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Title: Towards a cure for Human Immunodeficiency Virus

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Abstract:

Modern antiretroviral therapy (ART) confers a near-normal life expectancy to people living with Human Immunodeficiency Virus (HIV) and prevents its transmission. However, life-long treatment is required and there is no cure. A global concerted effort is underway to find a cure for HIV or a method to allow individuals to cease ART safely. We review current understanding of the major barriers to HIV cure and the strategies for cure being evaluated in animal models and clinical trials.

Key words:

HIV
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Towards a cure for Human Immunodeficiency Virus

The past three decades have seen spectacular advances in the management of Human Immunodeficiency Virus (HIV) infection. Modern antiretroviral therapy (ART) effectively suppresses the virus to undetectable levels in blood allowing for immune recovery and near-normal life expectancy for most people living with HIV[1]. Additionally, it is now clear that commencing ART immediately at any CD4+ T cell count reduces the risk of morbidity and mortality[2] and that HIV-infected individuals with an undetectable viral load on ART are unable to transmit virus sexually[3]. This has led to a global initiative to treat all people living with HIV upon diagnosis and for ART to complement other strategies to prevent new HIV infections[4]. However, in order ultimately to eliminate HIV at a population level, we will still need an effective prophylactic vaccine.

Despite the success of ART, if ART is ceased, virus returns rapidly in blood, usually within weeks. Treatment must therefore be continued life-long. There are now 19.5 million people living with HIV on ART but this accounts for only 53% of the world's 36.7 million people living with HIV. In Australia, uptake of ART is approaching 90%, one of the highest rates of treatment uptake globally. The estimated cost of achieving similarly high levels of treatment worldwide by 2020 is US$20 billion per year[4]. In many parts of the world, stigma, discrimination and punitive laws remain significant problems for people living with HIV. Therefore, lifelong treatment for HIV comes at a great economic and personal cost and there is now an accelerated global effort to find either a cure or a safe way for individuals to cease ART such that the virus stays at low levels[5]. This is also known as HIV remission.

ART works by interrupting viral replication within activated CD4+ T cells, the main target of HIV. However, in resting CD4+ T cells, HIV integrates into human DNA where it is silenced and cannot complete the viral replication cycle. This is known as latent infection and this integrated virus cannot be targeted by ART or the immune system. HIV latency is established within the first few days of infection and persists indefinitely due to the long life-span of infected memory CD4+ T cells and proliferation of these cells[6]. Although infrequent at one in 100,000 cells, these latently infected cells contain infectious virus that can reactivate upon ART cessation. In most people, virus will reactivate within 2-3 weeks of stopping ART[7].

The earlier ART is commenced, the fewer latently infected cells and the more intact an individual's immune system[8]. Interestingly, it is now recognised that a small proportion of individuals who commence ART during acute infection and then stop years later are able to maintain low to undetectable levels of virus for prolonged periods[9, 10]. These 'post-treatment controllers' are quite distinct from individuals who can naturally control virus without ART, called 'elite controllers'. Elite control occurs in <1% of people living with HIV while the frequency of post-treatment control may be as high as 5-15%[9]. Post-treatment control has also been demonstrated in children infected at birth who start ART within days to months[11, 12] or even several years[13]. Despite these encouraging but rare case reports, lifelong ART remains the recommendation for all HIV-infected individuals outside of clinical studies.

There are multiple approaches that are currently being evaluated to allow people to stop ART safely and achieve remission. These approaches can be broadly divided into four categories: bone marrow transplantation and gene editing, latency activation, latency silencing and immunomodulation. The common aims are to reduce the amount of HIV that persists on ART and/or to boost HIV-specific

immunity. Interventional studies in this area are performed in HIV-infected individuals, simian immunodeficiency virus (SIV)-infected macaques and HIV-infected humanised mice. The latter are highly immunodeficient mice which have been given a human haematopoietic stem cell transplant (HSCT) at birth and several months later infected with HIV. ART controls virus replication in all of these settings and therefore interventions to stop ART safely can be evaluated.

The gold standard test to determine if an intervention has worked is to stop ART with regular viral load monitoring. The primary end point for these studies is the time until virus becomes detectable and/or the frequency of achieving a low viral load steady state, often defined as <50 copies/ml. Many of the earlier studies of cure strategies have not stopped ART but have instead evaluated the effects of an intervention on the frequency of HIV-infected cells measured by polymerase chain reaction (PCR) quantification of HIV DNA or the frequency of infectious genomes[14, 15]. Unfortunately these parameters do not predict viral rebound off ART accurately; however, with frequent monitoring, stopping ART is considered to be safe and viral control off ART is now a frequent endpoint to these studies.

Bone marrow transplantation and gene editing

Only one person is known to have been completely cured of HIV. Timothy Brown, a US citizen living in Berlin with HIV suppressed on ART, required a HSCT in 2007 for acute myeloid leukaemia (AML)[16]. The stem cell donor was homozygous for the Δ32 mutation in CCR5, the chemokine receptor required for HIV entry into the cell. Homozygosity for this mutation means that cells are resistant to HIV infection.

Timothy ceased ART upon transplantation and has since remained off ART without any evidence of residual virus 10 years later despite extensive tissue sampling[16].

Multiple factors may have contributed to Timothy Brown's cure including myeloablative conditioning, graft-versus-host disease (which may also have had a graft-versus-virus effect) or a second transplant following AML relapse. Unfortunately, despite multiple attempts, no other recipient of a HSCT from a donor homozygous for the CCR5 Δ32 mutation has had long-term HIV-free survival. This has mostly been due to early fatal complications of the HSCT rather than HIV relapse[17]. Allogeneic HSCT from donors expressing wild-type CCR5 have been associated with marked depletion of residual HIV DNA to below detectable limits[18, 19]; however, in all such HSCT recipients to date, viral rebound has occurred post treatment interruption, albeit significantly delayed in some individuals[18]. Clearly HSCT has unacceptably high risks for a person living with HIV on ART who has a near-normal life expectancy. Therefore transplantation is only suitable for individuals who require it for another indication.

The case of Timothy Brown is proof of concept that HIV cure is possible and has spurred efforts to 'knock out' the CCR5 gene from HIV-infected individuals' haematopoietic stem cells using gene editing technologies such as CRISPR-Cas9 or zinc finger nucleases (ZFN). Clinical trials using infusions of autologous peripheral CD4+ T cells which have had CCR5 gene editing *ex vivo* using ZFN were shown to be safe in HIV-infected individuals on ART and 13.9% of circulating CD4+ T cells were gene modified[20]. This approach is unlikely to work for the small subset of individuals infected with HIV that uses CXCR4, another chemokine receptor that HIV can use to enter a cell. Work continues on nanoparticle or viral vectors for *in vivo* delivery of gene editing enzymes.

Latency activation

The most widely studied approach to HIV cure has been to activate latent HIV, often called 'shock and kill'. This approach attempts to force transcription and protein expression from an integrated virus in latently infected cells so that the immune system can recognise these cells as infected and kill them. ART is continued throughout to prevent infection of neighbouring cells.

Histone deacetylase inhibitors, licensed for use in haematologic malignancy, have been extensively evaluated as a strategy to activate latency. These drugs work by promoting a more open DNA structure which facilitates transcription of HIV DNA to RNA. Whilst these drugs have been shown in HIV-infected individuals on ART to be safe and to increase HIV RNA both inside cells and in plasma, they have not been shown to deplete residual HIV DNA[14]. It seems that forcing transcription of virus is not enough and a stimulus to help the immune system kill the expressed virus may also be required.

Other agents being evaluated for latency activation include drugs that modify chromatin through changes in DNA methylation. Most promising and least toxic of the latency activation drugs appear to be toll like receptor (TLR) agonists that activate latency by increasing interferon production. TLR7 and TLR9 agonists have recently been shown *in vivo* and *ex vivo* to activate latent infection and to enhance CD8+ T cell and NK cell activity[21, 22]. Therefore, TLR agonists may potentially both 'shock' and 'kill'.

Latency silencing

The opposite approach to activating latent infection is to induce a state of deep latency or put the virus to sleep permanently. This has been termed 'block and lock'. Didehydro-cortistatin A, a novel drug derived from a marine sponge, can induce a state of deep latency through changes in DNA structure both *in vitro* and in the humanised mouse model, delaying viral rebound off ART, but clinical trials in humans have not yet begun[23]. Gene therapy approaches could also be used to permanently silence HIV RNA using small interfering RNA (siRNA)[24].

Immunomodulation

Targeting the host immune system and not just the virus is also an active area of investigation. Therapeutic vaccination with constructs that boost HIV-specific T cell function in infected individuals has recently shown promise. Studies in a macaque model show that using a vaccine made from an adenovirus together with a TLR7 agonist could induce post-treatment control after ART cessation[25]. A similar strategy is now being tested in HIV-infected individuals (ClinicalTrials.gov Identifier NCT02616874).

Another promising area of therapeutic vaccination is the passive administration of antibodies that recognise multiple virus strains, known as 'broadly neutralising antibodies' (bNAbs). There are now many highly effective bNAbs that not only block viral infection of cells but also deplete infected cells and may also boost HIV-specific T cell function. Administration of bNAbs to HIV-infected individuals on ART has led to significant delays in viral rebound following ART interruption[7]. The challenge now is

to increase the half-life of these antibodies or find a strategy that will not require passive administration.

Immunomodulation can also be targeted at immune processes that are not HIV specific. These include (1) reducing chronic inflammation to improve immune responses, (2) altering migration of immune cells to tissue sites such as gut and lymph node where there is a high frequency of infected cells and (3) boosting T cell function through reversal of T cell exhaustion.

This rapidly expanding field has demonstrated some notable results in animal models that require further evaluation in humans. Interleukin 21 and anti-interferon α/β receptor antibody are examples of agents which have been shown to reduce inflammation and concomitantly deplete the viral reservoir and reduce or delay viral rebound upon ART interruption[26, 27].

$\alpha_4\beta_7$ integrin is a cell surface marker directing immune cells to the gut. A monoclonal antibody against $\alpha_4\beta_7$ integrin given to ART-suppressed SIV-infected macaques has induced post-treatment control for at least two years following cessation of both ART and antibody[28]. Although the exact mechanism of post-treatment control is not understood, there is some evidence to suggest that this is mediated by natural killer cell and humoral immunity. A single arm study using an anti-human $\alpha_4\beta_7$ monoclonal antibody, vedolizumab, licensed for use in inflammatory bowel disease, is currently underway to determine whether this finding can be replicated in HIV-infected humans on ART (ClinicalTrials.gov Identifier NCT02788175).

Finally, the recent substantial improvements in cancer outcomes with immunotherapy may also provide a promising approach in eliminating HIV persistence and inducing post-treatment control. Immune checkpoint blockers such

as anti-PD-1 and anti-CTLA-4 monoclonal antibodies, licenced for use in melanoma and other solid malignancies, boost cancer-specific T cells but can also boost HIV-specific T cells *ex vivo* and SIV-specific T cells in macaque models[29]. Furthermore, in individuals on ART, HIV is concentrated in CD4+ T cells expressing these receptors. Blocking these receptors may potentially increase HIV expression and relieve the block on CD8+ cytotoxic T cell function. Clinical trials evaluating the effect of these antibodies on HIV in the context of HIV-infected individuals requiring immune checkpoint blockade for malignancy have now begun (reviewed by Wykes and Lewin[29]).

Conclusion

There have been substantial advances in our understanding of how HIV persists on ART and in testing novel approaches to achieve HIV remission off ART. A wide variety of HIV cure strategies are being evaluated and there is a long list of human clinical studies in the pipeline. However, given that modern ART regimens are simple, non-toxic and confer a near-normal life expectancy and inability to transmit virus, a cure intervention must be both safe and highly effective. An optimal cure regimen would also need to be cheap, stable at room temperature and available orally to facilitate scale-up to developing countries where the epidemic is the worst. Thus the bar is set very high.

Unimaginable advances have been made in HIV treatment and prevention over the past three decades and the hope now is that similar great advances will be made towards achieving a cure. This will need significant funding, investment from the

private sector, widespread academic collaboration and most importantly community engagement at every level.

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