EDITORIAL REVIEW

HIV cure and eradication: how will we get from the laboratory to effective clinical trials?

Sharon R. Lewin\(^a,b,c\) and Christine Rouzioux\(^d,e\)

Combination antiretroviral therapy (cART) has led to a major reduction in HIV-related mortality and morbidity; however, HIV can still not be cured. Achieving either a functional cure (long-term control of HIV in the absence of cART) or a sterilizing cure (elimination of all HIV-infected cells) remains a major challenge. The most significant barrier to cure is the establishment of a latent or ‘silent’ infection in resting CD4\(^+\) T cells. Several randomized clinical trials have demonstrated that treatment intensification with additional antiretrovirals has little impact on latent reservoirs. Some potential other approaches that may reduce the latent reservoir include very early initiation of cART and the use of agents that could reverse latent infection. Drugs such as histone deacetylase inhibitors, currently used and licensed for the treatment of some cancers; methylation inhibitors; cytokines such as IL-7 or activators of nuclear factor kappa B (NF-\(\kappa B\)) such as prostratin, show promising activity in reversing latency \textit{in vitro} when used either alone or in combination. Alternate strategies include using gene therapy to modify expression of CCR5 and therefore make cells resistant to HIV. This review will primarily focus on the advantages and disadvantages of methods currently being used to quantify persistent virus \textit{ex vivo} in patients receiving cART and strategies aimed at cure that are being tested \textit{in vitro} or in early clinical development. In addition, we discuss key issues that need to be addressed to successfully move laboratory research to clinical trials aimed at curing HIV.

**Keywords:** eradication, HIV, latency, reservoirs

**Introduction**

Despite the significant reduction in morbidity and mortality following combination antiretroviral therapy (cART), cART cannot eradicate HIV. Recently, there has been a renewed scientific interest in developing new strategies to eventually find a cure for HIV. There have been several significant advances in our understanding of the major barriers to curing HIV. These barriers include long-lived latently infected cells and residual viral replication, at least in some patients. In addition, anatomical reservoirs including the gastrointestinal tract, lymphoid tissue and the central nervous system (CNS) may harbour unique long-lived infected cells and penetration of cART may be limited at these sites. The complex mechanisms of how latency is established and maintained in different T-cell subsets and the major cellular reservoirs that persist in patients on cART have recently been extensively reviewed elsewhere [1–5]. In this review, we will therefore focus on the key scientific and clinical variables that we need to understand in order to significantly expand the breadth and scope of clinical

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trials aimed at finding a cure for HIV. In addition we will focus on strategies for cure that are currently being or soon to be tested in clinical trials.

Why do we need a cure for HIV?

Even with the major successes of cART, full life expectancy for patients living with HIV has not been restored. Although some cohort studies have shown near normal life expectancy for a subset of patients [6], other studies have shown that life expectancy remains shortened [7,8]. In a prospective study of 3990 HIV-infected individuals in Denmark, the chance of a person with HIV reaching the age of 70 was 50% that of uninfected population controls [9]. The incidence of significant morbidity also remains elevated despite successful cART (reviewed in [10]), due to complex interactions between drug toxicity [11], persistent inflammation [12], and risk behaviours [13]. Finally, despite the clear need for universal access to cART, the lack of financial resources to support lifelong treatment, for everyone in need of treatment, is still a major challenge [14,15].

Functional or sterilizing cure?

There are two potential strategies for cure. The first is what could be considered an ‘infectious diseases model’ of cure which would require the elimination of all HIV-infected cells in all compartments and sanctuaries and for patients to have a plasma HIV RNA count of less than 1 copy/ml. This is now commonly referred to as a sterilizing cure. The alternative approach would be to aim for remission or what could be considered a ‘cancer model’ of cure, in which an individual would have long-term health in the absence of treatment, with low-level viraemia at less than 50 copies/ml. This is commonly referred to as a functional cure.

Sterilizing cure: elimination of HIV following bone marrow transplantation

The recent case report of a German patient with acute myeloid leukaemia, who received a bone marrow transplant from a donor who carried a 32-base pair deletion in the CCR5 gene, is the only current example of a sterilizing cure [16]. Following transplantation, the patient stopped cART and HIV RNA remained at below 1 copy/ml. In more detailed studies, including multiple biopsies of his gastrointestinal tract, analysis of his cerebrospinal fluid (CSF) and bone marrow and even a brain biopsy, neither HIV DNA or HIV RNA was detected [16,17]. The patient has now been off cART for over 45 months and HIV is still not detected. Reconstitution of circulating and mucosal CD4+ T cells that did not express CCR5 was observed [17]. CCR5+ macrophages were detected early post transplantation in the gastrointestinal tract but at later time points, all mucosal macrophages expressed the mutant CCR5 [17]. In addition, the patient’s peripheral blood mononuclear cells (PBMCs) were permissive to CXCR4 using laboratory isolates ex vivo, demonstrating that the patients CD4+ T cells were not resistant to HIV. Potential factors leading to the elimination of long-lived reservoirs in this patient could have included the specific chemotherapy administered, total body irradiation or low-grade graft-versus-host disease in addition to eliminating the capacity for any residual replication by removing target cells that express CCR5. Whereas a strategy of using bone marrow transplantation with a CCR5 mutant donor is not a realistic cure for HIV given the toxicity and complexity of the treatment, we need to continue to comprehensively study this patient to fully understand how and why HIV was eliminated.

Functional cure: elite controllers

Elite controllers represent a unique group of patients who are able to achieve a consistent and long-term control of viral replication with HIV RNA of less than 50 copies/ml in the absence of cART. In addition, the reservoir is significantly smaller in elite controllers with low concentration of HIV DNA in different subsets of circulating CD4+ T cells in blood [18,19] as well as in rectal tissue [20,21].

There have been multiple studies examining the role of genetics, the virus and the immune response in elite controllers [20,22–25]. One of the consistent results from this work is the clear association with HLA class one genes [26,27]. Recent work has also demonstrated the importance of an effective cytolytic CD8+ T-cell response in blood which has been associated with enhanced activity of the T-box transcription factor t-bet [28,29] and increased production of IL–21 [30]. Strong HIV-specific CD4+ and CD8+ T-cell responses were also identified in mucosal tissue from elite controllers [31,32]. The innate immune system may also be important with enhanced activity of myeloid dendritic cells [33]. These data provide supportive evidence that inducing an effective immune response, perhaps via vaccination, may be one strategy to achieve a functional cure.

As some elite controllers do not bear the protective alleles HLA B27 or HLA B57, mechanisms other than enhanced T-cell immunity have also been explored. Several investigators have demonstrated lower replicative capacity of the virus isolated from elite controllers [34–36], and very low level of viral replication soon after infection [37]. There is no evidence currently that activated CD4+ T cells from these patients are resistant to HIV [38].

Despite apparent ‘functional cure’ in elite controllers, it is important to remember that low-level viraemia and
infected resting CD4\(^+\) T-cells are detected [23,24]. Compared with patients receiving cART, PBMC from elite controllers have similar levels of total DNA, but significantly lower integrated DNA and higher 2-long terminal repeat (2-LTR) levels [39]. Immune activation is higher in elite controllers compared with healthy controls [22]. In contrast to patients on cART with HIV RNA below 50 copies/ml, there is evolution in HIV RNA sequences in elite controllers [40], and in approximately 7% of elite controllers, CD4\(^+\) T cells decline over time [24]. Because of the low total number of infected cells and robust HIV-specific immune responses, elite controllers could potentially be the best candidates to test strategies aimed at achieving a sterilizing cure.

**Measuring latently infected cells and the ‘reservoir’ in vivo**

The major reason why HIV cannot be cured is the persistence of HIV in a latent form in different cellular reservoirs. *In vivo*, HIV latency occurs in resting CD4\(^+\) T cells either as preintegration or postintegration latency. Preintegration latency refers to unintegrated HIV DNA that is unstable and will either degrade or will integrate into the host cell genome, usually following cell activation [41]. Postintegration latency refers to the presence of integrated HIV DNA in cells that are not actively producing viral particles [42].

The major reservoir of cells that harbour postintegration latency *in vivo* are resting central memory (CD45RA-CCR7\(^+\)CD27\(^+\)) and transitional memory (CD45RA-CCR7-CD27\(^+\)) CD4\(^+\) T cells [43,44]. Latent infection can also be established in other long-lived cells including naïve T cells [45,46], bone marrow progenitor cells [47], thymocytes [48], and astrocytes [49,50]. Other cells such as monocyte/macrophages can support long-lived low-level productive infection [51].

Together, these persistent infected cells constitute the ‘latent reservoir’. Latently infected cells can be detected in both blood and tissue, including the gastrointestinal tract [52], genital tract [53] and the central nervous system [50,54]. When activated, latently infected T cells can either release viral particles or become productively infected T cells. In the presence of treatment, further rounds of infection do not occur and there is no viral rebound but when treatment is stopped, viral rebound will occur. There are multiple methods currently used to quantify persistent HIV-infected cells in patients on cART (summarized in Fig. 1 and Table 1) [39,42,53,55–73].

**Replication-competent virus: cell associated**

The gold standard used to measure the frequency of resting CD4\(^+\) T cells carrying latent but replication-competent virus is based on co-culture of highly purified resting CD4\(^+\) T cells from the patient together with PBMCs from an HIV-negative donor and is measured as infectious units per million cells (IUPM). The major limitation of using this technique in large multisite clinical trials is the need for large blood volume, often requiring leukapheresis. In addition, the assay is labour-intensive, has a wide coefficient of variation and cannot be performed with tissue biopsies [42,55].

**HIV DNA: unintegrated, integrated and circular**

Within infected cells, HIV DNA can exist as linear unintegrated forms, circular forms and as an integrated provirus (Fig. 1). In patients receiving effective cART, the

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**Fig. 1.** The viral life cycle in a latently infected cell (left) and productively infected cell (right) is shown together with the current available tests that quantify different forms of HIV RNA (orange boxes and arrows) and HIV DNA (blue boxes and arrows). HIV DNA is shown in blue and HIV RNA is shown in black. *HIV DNA measures unintegrated and integrated DNA as well as 2-LTR circles.*
majority of HIV DNA is integrated in resting latently infected CD4\(^+\) T cells [74]. One popular and widely used technique to quantify the number of cells that contain integrated virus is called Alu-LTR PCR [56]. To improve sensitivity of this assay, many published methods use nested PCR, with the second round of amplification having both primers within the HIV LTR [57,58]. This approach increases sensitivity but also increases the complexity of the assay and multiple replicates and a large number of controls are needed to enhance the accuracy and reproducibility of this assay [57,58,74].

Quantification of 2-LTR circles that are episomal forms of nonintegrated HIV DNA containing two copies of the LTR is also a useful tool. 2-LTR circles are produced following infection of a cell and have a relatively short half-life. Therefore, detection of 2-LTR circles is generally considered to be a surrogate marker of recent infection, rather than a marker of the number of residual infected cells [59,60]. In a recent small study of six patients who ceased cART, envelope sequences derived from 2-LTR circles from PBMC collected prior to cART cessation were related phylogenetically to envelope sequences from the rebound virus in plasma following cessation of cART [73]. In contrast, envelope sequences from PBMC from proviral DNA clustered less frequently with the rebound virus providing further evidence that 2-LTR circles are a surrogate measure of replicating virus [73].

Total HIV DNA quantifies integrated and nonintegrated DNA as well as latent and defective virus. There is a

<table>
<thead>
<tr>
<th>Methods</th>
<th>Objective</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IUPM</td>
<td>Measures capacity of the cell to produce infectious virus</td>
<td>Gold standard to identify latently infected resting CD4(^+) T cells</td>
<td>Labour-intensive technique Large volume of fresh blood needed Unable to perform on tissue</td>
<td>[41,54]</td>
</tr>
<tr>
<td>Integrated HIV DNA</td>
<td>Measures integrated provirus</td>
<td>Marker of latency in sorted resting CD4(^+) T cells Can be performed on small volumes of frozen samples</td>
<td>Quantifies both replication competent and incompetent integrated virus Labour-intensive technique Multiple methods used in different studies Reproducibility across multiple labs unknown</td>
<td>[39,56–58]</td>
</tr>
<tr>
<td>2-LTR circles</td>
<td>Measures a labile byproduct of HIV integration</td>
<td>Marker of recent cycles of replication Can be performed on small volumes of frozen samples</td>
<td>Reproducibility across multiple labs unknown</td>
<td>[59,60,73]</td>
</tr>
<tr>
<td>Total cell-associated HIV DNA</td>
<td>Measures unintegrated, integrated linear DNA and 2-LTR circles</td>
<td>Good correlation with integrated DNA Can be performed on small volumes of frozen samples including blood and tissue</td>
<td>Includes quantification of replication competent and incompetent virus</td>
<td>[52,71,72,127,128]</td>
</tr>
<tr>
<td>Cell-associated US-RNA and MS-RNA</td>
<td>Measures HIV transcription in productively and latently infected cells</td>
<td>Useful to estimate residual productively infected cells in patients on cART Could be used to quantify any increase in transcription following activation strategies</td>
<td>Few published studies Reproducibility across multiple labs unknown</td>
<td>[66–69]</td>
</tr>
<tr>
<td>HIV RNA SCA</td>
<td>Ultrasensitive method to quantify HIV RNA in plasma which is not detected by conventional HIV assays</td>
<td>High throughput Not technically complicated, single round PCR Cell isolation not required</td>
<td>Large volume of plasma needed Reproducibility across multiple labs unknown Relationship between low level viraemia, viral persistence and viral rebound unknown</td>
<td>[62–65]</td>
</tr>
</tbody>
</table>

IUPM, infectious units per million cells; LTR, long terminal repeat; MS-RNA, multiply spliced RNA; SCA, single copy assay; US-RNA, unspliced RNA.

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strong correlation between total HIV DNA and integrated HIV DNA in patients on cART and therefore cell-associated HIV DNA is likely to be a good surrogate marker of the total number of latently infected cells [74]. In a recent small study, three of seven patients examined had an excess of unintegrated DNA compared with integrated DNA suggesting that total HIV DNA may not be an ideal way to quantify the reservoir in all patients [75]. Quantification of total cell-associated HIV DNA is likely to be the most feasible tool to evaluate the frequency of infected cells in large-scale clinical trials and cohorts.

HIV RNA: plasma
The quantification of low-level viraemia in patient plasma is now possible using an ultrasensitive PCR-based assay that can measure down to 1 copy/ml [61]. Using this assay, over 80% of patients on cART have detectable viraemia at around 3–5 copies/ml [62]. There are several limitations to this assay. First the assay requires a large volume of plasma (7 ml) and in 10% of patients amplification of the viral sequence is not possible (Dr Sarah Palmer, Karolinska Institute, Sweden, personal communication). It is currently unclear whether this low-level virus is indeed replication-competent and how this virus is related to rebound virus following treatment cessation. When HIV RNA is below 50 copies/ml the sequence of plasma virus over time is very stable and shares little homology with the sequence of HIV derived from resting CD4+ T cells or monocytes [63,64]. Further work is needed to understand the source of low-level viraemia and whether this virus contributes to viral rebound.

HIV RNA: cell associated
Measurement of cell-associated HIV RNA, includes quantification of extracellular or cell-associated unspliced and multiply spliced RNA (Fig. 1) [65,66]. In latently infected cells, one would expect no extracellular HIV RNA if ongoing viral replication has been blocked. However, ongoing production of virus from latently infected cells will produce both unspliced and multiply spliced RNA. Indeed, following cART in patients with chronic infection, extracellular HIV RNA rapidly declines, whereas unspliced RNA persists in approximately 70–80% of patients [65–67]. Multiply spliced RNA is critical for the production of tat, nef and rev, which are all required for efficient production of full-length unspliced RNA [68]. In latently infected cells, there is a block in nuclear export of multiply spliced RNA and inefficient production of unspliced RNA [76]. Therefore, detection of specific types of multiply spliced RNA differs in patients with productive infection and following suppressive cART [66].

Blood or tissue?
The highest concentration of HIV DNA and cell-associated unspliced HIV RNA in patients on cART is found in tissues such as lymphoid or gastrointestinal tract tissue, as recently demonstrated in both infected humans and macaques [69,70]. The concentration of HIV DNA and HIV RNA in the gastrointestinal tract is almost 10 times that in blood in patients on suppressive cART [52,69]. In a recent study of anatomical reservoirs in RT-SHIV-infected macaques on cART, lymphoid tissue (including spleen and lymph node) and gastrointestinal tract had the largest pool of infected cells (measured by cell-associated HIV DNA and unspliced RNA), whereas minimal residual infected cells were detected in the CNS or reproductive tract [70]. It is likely that factors that maintain and/or allow the establishment of latency may differ in blood and tissues. Therefore it is critical that for new interventions aimed at eradication, if at all possible, quantification of latently and productively infected cells, should include tissue as well as blood.

Current and future strategies aimed at eradicating HIV: advantages, disadvantages
A number of strategies aimed at eliminating persistent virus in patients receiving suppressive cART have been tested, are in early phases of development or are close to evaluation in clinical trials (summarized in Table 2).

Treatement intensification
There have been a number of studies that have looked at the effect of treatment intensification on residual virus, in patients receiving cART. These studies have included the addition of agents, such as enfuviritide, additional protease inhibitors (ritonavir-boosted atazanavir or lopinavir) or raltegravir, to an already suppressive regimen [77–82]. Disappointingly, none of these studies have demonstrated any decline in low-level viraemia, IUPM or cell-associated HIV DNA. In addition, two small, non-randomized studies showed no significant decline in cell-associated HIV DNA or unspliced RNA in the gastrointestinal tract following intensification with raltegravir (n = 7) [80] and no change in HIV RNA in the CSF following intensification with maraviroc, lopinavir/ritonavir or enfuviritide (n = 10) [83].

One randomized study using raltegravir intensification for 48 weeks in 69 patients showed no change in persistent low-level plasma HIV RNA or cell-associated DNA after 24 weeks but demonstrated clear evidence of residual viral replication in at least one-third of patients as seen by an early and rapid increase in 2-LTR circles [79]. Taken together these studies suggest that the addition of the potent antiretroviral raltegravir alone to suppressive cART has minimal effect on persisting low-level viraemia and HIV DNA in either blood or tissue and approaches other than intensification will be needed.
Antiretroviral agents from different classes may have unique activity on establishing, maintaining or eliminating latently infected cells. A recent small, nonrandomized intensification study with maraviroc, a CCR5 antagonist, demonstrated some intriguing findings [84]. In this Spanish study, maraviroc was added for 48 weeks to a suppressive cART regimen in 10 patients. They observed an increase in HIV RNA (measured by the single copy assay) and 2-LTR circles in association with a significant decrease in the IUPM. This is the only intervention to date that has shown a decrease in the number of latently infected cells (measured by IUPM) using intensification in patients on suppressive cART. The mechanism for how maraviroc may be working is unclear; however, investigation of tissue reservoirs, specifically gastrointestinal tract and lymphoid tissue and repeating this study in a larger randomized design will be important [16,17,55,66,77–83,85–107].

**Early treatment**

Early treatment may be a potential strategy to reduce or even control the number of persistent latently infected cells. Several groups have demonstrated that the number of infected cells, as measured by both cell-associated HIV DNA and HIV unspliced RNA, decreases to a significantly lower level if cART is initiated during acute rather than chronic infection [55,66,85,86].

In a recent longitudinal study of patients who initiated cART during very early acute infection and stayed on cART for a prolonged period, in five of 32 (16%) patients following cessation of cART, HIV RNA was maintained at below 50 copies/ml for a median of 77 months off cART [85]. The use of very early cART may have had a significant impact on the number of infected cells as measured by total HIV DNA (Fig. 2) [20,85,108–110]. However, the findings from this study were in contrast to many other studies of viral rebound in nearly all patients following cessation of cART, even when initiated during acute infection [87,111,112]. The role of very early treatment initiation in limiting seeding of the HIV reservoir, as well as preserving immune responses capable of controlling HIV replication, requires further investigation.

**Elimination of latently infected T cells via induction of virus production**

One strategy to eliminate latently infected cells is to induce virus production from latently infected cells. Further rounds of infection would be blocked by cART and the productively infected cell would die. This strategy is only viable if active viral replication is completely inhibited on cART.

**Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway**

IL-7 is a cytokine that can effectively induce productive infection from latently infected resting CD4+ T cells in vitro via activation of the JAK-STAT pathway [88]. IL-7 has also recently been shown to be well tolerated in patients with HIV infection and leads to the expansion of naive and memory CD4+ and CD8+ T cells following cessation of cART.

**Table 2. Current strategies that are in development or in clinical trials for eradicating residual virus in patients on cART.**

<table>
<thead>
<tr>
<th>Rationale</th>
<th>Strategy</th>
<th>Mechanism of action</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduce viral replication</td>
<td>Treatment intensification</td>
<td></td>
<td>No effect on residual HIV DNA or plasma HIV RNA.</td>
<td>[73–79]</td>
</tr>
<tr>
<td></td>
<td>Early treatment during acute infection</td>
<td>JAK-STAT activation, e.g. IL-7, NF-kB activation, e.g. prostratin, TNF-α, Chromatin modification, e.g. HDACi and methylation inhibitors Combination approach</td>
<td>Reduction in both HIV DNA and cell associated HIV RNA.</td>
<td>[54,67,81,82]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhance HIV-specific CD4+ and CD8+ T cells Enhance neutralizing antibody</td>
<td>Viral blips following IL-7, source unclear</td>
<td>[90]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>In-vitro studies only</td>
<td></td>
</tr>
<tr>
<td>Eliminate latently infected cells</td>
<td>Activate viral replication</td>
<td>JAK-STAT activation, e.g. IL-7, NF-kB activation, e.g. prostratin, TNF-α, Chromatin modification, e.g. HDACi and methylation inhibitors Combination approach</td>
<td>Valproic acid not active in vivo.</td>
<td>[90,96–101,108]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Methylion inhibitors in vitro only</td>
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<td></td>
<td></td>
<td></td>
<td>In-vitro studies only</td>
<td>[90,108–110]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No effect on viral rebound</td>
<td>[84,111,112]</td>
</tr>
<tr>
<td>Boosting immunity</td>
<td>Therapeutic vaccination</td>
<td></td>
<td>Some effect in SIV-infected macaques</td>
<td>[113]</td>
</tr>
<tr>
<td>Make cells resistant to HIV</td>
<td>Transplantation</td>
<td>Bone marrow donor CCR5Δ32 homozygous mutant</td>
<td>No HIV detected in plasma or tissue after 45 months off cART</td>
<td>[16,17]</td>
</tr>
<tr>
<td></td>
<td>Gene therapy</td>
<td>Zinc finger nuclease to reduce CCR5 expression</td>
<td>Reduces HIV replication in mice. Successful engraftment in humans.</td>
<td>[114,115]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RNA based gene therapy to reduce expression of CCR5 and HIV</td>
<td>Efficacy not yet studied</td>
<td></td>
</tr>
</tbody>
</table>

cART, combination antiretroviral therapy.
In these studies, a clear but transient increase in HIV RNA was observed, despite all patients receiving cART. The virus detected in plasma following IL-7 was similar phylogenetically to virus prior to IL-7 in both plasma and CD4\(^+\) T cells [91]. One concern with IL-7 is that this cytokine may potentially expand not only uninfected cells but also latently infected cells by inducing proliferation of all cells, specifically transitional memory T cells [43]. IL-7 is currently undergoing clinical trials (ERAMUNE, www.clinicaltrials.gov), as a strategy to reduce the size of the latent reservoir, and results of this trial are awaited with high interest.

**Activation of NF-κB: prostratin**

There are alternative compounds, such as prostratin, that can promote T-cell activation and HIV transcription in vitro [92]. The large diversity of latently infected T-cell subsets may differ in their capacity to proliferate, and/or uptake of these drugs. However, prostratin has not yet been assessed for safety and toxicity in humans and therefore is unlikely to enter clinical trials in the near future.

**Enhance histone acetylation: histone deacetylase inhibitors**

Many in-vitro studies have demonstrated that latency can be reversed, that is viral production can be activated by promoting histone acetylation [113]. Histone deacetylase inhibitors (HDACis) are drugs that can modify gene expression by changing the acetylation state of histones, leading to enhanced transcription from multiple genes including from the HIV LTR [114]. In cancer cells, HDACis induce cell death and cell cycle arrest of rapidly dividing malignant cells and many HDACis are now in advanced clinical development for the treatment of different cancers [115,116]. Following treatment of latently infected cells lines with a number of different HDACis, including valproic acid, MCT1, MCT3 and oxamflatin, we demonstrated that preferential apoptosis also occurred in cells that were producing virus [117].

Valproic acid, a relatively weak and non-toxic HDACi, showed promising effects in a small pilot study [93]; however, further retrospective studies failed to demonstrate any benefit from valproic acid in reducing the number of latently infected resting CD4\(^+\) T cells [94–96]. A far more potent HDACi, vorinostat (also called SAHA), is licensed for the treatment of cutaneous T-cell lymphoma, is relatively well tolerated in humans and has significant potency in promoting HIV replication from latently infected cells in vitro [97,98].

In patients treated for malignancy, the main adverse events from vorinostat were fatigue, diarrhoea and thrombocytopenia which occurred with severity of grade 3–5 in 3–5% [116,118]. The median time to onset of an adverse event requiring dose modification of vorinostat was 42 (17–267) days. Following the administration of panobinostat (LBH 257, Novartis), a pan HDACi similar to vorinostat, a change in gene activity in tumour cells and histone acetylation in circulating PBMC was observed within 2 h of administration and gene activity returned to baseline levels within 72 h [119]. Given that the onset of gene activation and suppression is extremely rapid with HDACi, it is possible that only a short course of these
drugs may be required for reversing HIV latency and this would significantly reduce the likelihood of toxicities and adverse events.

A theoretical risk of HDACis is that they will induce activation of other retroviruses and/or DNA viruses including cytomegalovirus (CMV), hepatitis B virus (HBV) and JC viruses which has been demonstrated in vitro [120–122]. However, to date there has been no evidence that the clinical use of HDACi is associated with reactivation of DNA viruses [reviewed in [116]]. There has only been a single published case series of three patients in which reactivation of EBV and HBV was reported following administration of an HDACi [123]. In this small case series, there was a temporal association with reactivation and administration of the HDACi but it is important to keep in mind that these patients were all patients with advanced cancer and immunosuppression. Therefore it remains unclear if HDACis were indeed the cause of any viral reactivation. The long-term impact of HDACi on enhancing the risk of malignancy and/or reactivation of oncogenes or endogenous retroviruses is also unknown.

Inhibit DNA methylation: methylation inhibitors

The methylation inhibitor 5-aza-deoxycytidine (decitabine; Dacogen, MGI Pharma Inc.) is a nucleoside analogue that promotes DNA cytosine methylation and also has a similar effect to HDACi in promoting HIV transcription in vitro, but only in a subset of latently infected resting CD4+ T cells [99]. Decitabine was approved by the FDA in 2006 for the treatment of myelodysplastic syndromes. In vitro, decitabine induces HIV transcription from latently infected cell lines and patient-derived latently infected cells; however, this drug was not active in all latently infected cells and the potency was greatest when used in combination with other drugs such as prostratin and an HDACi [92,99]. Other compounds that inhibit methylation include histone methyltransferase inhibitors (HMTis). HMTis are in advanced development for the treatment of cancer and further work is underway to determine if these compounds are also active in latently infected cells.

Combination strategies

Several studies have now demonstrated that the potency of different interventions that modulate HIV gene expression may vary in different latently infected cells, depending on the integration site, the degree of transcriptional interference, chromatin structure and methylation of the LTR in the particular cell [92,99–101]. In addition, a combination of strategies, for example SAHA together with prostratin, appears to have greatest potency in promoting HIV transcription, at least in vitro [92,100]. Most of the studies that evaluate a combination approach have been performed in latently infected T-cell and monocytic cell lines and it is currently unknown whether this approach will also enhance potency in latently infected primary T cells. In addition, it is currently unknown whether any of these compounds work in other latently infected cells such as astrocytes, naive T cells or stem cells. It is likely that the elimination of the diverse latently infected cells found in vivo will require a combination approach similar to what is currently used in cancer treatment.

Boosting immunity to HIV: therapeutic vaccination

Many natural history studies of HIV-infected patients have shown a clear relationship between virological control and a robust HIV-specific CD4 and CD8 T-cell response raising the possibility that induction of T-cell immunity via vaccination may potentially generate a functional cure. To date the use of therapeutic vaccination, in patients receiving cART, has not been successful [87,102,103]. In one of these studies, treatment interruption resulted in a significantly shorter time to viral rebound following therapeutic vaccination compared with placebo [102].

A recent intriguing study in SIV-infected Chinese rhesus macaques treated with two antiretroviral agents and a live inactivated SIV virus combined with a toll like receptor (TLR) agonist polyICLC applied to the tonsil resulted in significant control of viral replication following cessation of cART [104]. The animals who received the polyICLC vaccine had significantly elevated titres of neutralizing antibodies compared to the control groups suggesting that the generation of neutralizing antibodies may be important in preventing viral rebound following cessation of cART.

Making cells resistant to HIV

Future strategies aimed at making CD4+ T cells resistant to HIV are also currently being investigated. Some approaches that may potentially mimic HIV eradication in the HIV-infected German patient include gene therapy to reduce expression of CCR5. This has successfully been performed in mice through the introduction of a zinc finger nuclease into haematopoietic progenitor cells, which effectively disrupts the gene coding for CCR5 in all daughter cells [105]. This led to a reduction in the expression of CCR5 in a subset of transplanted cells. Following HIV infection of these mice, there was a selective advantage for the CCR5−/− cells which subsequently increased, HIV RNA remained low and CD4+ T cells were preserved in both blood and tissue [105].

A recent phase 2 study demonstrated that infusion of autologous T cells transduced with a zinc finger nuclease that inhibited expression of CCR5 was well tolerated in a small study of six HIV-infected patients on cART [106]. An alternative approach is to use RNA-based gene therapy to reduce CCR5 expression, as well as specifically inhibit HIV replication [107]. This approach was recently
investigated in HIV-infected patients and was shown to be well tolerated and that the transduced genes persisted in a subset of cells for 24 months. Although widespread use of these therapies is many years away, these results are encouraging for the possible development of a gene therapy-based treatment strategy that may achieve a functional cure.

**Moving toward clinical trials to test for eradication**

**Animal models**

Given the absence of a robust animal model of suppressive cART, it is currently unclear whether there is a real need for all interventions to first be trialled in macaques to determine efficacy. To date there have been a limited number of antiretroviral agents that are active or can be administered to SIV-infected macaques allowing durable control of SIV RNA to below 50 copies/ml for a prolonged period of time. We know from viral kinetic models of HIV infection that elimination of productively infected cells likely takes more than 3 years [62,124]. Given the high costs of animal maintenance it is difficult to maintain SIV-infected macaques on SIV cART for longer than 48 weeks. Finally most detailed analyses of factors that modify HIV transcription, for example, activity of HDACi and methylation inhibitors, have been performed in in-vitro models of latent HIV infection in human cells and it is unclear whether this can be translated to SIV infection.

Recent work has, however, demonstrated that infection of rhesus macaques with SIVmac239 that contains the HIV-1 RT (clone HXBc2) and envelope genes (RT-SHIV) treated with tenofovir, emtricitabine and efavirenz appears to be a very promising model of suppressive cART [125,126]. Using an ultrasensitive assay to measure RT-SHIV RNA in plasma, these animals had detectable but low-level viraemia of 2–58 copies/ml consistent with suppressive cART in humans [126]. Some recent small animal models also show promise, specifically the blood-liver-thymus (BLT) mouse that can be efficiently infected with R5 HIV and HIV RNA declines in response to antiretroviral drugs [127,128].

**Clinical trials**

Clinical trials testing strategies for eradication pose unique challenges that require consideration and further debate. First, measuring the reservoir is complex, can require large volumes of cells, can be invasive and no assays have yet been standardized across multiple laboratories (Table 1). In addition, it is currently unknown what assay will best predict the likelihood of viral rebound following cessation of cART. Second, most studies to date have been small, nonrandomized studies. This approach is appropriate to test the safety, feasibility and potential efficacy of new strategies; however, there is a need for larger randomized studies in this field. Third, most strategies identified to date, that may have an impact on latent reservoirs, have associated toxicities. Careful consideration of the risk benefit for any of these interventions remains challenging for investigators, patients and associated regulatory authorities. Finally, there remains a need for more multidisciplinary studies, including the use of mathematical models to study the dynamics of reservoirs, taking in account half-life of the subsets of infected cells and their homeostatic proliferation [129].

Although there are multiple ways to quantify residual infected cells in patients on cART (Fig. 1 and Table 1), it is currently unknown whether there is a critical threshold at which viral rebound does not occur or whether all infected cells need to be eliminated to prevent viral rebound. A recent study of a patient treated with suppressive cART during primary infection had extremely low levels of IUPM for prolonged periods of time; however, disappointing viral rebound still occurred in this patient following cessation of cART [112]. Therefore, eventually we will need to consider whether cART interruption is an appropriate clinical end point of these studies given the well documented risks of reactivation of viral replication [130]. Moreover, it is possible that reducing reservoirs to levels below the limit of detection of available assays, may not necessarily predict the likelihood of viral rebound after stopping cART.

**Conclusion**

There are multiple barriers to the eradication of HIV infection and despite some recent significant advances in in-vitro models of latency, better animal models and the identification of several compounds that can reverse latency in vitro, there is still a need for more research. Promising in-vitro strategies need to be tested in well designed and well tolerated clinical trials to demonstrate proof of concept and to determine whether further investment should be placed in these approaches. It is likely that multiple combined approaches will be needed to eradicate HIV given that HIV can persist in diverse cell populations in patients on cART. A well funded multidisciplinary approach that includes basic virologists, immunologists, clinicians, pharma and the infected community will be needed if we are ever going to meet this challenge.

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