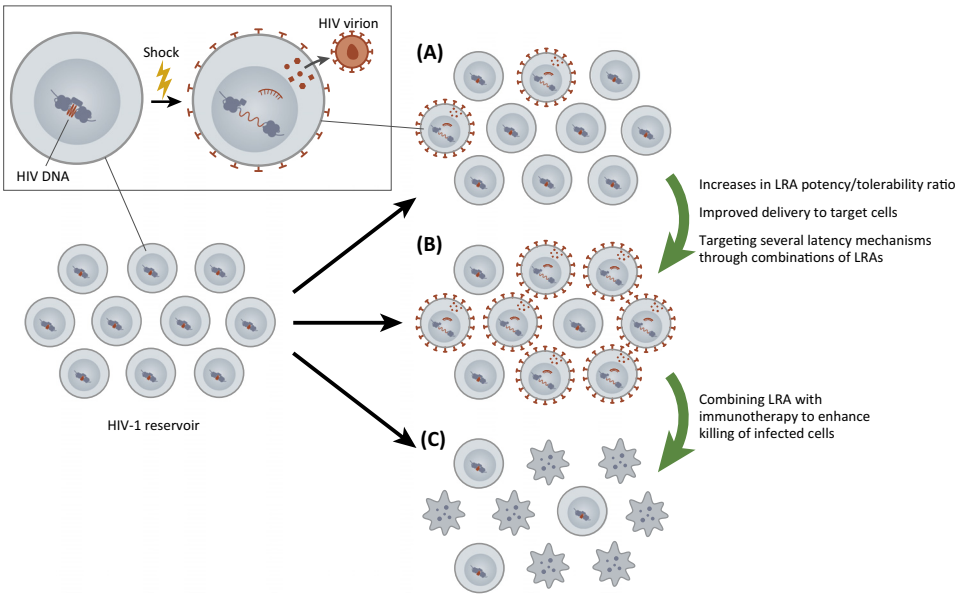

Figure 1. The figure illustrates the total pool of cells latently infected with replication-competent HIV-1 (left side), and how latency-reversing agents (LRAs) may activate production of HIV-1 transcripts and viral proteins (enlargement). Panels A, B, and C are schematic representations of shock-and-kill approaches with various degrees of impact on the latent HIV-1 reservoir and indication of factors that may lead to improvement in shock-and-kill approaches. (A) Illustrates the extent of latency reversal achieved with current LRA studies. (B) Illustrates how optimization of LRA interventions may enhance the magnitude of latency reversal. (C) Illustrates how combinations of immunotherapy and LRAs may augment killing of latently infected cells.

compromising safety is uncertain. Still, combining LRAs with different mechanisms of actions may significantly increase *in vivo* effects. In addition to HDACi and disulfiram, which are mainly discussed here owing to their inclusion in clinical trials, other classes of drugs capable of reversing HIV-1 latency are concurrently investigated in preclinical studies. These include the naturally occurring protein kinase C (PKC) agonist bryostatin-1, which can be isolated in small quantities from the marine bryozoan *Bugula neritina* [45] and which displayed high potency for inducing HIV-1 production *ex vivo* [44]. However, while bryostatin-1 has been employed in clinical trials for malignancies [46], the first study in humans infected with HIV-1 is still awaited (ClinicalTrials.gov, identifier NCT02269605). Other compounds showing promising potential for reversing latency include PKC agonistic derivatives of ingenol ester and bromodomain inhibitors, which exert their action by promoting recruitment of positive transcription elongation factor (p-TEFb) to the HIV-1 promoter [9,47–49]. Also, by depleting cellular inhibitors of apoptosis proteins, small-molecule antagonists referred to as Smac mimetics were recently shown to reverse HIV-1 latency *in vitro* [50]. Finally, the Toll-like receptor (TLR) 7 and 9 [51] agonists have attracted some interest in cure research lately. Specifically, the TLR7 agonist GS-9603 induced repeated episodes of plasma viremia in a nonhuman primate study (http://www.croiconference.org/sessions/treatment-tlr7-agonist-induces-transient-viremia-siv-infected-art-suppressed-monkeys). The critical unknown questions are, of course, to what extent these

molecules reverse latency *in vivo* and whether combinations of LRAs will maintain sufficient tolerability to allow coadministration in humans.

Overall, improvements in the magnitude and/or breadth of latency reversal may be achieved through:

- Dose adjustments
- Alternative dosing schedules
- Identification of more potent compounds
- Discovery of new latency mechanisms that can be targeted
- Indirect triggering of latently infected cells through activation of non-$CD4^+$ immune cells, for example, plasmacytoid dendritic cells
- Combination of LRAs with different mechanism of action.

We already have several indications that such adjustments could impact the success of latency-reversal strategies. Examples include the dose–response relationship observed in the disulfiram study [20], the significant increase in HIV-1 transcription from the first to the second dose of romidepsin [19], the apparent difference in potency between HDACi [40,52], preclinical data showing potent indirect latency reversal by TLR agonists (http://www.croiconference.org/sessions/treatment-tlr7-agonist-induces-transient-viremia-siv-infected-art-suppressed-monkeys), and *in vitro* studies demonstrating that combining LRAs with different mechanisms of action, compared to any LRA used alone, has a much more pronounced effect on latently infected cells [44,53]. However, for safety reasons, clinical testing of LRAs can only be conducted step by step, and promising novel compounds obviously have to go through extensive preclinical testing prior to being tested in humans. This latency-reversal optimization process requires extensive *in vitro*, preclinical, and clinical testing, all of which are labour-intensive and expensive. Without significant engagement from the pharmaceutical industry and funding agencies, this process will likely take many years to complete. In addition, there are important ethical considerations around whether or not new interventions require testing in animal models before human trials. While novel compounds and high-risk interventions obviously need to go through safety testing in animal models such as nonhuman primates or humanized mice, efficacy measures in these models must be interpreted cautiously. No current animal model can replicate the virological and immunological characteristics of HIV-1 latency as established during long-term suppressive therapy, nor the complex effects in human infection of interventions designed to reverse latency or enhance immune surveillance.

Combining Latency Reversal with Other Curative Strategies

While it seems questionable that short-term treatment with single LRAs is able to significantly reduce the HIV-1 reservoir, trials showing significant increases in viral transcription during HDACi treatment have provided grounds for optimism. This had led to the design and initiation of new clinical trials combining HDACi with therapeutic HIV-1 vaccination or other immune therapy. In line with the shock-and-kill approach, the idea here is to boost the ability of the immune system to kill virus-expressing cells either prior to or during reversal of latency. Enhancement of antiviral immune responses towards infected cells may be accomplished by boosting existing innate and adaptive immune effector mechanisms, inducing novel immune responses, or by passive immunization. Several approaches are being pursued. First, the recent discovery and use of broadly neutralizing antibodies (bNAbs) holds great promise as a new treatment modality for HIV-1 infections [54] and could facilitate the elimination of virus-expressing cells when combined with LRAs. Indeed, in humanized mice, coadministration of the bNAb 3BNC117 and a cocktail of LRAs delayed time to viral rebound, following ART discontinuation, as compared with antibody alone [55]. Second, advances in HIV vaccine development may provide new tools for viral clearance. Specifically, rhesus macaques vaccinated with rhesus cytomegalovirus vectors expressing simian immunodeficiency virus (SIV) protein attained durable virological control.

Remarkably, despite manifest SIV infection, immunological and virological signs of infection were lost during follow up, suggesting that viral reservoirs may be eliminated through vaccine-mediated continuous immune surveillance [56]. Third, bi-specific antibodies which stimulate T cell activation and HIV-1 expression through CD3 engagement while also facilitating immune-mediated lysis by binding HIV-1 antigens with high specificity, have the potential to be effective new agents to eliminate latently infected cells [57,58]. Finally, drugs capable of altering cell death pathways [59] and the use of monoclonal antibodies that block immune checkpoints are also explored as strategies to enhance elimination of infected cells [60]. Results of coming clinical trials testing these concepts will be extremely informative in terms of estimating the total impact of LRAs on the inducible HIV-1 reservoir as well as establishing the immune effector mechanisms necessary to eliminate infected cells expressing HIV-1 proteins.

Concluding Remarks

Nevertheless, the key to advancing latency reversal as part of a curative strategy for HIV-1 will be to demonstrate, in a randomized trial, persistent reduction in the size of the latent reservoir and a prolonged time to re-emergence of virus in plasma upon cART discontinuation. This would be considered an important indication of our ability to significantly impact the latent HIV-1 infection, but still only an intermediate result in the absence of a sustained clinical benefit for the infected patient. With the impressive efficacy of cART, the ultimate goal in cure-related research cannot be anything less than a complete cure for HIV-1 infection or at least a permanent state of ART-free disease remission. If reversal of HIV-1 latency is to be part of the strategy towards this goal, potent and well-tolerated LRAs as well as effective immune therapy must be developed.

Outstanding Questions

What proportion of the inducible latent HIV-1 reservoir is targeted by current LRAs *in vivo*?

What is the impact of LRAs on persistent HIV-1 in lymphoid tissues, other anatomical reservoirs, and/or immune privileged sites?

How do we best assess efficacy of LRAs in clinical trials?

What is the optimal dosing schedule for LRAs – continuous versus intermittent?

Will prolonged exposure to LRAs lead to an increased effect or will the effect wane over time (e.g., due to autoregulation of gene transcription)?

Which immune effector mechanisms represent the most optimal target for enhancement in order to augment killing of virus-expressing cells?

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