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Barriers and strategies to achieve a cure for HIV

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Abstract

Nine years following the report of a cure for HIV using CCR5-Δ32 stem cell transplantation, there have been no further cases of confirmed HIV cure despite much scientific effort. However, significant progress has been made in understanding the biology of the latent HIV reservoir and in measuring the amount of virus that persists on antiretroviral therapy (ART) using increasingly sophisticated tools. This knowledge is being translated into an increasingly long pipeline of clinical trials seeking to reduce viral persistence in participants on suppressive ART and ultimately to allow safe cessation of ART. Here we review the main barriers to HIV cure, methods to measure HIV persistence on ART and clinical strategies that are under evaluation to cure HIV. Finally, we highlight future directions for the field.

The report of Timothy Brown being cured of HIV following transplantation of stem cells from a CCR5-Δ32 donor in 2009 demonstrated for the first time that a cure for HIV was possible and provided great excitement in the HIV cure field.¹ Over the subsequent nine

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years there have unfortunately been no further reports of a true cure for HIV but there have been multiple other case reports of individuals who, after stopping antiretroviral therapy (ART), have achieved undetectable viral loads for months or even years prior to viral rebound^{2,3} or have controlled virus at low but detectable levels (<50 copies/ml).⁴ These cases have provided inspiration to people living with HIV, scientists and clinicians alike that perhaps ART may not be required life long, at least for some individuals. But unfortunately much more work is still needed.

Over the last decade, our understanding of where and how HIV persists on ART has transformed significantly with evidence that HIV persists in multiple cell types and tissue sites and in both quiescent and proliferating long lived latently infected cells. Assays that measure HIV persistence have improved but have also become more complex. There have been several single arm and very few placebo controlled interventional studies aimed at perturbing the reservoir but unfortunately no studies have yet been successful in inducing either remission or cure. In contrast, several interventions in ART treated simian immunodeficiency virus (SIV)-infected non-human primate (NHP) models have led to successful and sustained remission.^{5,6} Whether the successes in NHP models can be translated to success in people living with HIV remains unclear. Here we review the major advances in our understanding of how HIV persists on ART and the rationale and findings of strategies that have been tested to date. Finally, we highlight future directions and priorities for HIV cure research.

Main barriers to cure for HIV

In HIV-infected individuals on antiretroviral therapy (ART), HIV can persist in both a latent and transcriptionally active state, in quiescent or proliferating cells, in multiple T cell subsets and tissue sites and as both defective and intact virus (summarised in Figure 1).

HIV latency

HIV latency is defined as the integration of replication competent intact virus into the host genome in the absence of virus production. Latently infected cells differ from productively infected cells in multiple ways including the integration sites of the virus, the chromatin environment including the degree of histone acetylation and methylation, the frequency of transcription factors that can drive transcription of viral DNA into RNA and the expression of microRNAs that reduce translation of viral RNA into protein (Fig. 1a).

In both human and animal studies, latency is established extremely early, within days of acquisition of infection.^{7,8} Although latency was initially described in long-lived resting memory CD4⁺ T cells, it is now clear that HIV can persist on ART in multiple T cell subsets including naïve, stem cell memory and central, transitional and effector memory CD4⁺ T cells. Furthermore, in lymphoid tissue in individuals on ART, HIV is enriched in T follicular helper (Tfh) cells within B cell follicles⁹ and, in SIV-infected NHP on ART, SIV is enriched within T regulatory (Treg) cells at extrafollicular sites.¹⁰ Using an NHP model which allowed for extensive tissue analysis, the primary site of persistence of both SIV RNA and DNA was the gastrointestinal tract.¹¹ This study also demonstrated the spectrum of transcriptional activity of infected cells that persist on ART.¹¹ The relative contribution of

each of these infected cell types to viral rebound once ART is stopped remains poorly understood and is an important area of ongoing investigation.

Intact and defective virus

In individuals treated in both acute and chronic infection, a large proportion of the virus detected in latently infected cells has deletions or mutations and only a fraction of latently infected cells *in vivo* contain intact virus.¹² Unexpectedly, in contrast to relative concentrations of total HIV DNA,¹³ intact HIV DNA is more frequently detected in effector memory than central memory CD4+ T cells.¹⁴ Using full length virus sequencing, the frequency of peripheral blood resting CD4+ T cells carrying intact virus is on average around 60 per million cells¹⁵ which is significantly higher than previous estimates of inducible replication-competent virus at on average just one per million cells using a viral outgrowth assay (VOA).¹⁵ This discrepancy is because virus can be intact but either is not induced following T cell stimulation or requires multiple rounds of T cell stimulation to be induced.¹⁶ It is unclear whether non-induced intact virus represents latently infected cells that are less likely to reactivate *in vivo* and to contribute to rebound viraemia off ART.

Proliferation of latently infected cells

The importance of proliferation of latently infected cells to HIV persistence has been demonstrated by identifying multiple cells in individuals on ART that are infected with virus located at an identical integration site^{17,18} (Fig. 1b). These cells are referred to as expanded clones and in some individuals they can account for over half the number of latently infected cells.^{16,19} Expanded clones can contain defective as well as intact virus^{16,19,20} and can also express cell associated HIV RNA²¹ but surprisingly these cells don't die from virus mediated cytolysis. The driver for proliferation of an infected cell on ART remains unclear and is critically important to understand. Possible causes include T cell division in response to homeostatic proliferation,¹³ antigen recognition¹³ or integration within a gene that favours cell proliferation and survival.^{17,18}

Residual viral replication

Whether residual viral replication occurs on long-term ART remains controversial after almost two decades of research and debate (Fig. 1c). Arguments in favour of residual viral replication include poor penetration of many antiretroviral drugs into lymphoid tissue with an associated inverse correlation between ART penetration and detection of virus in lymph nodes.²² In some ART intensification studies, the addition of the integrase inhibitor raltegravir to a suppressive ART regimen led to a transient increase in 2-LTR circles, a byproduct of aborted HIV integration, in some participants consistent with residual viral replication in these individuals.²³ However, there is also a significant body of evidence against residual viral replication on ART. This includes the lack of evolution of viral sequences in plasma or cells over years on ART,²⁴ the absence of development of drug resistance on effective ART and the lack of effect of ART intensification on multiple markers of HIV persistence.²⁵ Ongoing detection of cell-associated HIV RNA on ART in CD4+ T cells from blood and tissue¹¹ and persistent low level viraemia in plasma does not provide proof of residual replication occurring as these findings may simply reflect ongoing

viral transcription and/or release from stable reservoirs, a process which is not affected by ART.

Tissue reservoirs

In individuals on ART, tissue sites such as lymph nodes and the gastrointestinal tract, compared to blood, contain a much higher frequency of HIV DNA and RNA per CD4+ T cell.^{11,26} In lymph node tissue from HIV-infected individuals on ART, infected cells are preferentially detected within B cell follicles which have low penetration by cytotoxic CD8+ T cells thereby providing an immune protected sanctuary^{9,27} (Fig. 1d). The gastrointestinal tract may also provide a unique environment for HIV persistence given the high frequency of Th17 cells which express high levels of CCR5 and are preferentially infected off and on ART²⁸ (Fig. 1e). Finally, tissue macrophages can be infected with HIV and, given that infection of macrophages is not cytopathic, these long lived infected cells may persist on ART in sites such as the brain.²⁹ Although the contribution of infected macrophages to HIV persistence on ART is still debated, using a unique mouse model, infected macrophages were the clear source of viral rebound off ART.³⁰

Measuring HIV persistence on ART

There is currently no phenotypic marker for a latently infected cell making quantification of virus persistence on ART challenging. The most recent discovery that CD32, a weak Fc receptor usually expressed on myeloid rather than lymphoid cells, identifies cells that are highly enriched for HIV in individuals on ART³¹ has unfortunately not been successfully repeated in other laboratories.³² For cure intervention studies, the most important clinical endpoint is a delay to viral rebound or control of virus to low or undetectable levels off ART. Ultimately we need a biomarker that could predict either clinical endpoint. The design of an HIV cure clinical trial incorporating an analytical treatment interruption (ATI) and its possible outcomes is depicted in Figure 2.

Virus detection

Initial studies of HIV persistence on ART quantified replication competent virus using quantitative viral outgrowth assays (QVOA) and polymerase chain reaction (PCR) based assays to detect cell-associated total and integrated HIV DNA and cell-associated and plasma HIV RNA. Recent advances include limiting dilution PCR based assays,³³ sequencing of cell-associated HIV DNA and RNA to detect intact virus^{14,34} and detection of viral proteins.³⁵ The main features of each of these assays and their advantages and disadvantages are summarised in Table 1.

Immune response based assays

Another approach to detect virus persistence is to quantify the immune response to the virus rather than quantifying the virus itself. Following stem cell transplantation there is a significant decrease in the number of infected cells together with a decline in titres and functionality of anti-HIV antibodies raising the possibility that antibody detection could be used as a sensitive marker of HIV persistence.^{43,44} However, stem cell transplantation itself

affects antibody titres independent of antigen persistence so this finding may not necessarily be replicated with other HIV cure strategies.

Imaging with radioactive labeled antibodies

Given the location of latently infected cells in tissue, a strategy to detect virus that doesn't require tissue biopsy would be highly desirable. A radioactive copper-labelled anti-SIV envelope antibody has been used in a Positron Emission Tomography-Computed Tomography (PET-CT) imaging study of the viral reservoir in SIV-infected macaques (immunoPET) and demonstrated extensive uptake of antibody in lymphoid tissue throughout the body which diminished but remained detectable after five weeks on ART.⁴⁵ As antibodies do not penetrate cells, this modality is only capable of detecting infected cells that express Env protein at the cell surface. Whether immunoPET has sufficient resolution to detect Env protein in individuals on long-term suppressive ART is currently being evaluated (NCT03063788).

Major clinical strategies being tested

Strategies targeting persistent HIV on ART can be divided into three groups: those that target the HIV replication cycle (Fig. 3a), those that enhance the HIV-specific immune response (Fig. 3b) and those that modulate the immune system in a general manner (Fig. 3c). These are discussed in further detail below. Table 2 summarises cure interventions that have entered or completed human clinical trials.

Strategies that target the HIV replication cycle

Transplantation—Only six HIV-infected individuals on ART who received haematopoietic stem cell transplants (HSCTs) from donors homozygous for CCR5^{Δ32} as part of management of haematological malignancy have been reported in the literature, apart from Timothy Brown.⁴⁶ Unfortunately virus rebounded in one case as a CXCR4-tropic strain and in the other cases there was early mortality due to graft complications or relapse of malignancy.⁴⁶ Interestingly, HSCTs from donors with wild-type CCR5+ cells to HIV-infected individuals on suppressive ART have also resulted in a dramatic reduction in the frequency of infected cells.^{2,43,44} In some of these individuals, cessation of ART has been associated with a prolonged time to viral rebound, ranging from three to ten months.^{2,44} However, current mortality rates of HSCT preclude this as a viable cure strategy for HIV-infected individuals without an independent clinical indication for transplantation.

It is unclear which component of HSCT contributes to the elimination of infected cells on ART. A similar effect has not been observed with chemotherapy alone.⁴⁷ The effect of total body irradiation on HIV persistence is less clear and should be further investigated in animal models. One hypothesis is that elimination of infected cells is a result graft versus host disease resulting in 'graft versus latent infection', but direct proof of this is still needed. Optimisation of HSCT in the SIV-infected macaque model will be important to understand this further.

Gene therapy—Gene editing of CCR5 or HIV itself has also been actively explored as a strategy to cure HIV. Clinical trials to date have used Zinc Finger Nucleases (ZFN) to target CCR5 but other gene editing approaches have been tested *in vitro* including transcription activator-like effector nucleases (TALEN) and Clustered Regularly Interspaced Short Palindromic Repeats/Cas-9 (CRISPR/Cas-9). In a clinical trial, 12 HIV-infected participants on suppressive ART were infused with autologous ZFN-mediated CCR5-modified CD4+ T cells.⁴⁸ The strategy was safe and resulted in 13.9% of circulating CD4+ T cells being gene modified.⁴⁸ Virus rebounded in all six participants who underwent ATI; there was a delay in rebound viraemia in one participant who was found to be heterozygous for CCR5 $\Delta 32$.⁴⁸ New strategies currently being tested include CCR5 modification of stem cells rather than T cells and different conditioning regimens. Multiple phase I and II clinical trials are currently underway assessing gene-edited CD4+ T cells as HIV cure strategies ([NCT03020524](#), [NCT02500849](#), [NCT00295477](#), [NCT01734850](#) [NCT02225665](#), [NCT01013415](#)).

In animal models and *in vitro*, CRISPR/Cas-9 technology has also been used to modify CCR5⁴⁹ and to target HIV directly; however, resistance mutations have occurred with the latter approach.⁵⁰ Gene editing of the HIV genome will likely require multiple targets to avoid resistance and will need enhanced delivery strategies to access rare infected cells in blood and tissue.

Latency reversal agents—One strategy that has been extensively investigated in clinical trials aims to reverse HIV latency to increase expression of viral antigens in latently infected cells leading to virus- or immune-mediated cell death. The first latency reversal agents (LRA) studied were histone deacetylase inhibitors (HDACi) which increase histone acetylation of the HIV promoter leading to enhanced transcription. Multiple HDACi have now been tested, including vorinostat, panobinostat and romidepsin with either single, intermittent or continuous dosing, and all studies have shown that HDACi can increase cell-associated unspliced HIV RNA within resting or total CD4+ T cells^{37–39} but none of these studies demonstrated a reduction in the frequency of infected cells. One of the main concerns of HDACi is safety given widespread off-target effects including sustained altered host gene expression³⁷ and suppression of HIV-specific T cell and natural killer (NK) cell function *ex vivo*^{51,52}.

Disulfiram, a drug used to treat alcoholism, has also been shown to induce an increase in cell-associated US and plasma HIV RNA in HIV-infected individuals on ART with the greatest increase in plasma HIV RNA seen at a higher dose of 2g/day.⁵³ Similar to HDACi, no effect on the frequency of infected cells has been observed.

Toll-like receptor (TLR) agonists have recently been studied in both human clinical trials and NHP as LRAs. The TLR9 agonist, MGN1703, was evaluated in HIV-infected individuals on ART and demonstrated an increase in plasma but not cell-associated HIV RNA, an increase in activation of CD8+ T cells, cytotoxic NK cells, activated dendritic cells and interferon-alpha2 (IFN- α 2) levels but no change in the frequency of infected cells.⁵⁴ TLR7 agonists have been found to increase interferon-mediated depletion of infected cells *in vitro*.⁵⁵ In SIV-infected NHP on suppressive ART, the TLR7 agonist vesatolimod (formerly GS-9620) and GS986 caused transient plasma viraemia following each dose, reduced SIV

DNA levels in blood and tissue and, in two macaques, led to sustained virologic remission.^{6,56} These data have led to the evaluation of vesatolimod in HIV-infected human participants (clinical trials ID [NCT02858401](#), [NCT03060447](#)).

Clinical trials of LRAs have clearly demonstrated a need for more potent, less toxic and more specific agents. Some combinations of LRAs *in vitro* have demonstrated synergism⁵⁷ but clinical trials of combination LRAs are awaited.

Kill agents—Given that LRAs alone appear not to deplete latently infected cells, new approaches are being developed that take advantage of the interaction between a range of HIV proteins and cell death pathways (reviewed by Kim et al).⁵⁸ One example of this is the interaction between the anti-apoptotic BCL-2 pathway and HIV protease. During active HIV replication, HIV protease cleaves procaspase 8 leading to apoptosis. However, in central memory CD4+ T cells, high levels of BCL-2 interfere with this pathway thus preventing apoptosis following cellular activation.⁵⁹ Using CD4+ T cells from HIV-infected individuals on ART *ex vivo*, the combination of T cell activation and the recently licensed pro-apoptotic BCL-2 inhibitor, venetoclax, resulted in a decrease in HIV DNA suggesting selective depletion of infected cells.⁵⁹

Latency silencing—In contrast to activating latency, another feasible approach is to silence HIV transcription permanently, now called ‘block and lock’. Following a four week exposure in an infected ART-treated humanised mouse model, the Tat inhibitor, didehydro-Cortistatin A (dCA), led to a reduction in cell-associated HIV RNA expression in tissue and to delayed viral rebound following ART cessation.⁶⁰ dCA-mediated Tat inhibition promotes epigenetic silencing of HIV transcription and thus a longer period of exposure to dCA may confer a deeper state of latency as shown *in vitro*. Another method to silence latent infection is to use RNA inhibition (RNAi) with either small interfering RNA (siRNA) or short hairpin RNA (shRNA) that can target the virus within the nucleus causing silencing of the viral promoter or within the cytoplasm causing degradation of complementary viral mRNA.^{61,62} The main challenge with RNAi technology is the difficulty in delivering it into all cells infected with replication-competent virus *in vivo*.

HIV specific immune enhancement

T cell vaccines

While initially designed to prevent transmission, HIV vaccines are likely to be important in boosting host immune responses, depleting virus-infected cells and potentially achieving virological control off ART. A number of studies have assessed the combination of an LRA with a T cell vaccine. The administration of six Vacc-4x intradermal immunisations with granulocyte-macrophage colony-stimulating factor (GM-CSF) as an adjuvant followed by three weekly intravenous infusions of the LRA romidepsin demonstrated a reduction in total HIV DNA at six weeks.⁶³ Another clinical trial evaluated romidepsin with an HIV-modified vaccinia virus Ankara vector expressing HIV-1 antigens and chimpanzee adenovirus and demonstrated viraemic control to <1000 copies/ml following ART cessation in four of 11 participants.⁶⁴ Of note, neither of these studies had a placebo control arm which is an important consideration as post treatment control of virus can occur following ART alone.

^{4,65} Other vectors that stimulate HIV-specific T cells that look promising, at least in NHP models, include the use of cytomegalovirus (CMV) as a vector⁶⁶ and the combination of an adenovirus vector with a TLR7 agonist as an adjuvant.⁶⁷

Sequencing of virus in latently infected cells identified multiple mutations consistent with immune escape suggesting that vaccine-induced HIV-specific T cells may not recognise the virus in latently infected cells.⁶⁸ Strategies that promote a broad cytotoxic CD8+ T cell response that harnesses the genetic diversity of Human Leukocyte Antigen (HLA) alleles without overreliance on immunodominant epitopes may have more success.⁶⁹ Given that significant immune perturbations can persist on ART, including T cell exhaustion, T cell activation and generalised inflammation, it remains unclear whether promoting activation of HIV-specific T cells with therapeutic vaccines will be effective for cure but this remains an active area of investigation.

Broadly neutralising antibodies

Broadly neutralising antibodies (bNAbs) are antibodies capable of neutralising a wide range of virus strains. In addition to neutralisation of free virus, these antibodies can also clear cells expressing viral antigen on the cell surface^{70,71} likely through interactions between the Fc component of the antibody and its receptor on phagocytic and NK cells.⁷² bNAbs may also, unexpectedly, enhance CD8+ T cell function, as recently demonstrated in an NHP model,⁷³ presumably by binding antigen and activating Fc receptors on antigen-presenting cells which can then process, present and cross-present antigen to CD4+ and CD8+ T cells.

Administration of the bNAbs 3BNC117 and VRC01 to HIV-infected adults on ART, followed by cessation of ART, resulted in a delay in time to viral rebound compared to historical controls.^{74,75} The time to viral rebound in these studies was most likely related principally to the pharmacokinetics of the antibodies and the rate of generation of viral resistance to the bNAbs.^{74,75} Perhaps the most promising application of bNAbs for remission may be in neonatal infection. The early administration of the bNAbs VRC07–523 and PGT121 to NHPs infected at one month of age with SIV containing an HIV envelope (SHIV) resulted in complete clearance of SHIV-infected cells.⁷⁶ A similar approach is now being evaluated in HIV-infected neonates who are receiving ART together with VRC01 or VRC01LS antibody within 72 hours or five days of birth ([NCT02256631](#)).

Antibody-based molecules with multiple specificities

bNAbs will most likely need to be used in combination to prevent resistance. In addition, new generation antibodies have been engineered that incorporate the antigen specificity of different bNAbs and bind to multiple different non-overlapping sites on the virus. These bispecific and trispecific antibodies exhibit significant breadth and potency in their capacity to neutralise HIV *in vitro*^{77,78} with synergy demonstrated compared to a combination of the individual parent antibodies.⁷⁷ Bispecific antibodies have also been designed that bind to both virus and CD4 thereby docking the antibodies at the site of, and inhibiting, viral entry. These antibodies also have improved potency and breadth compared to their parent antibodies⁷⁹.

Dual-Affinity Re-Targeting molecules (DARTs) are engineered disulfide-linked heterodimers comprising the variable domains of two different monoclonal antibodies. DARTs have been designed with specificity against HIV envelope and CD3 with the aim to bring cytotoxic CD8+ T cells into close proximity with infected cells expressing HIV envelope on their surface. These DARTs have been demonstrated to facilitate clearance of infected cells *in vitro*.⁸⁰

Neither bispecific or trispecific antibodies nor DARTs have yet entered clinical trials.

Chimeric Antigen Receptor T cells

Chimeric antigen receptors (CARs) are engineered receptors comprising an extracellular domain, usually either a single chain variable fragment derived from a monoclonal antibody or a cell surface receptor or ligand, a transmembrane domain and an intracellular domain comprising the zeta signalling chain of CD3 with or without other intracellular domains of costimulatory receptors such as CD28. In recent years, primary human T cells have been engineered to express CAR with a bNAb single chain variable fragment in the extracellular domain and have been shown to lyse HIV infected cells effectively *in vitro* and *ex vivo*.^{81,82} Clinical trials are underway ([NCT03240328](#)).

Immune modulation

Immune checkpoint blockers

Immune checkpoints are immune modulating proteins that trigger regulatory pathways which reduce T cell activity and have been associated with immune exhaustion and impaired function (reviewed by Wykes and Lewin).⁸³ CD4+ T cells in blood and lymphoid tissue that express the immune checkpoint markers programmed cell death protein 1 (PD-1), cytotoxic T lymphocyte antigen 4 (CTLA-4) and others are enriched for HIV infection.^{10,13,84} In addition, HIV-specific CD4+ and CD8+ T cells express high levels of immune checkpoints in untreated and treated HIV infection and, *ex vivo*, antibodies against immune checkpoint markers alone or in combination can enhance HIV-specific T cell function.⁸⁵ Therefore, immune checkpoints may be attractive targets both to boost T cell function and also to induce latency reversal.⁸³

A randomised controlled dose escalation trial of an antibody against PD-L1 (BMS-936559) in HIV-infected individuals without malignancy demonstrated an increase in HIV-specific T cell responses in two of six participants but the trial was ceased early due to new data showing retinal toxicity in animals.⁸⁶ Given the significant immune related adverse effects of these antibodies, including myocarditis, pneumonitis and others these agents have to date only been examined in the context of people living with HIV with concomitant malignancy. In a single case report of anti-CTLA-4 antibody (ipilimumab) in an individual with metastatic melanoma, a significant increase in cell-associated HIV RNA was observed consistent with latency reversal.⁸⁷ More recently, repeated doses of anti-PD-1 antibody (pembrolizumab) were associated with a significant decline in cell-associated HIV DNA, a modest increase in plasma HIV RNA and an increase in functional HIV-specific CD8+ T cells.⁸⁸ Larger observational clinical trials of immune checkpoint blockers in ART-

suppressed HIV-infected individuals with malignancy are underway ([NCT02408861](#), [NCT02595866](#), [NCT03304093](#), [NCT03354936](#), [NCT03367754](#), [NCT03426189](#)); these are needed to understand fully the safety and HIV-specific activity of these antibodies.

T cell trafficking

Inhibition of T cell trafficking to tissue sites which may favour HIV infection or persistence is another potential cure strategy. $\alpha_4\beta_7$ integrin is a gut-homing marker expressed on the surface of CD4+ T cells that are highly susceptible to HIV/SIV infection. SIV-infected ART-suppressed macaques administered anti- $\alpha_4\beta_7$ integrin monoclonal antibody subsequently remained aviraemic for at least two years post ATI.⁵ Although the initial goal of this study was to interrupt CD4+ T cell trafficking to the gut, trafficking was in fact enhanced and the beneficial effects of the antibody may instead have been a result of a direct antiviral effect or enhanced antigen presentation through formation of immune complexes. The monoclonal antibody vedolizumab, which targets human $\alpha_4\beta_7$ integrin, is currently being investigated in HIV-infected individuals on suppressive ART ([NCT03147859](#), [NCT02788175](#)).

Alteration of T cell trafficking can also facilitate the migration of HIV-specific T cells to sites of HIV persistence. For example, increasing the expression of the chemokine receptor CXCR5 on CD8+ T cells could increase trafficking of these cells into B cell follicles within lymph nodes where HIV can persist.²⁷ This has been successfully demonstrated using passive administration of CD8+ T cells transduced to express CXCR5 in an NHP model.⁸⁹ Additionally, administration of an IL-15 superagonist to NHP has been shown to increase CXCR5 expression on CD8+ T cells and to promote their migration into lymph node B cell follicles.⁹⁰ A phase 1 clinical trial is currently examining the safety of this superagonist in humans ([NCT02191098](#)).

Reducing inflammation

Given the associations between chronic inflammation, immune exhaustion and HIV persistence,²⁶ there is interest in using anti-inflammatory agents as a strategy to reduce HIV persistence. One candidate drug for this purpose is the mTOR inhibitor sirolimus/rapamycin, use of which has been associated with a reduction in HIV DNA levels following kidney transplantation.⁹¹ Clinical studies are currently evaluating the effect of sirolimus on the reservoir alone or in combination with maraviroc in individuals on ART ([NCT02440789](#), [NCT02990312](#)).

Several studies of other agents that reduce inflammation have demonstrated encouraging results in animal models but have not yet been performed in humans. An IL-21 superagonist restored Th17 and Th22 cells in the gut of SIV-infected macaques on ART with associated reduction in gut inflammation and CD4+ and CD8+ T cell activation and a reduction in viral set-point following ATI.⁹² Anti-interferon α/β receptor antibody in HIV-infected ART-suppressed humanised mice reduced immune activation, improved the CD4+ and CD8+ T cell HIV-specific immune response, reduced viral reservoirs in lymphoid tissue and also caused a delay in viral rebound following ATI.⁹³

Specific populations of interest

Acute infection

Initiation of ART in early HIV infection reduces the frequency of infected cells that persist on ART and reduces inflammation. In addition, the quality of the HIV-specific CD8+ T cell response depends on the time of initiation of ART and the capacity for these cells to persist is inversely related to the size of the reservoir.⁹⁴ Therefore, there has been great interest in performing cure studies in individuals commenced on ART early after HIV infection. Early ART by itself is insufficient to effect a cure with multiple studies showing that, whilst very early initiation of ART will reduce HIV persistence, viral rebound still occurs, consistent with findings in an NHP model.^{7,8,95}

Elite control

It has long been recognised that about 1% of individuals infected with HIV can maintain suppressed viral loads without receiving ART. These individuals, known as elite controllers, have fewer latently infected cells, particularly infected peripheral T follicular helper cells,⁹⁶ increased HIV-specific CD8+ T cell and polyfunctional antibody responses⁹⁷ and are more likely to have protective HLA class I alleles. Although understanding the host and viral factors leading to elite control is highly relevant to strategies for HIV remission, it is important to emphasise that there are clear differences between elite control and individuals on suppressive ART. For instance, compared to individuals on ART, elite controllers have higher levels of immune activation⁹⁸, lower levels of immune exhaustion⁹⁹ and, most importantly, increased incidence of non-AIDS events, specifically hospitalisation for cardiovascular events.¹⁰⁰ Some elite controllers become viraemic over time; this is associated with fewer polyfunctional Gag-specific CD8+ T cells and higher proinflammatory cytokine levels in the year prior to loss of viraemic control.¹⁰¹

Post treatment control

There has also been great interest in individuals able to maintain viral loads at <50 copies/ml for a prolonged period after cessation of ART, known as post treatment control (PTC). PTC has been described in individuals treated during acute and chronic infection and as adults,^{4,102} children¹⁰³ and infants.^{104,105} Individuals with PTC do not exhibit specific HLA types or elevated HIV-specific T cells typical of HIV elite controllers.⁴ Possible mechanisms of PTC include an altered distribution of latency in T cell subsets, with a lower frequency of infection in long-lived naïve and central memory CD4+ T cells,¹⁰⁶ or increased NK cell cytotoxic activity.¹⁰⁷ The true incidence of PTC remains unclear; in a post hoc analysis of a prospective clinical trial of individuals with acute infection who were randomised to either early ART or no ART, transient virological control was achieved in 15.1% and 7.9% of participants respectively.¹⁰⁸ This was not due to an elite controller genotype in the ART-naïve group¹⁰⁸ which suggests that a proportion of individuals may control virus even following a period of viremia and without exposure to ART.

Children and infants

Several perinatally-infected infants treated early following infection have achieved viral control after ART cessation. One of these children, the Mississippi baby, had sustained virological control for 28 months off ART before virological rebound.³ Multiple potential explanations have been proposed in these cases which include defective non-inducible provirus being favoured by early ART¹⁰⁹ or, possibly, fewer long-lived central memory CD4+ T cells being infected given that these cells comprise a lower proportion of CD4+ T cells in children compared to adults.

Future directions

Despite a clear need and commitment to find a cure for HIV or a strategy to allow individuals to stop ART safely, there are no interventions that have yet been shown to achieve HIV remission or cure. Our understanding of how, where and why HIV persists on ART has advanced significantly but there remains a need to identify a phenotypic marker of latently infected cells and a biomarker to predict outcomes post ART cessation. Positive findings following clinical trials of cure interventions have been slower than hoped. A combination approach will most likely be needed but evaluation of the safety of individual interventions is important as people living with HIV have a near normal life expectancy and the acceptability of toxicity is therefore much lower in comparison to other diseases.

More work is needed to understand HIV persistence in people in low income countries who have different viral and host characteristics and a greater frequency of co-infection compared to individuals in high income countries. Successful examples of HIV cure focused research infrastructure in low income settings have employed effective socioeconomic interventions to recruit participants;¹¹⁰ this provides an encouraging model for other low income settings. Finally, individuals with co-infections such as viral hepatitis and tuberculosis are often excluded from cure focused trials and women are often underrepresented. Future work should specifically address these populations. Given that over 30 of the 36.7 million people living with HIV live in low- and middle-income countries, any HIV cure regimen needs to be scalable, cost-effective and able to be implemented in diverse clinical settings.

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References

1. Hutter G, Nowak D, Mossner M, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med* 2009; 360(7): 692–8. [PubMed: 19213682]
2. Henrich TJ, Hanhauser E, Marty FM, et al. Antiretroviral-free HIV-1 remission and viral rebound after allogeneic stem cell transplantation: report of 2 cases. *Ann Intern Med* 2014; 161(5): 319–27. [PubMed: 25047577]
3. Luzuriaga K, Gay H, Ziemniak C, et al. Viremic relapse after HIV-1 remission in a perinatally infected child. *N Engl J Med* 2015; 372(8): 786–8. [PubMed: 25693029]
4. Saez-Cirion A, Bacchus C, Hocqueloux L, et al. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI Study. *PLoS Pathog* 2013; 9(3): e1003211. [PubMed: 23516360]
5. Byrreddy SN, Arthos J, Cicala C, et al. Sustained virologic control in SIV+ macaques after antiretroviral and alpha4beta7 antibody therapy. *Science* 2016; 354(6309): 197–202. [PubMed: 27738167]
6. Lim SY, Osuna C, Hesselgesser J, et al. TLR7 agonist treatment of SIV+ monkeys on ART can lead to complete viral remission. Conference on Retroviruses and Opportunistic Infections. Seattle, Washington; 2017.
7. Henrich TJ, Hatano H, Bacon O, et al. HIV-1 persistence following extremely early initiation of antiretroviral therapy (ART) during acute HIV-1 infection: An observational study. *PLoS Med* 2017; 14(11): e1002417. [PubMed: 29112956]
8. Whitney JB, Hill AL, Sanisetty S, et al. Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. *Nature* 2014; 512(7512): 74–7. [PubMed: 25042999]
9. Banga R, Procopio FA, Noto A, et al. PD-1(+) and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals. *Nat Med* 2016; 22(7): 754–61. [PubMed: 27239760]
10. McGary CS, Deleage C, Harper J, et al. CTLA-4(+)PD-1(–) Memory CD4(+) T Cells Critically Contribute to Viral Persistence in Antiretroviral Therapy-Suppressed, SIV-Infected Rhesus Macaques. *Immunity* 2017; 47(4): 776–88.e5. [PubMed: 29045906]
11. Estes JD, Kityo C, Ssali F, et al. Defining total-body AIDS-virus burden with implications for curative strategies. *Nat Med* 2017; 23(11): 1271–6. [PubMed: 28967921]
12. Bruner KM, Murray AJ, Pollack RA, et al. Defective proviruses rapidly accumulate during acute HIV-1 infection. *Nat Med* 2016; 22(9): 1043–9. [PubMed: 27500724]
13. Chomont N, El-Far M, Ancuta P, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med* 2009; 15(8): 893–900. [PubMed: 19543283]
14. Hiener B, Horsburgh BA, Eden JS, et al. Identification of Genetically Intact HIV-1 Proviruses in Specific CD4(+) T Cells from Effectively Treated Participants. *Cell Rep* 2017; 21(3): 813–22. [PubMed: 29045846]
15. Ho YC, Shan L, Hosmane NN, et al. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell* 2013; 155(3): 540–51. [PubMed: 24243014]
16. Hosmane NN, Kwon KJ, Bruner KM, et al. Proliferation of latently infected CD4+ T cells carrying replication-competent HIV-1: Potential role in latent reservoir dynamics. *J Exp Med* 2017; 214(4): 959–72. [PubMed: 28341641]
17. Maldarelli F, Wu X, Su L, et al. HIV latency. Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. *Science* 2014; 345(6193): 179–83. [PubMed: 24968937]
18. Wagner TA, McLaughlin S, Garg K, et al. HIV latency. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. *Science* 2014; 345(6196): 570–3. [PubMed: 25011556]
19. Bui JK, Sobolewski MD, Keele BF, et al. Proviruses with identical sequences comprise a large fraction of the replication-competent HIV reservoir. *PLoS Pathog* 2017; 13(3): e1006283. [PubMed: 28328934]

20. Lorenzi JC, Cohen YZ, Cohn LB, et al. Paired quantitative and qualitative assessment of the replication-competent HIV-1 reservoir and comparison with integrated proviral DNA. *Proc Natl Acad Sci U S A* 2016; 113(49): E7908–e16. [PubMed: 27872306]
21. Wiegand A, Spindler J, Hong FF, et al. Single-cell analysis of HIV-1 transcriptional activity reveals expression of proviruses in expanded clones during ART. *Proc Natl Acad Sci U S A* 2017; 114(18): E3659–e68. [PubMed: 28416661]
22. Fletcher CV, Staskus K, Wietgreffe SW, et al. Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. *Proc Natl Acad Sci U S A* 2014; 111(6): 2307–12. [PubMed: 24469825]
23. Buzon MJ, Massanella M, Llibre JM, et al. HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects. *Nat Med* 2010; 16(4): 460–5. [PubMed: 20228817]
24. Van Zyl GU, Katusiime MG, Wiegand A, et al. No evidence of HIV replication in children on antiretroviral therapy. *J Clin Invest* 2017; 127(10): 3827–34. [PubMed: 28891813]
25. Lafeuillade A, Assi A, Poggi C, Bresson-Cuquemelle C, Jullian E, Tamalet C. Failure of combined antiretroviral therapy intensification with maraviroc and raltegravir in chronically HIV-1 infected patients to reduce the viral reservoir: the IntensHIV randomized trial. *AIDS Res Ther* 2014; 11(1): 33. [PubMed: 25320633]
26. Khoury G, Fromentin R, Solomon A, et al. HIV persistence and T-cell activation in blood, rectal and lymph node tissue in HIV-infected individuals receiving suppressive ART. *J Infect Dis* 2017.
27. Fukazawa Y, Lum R, Okoye AA, et al. B cell follicle sanctuary permits persistent productive simian immunodeficiency virus infection in elite controllers. *Nat Med* 2015; 21(2): 132–9. [PubMed: 25599132]
28. Planas D, Zhang Y, Monteiro P, et al. HIV-1 selectively targets gut-homing CCR6+CD4+ T cells via mTOR-dependent mechanisms. *JCI Insight* 2017; 2(15).
29. Castellano P, Prevedel L, Eugenin EA. HIV-infected macrophages and microglia that survive acute infection become viral reservoirs by a mechanism involving Bim. *Sci Rep* 2017; 7(1): 12866. [PubMed: 28993666]
30. Honeycutt JB, Thayer WO, Baker CE, et al. HIV persistence in tissue macrophages of humanized myeloid-only mice during antiretroviral therapy. *Nat Med* 2017; 23(5): 638–43. [PubMed: 28414330]
31. Descours B, Petitjean G, Lopez-Zaragoza JL, et al. CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses. *Nature* 2017; 543(7646): 564–7. [PubMed: 28297712]
32. Bertagnolli LN, White JA, Beg SA, et al. Majority of the latent reservoir resides in CD32a negative CD4+ T cells. *Journal of Virus Eradication* 2017; 3(Suppl 5: Abstracts of the Eighth International Workshop on HIV Persistence during Therapy).
33. Procopio FA, Fromentin R, Kulpa DA, et al. A Novel Assay to Measure the Magnitude of the Inducible Viral Reservoir in HIV-infected Individuals. *EBioMedicine* 2015; 2(8): 872–81.
34. Barton K, Hiener B, Winckelmann A, et al. Broad activation of latent HIV-1 in vivo. *Nature communications* 2016; 7: 12731.
35. Passaes CP, Bruel T, Decalf J, et al. Ultrasensitive HIV-1 p24 Assay Detects Single Infected Cells and Differences in Reservoir Induction by Latency Reversal Agents. *J Virol* 2017; 91(6).
36. Metcalf Pate KA, Pohlmeier CW, Walker-Sperling VE, et al. A Murine Viral Outgrowth Assay to Detect Residual HIV Type 1 in Patients With Undetectable Viral Loads. *J Infect Dis* 2015; 212(9): 1387–96. [PubMed: 25883388]
37. Elliott JH, Wightman F, Solomon A, et al. Activation of HIV transcription with short-course vorinostat in HIV-infected patients on suppressive antiretroviral therapy. *PLoS Pathog* 2014; 10(10): e1004473. [PubMed: 25393648]
38. Rasmussen TA, Tolstrup M, Brinkmann CR, et al. Panobinostat, a histone deacetylase inhibitor, for latent-virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase 1/2, single group, clinical trial. *Lancet HIV* 2014; 1(1): e13–21. [PubMed: 26423811]
39. Sogaard OS, Graversen ME, Leth S, et al. The Depsipeptide Romidepsin Reverses HIV-1 Latency In Vivo. *PLoS Pathog* 2015; 11(9): e1005142. [PubMed: 26379282]

40. Baxter AE, Niessl J, Fromentin R, et al. Single-Cell Characterization of Viral Translation-Competent Reservoirs in HIV-Infected Individuals. *Cell Host Microbe* 2016; 20(3): 368–80. [PubMed: 27545045]
41. Imamichi H, Dewar RL, Adelsberger JW, et al. Defective HIV-1 proviruses produce novel protein-coding RNA species in HIV-infected patients on combination antiretroviral therapy. *Proc Natl Acad Sci U S A* 2016; 113(31): 8783–8. [PubMed: 27432972]
42. Clarridge KE, Blazkova J, Einkauf K, et al. Effect of analytical treatment interruption and reinitiation of antiretroviral therapy on HIV reservoirs and immunologic parameters in infected individuals. *PLoS Pathog* 2018; 14(1): e1006792. [PubMed: 29324842]
43. Koelsch KK, Rasmussen TA, Hey-Nguyen WJ, et al. Impact of Allogeneic Hematopoietic Stem Cell Transplantation on the HIV Reservoir and Immune Response in 3 HIV-Infected Individuals. *J Acquir Immune Defic Syndr* 2017; 75(3): 328–37. [PubMed: 28350553]
44. Cummins NW, Rizza S, Litzow MR, et al. Extensive virologic and immunologic characterization in an HIV-infected individual following allogeneic stem cell transplant and analytic cessation of antiretroviral therapy: A case study. *PLoS Med* 2017; 14(11): e1002461. [PubMed: 29182633]
45. Santangelo PJ, Rogers KA, Zurla C, et al. Whole-body immunoPET reveals active SIV dynamics in viremic and antiretroviral therapy-treated macaques. *Nat Methods* 2015; 12(5): 427–32. [PubMed: 25751144]
46. Hutter G More on shift of HIV tropism in stem-cell transplantation with CCR5 delta32/delta32 mutation. *N Engl J Med* 2014; 371(25): 2437–8.
47. Cillo AR, Krishnan S, McMahon DK, Mitsuyasu RT, Para MF, Mellors JW. Impact of chemotherapy for HIV-1 related lymphoma on residual viremia and cellular HIV-1 DNA in patients on suppressive antiretroviral therapy. *PLoS One* 2014; 9(3): e92118. [PubMed: 24638072]
48. Tebas P, Stein D, Tang WW, et al. Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. *N Engl J Med* 2014; 370(10): 901–10. [PubMed: 24597865]
49. Liu Z, Chen S, Jin X, et al. Genome editing of the HIV co-receptors CCR5 and CXCR4 by CRISPR-Cas9 protects CD4(+) T cells from HIV-1 infection. *Cell Biosci* 2017; 7: 47. [PubMed: 28904745]
50. Wang Z, Pan Q, Gendron P, et al. CRISPR/Cas9-Derived Mutations Both Inhibit HIV-1 Replication and Accelerate Viral Escape. *Cell Rep* 2016; 15(3): 481–9. [PubMed: 27068471]
51. Jones RB, O'Connor R, Mueller S, et al. Histone deacetylase inhibitors impair the elimination of HIV-infected cells by cytotoxic T-lymphocytes. *PLoS Pathog* 2014; 10(8): e1004287. [PubMed: 25122219]
52. Pace M, Williams J, Kurioka A, et al. Histone Deacetylase Inhibitors Enhance CD4 T Cell Susceptibility to NK Cell Killing but Reduce NK Cell Function. *PLoS Pathog* 2016; 12(8): e1005782. [PubMed: 27529554]
53. Elliott JH, McMahon JH, Chang CC, et al. Short-term administration of disulfiram for reversal of latent HIV infection: a phase 2 dose-escalation study. *Lancet HIV* 2015; 2(12): e520–9. [PubMed: 26614966]
54. Vibholm L, Schleimann MH, Hojen JF, et al. Short-Course Toll-Like Receptor 9 Agonist Treatment Impacts Innate Immunity and Plasma Viremia in Individuals With Human Immunodeficiency Virus Infection. *Clin Infect Dis* 2017; 64(12): 1686–95. [PubMed: 28329286]
55. Tsai A, Irrinki A, Kaur J, et al. Toll-Like Receptor 7 Agonist GS-9620 Induces HIV Expression and HIV-Specific Immunity in Cells from HIV-Infected Individuals on Suppressive Antiretroviral Therapy. *J Virol* 2017; 91(8).
56. Whitney JB, Lim SY, Osuna CE, et al. Repeated TLR7 agonist treatment of SIV+ monkeys on ART can lead to viral remission. *Conference on Retroviruses and Opportunistic Infections*. Boston, Massachusetts; 2016.
57. Laird GM, Bullen CK, Rosenbloom DI, et al. Ex vivo analysis identifies effective HIV-1 latency-reversing drug combinations. *J Clin Invest* 2015; 125(5): 1901–12. [PubMed: 25822022]
58. Kim Y, Anderson JL, Lewin SR. Getting the “Kill” into “Shock and Kill”: Strategies to Eliminate Latent HIV. *Cell Host Microbe* 2018; 23(1): 14–26. [PubMed: 29324227]

59. Cummins NW, Sainski AM, Dai H, et al. Prime, Shock, and Kill: Priming CD4 T Cells from HIV Patients with a BCL-2 Antagonist before HIV Reactivation Reduces HIV Reservoir Size. *J Virol* 2016; 90(8): 4032–48. [PubMed: 26842479]
60. Kessing CF, Nixon CC, Li C, et al. In Vivo Suppression of HIV Rebound by Didehydro-Cortistatin A, a “Block-and-Lock” Strategy for HIV-1 Treatment. *Cell Rep* 2017; 21(3): 600–11. [PubMed: 29045830]
61. Ahlenstiel C, Mendez C, Lim ST, et al. Novel RNA Duplex Locks HIV-1 in a Latent State via Chromatin-mediated Transcriptional Silencing. *Molecular therapy Nucleic acids* 2015; 4: e261. [PubMed: 26506039]
62. Centlivre M, Legrand N, Klamer S, et al. Preclinical in vivo evaluation of the safety of a multi-shRNA-based gene therapy against HIV-1. *Molecular therapy Nucleic acids* 2013; 2: e120. [PubMed: 24002730]
63. Leth S, Schleimann MH, Nissen SK, et al. Combined effect of Vacc-4x, recombinant human granulocyte macrophage colony-stimulating factor vaccination, and romidepsin on the HIV-1 reservoir (REDUC): a single-arm, phase 1B/2A trial. *Lancet HIV* 2016; 3(10): e463–72. [PubMed: 27658863]
64. Mothe B, Moltó J, Manzardo C, et al. Viral control induced by HIVconsv vaccines & romidepsin in early treated individuals. Conference on Retroviruses and Opportunistic Infections. Seattle, USA; 2017.
65. Sneller MC, Justement JS, Gittens KR, et al. A randomized controlled safety/efficacy trial of therapeutic vaccination in HIV-infected individuals who initiated antiretroviral therapy early in infection. *Sci Transl Med* 2017; 9(419).
66. Hansen SG, Ford JC, Lewis MS, et al. Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. *Nature* 2011; 473(7348): 523–7. [PubMed: 21562493]
67. Borducchi EN, Cabral C, Stephenson KE, et al. Ad26/MVA therapeutic vaccination with TLR7 stimulation in SIV-infected rhesus monkeys. *Nature* 2016; 540(7632): 284–7. [PubMed: 27841870]
68. Deng K, Perteu M, Rongvaux A, et al. Broad CTL response is required to clear latent HIV-1 due to dominance of escape mutations. *Nature* 2015; 517(7534): 381–5. [PubMed: 25561180]
69. Hancock G, Yang H, Yorke E, et al. Identification of effective subdominant anti-HIV-1 CD8+ T cells within entire post-infection and post-vaccination immune responses. *PLoS Pathog* 2015; 11(2): e1004658. [PubMed: 25723536]
70. Lu CL, Murakowski DK, Bournazos S, et al. Enhanced clearance of HIV-1-infected cells by broadly neutralizing antibodies against HIV-1 in vivo. *Science* 2016; 352(6288): 1001–4. [PubMed: 27199430]
71. Schoofs T, Klein F, Braunschweig M, et al. HIV-1 therapy with monoclonal antibody 3BNC117 elicits host immune responses against HIV-1. *Science* 2016; 352(6288): 997–1001. [PubMed: 27199429]
72. Bruel T, Guivel-Benhassine F, Amraoui S, et al. Elimination of HIV-1-infected cells by broadly neutralizing antibodies. *Nature communications* 2016; 7: 10844.
73. Nishimura Y, Gautam R, Chun TW, et al. Early antibody therapy can induce long-lasting immunity to SHIV. *Nature* 2017; 543(7646): 559–63. [PubMed: 28289286]
74. Bar KJ, Sneller MC, Harrison LJ, et al. Effect of HIV Antibody VRC01 on Viral Rebound after Treatment Interruption. *N Engl J Med* 2016; 375(21): 2037–50. [PubMed: 27959728]
75. Scheid JF, Horwitz JA, Bar-On Y, et al. HIV-1 antibody 3BNC117 suppresses viral rebound in humans during treatment interruption. *Nature* 2016; 535(7613): 556–60. [PubMed: 27338952]
76. Hessel AJ, Jaworski JP, Epton E, et al. Early short-term treatment with neutralizing human monoclonal antibodies halts SHIV infection in infant macaques. *Nat Med* 2016; 22(4): 362–8. [PubMed: 26998834]
77. Huang Y, Yu J, Lanzi A, et al. Engineered Bispecific Antibodies with Exquisite HIV-1-Neutralizing Activity. *Cell* 2016; 165(7): 1621–31. [PubMed: 27315479]
78. Xu L, Pegu A, Rao E, et al. Trispecific broadly neutralizing HIV antibodies mediate potent SHIV protection in macaques. *Science* 2017; 358(6359): 85–90. [PubMed: 28931639]

79. Pace CS, Song R, Ochsenbauer C, et al. Bispecific antibodies directed to CD4 domain 2 and HIV envelope exhibit exceptional breadth and picomolar potency against HIV-1. *Proc Natl Acad Sci U S A* 2013; 110(33): 13540–5. [PubMed: 23878231]
80. Sloan DD, Lam CY, Irrinki A, et al. Targeting HIV Reservoir in Infected CD4 T Cells by Dual-Affinity Re-targeting Molecules (DARTs) that Bind HIV Envelope and Recruit Cytotoxic T Cells. *PLoS Pathog* 2015; 11(11): e1005233. [PubMed: 26539983]
81. Hale M, Mesojednik T, Romano Ibarra GS, et al. Engineering HIV-Resistant, Anti-HIV Chimeric Antigen Receptor T Cells. *Mol Ther* 2017; 25(3): 570–9. [PubMed: 28143740]
82. Liu B, Zou F, Lu L, et al. Chimeric Antigen Receptor T Cells Guided by the Single-Chain Fv of a Broadly Neutralizing Antibody Specifically and Effectively Eradicate Virus Reactivated from Latency in CD4+ T Lymphocytes Isolated from HIV-1-Infected Individuals Receiving Suppressive Combined Antiretroviral Therapy. *J Virol* 2016; 90(21): 9712–24. [PubMed: 27535056]
83. Wykes MN, Lewin SR. Immune checkpoint blockade in infectious diseases. *Nat Rev Immunol* 2017.
84. Fromentin R, Bakeman W, Lawani MB, et al. CD4+ T Cells Expressing PD-1, TIGIT and LAG-3 Contribute to HIV Persistence during ART. *PLoS Pathog* 2016; 12(7): e1005761. [PubMed: 27415008]
85. Chew GM, Fujita T, Webb GM, et al. TIGIT Marks Exhausted T Cells, Correlates with Disease Progression, and Serves as a Target for Immune Restoration in HIV and SIV Infection. *PLoS Pathog* 2016; 12(1): e1005349. [PubMed: 26741490]
86. Gay CL, Bosch RJ, Ritz J, et al. Clinical Trial of the Anti-PD-L1 Antibody BMS-936559 in HIV-1 Infected Participants on Suppressive Antiretroviral Therapy. *J Infect Dis* 2017; 215(11): 1725–33. [PubMed: 28431010]
87. Wightman F, Solomon A, Kumar SS, et al. Effect of ipilimumab on the HIV reservoir in an HIV-infected individual with metastatic melanoma. *AIDS* 2015; 29(4): 504–6. [PubMed: 25628259]
88. Guihot A, Marcelin AG, Massiani MA, et al. Drastic decrease of the HIV reservoir in a patient treated with nivolumab for lung cancer. *Ann Oncol* 2017.
89. Ayala VI, Deleage C, Trivett MT, et al. CXCR5-Dependent Entry of CD8 T Cells into Rhesus Macaque B-Cell Follicles Achieved through T-Cell Engineering. *J Virol* 2017; 91(11).
90. Webb G, Li S, Greene J, et al. The human IL-15 superagonist ALT-803 activates NK and memory T cells, reactivates latent SIV and drives SIV-specific CD8+ T cells into B cell follicles 9th IAS Conference on HIV Science. Paris, France; 2017.
91. Stock PG, Barin B, Hatano H, et al. Reduction of HIV persistence following transplantation in HIV-infected kidney transplant recipients. *Am J Transplant* 2014; 14(5): 1136–41. [PubMed: 24698537]
92. Micci L, Ryan ES, Fromentin R, et al. Interleukin-21 combined with ART reduces inflammation and viral reservoir in SIV-infected macaques. *J Clin Invest* 2015; 125(12): 4497–513. [PubMed: 26551680]
93. Cheng L, Ma J, Li J, et al. Blocking type I interferon signaling enhances T cell recovery and reduces HIV-1 reservoirs. *J Clin Invest* 2017; 127(1): 269–79. [PubMed: 27941247]
94. Takata H, Buranapraditkun S, Kessing C, et al. Delayed differentiation of potent effector CD8(+) T cells reducing viremia and reservoir seeding in acute HIV infection. *Sci Transl Med* 2017; 9(377).
95. Ananworanich J, Chomont N, Eller LA, et al. HIV DNA Set Point is Rapidly Established in Acute HIV Infection and Dramatically Reduced by Early ART. *EBioMedicine* 2016; 11: 68–72. [PubMed: 27460436]
96. Garcia M, Gorgolas M, Cabello A, et al. Peripheral T follicular helper Cells Make a Difference in HIV Reservoir Size between Elite Controllers and Patients on Successful cART. *Sci Rep* 2017; 7(1): 16799. [PubMed: 29196729]
97. Ackerman ME, Mikhailova A, Brown EP, et al. Polyfunctional HIV-Specific Antibody Responses Are Associated with Spontaneous HIV Control. *PLoS Pathog* 2016; 12(1): e1005315. [PubMed: 26745376]
98. Jacobs ES, Keating SM, Abdel-Mohsen M, et al. Cytokines Elevated in HIV Elite Controllers Reduce HIV Replication In Vitro and Modulate HIV Restriction Factor Expression. *J Virol* 2017; 91(6).

99. Noyan K, Nguyen S, Betts MR, Sonnerborg A, Buggert M. Human Immunodeficiency Virus Type-1 Elite Controllers Maintain Low Co-Expression of Inhibitory Receptors on CD4+ T Cells. *Front Immunol* 2018; 9: 19. [PubMed: 29403500]
100. Crowell TA, Gebo KA, Blankson JN, et al. Hospitalization Rates and Reasons Among HIV Elite Controllers and Persons With Medically Controlled HIV Infection. *J Infect Dis* 2015; 211(11): 1692–702. [PubMed: 25512624]
101. Pernas M, Tarancon-Diez L, Rodriguez-Gallego E, et al. Factors Leading to the Loss of Natural Elite Control of HIV-1 Infection. *J Virol* 2017.
102. Assoumou L, Weiss L, Piketty C, et al. A low HIV-DNA level in peripheral blood mononuclear cells at antiretroviral treatment interruption predicts a higher probability of maintaining viral control. *AIDS* 2015; 29(15): 2003–7. [PubMed: 26355572]
103. McMahon JH, Chang J, Tennakoon S, et al. Post-treatment control in an adult with perinatally acquired HIV following cessation of antiretroviral therapy. *AIDS* 2017; 31(9): 1344–6.
104. Frange P, Faye A, Avettand-Fenoel V, et al. HIV-1 virological remission lasting more than 12 years after interruption of early antiretroviral therapy in a perinatally infected teenager enrolled in the French ANRS EPF-CO10 paediatric cohort: a case report. *Lancet HIV* 2016; 3(1): e49–54. [PubMed: 26762993]
105. Persaud D, Gay H, Ziemniak C, et al. Absence of detectable HIV-1 viremia after treatment cessation in an infant. *N Engl J Med* 2013; 369(19): 1828–35. [PubMed: 24152233]
106. Cheret A, Bacchus-Souffan C, Avettand-Fenoel V, et al. Combined ART started during acute HIV infection protects central memory CD4+ T cells and can induce remission. *J Antimicrob Chemother* 2015; 70(7): 2108–20. [PubMed: 25900157]
107. Jensen SS, Fomsgaard A, Borggren M, et al. HIV-Specific Antibody-Dependent Cellular Cytotoxicity (ADCC) -Mediating Antibodies Decline while NK Cell Function Increases during Antiretroviral Therapy (ART). *PLoS One* 2015; 10(12): e0145249. [PubMed: 26696395]
108. Martin GE, Gossez M, Williams JP, et al. Post-treatment control or treated controllers? Viral remission in treated and untreated primary HIV infection. *AIDS* 2017; 31(4): 477–84. [PubMed: 28060012]
109. Rainwater-Lovett K, Ziemniak C, Watson D, et al. Paucity of Intact Non-Induced Provirus with Early, Long-Term Antiretroviral Therapy of Perinatal HIV Infection. *PLoS One* 2017; 12(2): e0170548. [PubMed: 28178277]
110. Dong KL, Moodley A, Kwon DS, et al. Detection and treatment of Fiebig stage I HIV-1 infection in young at-risk women in South Africa: a prospective cohort study. *Lancet HIV* 2018; 5(1): e35–e44. [PubMed: 28978417]

Figure 1. Mechanisms of HIV persistence in cells and tissue.

(a) Latency is established in long-lived CD4⁺ T cells through mechanisms such as epigenetic modifications that reduce HIV transcription, including reduced acetylation and enhanced methylation, a lack of HIV transcription factors, inhibition of RNA export and inhibition of protein translation by microRNAs. (b) Integrated latent HIV is replicated upon cellular division. (c) Residual viral replication despite antiretroviral therapy may contribute to HIV persistence on ART. (d) B cell follicles of lymph nodes and other lymphoid tissue provide an immune sanctuary for HIV both within CD4⁺ T cells (T follicular helper cells) and on the follicular dendritic cell network by excluding cytotoxic CD8⁺ T lymphocytes. (e) Th17 cells in the gastrointestinal tract are infected and depleted leading to loss of gut barrier integrity, microbial translocation and immune activation. Subsequent chronic inflammation may promote HIV persistence on ART through cellular proliferation, CD8⁺ T cell

exhaustion and possible residual viral replication. Integrated viral DNA is shown in green for latently infected cells and in red for productively infected cells; genomic DNA is blue.

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Figure 2. HIV cure clinical trial design.

Interventions are usually administered to HIV infected individuals whilst on suppressive antiretroviral therapy (ART). These interventions may also be continued into the analytical treatment interruption (ATI) period. Several potential plasma viral outcomes are depicted. Yellow line: lack of response to the intervention with rapid viral rebound to a high level and high subsequent viral set point. Red line: rapid but low level viral rebound followed by viraemic control to between 50 and 1000 copies/ml. Green line: viral rebound delayed but virus rebounds to a high level. Blue line: No viral rebound to end of monitoring period. The primary endpoint may be defined as the time to viral rebound, the viral set-point reached following rebound or the area under the plasma virus curve which integrates both of these.

Figure 3. Strategies for HIV cure.

(a) Strategies that target the viral replication cycle. (b) HIV specific immune enhancement. (c) Immune modulation that is not specifically targeted at HIV. DARTs = Dual-Affinity Re-Targeting molecules. CAR = Chimeric Antigen Receptor. Integrated latent viral DNA is shown in green and genomic DNA is blue.

Table 1:

Quantifying HIV persistence on ART in clinical trials

Assay	Method	Advantages	Disadvantages
Quantitative Viral Outgrowth Assay (QVOA)	Uses limiting dilutions of CD4+ T cells cocultured with uninfected cells that support active HIV replication in a highly activating environment that promotes cell activation, division and HIV replication. The cocultured cells amplify the infection allowing ready detection of infection using reverse transcription PCR (RT-PCR) or p24 ELISA.	Most specific measure of the inducible replication-competent HIV reservoir. Advances on QVOA include: - MS-VOA: Multiple stimulation VOA ¹⁶ - Q ² VOA: Sequencing of cell-associated and supernatant virus following QVOA to quantify expanded clones ²⁰ - MVOA (mouse VOA): Administration of patient derived cells to an uninfected humanised mouse for amplification of virus <i>in vivo</i> (highly sensitive but not quantitative). ³⁶	Low sensitivity because a cell which does not produce virus after one round of stimulation may produce virus with a subsequent round. ¹⁶
Total and integrated HIV DNA	PCR	Sensitive measure of HIV persistence.	Quantifies both intact and defective virus and therefore will overestimate the size of the reservoir.
2-LTR circles	PCR	Formed when HIV DNA fails to integrate into the host genome. Diluted upon cell division as, unlike integrated HIV DNA, 2-LTR circles are not replicated upon cell division.	Long-term stability of 2-LTR circles within non-dividing cells is controversial.
Plasma HIV RNA	RT-PCR and single copy assay (sensitivity of 1 copy/ml)	Primary endpoint for latency reversal studies and analytical treatment interruptions.	It remains unclear which cells produce HIV RNA in plasma on ART and whether this virus contributes to viral rebound off ART.
Cell-associated unspliced (US) HIV RNA	RT-PCR	Changes in US RNA have been used to assess activity of latency reversing agents <i>in vivo</i> . ³⁷⁻³⁹	US RNA can either be virus initiated or driven by a host gene promoter.
Cell-associated multiply spliced (MS) HIV RNA	RT-PCR	MS RNA generally reflects a cell that is productively infected.	Detection of MS RNA on ART is infrequent.
<i>Tat/rev</i> Induced Limiting Dilution Assay (TILDA)	Uses RT-PCR to quantify multiply spliced HIV <i>tat/rev</i> transcripts using limiting dilutions of CD4+ T cells that are unstimulated or have been stimulated with a mitogen. ³³	Provides a measure of the frequency of cells with constitutive and/or inducible virus expression.	Measured transcripts may not represent replication-competent virus.
Viral DNA sequencing	Sanger sequencing ¹² or next generation sequencing ¹⁴	The frequency of genetically intact genomes provides an upper limit to the frequency of replication competent virus.	Cannot determine if virus is inducible. Expensive and time-consuming.
Viral RNA sequencing	Sanger sequencing ³⁴	Can determine the diversity of RNA transcripts and virions induced by latency reversing agents. ³⁴	Expensive and time-consuming.
Fluorescent probes to detect HIV DNA, RNA and protein	Fluorescent DNA and RNA probes use signal amplification. Detection uses microscopy or flow cytometry.	Can detect simultaneous expression of HIV DNA, RNA and/or protein in the same cell. Microscopy on tissue allows assessment of HIV DNA and RNA within the tissue microenvironment. ¹¹ Recent advances in using these probes with flow cytometry. ⁴⁰	Cannot determine if virus is replication competent. Tissue analysis requires specialised collection and storage of tissue for optimal staining.
Viral protein	Single Molecule Array (Simoa) ³⁵	Substantially improved sensitivity over ELISA assays allowing for the detection of p24 on ART or	Production of p24 Gag protein does not require full length intact virus so this assay may still detect defective virus. ⁴¹

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Analytical treatment interruption (ATI)	Plasma HIV RNA (RT-PCR)	Gold standard test of cure. No evidence of an increase in the size of the reservoir following ART recommencement. ⁴²	Potential risk of clinical symptoms at rebound ² and/or virus transmission while viraemic, although latter not reported to date.

Table 2:

HIV cure interventions that have entered or completed human clinical trials

Strategy group	Strategy	Rationale	Key findings	Future directions
Targeting the viral replication cycle	Bone marrow transplantation	Transplanted cells from a CCR5-32 donor are resistant to HIV infection. Transplanted cells may eliminate infected cells in the recipient via graft versus host disease.	Transplantation with CCR5-32 donor cells has cured one individual ¹ but has not been successful in other recipients with high rates of mortality. ⁴⁶ Transplantation of wild type CCR5 donor cells resulted in reduction in HIV persistence on ART and delayed viral rebound once ART was stopped. ²	Large observational cohort studies of transplant recipients. Optimisation of transplantation with modified and wild type donor cells in NHP models.
	Gene editing	Alter host proteins such as CCR5 so that T cells are resistant to HIV infection.	Transfusion of genetically modified T cells that don't express CCR5 is safe and modified cells persist <i>in vivo</i> but at low levels. ⁴⁸ Transfusion of modified CD4+ T cells did not delay viral rebound once ART was stopped. ⁴⁸	Enhance frequency and tissue penetration of gene modified cells through gene editing of stem cells, novel conditioning regimens and newer gene editing technologies. ⁴⁹ Use gene editing strategies to target HIV itself and/or other host proteins such as HIV restriction factors.
	Latency Reversal Agents (LRAs)	Increase transcription of HIV to induce virus- or immune-mediated cytolysis.	LRAs can increase cell-associated and in some cases plasma HIV RNA but have so far not been found to decrease the frequency of infected cells in humans. ^{37-39,53,54} Although safe, some concerning adverse effects of HDACi on host gene transcription ³⁷ and T cell function. ^{51,52}	Combine with agents that can enhance clearance of infected cells. Increase potency and specificity, reduce toxicity and enhance delivery to tissue sites.
HIV-specific immune enhancement	T cell vaccines	Increase HIV-specific T cell immunity to maintain long term remission and kill latently infected cells.	Multiple studies have shown an increase in the number and function of HIV-specific T cells post vaccination. The combination of T cell vaccines with the LRA romidepsin led to enhanced virus control post ART cessation ⁶⁴ but, in the absence of a placebo arm, it is difficult to	Combine T cell vaccines with new adjuvants eg TLR7 agonists ⁶⁷ and/or use new antigens eg mosaic proteins, new delivery systems eg electroporation and new vectors eg CMV vector ⁶⁶ or RNA vaccine.

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Immune modulation	Broadly Neutralising Antibodies	Antibodies that target a large number of HIV strains administered by passive infusion. Can bind and clear free virions and potentially infected cells.	interpret these findings. ⁶⁵ Administration of a single bNAb to HIV-infected individuals on ART resulted in a delay to rebound after ART cessation compared to historical controls. ^{74,75} Rebound virus was often resistant to the bNAb. ^{74,75} Unclear if bNAbs eliminate infected cells <i>in vivo</i> or just neutralise rebounding virus.	Evaluate combination bNAbs to reduce emergence of resistance, newer longer acting antibodies, bispecific and trispecific bNAbs, combine bNAbs with LRAs.
	Chimeric antigen receptor (CAR) T cells	Allow strong antigen-specific T cell responses that are independent of major histocompatibility complex (MHC) restriction.	CAR T cells with a bNAb single chain variable fragment in the CAR extracellular domain can lyse HIV infected cells <i>ex vivo</i> . ⁸²	Minimise toxicity from cytokine release.
	Immune checkpoint blockers	Enhance the HIV-specific functional immune response and potentially reverse HIV latency.	Safety profile of some concern, particularly immune related adverse events. Case reports show perturbation of the reservoir but very limited numbers of individuals assessed. ^{87,88}	Large observational cohort studies of individuals with HIV infection and cancer who receive immune checkpoint blockers. Dose escalation studies in HIV-infected individuals without cancer to assess safety. Evaluation of immune checkpoint blockers alone and in combination in animal models.
	Vedolizumab	Reduce trafficking of highly susceptible CD4+ T cells to the gut HIV reservoir.	Prolonged viraemic control following ATI in NHP. ⁵	Results of clinical trials in humans awaited.
	IL-15 superagonist	Improve trafficking of CD8+ T cells into lymphoid tissue B cell follicle reservoirs.	Increased CD8+ T cell CXCR5 expression and infiltration into B cell follicles of lymph nodes in macaques. ⁹⁰	Results of clinical trial in humans awaited.
	Sirolimus	To reduce HIV-associated inflammation and thereby improve the HIV-specific immune response.	Associated with reduced HIV DNA levels in renal transplant cohort study. ⁹¹	Results of clinical trial awaited.