

Toward the Cure of HIV-1 Infection: Lessons Learned and Yet to be Learned as New Strategies are Developed

Jeffrey M. Jacobson^{1,2} and Kamel Khalili¹

¹Department of Neuroscience, Center for Translational AIDS Research; ²Department of Medicine, Lewis Katz School of Medicine, Temple University, Philadelphia, USA

Abstract

Here, we review the progress that has been made in achieving a cure of HIV-1 infection. To date, this has only occurred in one person after he received allogeneic stem cell transplants from a CCR5 Δ32 homozygous donor in addition to chemotherapy and radiation to treat his acute myelocytic leukemia. The general consensus is that achieving a sustained remission of infection in the absence of antiretroviral therapy will involve a combination of strategies that involve both the targeting of the latent proviral genome and the induction of more effective anti-HIV-1 immune responses. Efforts to reverse HIV-1 proviral DNA integration in the host cell genome and those to enhance anti-HIV immunity have been disappointing thus far. The lack of clinically validated assays to measure both effects has hampered the development of effective therapies. We suggest the consideration of genome editing as a new approach to reduce the latently integrated proviral genome. In addition, new approaches to therapeutic immunization, alterations of immuno-regulatory pathways, anti-HIV-1 antibodies, and anti-HIV-1 chimeric antigen receptor T lymphocytes are in development. (AIDS Rev. 2018;20:220-225)

Corresponding author: Jeffrey M. Jacobson, jeffrey.jacobson@temple.edu

Key words:

HIV cure. Gene therapy. Immune-based therapy. Therapeutic HIV vaccine. Anti-HIV antibodies. Latent HIV reservoir.

Introduction

More than 10 years have passed since the “Berlin Patient” was cured of his HIV-1 infection when he received allogeneic stem cell transplants from a CCR5 Δ32 homozygous donor in addition to chemotherapy and radiation to treat his acute myelocytic leukemia¹.

This event stimulated great hope and a massive research effort toward developing a more generalizable strategy for achieving a cure of HIV-1 infection. Much has been learned, but little therapeutic progress has been made. Here, we review the lessons learned and the challenges that lay ahead for the field, with new potential approaches that can be taken to advance our ability to eliminate active infection in an individual.

Correspondence to:

Jeffrey M. Jacobson

Department of Neuroscience, Center for Translational AIDS Research, Lewis Katz School of Medicine, Temple University 3500 N, Broad Street, Philadelphia, PA 19140, USA
E-mail: jeffrey.jacobson@temple.edu

Received in original form: 27/09/2018

Accepted in final form: 05/11/2018

DOI: 10.24875/AIDSRev.18000027

Background

In the life cycle of HIV-1, proviral DNA becomes integrated into the host cell genome, and these cells remain latently infected, even when viral replication is suppressed with antiretrovirals. When antiretroviral therapy (ART) is discontinued, viral replication resumes.

These latently infected cells, largely memory CD4 T lymphocytes, persist, perhaps for a life-time, as they were inherently designed to do². Although the CD4 T lymphocyte is the major “reservoir” of latent infection, other cells such as the monocyte/macrophage series may have some importance, particularly in the central nervous system³.

Anatomic considerations may be important in that certain tissues may be more impenetrable to immune responses and drugs. For example, CD8 cytolytic T lymphocytes do not have ready access to the B cell follicles of lymph nodes, where T follicular helper cells laden with latent virus reside⁴. Other possible sanctuaries include the brain, gastrointestinal lymphatic tissue, and genitourinary tract. Various antiretroviral drugs achieve lower levels in lymph nodes and other tissues than in blood⁵.

Definitions

To review definitions, two types of cure of HIV-1 infection have been envisioned. An eradication cure would entail the complete elimination of all replication-competent HIV-1 DNA and RNA in blood and tissues. This would be difficult to establish definitively because of diagnostic limitations detailed below. Short of making that determination, an achieved remission from HIV-1 disease, a functional cure, would be demonstrated by the sustained absence of viral replication, as represented by assays of plasma HIV-1 RNA, off ART. Conceivably, this could be accomplished without the complete elimination of replication-competent virus and would be aided, or even fully effected, by the induction of more effective immune responses to the virus. In this situation, an important consideration would be the potential continued risk for HIV-related clinical disease from the effects of residual immune activation and inflammation despite control of HIV-1 replication, as noted in natural “elite controllers” of HIV-1 infection⁶.

The cure experience

The possibility of curative treatment was energized by the case of the “Berlin patient” an HIV-infected individual who received stem cell transplants from a

CCR5 Δ32 homozygous donor after radiation and chemotherapy for his acute myelocytic leukemia. This patient has maintained undetectable virus in his blood and tissues for > 10 years after ART was stopped (although recently he has been taking antiretrovirals for pre-exposure prophylaxis to prevent a new infection). Importantly, the Berlin patient has manifested some degree of graft versus host disease. How much that has contributed to maintaining the apparent absence of active HIV-1 infection is unclear^{1,7}.

Two individuals in Boston with HIV infection and lymphoma, themselves heterozygous for the CCR5 Δ32 polymorphism, received stem cell transplants from donors without the CCR5 Δ32 polymorphism and had evidence of graft versus host disease. Continuing ART after the transplant, both individuals remained undetectable for HIV RNA and DNA in blood (rectal lymph tissue in one), had negative viral outgrowth assays, and lost HIV antibody seropositivity 4.3 years later in one individual and 2.6 years in the other⁸. In a similar Spanish experience reported recently, 5 of 6 recipients of CCR5 wild-type stem cell transplants who were followed while continuing to receive ART for at least 2 years were reported to have undetectable HIV DNA and RNA in blood, negative viral outgrowth assays, and undetectable virus in the cerebrospinal fluid, bone marrow, lymph nodes, and ileal biopsies, with one individual losing HIV-1 antibody. (The one transplant recipient with detectable virus received the more immature cord blood stem cells, was exposed to a more immunosuppressive conditioning regimen, did not develop graft vs. host disease, and did not achieve chimerism as early and completely as the others)⁹. Nevertheless, when ART was stopped in the Boston patients, viremia returned⁸.

Limitations of the laboratory assays

These cases illustrate the point that our current laboratory assays are not adequate for determining the loss of the latent HIV-1 reservoir. A major limitation of HIV-1 cure research is the absence of a clinically validated assay that reliably, with good reproducibility, measures the size of the latent cell reservoir; one that is comparable to the plasma HIV-1 RNA assays (“viral load”) that are available to measure active viral replication and have become the mainstay of the clinical and investigational assessment of the activity of disease and response to treatment. Assays measuring cell-associated viral DNA and RNA overestimate the size of the reservoir because most of what is measured are defective and non-replicating viral genetic elements. Assays measuring inducible virus or viral elements underestimate the size of the reservoir because

the efficiency of induction may not be complete. Recently developed whole genome assays have been employed to measure intact viral genomes as surrogates of replication-competent virus, but these assays have not been clinically validated yet¹⁰. Furthermore, ascertaining the complete elimination of replicative virus is limited by the ready, safe accessibility of all potential tissues that might harbor virus. Thus, a pause in ART remains the best currently available tool to assess whether the intervention being tested has done anything clinically meaningful to effect a cure, however defined. This pause should be monitored with blood antiretroviral drug testing to ensure the reliability of the virological findings.

Reducing the latent viral reservoir

For a clinically meaningful cure, does the elimination of replication-competent proviral genome need to be complete? Early treatment with ART soon after exposure, whether it be within hours of childbirth to an infected mother or within 1-2 days of sexual exposure, would seem to minimize the size of the proviral reservoir established, but viremia returns in most individuals when ART is discontinued^{11,12}. On the other hand, in individuals treated with long-term ART either soon after initial infection or during chronic infection, two studies found that post-treatment control of viremia when ART is stopped is associated with lower HIV DNA levels^{13,14}. Another study in persons treated during chronic infection found an association with lower cell-associated RNA, not with lower HIV DNA¹⁵.

Efforts to reverse integration of the HIV genome in latently infected cells of HIV-infected persons receiving ART have only attained a modest level of efficiency in clinical studies, not enough to affect the size of the pro-viral DNA pool when administered alone¹⁶⁻¹⁹. *Ex vivo* data suggest that a targeted immune response must be on the ready to eliminate latently infected cells when they are induced to express HIV antigens²⁰.

Unless the efficiency of latency reversal agents, alone or in combination, is improved, alternative strategies to reduce the latent pro-viral DNA burden will be needed. Several genome-based approaches are being explored. Promising candidates include those that ablate, permanently inactivate, or silence essential components of the integrated HIV-1 genome or host cell genetic elements critical for enabling HIV-1 infection of cells. In studies to date, autologous CD4 T lymphocytes or stem cells from HIV-infected persons are modified *ex vivo* to make these cells resistant to HIV-1 infection and subsequently reinfused into the same individuals with the goal of creating a population of protected cells, perhaps ultimately re-

placing those latently infected. The latter scenario would probably need an additional immunological strategy component that supports the targeted killing of the infected cells. In one uncontrolled clinical study of the *ex vivo* modification of CCR5 on CD4 T lymphocytes with zinc finger nuclease technology, total CD4 T lymphocyte counts increased and the CCR5 gene-modified cells persisted, declining at a slower rate than other CD4 T cells during an interruption of ART²¹.

Promising as this might be, the more efficient delivery of gene-modifying technologies would occur through direct *in vivo* administration utilizing viral vectors, nanoparticles, or other carrier constructs. This method would allow for widespread distribution to the cells and tissues to be targeted, albeit at greater risk for systemic toxicity. Replication incompetent lentiviral vectors are non-pathogenic and can efficiently deliver large amounts of genetic material that is stably expressed in targeted cells^{22,23}. The risk of immunogenicity is low. However, they can integrate into the host cell genome, and there is a theoretical risk of insertional mutagenesis. Non-integrating lentiviruses are available, but they are less efficient at gene delivery. Non-pathogenic adeno-associated viruses (AAVs) are non-integrating; the durable, stable gene expression occurs on episomes of targeted cells^{24,25}. An advantage of AAVs is that their carbohydrate-binding capsid sequences can be modified to optimize the desired cell type and tissue tropism as well as influence penetration across the blood-brain barrier. Unfortunately, anti-AAV immune responses readily occur after administration, and pre-existing immunity is not uncommon. Thus, additional administrations of a specific AAV vector strain may lose effectiveness. The use of multiple different AAV vector strains, natural and engineered, or the combination of AAV delivery with nanoparticle or another enhanced delivery technique, may allow for repeat administrations and enable the targeting of different cells and tissues.

The clustered regularly interspaced short palindromic repeats (CRISPR) technology is derived from a bacterial host defense system that consists of RNA complements of DNA sequences that contain elements of the bacteriophages that have infected the bacterium previously²⁶. The bacterium can then recognize DNA from a subsequent infection with a similar virus and use CRISPR-associated proteins, Cas, to recognize and cleave the newly invading viral DNA. The CRISPR-Cas complex can be adapted using specific "guide RNAs" (gRNAs) in a new potent technology to cleave a specific targeted site on a cell gene and disrupt the function of that gene or to introduce new genetic components at that site. Several laboratories have successfully employed this tech-

nology to introduce mutations in the non-coding HIV-1 long terminal repeat promoter regions as well as the coding sequences for various viral proteins, causing permanent inactivation of viral gene expression and replication in cell cultures and small animal models²⁷⁻³³. Simultaneous use of multiple gRNAs for targeting and editing various regions within the viral genome has led to the removal of large intervening segments of viral DNA and reduces the risk of mutant “escape” virus emerging³⁴⁻³⁶. Other safety concerns, including off-target effects and other potentially undesired changes in chromosome and cell homeostasis caused by the presence of CRISPR/Cas, need close attention in the design and implementation of this strategy for targeting the viral genome³⁷. As noted above, effective delivery of the CRISPR/Cas construct to the sites of virus latency presents another challenge under intense investigation³⁸. Recent studies have shown a widespread distribution of a non-integrating AAV vector in mouse models harboring the HIV-1 genome, with the efficient editing of the viral DNA in various sites including lymphoid organs^{31,39}. Nanoparticles and extracellular vesicles are alternative promising methods for targeted delivery of CRISPR/Cas^{38,40,41}. Regardless of the method of delivery, one important issue relates to the genetic variations seen in the patient-derived HIV-1 sequence and how that would affect the creation of sets of universal gRNAs for this therapeutic strategy. Indeed, as the technology advances, one may begin to personalize the strategy for the elimination of replication-competent viral quasispecies present in the patient⁴². Nevertheless, it remains to be seen whether the CRISPR/Cas technology, alone or in combination with other strategies, can eliminate replication-competent virus in chronically infected non-human primates (NHP) and humans.

Immunological strategies

Virus-targeting strategies are unlikely to completely eliminate all latent viral elements on their own. Potent immunological targeting of residual cells harboring latent virus will be needed to provide synergy and contain the potential re-emergence of replication-competent virus from hidden sanctuaries (Fig. 1). There is widespread belief that cells harboring latent viral genomes do not express viral antigens and avoid being subject to immune recognition and attack, but whether partial or complete expression of viral antigens occurs intermittently has not been fully explored. HIV-1 vaccines designed to improve HIV-specific immune responses in individuals already infected with HIV-1 have yet to demonstrate substantial potency at controlling viremia, although there have been some hints of activity⁴³.

Most therapeutic HIV-1 vaccines, whether protein-, peptide-, or DNA-based, have been based on consensus HIV-1 antigens. Newer strategies are exploring conserved epitopes that, during natural infection, elicit subdominant immune responses that are overshadowed by dominant immune responses to variable epitopes to which the virus readily escapes⁴⁴. Acknowledging the wide genetic variability of HIV-1 in the infected population, a study that provided pulsed exposure to autologous viral antigens with brief pauses of ART after long-term ART suppression of virus demonstrated evidence of containing viral replication during a subsequent longer analytical interruption of ART⁴⁵, but attempts to build on this finding with studies of autologous viral antigens presented on autologous dendritic cells have been disappointing⁴⁶⁻⁴⁸.

As with the laboratory assays measuring the replication-competent HIV-1 latent cell reservoir, there is no clinically reliable laboratory surrogate of improved host control of viral replication as a result of an immunological intervention that can predict the viral kinetics observed (compared to those in controls) during a subsequent antiretroviral drug interruption or measure an improved immunological effect at enhancing elimination of the latent cell reservoir. There is an urgent need to develop and clinically validate such assays.

Using antigen-specific single B-cell sorting techniques with subsequent cloning of the antibody gene, a newer generation of more potent broadly active neutralizing antibodies that target conserved neutralization-sensitive regions of the HIV-1 envelope spike has been generated from infected persons. The relevant epitopes identified reside in the CD4 binding site, the V1/V2 loop, the base of the V3 loop, the membrane proximal external region of gp41, the gp120/gp41 interface, and HIV-1 envelope glycans⁴⁹. Initial clinical studies have demonstrated persistent antiviral suppressive activity when substituted as maintenance therapy in HIV-infected individuals receiving ART^{50,51}. Due to some degree of baseline and treatment-emergent resistance, these antibodies need to be given in combination. To effect a cure of HIV-1 infection by targeting latently infected cells that are induced to present HIV-1 antigens, it will probably be necessary to engineer the Fc activities of these antibodies or other antibodies to engage natural killer cell or phagocytic cell functions⁵². With the same goal, other bispecific and trispecific antibodies are being designed to combine anti-HIV envelope specificity with cytolytic cell binding specificity⁵³. Finally, genetically modified chimeric antigen receptor (CAR) CD8 T lymphocytes are being engineered with MHC-independent receptors capable of binding HIV-1 envelope such as CD4 or anti-envelope antibodies. Coupled to an intracellular signaling mole-

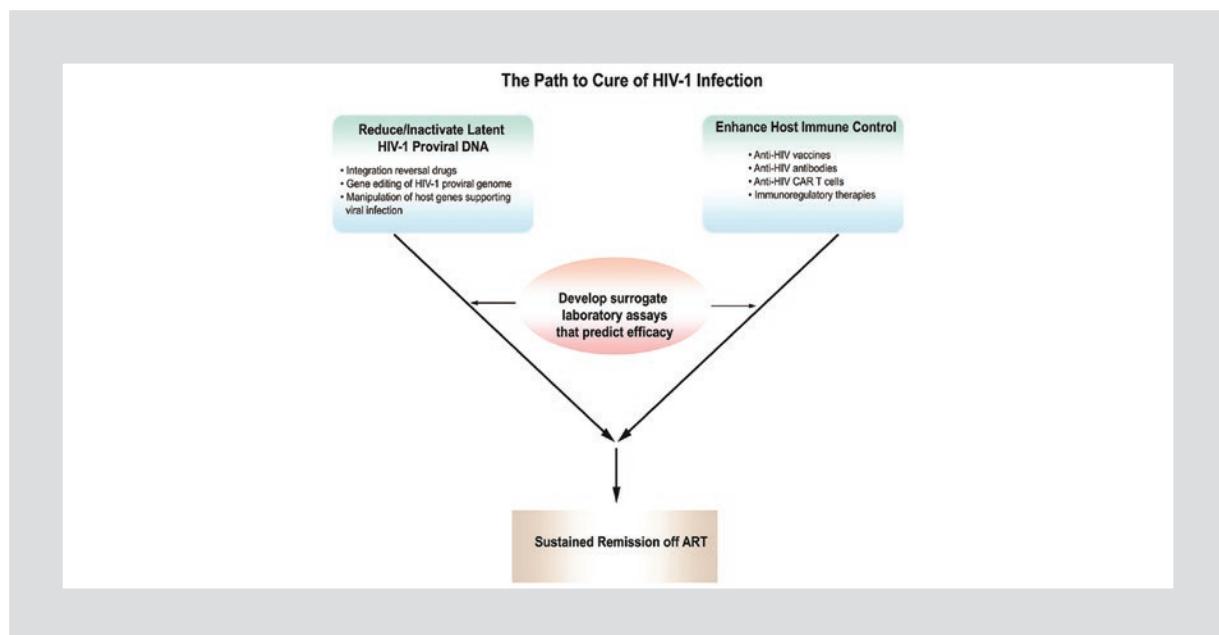


Figure 1. Illustration of the combined strategy of reducing the latent proviral reservoir and/or eliminating proviral DNA by gene editing together with enhancing immune activity against HIV-1 to achieve a sustained remission of active infection off antiretroviral therapy (ART). The several approaches to target the latent proviral genome, as well as measures to improve anti-HIV-1 immune function, are listed. To expedite the development of both types of cure strategies, laboratory assays that measure the size of the latent proviral reservoir and assays that measure anti-HIV host immune responses proven to contribute to reducing the latent proviral reservoir and/or to controlling viral replication in the absence of ART need to be developed and clinically validated as predictive of sustained remission of active infection.

cycle activated on binding, these cytolytic cells would serve to target HIV-1 antigen expressing cells⁵⁴.

An anti- $\alpha 4\beta 7$ antibody, an inhibitor of cell trafficking to the gastrointestinal tract, induced long-term remission of SIV infection off ART in a NHP model⁵⁵, but these promising results were not repeated in follow-up NHP and human studies^{56,57}.

Finally, an alternative immunological strategy that might reduce the size of the latently infected cell reservoir and contribute to a cure of HIV-1 infection involves the reversal of immune cell exhaustion and the restimulation of effective HIV-directed responses with checkpoint inhibitors and anti-regulatory T-cell therapies⁵⁸. These broadly active techniques risk off-target adverse autoimmune reactions in individuals otherwise faring well on suppressive ART⁵⁹.

Conclusions

A much greater understanding of both the promise and the difficulties of the path toward curing HIV-1 infection has become apparent. Much has yet to be learned. From the current vantage point, it would appear that the most effective strategy is likely to combine the genetic inactivation of latent viral genomes with efficient HIV-directed immune attack. That said, if a cure of HIV-1 infection is

achieved, questions will remain as to the residual immune damage left in its wake and its reversibility^{60,61}.

Acknowledgments

Both authors contributed to the writing of the manuscript. This work was made possible by the Comprehensive NeuroAIDS Center grant (P30MH092177) awarded by NIH to KK.

Conflicts of interest

KK is named on patents related to viral gene editing. KK is a co-founder, board member, scientific advisor, and holds equity in Excision Biotherapeutics, a biotech start-up. JMJ is currently receiving a clinical protocol development grant from Excision Biotherapeutics. The authors declare that this work was produced solely by the authors and that no other individuals or entities influenced any aspect of the work.

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