Wake me up before you go: a strategy to reduce the latent HIV reservoir

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Targeting the reservoir to cure HIV infection

Antiretroviral therapy (ART) does not eradicate HIV as demonstrated by the rapid return of viremia whenever treatment is interrupted. The possible long-term toxicity of ART, viral resistance, stigma, and cost all contribute to the necessity of finding a cure. In individuals on ART, HIV primarily persists in CD4\textsuperscript{+} T cells that do not spontaneously produce HIV particles unless activated. The few HIV remission cases reported to date were adults or children with extremely low reservoir either by initiating ART in the first hours of life, or by immune ablative treatment and allogeneic stem cell transplant [1–3]. These long-lived resting CD4\textsuperscript{+} latently infected cells are often considered as the major obstacle to HIV eradication, although low-level cryptic replication in tissue sanctuaries may be a contributing factor [4,5]. Overall, it is assumed that eliminating, or at least reducing significantly the size of the HIV reservoir is a prerequisite to the development of a cure for HIV infection.

Early antiretroviral therapy: good but not enough

The only intervention to date that has been shown to significantly affect the size of the latent reservoir is the early initiation of ART, within the first weeks/months following infection [3,6–11]. Although early ART initiation has a dramatic impact on the size of the reservoir [12–15], it does not prevent the establishment of a small pool of persistently infected cells. In nonhuman primate (NHP), starting ART as early as 3 days after infection does not prevent viral rebound after ART interruption [16]. In humans, recent data also suggest that ART initiation at the earliest stage of acute infection (Fiebig I) does not clear HIV from the body and does not result in a clinically significant delay in viral rebound [17]. Altogether, these studies indicate that while early ART is very efficient at limiting the seeding of the latent reservoir, it will neither achieve HIV clearance nor natural control of HIV replication. They also imply that efforts gearing toward HIV cure by solely eliminating latently infected cells will require reducing the HIV
reservoir to magnitudes lower than achieved in people treated within days of detectable infection.

**Shock and kill strategies**

Inducing HIV expression combined with ART has been proposed as a strategy to eliminate persistently infected cells that could ultimately lead to viral eradication [18–20]. It is assumed that upon viral reactivation, infected cells will be eliminated through cytopathic effect caused by HIV or following recognition and killing by immune cells [including cytotoxic t lymphocytes (CTL) and natural killer cells]. Several molecules with such potential for viral induction have been identified in in-vitro models of HIV latency. They include protein kinase C activators [21], histone deacetylase inhibitors (HDACi) [19,22,23], toll-like receptor (TLR) ago-nists [24,25] and common γ-chain cytokines [26,27]. The majority of these molecules display highly variable activities between the in-vitro latency model tested [19,28,29], are often inefficient at inducing the production of viral particles in patient’s cells [30] and do not induce a measurable decrease in the size of the HIV reservoir *in vivo* [19,31,32]. Notably, a few recent studies using latency reversing agents (LRAs) *in vivo* reported a significant increase in plasma viral load [24,33]. Although large efforts in developing new classes of LRAs to potently reactivate the latent HIV reservoir progress rapidly, the means of how to eliminate efficiently the reactivated cells are still to be determined.

**Waking-up latent HIV before cytotoxic t lymphocytes are gone**

Purging the latent HIV reservoir requires both the induction of viral replication from its latent state and the elimination of these reactivated latently infected cells by either viral cytopathic effect or immune cell-mediated killing. Killing is quite inefficient when LRAs are administered to HIV-infected individuals on suppressive ART for several years. This may be because of low level of induced antigen expression, negative impact of LRAs on clearance mechanisms, or the very low number of effective HIV-specific CD8+ T cells.

Here, we provide rationale for a strategy in which an LRA would be administered at the time of ART initiation before CTL responses wane. This alternative timing of intervention during the ‘window of opportunity,’ where the immune responses are still present and the latent reservoir may be easier to reactivate was proposed previously [34] and has been validated theoretically by in-silico modeling [35]. We provide a rationale supporting the concept that this approach may improve the efficacy of both components of a shock and kill approach (Fig. 1).

![Fig. 1. A strategy to wake up latent HIV before CTLs are gone.](image)

(a) Current strategy to reduce the HIV reservoir in humans, where LRAs are administered during suppressive ART while CTL numbers are low (blue line) and latent HIV reservoir is stable (green line) resulting in limited effect on reservoir size and viral rebound upon ART cessation (red line). (b) Strategy proposed to reduce the HIV reservoir in humans, where LRAs are administered at time of ART initiation, whereas CTLs are still present and HIV reservoir is less stable resulting in a decrease in HIV reservoir and potentially a delay (dashed lines) or control (dotted lines) of viral rebound after ART cessation. ART, antiretroviral therapy; CTLs, cytotoxic t lymphocytes; LRA, latency reversing agent.
In addition, we discuss preclinical and clinical considerations for testing this strategy.

**Improved viral reactivation from latency**

The size of the latent HIV reservoir is maximal when ART is initiated. We previously demonstrated that a significant fraction of infected cells in untreated individuals do not spontaneously produce HIV but have the capacity to do so when stimulated in vitro [36]. On average, the frequency of latently infected cells was ten and four times higher than the frequency of productively infected cells in virally suppressed and viremics individuals, respectively, demonstrating that the latent viral reservoir is already established before ART and that latency can be reverted in these cells.

‘The degree’ of HIV latency during untreated HIV infection is hard to evaluate as latently infected cells represent only a fraction of the entire pool of infected cells [37], and latency is occluded by the ‘noise’ attributed to productively infected cells. In addition, the activation status of a cell does not necessarily correlate with the transcriptional status of the provirus within this cell, at least in vitro [38]. Whether latently infected cells in treated and untreated individuals are equally responsive to latency reversal by shocking agents is currently unknown. Nonetheless, it is reasonable to think that several cellular factors contributing to the silencing of HIV transcription during ART may not exert their latracy activity optimally when viremia in not suppressed, that is, when global T-cell activation is not controlled. Indeed, untreated HIV infection is characterized by a proinflammatory environment stemming from high antigen load and microbial translocation [39], which leads to T-cell activation directly through T-cell receptor and TLR engagement and indirectly through the production of proinflammatory cytokine [40,41]. Several of these factors have been shown to facilitate latency reversal, including bacterial products [42], TLR ligands [24,25,43,44], and proinflammatory cytokines [45]. Therefore, one can hypothesize that the proinflammatory environment characteristic of untreated HIV infection will favor latency reversal, although this has never been directly evaluated.

**Increased cytotoxic t lymphocyte-mediated killing**

HIV-specific CD8$^+$ T cells become exhausted by chronic antigen exposure but still retain cytotoxic capacity as they maintain an equilibrium with viral replication in untreated infection at viral set point. In people on suppressive ART, CTLs directed against HIV have been quantified using cytokine secretion after peptide stimulation or tetramer staining and showed that their frequency is at least 10 times lower than before ART, although it has not been exhaustively quantified for all HIV epitopes [46–50]. Even though they lack persistence on ART, these cells are still able to control viral production as demonstrated by the resurgence of viral load after CD8$^+$ depletion in NHP on suppressive ART [51]. Recent studies in vitro or in the NHP model have provided the rational for boosting the numbers, enhancing the function and targeting the location of HIV-specific CD8$^+$ T cells to purge HIV-infected cells [52,53]. Enhanced CTL activity induced by vaccination can have a profound effect on the levels of simian immunodeficiency virus (SIV)-infected T cells in macaques on suppressive ART as demonstrated by a significant reduction in SIV DNA in peripheral blood mononuclear cells and lymph nodes in animals that received Ad26/modified Vaccinia Ankara vaccination (with or without TLR7), in comparison with unvaccinated controls [54]. Strategies aiming at boosting CTLs on ART would need to induce responses in the same order of magnitude or even higher, but the frequencies of CTLs needed to have a measurable impact on the HIV reservoir remain unknown. Therapeutic vaccines have been tested for many years with no success on reducing the HIV reservoir. Novel therapeutic approaches including novel vectors delivering persistent antigens are being developed but are still far from being implemented into the clinic [55]. The alternative to these approaches is to reactivate the latent HIV reservoir with LRAs at time of ART initiation when the HIV-specific CD8$^+$ T-cell responses are still present in high numbers and would not require additional intervention. In the context of chronic HIV infection, although HIV-specific CTLs are present at high numbers, their survival capacity may not be optimal because of antigen driven exhaustion [56]. However, pharmodynamic–1 high CTLs have an in vivo higher turnover rate to maintain their numbers that could compensate their loss [57]. Of note, other immune subsets might also contribute to cytokytic-mediated clearance of virus infected cells and control of viral rebound in these interventions.

**Testing the concept in nonhuman primate models**

In recent years, significant effort and resources have been invested in developing relevant, state-of-the-art NHP models for HIV cure-related studies. For instance, several ART regimens have been optimized and validated that are capable of safely and sustainably achieving clinically relevant levels of virus suppression in SIV-infected rhesus macaques [58,59]. In addition, assays to characterize the
residual virus remaining in such rhesus macaques have demonstrated that where relevant comparisons can be made, the levels and dynamics of residual virus in SIV-infected rhesus macaques on long-term ART are quite comparable with similar measurements in ART-suppressed HIV-infected humans, including lower reservoirs in rhesus macaques in which ART was initiated earlier in infection [16,53,54]. The NHP model has been used successfully to evaluate how HDACi such as romidepsin and vorinostat affect levels of residual virus in ART suppressed infections with results similar to observations in humans [60–62]. However, the ability to quantify and characterize viral reservoirs not just in blood but also in tissues with longitudinal biopsies and extensive sampling at necropsy further strengthens the application of the model to test HIV cure interventions, as variables such as the time of ART initiation (i.e. early ART or late ART), route and frequency of LRA administration and time of ART cessation can be easily controlled. In addition, animals can be selected to specifically express certain major histocompatibility complex haplotypes (Mamu-A*01, B*08, B*17), which upon SIV infection can elicit immunodominant epitope-specific CD8\(^+\) T-cell responses that can be followed with tetrators, allowing their precise quantification in cell suspensions by flow cytometry and in tissue sections by in-situ tetramer analysis. As such, the impact of LRA administration at time of ART initiation on tissue reservoirs and virus rebound dynamics after ART cessation can be thoroughly evaluated in the NHP model.

**Translating into humans**

Although several LRAs show latency disruption in resting \(CD4^+\) T cells *in vitro*, such effect has yet to be demonstrated in preclinical or clinical setting. The current ‘shock and kill’ paradigm states that latency disruption (‘shock’) will need to be paired with clearance functions (kill) likely provided by immune therapies (checkpoint blockers, broadly neutralizing antibodies with antibody-dependent cell-mediated cytotoxicity function), therapeutic vaccine, adoptive transfer of ex-vivo expanded T cells, redirected T-cell therapies (dual-affinity re-targeting), chimeric-antigen receptor T cells, and so on. Not only these combination strategies are complex to develop, but the efficacy of immunotherapy or cell transfer can be challenged by the immunomodulatory activities of some epigenetic LRAs (HDACi/bromodomain and extraterminal domain inhibitors/histone methyltransferase inhibitor). Some molecules might eliminate HIV-specific CD8\(^+\) T cells and inhibit CTL-mediated killing calling for a careful choice of LRAs to be administered [63–65]. We posit here that, in addition to the choice of LRAs, the timing of administration is critical for the clearance of the reservoir. The choice of ART regimen might also be carefully considered as an intensified regimen to blunt heightened viral expression and prevent reservoir replenishment when induced by protein kinase C agonist or other LRAs. As some LRAs will also potentiate immune responses, this intervention might generate a potential risk for IRIS that would need to be evaluated upon enrolment of clinical trial participants. As many modalities can be tested in the preclinical setting, choices have to be made based on current knowledge and available LRAs. A potential setting to define if truly a ‘window of opportunity’ exists at time of ART initiation would be to use an ingenoid in *Mamu-A*\(^*\)01 rhesus macaques, serially administered at ART initiation until viremia is fully suppressed. Once preclinical studies validate the strategy, clinical trials can be rapidly designed to test selected LRAs during ART initiation to reduce the HIV reservoir to levels lower than those achieved by treating in the earliest stages of acute HIV infection with ART alone.

Although the definitive test of a cure will require interruption of ART, it is necessary, however, to measure the impact of eradication strategies on the size of the viral reservoir even as therapy is continued if clinical trials are to proceed in an efficient and ethical manner.

In the strategy described herein, monitoring the efficacy of a shock approach during the viremic phase of the disease will be extremely challenging. The number of productively infected cells is likely to outweigh the number of cells in which latency is reversed, even with a potent LRA. Alternatively, rather than measuring latency reversal during the viremic phase, we propose to measure the size of the viral reservoir after several months of ART, when viremia is fully suppressed. Although this represents a less direct assessment of the efficacy of latency reversal, measurement of the reservoir provides an estimate of the overall efficacy of this approach by integrating both the shock and kill aspects of this strategy. After ART interruption, different scenarios could be expected depending on the levels of cleared HIV reservoir and immune control, with no viral rebound, or transient viral rebound with subsequent immune control of viral replication leading to long-term remission.

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Shock during antiretroviral therapy initiation

Chomont et al.

Conflicts of interest
There are no conflicts of interest.

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