CellPress

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