

Eradication of HIV: current challenges and new directions

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Highly active antiretroviral therapy (HAART) can potently suppress human immunodeficiency virus (HIV) replication and prevent progression to AIDS. However, HAART does not cure infected patients. Instead, HIV persists in latently infected CD4+ T cells and various cryptic cellular reservoirs. Hence, under current therapy regimens, patients must continue taking HAART for the remainder of their lives. Eliminating residual replication-competent virus is critical if eradication of HIV is to be achieved. While this challenge is formidable, we describe here a number of innovative approaches intended to further deplete HIV in HAART-treated patients. New antiretroviral drugs that target different viral proteins and stages of the virus life cycle, compounds that enhance anti-HIV immune responses and novel gene therapy approaches may each play a role in improving long-term suppression of the virus. Moreover, methods for more specifically and efficiently inducing HIV from latency and eliminating the newly activated host cells are also under development.

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Introduction

Human immunodeficiency virus (HIV) establishes a persistent, lifelong infection, which if left untreated almost invariably leads to acquired immunodeficiency syndrome (AIDS) and death of the infected individual. Over the past 25 years, significant advances have been made in the development of antiretroviral agents that can potently suppress viral replication and prevent progression to AIDS. However, the drugs used in combination during highly active antiretroviral therapy (HAART) are associated with significant problems, including toxic side effects, high pill burden, development of virological resistance and great financial expense. More importantly, HAART does not completely eliminate HIV from the body, meaning that if treatment is stopped, residual virus present in several potential reservoirs rapidly expands allowing disease progression to continue.^{1,2} Understanding the sources of replication-competent HIV that persist during therapy and developing methods to purge these viral reservoirs have therefore become important areas of research focus.

Mechanisms of HIV persistence during HAART

Perhaps, the most well-defined viral depository during therapy is the 'latent reservoir' within memory CD4+ T cells.^{3,4} It is

believed that HIV latency is generally established when an activated CD4+ T cell becomes infected by HIV but transitions to a memory cell before it can be killed by the virus. This transition is associated with a number of cellular changes, including a reduction in the levels of several transcription and other factors that are required by HIV for its replication. Because memory cells are by nature very long-lived, the host cell can then persist for decades with its silent viral cargo before receiving a stimulatory signal that activates the cell and concomitantly induces virus production from the latent HIV genome. Latently infected cells are rare, representing around one per million CD4+ T cells, which translates to a total of ~1 million latently infected cells in the body as a whole.⁵ These cells also decay very slowly during HAART, with an average half-life of ~44 months. It is therefore estimated that under current antiretroviral regimens, complete depletion of this reservoir would take over 60 years,⁴ even if there were no further viral replication or replenishment of the reservoir during therapy.

The situation is further complicated because in addition to the latent reservoir, other cryptic viral sources exist during HAART, including a very small amount of active virus replication that occurs even during therapy.⁶ It is not clear whether this is due to poor antiretroviral drug penetration into the sites of virus replication or because of residual replication that occurs even under 'optimal' drug concentrations. Even more enigmatic

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sources of virus have also been identified in some patients, although their precise cellular origin is unclear.^{7,8}

The issue of latency and these other obstacles have led some researchers to voice uncertainty over whether HIV infection can ever be cured. However, a number of strategies are currently being investigated that, if successful, may ultimately aid in the durable, lifelong suppression of HIV, or perhaps even the elimination of the virus altogether.

Improving long-term virus suppression

In order to cure an HIV-infected individual, all sources of replication-competent HIV must be eliminated. Achieving this goal may require a combination of different approaches, reflecting the variety of reservoirs that exist in infected patients. With this in mind, perhaps the most tractable hurdle is the persistent replication that occurs during HAART. It is possible, for example, that residual replication could be further suppressed simply by strengthening standard therapy regimens. This may be achieved by enhancing currently available antiretrovirals, either by improving bioavailability and pharmacokinetic properties of current drugs or by developing new ones that target different viral proteins and stages of the virus life cycle. Recent examples of newly approved antiretrovirals are the entry inhibitor maraviroc (UK-427857)⁹ and the integrase inhibitor raltegravir (MK-0518).¹⁰ The field is eagerly awaiting the results of studies investigating whether addition of these or other novel agents^{11,12} to current HAART regimens will further suppress viraemia, which would indicate that residual replication can indeed be inhibited by intensification of HAART. One interesting observation in this area is that raltegravir treatment appears to suppress plasma viral loads in infected patients more rapidly than treatment with other antiretrovirals.¹³ While the direct cause of this phenomenon is not clear, it does demonstrate that antiretrovirals with distinct modes of action can influence viral replication in different ways and may thus be differentially affecting the underlying HIV cellular reservoirs.

Other approaches for reducing viraemia include enhancing adaptive anti-HIV immune responses, which are arguably sub-optimal from the beginning of infection and may have further waned because of lack of sufficient antigen exposure during HAART. Anti-HIV vaccine development has proved challenging, and induction of sterilizing immunity via a vaccine is unlikely to be achieved in the near future. However, immunization may still yield benefits if therapeutic vaccines can be improved to the point where administration to infected individuals boosts immune responses and inhibits virus spread beyond that achievable with HAART alone. Alternatively, more efficient post-exposure vaccines may suppress viraemia sufficiently for HAART to be needed only periodically or not at all, thereby reducing the long-term side effects of therapy. Significant advances in both prophylactic and therapeutic vaccine development are likely to be closely associated with improvements in our basic understanding of HIV immunology, and in particular our understanding of what constitutes a truly effective anti-HIV immune response.

Gene therapy may also prove beneficial for HIV-infected patients. A strategy that is being actively pursued in this area is to develop methods that provide patients with immune cells that are resistant to infection with HIV. For example, our laboratory

has been involved in a recently completed Phase 1 clinical trial involving introduction of an anti-HIV ribozyme into autologous haematopoietic stem cells in HIV-infected patients,¹⁴ and a Phase 2 trial with the same ribozyme is currently underway. Various other approaches using either individual or multiple anti-HIV genes with different viral or cellular targets are also being developed.^{15–18} An exciting advancement in this area involves improvements in human embryonic stem cell (hESC) technology. Unlike haematopoietic stem cells, hESCs are exceptionally amenable to genetic modification, expansion *in vitro* and differentiation into a wide range of cellular lineages. As such, they may be useful for anti-HIV gene therapy. For example, if hESCs were to be genetically modified to include an anti-HIV gene then differentiated to the haematopoietic stem cell stage *in vitro*, they could be re-introduced to patients where HIV-resistant T cells and macrophages would be produced. While this approach may be years from the clinic, the ability to produce viable genetically modified T cells¹⁹ and macrophages²⁰ originally derived from hESCs has recently been established. The concept that cells with stem-cell-like properties [induced pluripotent stem (iPS) cells] can be derived from mature human cell types^{21–24} provides the additional advantage of generating patient-specific cells, which theoretically could be used in autologous transplant scenarios without the fear of immune rejection. Clearly, the safety and efficacy of therapies based on hESCs/iPS cells must be thoroughly scrutinized before these approaches can be advanced to clinical trials.

Potential approaches for eliminating HIV reservoirs

Given our current understanding of the latent reservoir, it is difficult to envisage a strategy for eradicating HIV that does not contain a component intended to specifically target latently infected cells. Most proposed strategies involve activating these cells in some way to induce expression from the HIV genome. The cell would then be killed either by viral cytopathic effects or by immune effector mechanisms, and virus spread would be prevented by maintenance of HAART throughout the stimulation period. Stimulants that have proved effective in activating HIV from latency in these cells include cytokines such as interleukin (IL)-2²⁵ and IL-7.²⁶ Other molecules, such as the non-tumour-inducing phorbol ester prostratin,²⁷ histone deacetylase inhibitors like valproic acid²⁸ and certain modulators of cellular microRNAs,²⁹ are also capable of activating a latent provirus. Unfortunately, none of the strategies that has been tested so far is capable of purging all latent virus, and those techniques that lead to the most robust viral activation are also associated with inducing undesirable generalized immune activation. These limitations highlight the need for further research into the molecular mechanisms associated with activation of HIV from latency. The development of *in vitro* primary cell models for latent HIV^{30,31} may provide the opportunity to identify small molecules that could improve the ability to activate and then purge these viral reservoirs. Along these lines, the recently reported³² chemical synthesis of prostratin and its analogues is promising because it should facilitate the development of phorbol esters that activate latent HIV with greater specificity and efficacy than naturally available compounds.

Methods for enhancing killing of recently activated HIV-infected cells are also under investigation. One example of

this is the use of immunotoxins. These are molecules composed of a targeting domain derived from a monoclonal antibody linked to a toxic moiety. An immunotoxin specifically targeting cells expressing the HIV envelope protein has been used to deplete both latently infected T cells³³ and infected macrophages³⁴ after up-regulation of HIV gene expression with stimulants. ‘Activation-elimination’ strategies such as this may therefore accelerate clearance of HIV from its various cellular reservoirs. Moreover, if this type of approach were used in conjunction with post-exposure vaccination or genetically modified stem cell immune reconstitution strategies, it might prove even more effective in decreasing or eliminating latent and persistent viral reservoirs.

While many of the approaches outlined above are still at the developmental stage and are not without limitations, it is hoped that some of these nascent strategies will be rapidly advanced to the point that they can provide benefits to patients.

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