Challenges, progress and strategies in the search for a cure for HIV

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The past three decades has seen a major transformation in the understanding and management of HIV infection. Effective combination antiretroviral therapy (cART) has transformed HIV from a universal death sentence to a chronic manageable disease. Current cART regimens are simpler - often only a single daily (combination) pill, but treatment must be taken life-long. Life expectancy of an HIV-infected person who receives effective cART, is now similar to a person without HIV. The cost of long-term treatment is significant. There is now intense scientific interest in finding a cure for HIV infection or a way to allow patients to safely stop cART and remain healthy with the virus under control. A cure for HIV could either be a ‘sterilising cure’ – where there is no evidence of persistent HIV infection or a ‘functional cure’ where HIV is still present at low levels and health is maintained in the absence of cART.

Why is curing HIV so difficult?

HIV replicates at high levels in activated CD4+ T-cells. In the absence of treatment, there is a slow and gradual loss of CD4+ T-cells from blood and tissue and associated immune dysfunction. cART effectively stops active virus replication and within just weeks of starting treatment, there is a rapid decline in HIV RNA in blood to undetectable levels (<20 copies/ml). However, in nearly all patients, HIV RNA rebounds rapidly when treatment is stopped, usually within 2–3 weeks. HIV can persist in patients on suppressive ART as a result of long lived latently infected cells, low level virus replication in some patients and anatomical reservoirs. Definitions of each of these terms are shown in Table 1 (adapted from Eisele and Siliciano).

HIV latency and factors that regulate virus expression

HIV, similar to other retroviruses is able to establish latent infection in resting CD4+ T-cells. Latency occurs when HIV enters a cell and integrates into the host cellular DNA but there is absence of virus production. However, when given the appropriate stimuli, infectious virus can re-emerge. Latency primarily occurs in long-lived central memory and transitional memory T-cells and less frequently in naive T-cells and cells of other lineages including monocytes, macrophages and astrocytes. Latently infected cells can also be maintained through homeostatic proliferation and recently, latency has also been demonstrated in memory stem cells which although infrequent, have the capacity to undergo self-renewal and expand over time on ART. Latently infected resting CD4+ T-cells in patients on cART are estimated to occur at a frequency of 60 per million CD4+ T-cells and there is likely to be some inter-patient variability. Unfortunately there are currently no phenotypic markers of a latently infected cell in vivo and this is a top priority for research currently.

Latent HIV infection in CD4+ T-cells is established early in HIV infection and is seen even in patients who commenced cART within the first week of HIV infection. Latency can be established in CD4+ T-cells via two pathways: first, when the virus infects an activated CD4+ T-cell and the infected cell survives and reverts to a resting memory T-cell carrying an integrated provirus. This is referred to as post-activation latency. Second, via direct infection of resting CD4+ T-cells, which in vitro requires additional stimuli such as chemokines, dendritic cell (DC) contact or a high level of infecting virus and spinoculation, termed pre-activation latency. The relative contribution of pre- and post-activation latency in vivo is unknown.
Once the virus integrates into the host genome, latency is maintained via multiple molecular mechanisms. These include transcriptional interference due to the site and orientation of the provirus in the cell chromosome; epigenetic silencing by post-transcriptional modifications of the histone tails thereby modulating the chromatin structure; absence of nuclear host transcription activators required for HIV expression (e.g. Nuclear factor-kB (NF-kB)); presence of nuclear transcription repressors; inefficient elongation of HIV transcripts related to absence of Tat protein; nuclear retention of multiply spliced RNA; and impaired translation of virus transcripts due to short-interfering RNAs (siRNA) and microRNAs (reviewed in Coiras et al.).

**Residual virus replication**

Whether there is residual virus replication (i.e. new rounds of HIV infection) in patients on cART remains controversial. Traditional methods of measuring sequence evolution over time suggest there is minimal change in plasma virus or cell-associated HIV DNA in patients on suppressive cART arguing that there is minimal virus replication. In addition, development of drug resistance is not observed in patients on cART arguing against any replication. However, recent evidence suggest that drug penetration in tissue sites such as the gastrointestinal tract and lymphoid tissue is sub-optimal in patients on cART which could potentially favour some residual replication in these tissue sites. Two randomised studies of patients on suppressive cART who intensified their cART regimen with the addition of an integrase inhibitor, raltegravir demonstrated that residual replication occurred in roughly 30% of patients. The persistent detection of low level viraemia at 1–3 copies/mL in nearly all patients on cART may represent either release of virus from long-lived latency infected cells or residual virus replication. The former is more likely given the relative homogeneity of genetic sequences seen in low-level viraemia over time.

**Anatomical reservoirs**

HIV can also persist in distinct anatomical sites – due to long-lived latency infected cells or residual virus replication. Best examples of this are studies of the central nervous system (CNS). Patients well-suppressed on cART may rarely have detectable HIV RNA in the cerebrospinal fluid (CSF) and the genetic resistance patterns in these compartments may also differ. Within the CNS, long-lived latency infected cells such as microglial cells and astrocytes have also been detected. Other anatomical reservoirs such as gastrointestinal tract and lymphoid tissue are likely to also be important.

**Is a cure possible?**

Over the last few years, several cases of HIV cure have been reported. The only case of a sterilising cure is Timothy Brown, also known as the ‘Berlin patient’. Timothy Brown received two allogeneic stem cell transplants for acute myeloid leukaemia from a donor with a homozygous CCR5 delta32 deletion. CCR5 is a co-receptor for HIV and is required for HIV to enter a cell. In the absence of CCR5, a T-cell is resistant to infection with CCR5-using strains of HIV. Remarkably, Mr Brown remains cured of HIV six years after cessation of cART with no detectable infectious virus in blood or tissue.

The other cases of HIV cure are all cases of functional cure – or long-term control in the absence of cART. These occur largely in the setting of early cART treatment – perhaps early enough to limit the number of latency infected cells and or to preserve an adequate HIV-specific T-cell response. Indeed, a group of 14 post-treatment controllers (called the VISCONTI cohort) demonstrated controlled viraemia for a median of 89 months after cART interruption, which was initiated during primary infection. Other studies of treatment interruption following initiation of cART in acute infection have found that post-treatment control varies from 2–15%.

The variation is most likely related to timing of initiation of cART, duration of cART and other still unknown factors.

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**Table 1. Definitions of commonly used terms in HIV cure research. Adapted from Eisele and Siliciano.**

<table>
<thead>
<tr>
<th>Latency</th>
<th>A reversible but non-productive infection. Latently infected cells do not produce infectious virus but retain the capacity to do so</th>
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<tr>
<td>Post integration latency</td>
<td>A latently infected cell that contains an integrated virus that persists for the life of that cell</td>
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<tr>
<td>Reservoir</td>
<td>An infected cell population that can persist in HIV-infected patients on cART. A reservoir can be cellular (e.g. resting memory T-cells or macrophages) or anatomical (e.g. the central nervous system, testis or lymphoid tissue)</td>
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<tr>
<td>Compartment</td>
<td>An anatomical site where there is limited exchange of viral genetic information with other sites</td>
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<tr>
<td>Sanctuary</td>
<td>An anatomical site with sub-optimal levels of antiretroviral therapy compared to blood</td>
</tr>
<tr>
<td>Cure – sterilising</td>
<td>Elimination of all HIV-infected cells and infectious virus from an individual.</td>
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<tr>
<td>Cure – functional</td>
<td>Long term health in the absence of cART. HIV RNA is present but at low levels (&lt;50 copies/mL)</td>
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The ‘Mississippi baby’ born to an HIV-infected woman who did not receive pre-natal care was commenced on cART at 30 hours of birth, but discontinued cART at 18 months. Twenty-four months after cessation of ART, neither HIV RNA nor HIV antibodies have been detected. Residual HIV DNA has since been detected in both CD4+ T-cells and monocytes but at very low levels. Identifying patients who are acutely infected with HIV is difficult in everyday practice and commencing immediate cART has its challenges. A new study, Eramune-03 (ULTRA-STOP) NCT01876862 will scrutinise factors that predict safe treatment interruption in patients treated during chronic infection with favorable profiles that are consistent with a small reservoir.

Current research strategies towards a cure

Haematological stem cell transplantation (HSCT)

It remains unclear what led to Timothy Brown’s cure and whether transplantation plays a role in the cure agenda. Was it the HIV-resistant CCR5 delta32 deletion donor morrow? Or was it the conditioning prior to transplant? Was it, in fact, graft-versus-host-disease (GVHD) that eliminated residual infected cells? The recent report of two patients from Boston who received a CCR5 wild-type HSCT and who had a prolonged period of undetectable HIV DNA following transplantation (2 and 4 years), unfortunately experienced viral rebound 12 and 32 weeks after cessation of cART. The time to viral rebound may be an indirect measure of the number of residual infected cells. Mathematical modelling suggests that if only one HIV-infected cell remains, the chance of cure is high. In comparison, viral rebound is still expected in 3 and 10 years, respectively, if 100 or 10 infected cells remain. In HSCT and who had a prolonged period of undetectable HIV DNA following transplantation (2 and 4 years), unfortunately experienced viral rebound 12 and 32 weeks after cessation of cART. The time to viral rebound may be an indirect measure of the number of residual infected cells. Mathematical modelling suggests that if only one HIV-infected cell remains, the chance of cure is high. In comparison, viral rebound is still expected in 3 and 10 years, respectively, if 100 or 10 infected cells remain.

Clearly the toxicity, safety and ethics of HSCTs preclude this as a practical HIV cure for all. Further, finding a CCR5 delta32 deletion donor is extremely difficult as this is exceedingly rare globally. Stem cell donor registries do not currently routinely test for CCR5 status. A database of CCR5 delta32 negative cord blood exists and dual transplantation (cord blood and HSCT) is being considered.

Gene therapy

A variety of stem-cell based gene therapy are being explored – targeting cellular genes necessary for viral replication (such as CCR5 co-receptor), directly against HIV gene expression (such as tat and rev protein) and those that introduce genes to interfere with HIV replication (such as host restriction factors or fusion inhibitors). Zinc finger proteins are sequence-specific DNA binding proteins that can be coupled to a DNA endonuclease (zinc finger nuclease (ZFN)) to cut DNA at specific sites. CCR5 ZFNs aim to eliminate CCR5 expression thus gene-modifying the cell to be resistant to HIV. This is currently performed using an adenovirus delivery vector to T-cells ex vivo and future studies will use a similar approach for stem cells. This approach was shown to be safe in a recent study of 12 patients who received CCR5-modified cells. Indeed, CCR5-modified CD4+ T-cells peaked one week after engraftment and constituted a median frequency of 8.8% of peripheral blood mononuclear cells and 13.9% of total circulating CD4+ T-cells. The modified cells declined but persisted for up to 48 weeks and were also detectable in the rectal mucosa. Even though HIV viral load rebounded following cART treatment interruption, the decline in CCR5-modified CD4+ T-cells was less than in the unmodified CD4+ T-cell population suggesting some survival advantage.

The other approach is to target the virus itself. A lentiviral vector to introduce a gene encoding a short hairpin RNA (shRNA) against CCR5 is being combined with a HIV fusion inhibitor (C46). This LVshRNA/C46 vector is being trialled as a Phase I/II clinical trial (CAL-USA-11, NCT01734850) in HIV-infected patients. A phase II gene therapy trial of a tat-ribozyme specific anti-HIV ribozyme (OZ1) showed no difference in plasma HIV viral load but the OZ1 treated group demonstrated higher CD4+ T-cell counts compared to the placebo group.

Newer site-specific gene editing techniques include homing endonucleases, transcription activator-like effectors nucleases (TALENs) which offer more DNA recognition specificity, and the more versatile cluster regularly interspaced palindromic repeats (CRISPR) locus and their surrounding cohort of CRISPR-associated (Cas) genes, termed CRISPR/Cas9 system (reviewed in Manjunath and Choudhary and Margolis). A new CRISPR/Cas9 system targeting the HIV LTR could potentially be used to remove integrated virus from long-lived latently infected cells.

Activating latent HIV

Activating transcription from latently infected cells is being investigated as part of a ‘kick and kill’ strategy, which aims to induce production of virus from latency (the kick), thus making the recently activated latently infected cell susceptible to virus-induced cytolysis or HIV-specific immunity (the kill). Some approaches include histone deacetylase inhibitors (HDACi), disulfiram, methylation inhibitors, cytokines and immune modulators (reviewed in Wightman et al.).

Histone deacetylase inhibitors (HDACi)

Histone acetyltransferase leads to a hyperacetylated euchromatin state which is a transcriptionally-active, open state while histone
deacetylase (HDAC) leads to a closed, transcriptionally-repressed, heterochromatin state\textsuperscript{[66]}. HDACis can activate latent HIV \textit{in vitro} (reviewed in Wightman \textit{et al.}\textsuperscript{[65]}) in latently infected T-cell lines, primary models of latency and resting CD4\textsuperscript{+} T-cells from HIV-infected patients on cART\textsuperscript{[67,68]}. Some recent work using CD4\textsuperscript{+} T-cells from HIV-infected patients on ART demonstrated minimal release of virus into supernatant following stimulation with an HDACi alone\textsuperscript{[69,70]}. Furthermore, using an \textit{in vitro} model of latency, stimulation with an HDACi did not lead to cell death\textsuperscript{[70]}. In HIV-infected patients on cART, administration of the HDACi vorinostat clearly induced HIV transcription, as measured by an increase in cell-associated unspliced HIV RNA\textsuperscript{[71–73]}. However, neither the single dose nor the repeated dose strategy induced a significant increase in plasma RNA, nor were the number of latently infected cells reduced\textsuperscript{[71–73]}.

Recently, a more potent pan-HDACi, panobinostat was administered 3-times a week, fortnightly for 8 weeks in a Danish trial of HIV-infected adults on suppressive cART. An increase in HIV transcription and a possible increase in transient viraemia was seen\textsuperscript{[74]}. A single-dose, placebo-controlled, dose-escalation study of another HDACi romidepsin NCT01933594 in HIV-infected patients is being planned\textsuperscript{[51]}. Other HDACis in early clinical development but with likely potential activity against latent HIV include belinostat, givinostat and entinostat\textsuperscript{[22]}. Whether HDACi alone will lead to a significant reduction in latently infected cells remains unknown and further trials are needed to address this.

\textbf{Disulfiram}

Disulfiram is a member of the dithiocarbamate family, a broad class of metal-chelators, used previously to deter alcohol abuse\textsuperscript{[75]}. Disulfiram activated viral production from latent HIV \textit{in vitro}, in some but not all laboratory models of latency\textsuperscript{[70]} and it was recently demonstrated that activation was secondary to modification of the PTEN (phosphatase and tensin homologue) pathway\textsuperscript{[77]}. An open label study of daily disulfiram given for 14 days recorded a transient rise in HIV RNA in a subset of patients with high disulfiram levels without a reduction in the number of latently-infected cells\textsuperscript{[78]}. A dose-escalation study is currently ongoing (NCT01944371)\textsuperscript{[51]}.

\textbf{IL-7}

IL-7 has recently been investigated as an agent that could enhance T-cell proliferation\textsuperscript{[79]} and potentially activate HIV transcription from latency, but recent studies suggested that IL-7 causes proliferation of both infected and uninfected cells and an expansion of the HIV reservoir\textsuperscript{[80]} and an increase in HIV DNA (Eramune 01, NCT01019551)\textsuperscript{[51]}.

\textbf{Reducing virus replication and/or immune activation}

Persistent immune activation and inflammation in HIV-infected patients well-suppressed on cART has been associated with HIV persistence\textsuperscript{[81]}. Whether this means that virus persistence drove immune activation or whether immune activation drove virus persistence is unknown. Previous attempts of adding additional antiretroviral agents to a standard cART regimen – ‘cART intensification’ – have failed to reduce the number of latently infected cells when measured as either low level viraemia or cell-associated HIV RNA or HIV DNA\textsuperscript{[30,31,82]}. However, some intensification studies, specifically with raltegravir have demonstrated a reduction in markers of T-cell activation\textsuperscript{[40]} and d-dimer\textsuperscript{[31]} in a subset of patient. \textbf{Anti-inflammatory agents} such as statins, chloroquine and hydroxychloroquine, selective COX-2 inhibitors, and leflunomide are being trialled to reduce immune activation but the effect of these agents on virus persistence is unknown (reviewed in Deeks \textit{et al.}\textsuperscript{[83]}).

\textbf{Enhancing HIV-specific immunity}

\textbf{Therapeutic vaccination}

It is likely that boosting immunity will be required in combination with strategies that activate latent HIV. To date, very few therapeutic vaccines have shown any efficacy. More promising approaches include modifying peripheral blood cells or stem cells with a molecularly-cloned T-cell receptor or a chimeric molecule to redirect them against HIV antigens\textsuperscript{[58]}. A vaccine composed of a fusion protein containing a human monoclonal antibody specific for the dendritic cell receptor, DEC-205 (CD205) and the HIV gag p24 protein is being tested in a clinical trial NCT01127464\textsuperscript{[51]}. Autologous monocyte-derived dendritic cells (DCs) pulsed with heat-inactivated whole HIV showed reduction in plasma HIV viral loads at week 12 and week 24 after cART interruption but rebounded at week 48\textsuperscript{[84]}. The sustained reduction in plasma HIV viral set-point at week 48 in this study was of some promise\textsuperscript{[84]}. Recently a novel construct using a live CMV vector that expresses SIV proteins (RhCMV/SIV) demonstrated excellent protection against SIV challenge\textsuperscript{[35,86]}. This construct may have potential application as a therapeutic vaccine given it can elicit such broad and potent antigen-specific CD8\textsuperscript{+} T-cells.

\textbf{Immunomodulation}

Agents that modulate the HIV-specific immune response including antibodies to programmed death (PD)-1 and its ligand, PDL-1, are being investigated for their ability to reverse immune exhaustion and possibly also activate HIV transcription. A clinical trial utilising BMS-936559 (an antibody to PDL-1) is in development (NCT02028403)\textsuperscript{[51]}. A study on interferon-alpha-2b intensification is ongoing (NCT01295515)\textsuperscript{[51]}.
Challenges to HIV cure research

There are considerable challenges ahead. Development of new drugs will depend on having a biomarker that can predict long term virological control following cessation of cART. Better in vitro models are needed to effectively screen compounds that activate latent infection\(^7\)–\(^8\). We need more accurate and reproducible assays to quantify virus in both blood and tissue. Involvement of the private sector to date has been limited largely due to the absence of good biomarkers, in vitro and animal models of HIV latency. Novel strategies or collaborative programs are needed that can enhance engagement of private industry. Involving people living with HIV will be essential in all aspects of study design and implementation. Lastly, HIV cure strategies must be equitable and accessible to all including those in resource-limited settings — accordingly, clinical HIV cure trials in these settings should be urgently pursued.

Conclusion

Despite the recent excitement related to reports that an HIV cure is possible and the increasing scientific interest in finding a cure, a cure is many years away. Finding a cure for HIV will require innovation, collaboration and significant funding. The task is too great for any single laboratory or country. A number of collaborative initiatives focusing on cure are now established. The lessons learnt, and hopefully successes gained from these consortia are eagerly awaited.

References

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Biographies

Professor Sharon Lewin, FRACP, PhD is an infectious diseases physician and basic scientist. She is Director of the Department of Infectious Diseases at The Alfred Hospital and Monash University and co-head of the Centre for Biomedical Research, Burnet Institute, Melbourne, Australia and an Australian National Health and Medical Research Council (NHMRC) Practitioner Fellow. Her laboratory focuses on strategies to cure HIV infection. Together with Nobel Laureate Francoise Barre Sinoussi she will co-chair the XXth International AIDS Conference (AIDS2014), which will be held in Melbourne July 2014.

Christina C Chang, MBBS, FRACP is an infectious diseases physician based at The Alfred Hospital, Melbourne. She recently completed her PhD on cryptococcosis-associated immune reconstitution inflammatory syndrome in patients living with HIV and will now undertake postdoctoral research in HIV cure.