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Experimental Approaches for Eliminating Latent HIV

Matthew D. Marsden^a and Jerome A. Zack^{a,b,*}

^aDepartment of Medicine, Division of Hematology and Oncology, University of California, Los Angeles, CA

^bDepartment of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA

Abstract

Antiretroviral therapy (ART) can reduce HIV viral loads to undetectable levels and prevent disease progression. However, HIV persists in rare cellular reservoirs within ART-treated patients and rapidly reemerges if ART is stopped. Latently infected CD4⁺ T cells represent a major reservoir of HIV that persists during ART. Therefore, a cure for HIV must include methods that either permanently inactivate or eliminate latent virus. Experimental methods under investigation for eliminating latently infected cells include transplantation/gene therapy approaches intended to deplete the infected cells and replace them with HIV-resistant ones, and DNA editing strategies that are capable of damaging or excising non-expressing HIV proviruses. Alternatively, “activation-elimination,” also known as “shock and kill,” approaches aim to induce expression of latent virus, allowing the virus to be eliminated by viral cytopathic effects, immune effector mechanisms, or additional cells/antibodies that specifically target and kill cells expressing HIV proteins. Here, we describe these experimental approaches for eliminating latent HIV along with other recent advances in HIV cure research.

Keywords

activation; latency; reservoir

I. INTRODUCTION

Human immunodeficiency virus (HIV) infects and kills CD4⁺ T cells. The continuous depletion of these cells over years eventually overcomes the regenerative capacity of the immune system, resulting in the development of acquired immunodeficiency syndrome (AIDS). Potent combinations of antiretroviral drugs have been developed to combat HIV, which inhibit virus replication and often suppress plasma viral loads to undetectable levels. Yet the required continuous daily use of antiretroviral therapy (ART) is complicated by side effects of the drugs, adherence issues, the development of resistant viruses, and high financial costs. Most importantly, ART alone does not cure HIV infection. This is because replication-competent HIV is maintained in rare viral reservoirs that persist throughout ART.

*Address all Correspondence to: Jerome Zack, PhD, David Geffen School of Medicine at UCLA, 615 Charles E Young Drive South, BSRB 173, Los Angeles, CA 90095; Tel.: 310-825-0876; Fax: 310-267-1875; jzack@ucla.edu.

The best characterized and probably the largest source of replication-competent HIV during ART is the latent HIV reservoir within resting CD4⁺ T cells.¹⁻⁴ There is some debate over whether replication-competent HIV can also survive via other mechanisms over many years of ART. For example, virus might in some cases be maintained through very low levels of ongoing virus replication in tissues with poor antiretroviral drug penetration, such as the central nervous system or deep within lymphoid organs.^{5,6} Persistence of HIV in these organs may be further aided by infection of cells such as macrophages, which have longer half-lives than HIV-infected activated CD4⁺ T cells⁷ and might support rare chains of infection, where virus is transferred from one cell to another very infrequently, perhaps only once every few weeks or months. However, even if other sources of persistent HIV are present during ART, latently infected CD4⁺ T cells represent a key barrier to HIV cure that appears to be present in all patients, and are therefore a major focus of HIV cure research.

Latently infected cells are resting, primarily central and transitional memory CD4⁺ T cells that harbor intact HIV genomes integrated within their chromosomes, but express little or no viral RNA and no viral proteins.^{1,3,8,9} Virus within these cells can persist in a latent state for decades,⁹ but then revert to producing infectious virions if the host cell becomes stimulated, for example, through an encounter with its cognate antigen or exposure to a pro-inflammatory cytokine environment. The latent proviruses are not affected by ART (which only inhibits actively replicating virus) and are invisible to the immune system due to their lack of viral protein expression. Therefore, these latently infected cells can persist during ART and generate virus that rapidly spreads if ART is stopped. Resting CD4⁺ T cells cannot usually be productively infected with HIV, and exposure of these cells to virus typically results in abortive infection prior to integration.¹⁰ Hence, it is believed that latently infected cells are probably formed when an overtly activated or cytokine-stimulated CD4⁺ becomes infected by HIV and then transitions to a resting memory cell before it can be killed by the immune response or viral cytopathic effects.

Historically, the latent reservoir has been quantified by observing viral outgrowth during a single round of stimulation in a limiting dilution coculture assay. Based on this assay, it is estimated that each infected individual harbors approximately a million latently infected cells, although this number can vary substantially from person to person.^{2,11} This translates into around 1 per million resting CD4⁺ T cells containing an activation-inducible latent provirus in each infected individual. A more recent study based on the quantification of apparently intact HIV proviral sequences within resting CD4⁺ cells suggests that the reservoir of replication-competent proviruses might be in some cases 60-fold higher than calculated using assays involving a single round of stimulation.¹² Regardless of the precise size of the latent reservoir, it is known to decay very slowly during ART, with a half-life of over 40 months.¹ This slow rate of decay is likely sufficient to ensure lifelong infection despite continuous ART. The latent reservoir is also seeded very soon after primary infection, and is detectable even in patients who initiated ART within weeks of infection.¹³ In addition to serving as a persistent source of virus, the latent reservoir can also serve as an archive of viral variants. This can complicate treatment options since antiretroviral drug resistant variants can be maintained in the reservoir. It also poses challenges for natural immune control of virus and the use of cytotoxic T lymphocyte (CTL)-based

immunotherapies to combat HIV, because CTL-resistant viruses are similarly deposited in the reservoir early in the course of infection.^{14,15}

Efforts to eliminate the latent reservoir of HIV have historically been focused on one of several individual strategies, as we have reviewed more completely elsewhere.^{16,17} One potential approach involves ablation/transplantation, whereby both latently infected and uninfected cells are eliminated by powerful ablative chemotherapy/radiotherapy. Hematopoietic stem cells from an individual who is resistant to HIV (e.g., due to a lack of expression of the CCR5 coreceptor) are then introduced into the patient, which repopulate the immune system with mature HIV-resistant cells. This approach appears to have been successful in the case of the “Berlin patient,” Timothy Ray Brown, who remains free of HIV after receiving aggressive therapy to treat leukemia and two bone marrow transplants using cells from a CCR5 32 homozygous individual.^{18,19} While the health risks, financial costs, and logistical problems associated with this treatment would preclude its use in the overwhelming majority of HIV-infected individuals, safer alternatives are being investigated. These include the genetic modification of a patient’s own stem cells to reduce or eliminate CCR5 expression, which should circumvent the significant problem of finding an appropriate CCR5-deficient donor match for transplantation.²⁰ Safer, more gentle conditioning procedures that make space for the donor cells but fall short of complete myeloablation are also being investigated.²¹ Without the fully ablative therapy and graft versus host disease that occurred in the Berlin patient, it is perhaps more likely that these milder approaches would lead to extended periods of drug-free virus control, or a “functional cure,” rather than a complete sterilizing cure whereby no replication-competent HIV is detectable.

Alternative approaches directed toward eliminating latently infected cells are also being developed. Perhaps the most conceptually straightforward method to reduce the latent reservoir would be to excise or otherwise permanently inactivate the non-expressing latent HIV proviruses. In recent years, a range of approaches involving DNA-editing enzymes have been developed to achieve precisely this goal (Fig. 1). Homing endonuclease,²² zinc finger nuclease,²³ evolved recombinase,²⁴ and CRISPR/Cas9 (Ref. 25) systems have each been developed that can disrupt HIV-derived proviruses in cell lines or activated primary cells. Challenges associated with applying these methods to latently infected resting CD4⁺ T cells *in vivo* include the difficulty of expressing exogenous DNA or RNA encoding the editing enzymes in resting CD4⁺ T cells. Delivering native therapeutic proteins into resting cells in sufficient quantities to be active would also be problematic. It is possible that nanoparticle-mediated delivery systems could aid in this process by protecting the therapeutic DNA/RNA/proteins during *in vivo* delivery and help to deliver them across the plasma membrane into cells.

Additional challenges associated with directly editing latent HIV genomes include the fact that latent proviruses can be integrated in relatively inaccessible regions of the chromosomal DNA, which could afford them some protection from exogenous DNA editing enzymes faced with the already difficult task of targeting a single 10 kilobase pair HIV genome within approximately 3 billion base pairs of human DNA present in each cell. Indeed, one of the factors that might contribute to establishment of HIV latency is the integration of virus

into non-expressing regions of cellular DNA such as heterochromatin.²⁶ Finally, any therapy directly targeting the HIV DNA genome will have to contend with the extremely large genetic diversity of HIV, which is a well-known challenge in the field of vaccine development, and is critically important for the ability of HIV to evade the immune response and evolve resistance to antiretroviral drugs. This genetic diversity will also be a challenge when designing DNA editing approaches for eliminating latent HIV from primary cells, although targeting conserved regions of the virus and the simultaneous editing of multiple regions of the viral genome are feasible approaches to circumvent these problems. Hence, the approach of directly editing the non-expressing HIV genome is not without challenges. Nevertheless, editing of latent proviral HIV DNA represents an attractive and direct potential pathway to eliminating the latent reservoir.

If the HIV DNA cannot be targeted directly, then an alternative approach for eliminating latently infected cells would be to selectively kill only those cells harboring HIV genomes (Fig. 1). This could potentially be achieved if latently infected cells bear any external features that distinguished them from their uninfected counterparts. However, beyond the broad phenotype of predominantly memory CD4⁺ T cells, no definitive markers of latently infected cells have yet been found. Research in this area is continuing, with membrane proteomics being coupled with HIV latency models to try to identify markers that are enriched in latently infected cells. Along these lines, high levels of CD2 expression have recently been associated with latently infected cells in both an *in vitro* model system and ART-treated patient samples.²⁷ Alternative surrogate markers for latently infected cells are also being searched for. For example, while latently infected cells do not express viral proteins, they might under some circumstances express short transcripts such as the TAR stem loop that is present in all HIV RNA, including abortive short transcripts. TAR RNA has been shown to be present in exosomes that are released from infected cells.²⁸ Therefore, efforts are underway to identify whether latently infected cells produce exosomes containing HIV RNA that could aid in their identification, quantification, and elimination.

A third potential method for eliminating latent virus has variously been termed an “activation-elimination,” “shock and kill,” or “kick and kill” approach (Fig. 2). This involves first flushing the virus out of latency leading to the expression of viral proteins. Once this has been achieved, the host cell would potentially become susceptible to viral cytopathic effects, immune effector mechanisms, or therapeutics that can target cells producing viral proteins. If this were performed under the cover of antiretroviral therapy, then any newly produced virions should be inhibited by the antiretroviral drugs and therefore should not be able to initiate new rounds of replication.

Methods for inducing expression of latent HIV in a safe and effective manner are currently under intense investigation (Fig. 2). Approaches that have been tested in ART-treated patients include the use of antibodies (anti-CD3), cytokines (IL-2), and histone deacetylase inhibitors (e.g., valproic acid and vorinostat).^{29–32} These efforts produced important information regarding the feasibility of latency activating approaches. However, they had varying effects on *in vivo* HIV expression and latent reservoir size, and none of the tested approaches have successfully eliminated persistent HIV reservoirs entirely or prevented viral rebound if therapy is stopped. Additional agents that are under investigation for inducing

latent HIV expression include small molecules such as protein kinase C (PKC) modulators. These include prostratin, bryostatin, and synthetic analogs based on these compounds, which have greatly improved characteristics compared with the parental compounds.^{33–37} BET bromodomain inhibitors and farnesyl transferase inhibitors have also been shown to be capable of activating latent HIV in some circumstances, and are therefore being further pursued.^{38,39} Immunomodulatory antibodies including anti-PD1 and anti-CTLA4 might overcome inhibitory signals in latently infected cells and thereby help in efforts to activate the host cell and induce latent virus expression.^{8,40–42}

There is also some concern that HIV latency activating agents could have unwanted side effects on cells that are not targets for HIV infection. Therefore, we have investigated nanoparticle delivery systems for latency reversing agents.^{43,44} These are still under development but offer potential advantages including the possibility of targeting latency reversing agents to CD4⁺ T cells by attaching antibodies or other targeting domains to the nanoparticles, which may reduce the possibility of toxic effects mediated through non-HIV target cells. Nanoparticles also allow for the co-packaging of multiple latency reversing agents into single particles, and simultaneous delivery of latency reversing agents and antiretroviral drugs such as protease inhibitors to latently infected cells, which could serve as a safety feature that prevents the newly activated cell from producing infectious virions.⁴⁴

If a high level of virus expression is produced by latency reversing agents, then the host cell could be killed by viral cytopathic effects. However, cell damage induced by virus production might not be sufficient if latency reversing agents only induce low levels of viral protein production. The natural antiviral immune response might also be insufficient in these circumstances, particularly since the latent reservoir is rapidly populated by virus that is resistant to immunodominant CTL epitopes.^{14,15,45} Therefore, augmentation of cell killing by infusion of peptide stimulated CTL, which are specific for subdominant HIV epitopes,¹⁴ or CTL that have been genetically engineered with T cell receptors that are specific for conserved HIV epitopes,⁴⁶ might help in rapidly eliminating latently infected cells (Fig. 2). Natural killer cells could also serve a similar function but have not yet been thoroughly evaluated in relevant *in vivo* latency models.

HIV envelope proteins are expressed on the surface of productively infected cells. Therefore, another approach for rapidly killing latently infected cells that have been induced to express viral proteins by a “kick and kill” strategy would be to use broadly neutralizing antibodies that target HIV gp120 and can facilitate antibody-dependent cell mediated cytotoxicity, or immunotoxins that can bind to Env-expressing cells and directly kill them with the toxic moieties they carry.^{41,47} These targeted, augmented killing approaches are also attractive because if any ongoing virus replication does occur in ART-treated individuals, then chronically/productively infected cells could be eliminated at the same time as the latently infected cells that have been induced to express virus.

II. CONCLUSION

The challenges to developing an HIV cure are formidable. However, over the last several years there has been a growing sentiment in the field that a cure for HIV is possible. This has

been fueled by the (so far) unique case of the Berlin patient,¹⁸ but also additional cases where a shrinking of the latent reservoir by bone marrow transplants in two adults or through very early and intensive ART initiation in an infant each resulted in delayed viral rebound on stopping therapy.^{48,49} It is hoped that advances in therapeutic approaches for eliminating latent HIV described herein, coupled with the availability of relevant *in vivo* models for evaluating their effects latent HIV,^{50–52} will provide a pathway for the development of scalable and safe methods for eliminating persistent reservoirs of HIV in the future.

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ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
ART	antiretroviral therapy
CTL	cytotoxic T lymphocytes
HIV	human immunodeficiency virus

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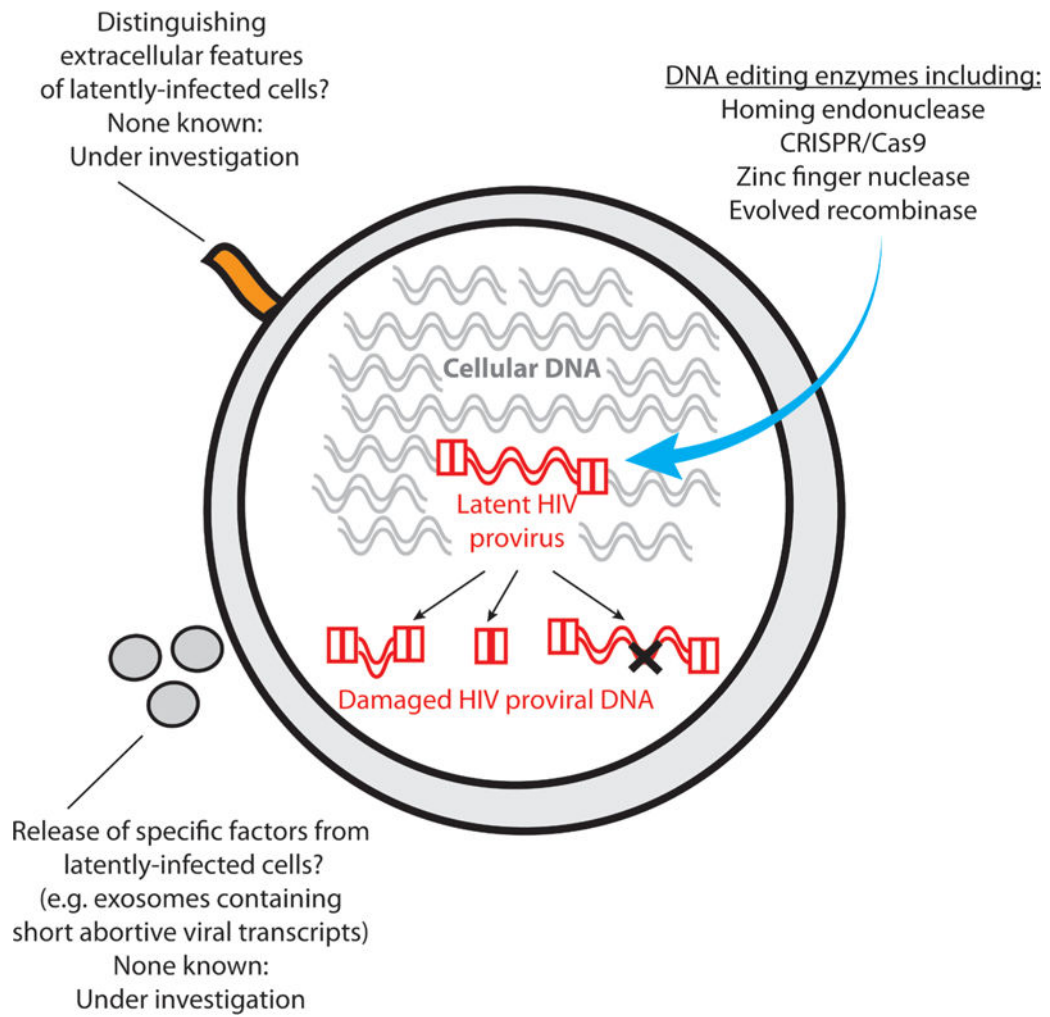


FIG. 1. Methods under investigation for eliminating latent HIV proviruses without first inducing HIV expression

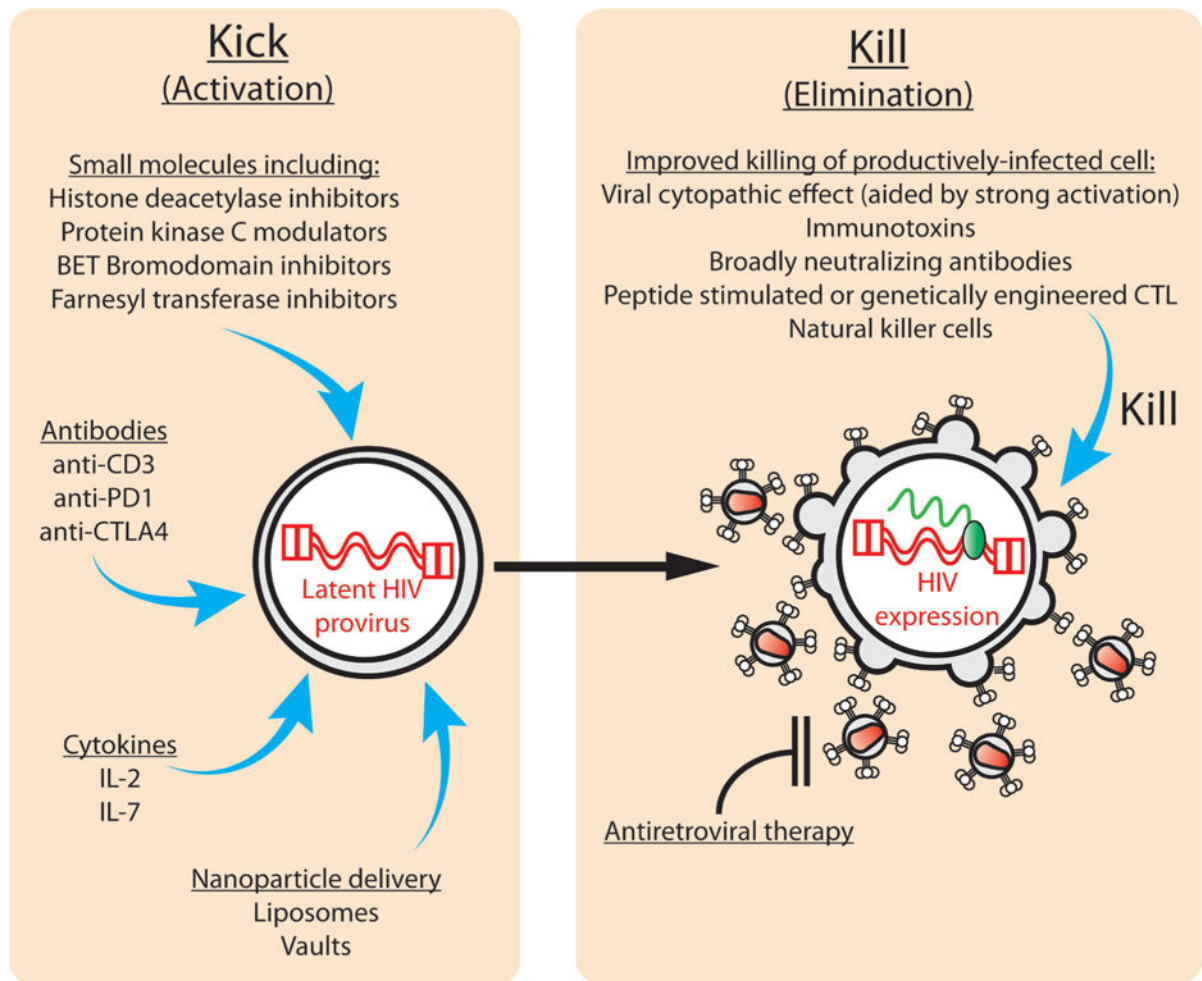


FIG. 2. “Kick and kill” approaches for inducing expression of latent HIV then killing of the virus expressing cell. In the left-hand panel several experimental approaches for inducing expression of latent HIV are shown. The right-hand panel lists possible approaches for accelerating the killing of cells induced to express viral proteins.