Barriers to a cure for HIV: new ways to target and eradicate HIV-1 reservoirs

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Antiretroviral therapy for HIV infection needs lifelong access and strict adherence to regimens that are both expensive and associated with toxic effects. A curative intervention will be needed to fully stop the epidemic. The failure to eradicate HIV infection during long-term antiretroviral therapy shows the intrinsic stability of the viral genome in latently infected CD4T cells and other cells, and possibly a sustained low-level viral replication. Heterogeneity in latently infected cell populations and homoeostatic proliferation of infected cells might affect the dynamics of virus production and persistence. Despite potent antiretroviral therapy, chronic immune activation, inflammation, and immune dysfunction persist, and are likely to have important effects on the size and distribution of the viral reservoir. The inability of the immune system to recognise cells harbouring latent virus and to eliminate cells actively producing virus is the biggest challenge to finding a cure. We look at new approaches to unravelling the complex virus–host interactions that lead to persistent infection and latency, and discuss the rationale for combination of novel treatment strategies with available antiretroviral treatment options to cure HIV.

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Introduction

Antiretroviral therapy (ART) is one of the major medical successes of the late 20th century. Effective ART results in indefinite viral suppression, restored immune function, improved quality of life, the near normalisation of expected lifespan, and reduced viral transmission. Despite the inherent potency of ART to suppress virus replication, treatment approaches have limitations; ART does not eliminate viral reservoirs, and needs lifelong adherence to expensive regimens that have potential short-term and long-term toxic effects. In 2010, more than 34 million people were estimated to be living with HIV, an increase of 17% over the past 10 years, and the number is expected to increase. Will we have enough resources worldwide to provide treatment and monitoring to all who need them? Even for those with access to ART, individual adherence is a big drawback. Additionally, despite virus control, HIV-associated complications persist, including a higher than normal risk of cardiovascular disease, cancer, osteoporosis, and other end-organ diseases. This increased risk might be due to the toxic effects of treatment or the consequences of persistent inflammation and immune dysfunction associated with HIV. Therefore, novel treatment approaches that eliminate persistent virus and do not need lifelong adherence to expensive and potentially toxic antiretroviral drugs are needed.

Two broadly defined categories of a cure for HIV infection exist—a functional cure and a sterilising cure. A functional cure is defined as host-mediated control of HIV replication, in the absence of ART. A functional cure suppresses viral replication for a pre-defined period of time (eg, 5 years) in the absence of treatment, restores and stabilises effective immune function, and decreases both HIV-induced inflammation (which could increase the risk of AIDS or non-AIDS morbidity) and in those individuals that maintain stable low-level plasma viral loads, reduces the risk of virus transmission to others. A functional cure is achieved spontaneously by a rare group of individuals who naturally control HIV replication without treatment (so-called elite controllers). These patients are characterised by a favourable HLA profile and potent HIV-specific CD8 T-cell responses that are associated with a low viral DNA reservoir. In 2010, a second group of patients who initiated ART during acute infection, and controlled HIV for several years after interruption of ART was identified.[1] These so-called post-treatment controllers are very rare, and unlike elite controllers, do not show strong HIV-specific CD8 T cell responses or have protective HLA alleles.[2,3]

A sterilising cure needs the complete elimination of replication-competent virus. Complete elimination of virus was probably first achieved after myeloablative chemotherapy, whole-body irradiation, and successful transplantation of haemopoietic stem cells from a CCR5

Search strategy and selection criteria

Our search criteria was to include a comprehensive list of references based upon each author's research and knowledge database and then to reduce the number of references cited to include either a seminal paper or a more recent published paper that best reflects our current understanding of the points discussed. We also opted for human clinical research data to support our hypotheses and selected key references from individual laboratories to balance contributions from many laboratories. We also added references based on the comments and suggestions of the reviewers. This helped us to provide a more balanced representation of the contributions of many laboratories and discussion of different interpretations of the data. Select citations of abstracts from recent conferences were included only if they provided sufficient detail and accessibility to the reader.

We searched PubMedfor articles published in English between Jan 1, 1990, and Jan 31, 2013, with the following search terms: "antiretroviral therapy", "highly active/methods", "HIV infections/therapy", "Virus latency/immunology", "HIV-1/pathogenicity", "CD4-Positive T-Lymphocytes/immunology", "Hematopoietic Stem Cell Transplantation", "Humans". We used the following keywords: "Histone Deacetylases", "Epigenetics", "Gene Silencing", "HIV-1/immunology". NIH-funded human clinical trial identifiers were obtained from a search of www.clinicaltrials.gov.

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delta32 homozygous donor into an HIV-infected individual with acute myelogenous leukaemia (the so-called Berlin patient).[4,5] In a recent report[6] from Boston, USA, two patients with relapsed Hodgkin's lymphoma treated with ART who received a CCR5+/+ haemopoietic stem-cell transplant, had undetectable pro-viral DNA and replication-competent HIV 8–17 months after transplantation. These findings suggest that ablative conditioning, immunosuppressive treatment, and post-transplant graft-versus-host disease—all of which were common in the Berlin patient and the Boston cases—might cause substantial and perhaps curative reductions in the size of the reservoir.[6] To establish whether the Boston individuals were truly cured, ART will need to be interrupted. Efforts to pursue both functional and sterilising cures are in progress. An effective cure might need a combination of approaches For example, attempts to eradicate the reservoir might not work unless the capacity of the immune system to clear and control the virus is enhanced.

HIV reservoirs: obstacles to cure

Establishment and maintenance of HIV latency

The HIV reservoir is established during primary infection. Administration of ART in very early acute infection seems to result in a low post-treatment total, and integrated DNA and HIV RNA concentrations, suggesting that aggressive treatment can decrease the size of the viral reservoir.[1,7–9] Although early treatment can substantially reduce the size of the total reservoir, a stable population of latently infected CD4 cells transits into the long-lived latent reservoir, and is unaffected by early combination ART (cART).[10] Most HIV pro-viral DNA is detected in CD4 T lymphocytes in lymphoid tissue.[11,12] In blood, most HIV DNA is found in central memory and transitional memory T cells, which maintain the reservoir because of their intrinsic capacity to persist through homoeostatic proliferation and renewal.[13] Other cellular reservoirs that might exist include naive CD4 T cells, monocytes and macrophages, astrocytes, and microglial cells.[14] During long-term effective ART, steady-state, low-level plasma HIV RNA is achieved, typically from less than one to three copies of RNA per mL.[15] Chronic production of HIV from a stable reservoir of long-lived infected cells (the so-called latent reservoir) is probably the main source of this persistent HIV. However, persistent low-level replication could also play a part, especially in tissues in which continuing persistent viral replication, despite ART, might be caused by cell-to-cell spread and insufficient drug penetrance in tissues.[16,17]

A prerequisite for the establishment of HIV latency is the integration of viral DNA into the host chromatin and epigenetic silencing of active viral transcription. Two models have been proposed to explain latent infection in memory cells. The pre-activation latency model relies on the idea that HIV can directly infect a subset of resting CD4 T cells.[18,19] The post-activation latency model proposes that activated antigen-specific CD4 memory T cells become preferentially infected, but avoid cell death, and then revert to a resting state.[20] The number of resting CD4 T cells that become latently infected varies widely between individuals, and could be as few as 1 per million CD4 T cells.

The molecular mechanisms contributing to the silencing of HIV DNA are complex.[21] Infected cells with replication-competent pro-virus are transcriptionally silenced by corepressor complexes that contain histone deacetylases, histone methyltransferases, and heterochromatin proteins; active methylation of the long terminal repeat might also play a part.[22,23] Epigenetic silencing of a pro-virus can be reversed by agents that mobilise chromatin remodelling complexes to replace repressive complexes poised at the viral long terminal repeat.[24] Signals delivered through the T cell receptor (TCR–CD3 complex , and CD28 co-stimulation can drive productive transcription, suggesting that physiological activation of memory CD4 T cells can lead to virus production in vivo.[25] Activated CD4 T cells are the most permissive target for HIV infection. How recently infected activated cells become long-lived latently infected resting memory cells is not fully understood. Many regulatory pathways designed to blunt the effect of cell activation are turned on during T-cell activation, including the upregulation of negative regulators of T cell activation—eg, PD-1, CTLA-4, TIM-3, LAG3, CD160, and 2B4 cell-surface receptors. Cells expressing these receptors could be preferentially latently infected with HIV. In a cross-sectional study of long-term treated individuals, PD-1-expressing cells were enriched with HIV DNA.[13] Drugs that interrupt the molecular pathways associated with these negative regulators could help clear the reservoir.

Novel strategies to cure HIV

Treatment intensification of ART

If persistent low-level replication of HIV or de-novo infection of new target cells continues during treatment, the addition of potent agents (so-called intensification) to a stable regimen could help reduce reservoir size. In treatment-intensification studies up to now, new classes of drugs such as the HIV integrase inhibitor raltegravir or the CCR5 inhibitor maraviroc have not reduced residual viraemia.[26] Some reports have shown that intensification with an integrase inhibitor leads to transient accumulation of pre-integration episomal DNA (2-long terminal repeat circles), and simultaneous reductions in CD8 T cell activation markers, suggesting inhibition of low-level viral replication.[26,27] Findings from another study showed that intensification with raltegravir reduced concentrations of HIV RNA in the ileum, which is rich in infected target cells, and a potentially ideal environment to support cell-to-cell transfer of virus during ART. However, reduction in HIV-1 DNA or residual viraemia was not seen.[28] ART might be unable to fully penetrate all tissues. Persistence of HIV replication in tissues justifies efforts to optimise drug delivery to

enhance penetration into cells in tissues that present drugs fail to reach, including the CNS and possibly lymphoid tissues.[29] Virus activity needs to be measured in lymphoid tissue and other organs such as the gut, reproductive organs, and the brain to better understand the limitations of ART intensification and to eradicate residual virus production.

Gene therapy

The case of the Berlin patient generated widespread interest in cell-based curative interventions. Allogeneic transplantation with stem cells from very rare donors who are naturally resistant to HIV is not a feasible strategy; therefore, much interest is now focused on gene therapy to delete the virus from infected cells or to produce cells resistant to HIV infection.[30] Three classes of DNA-editing enzymes are being studied for safety and efficacy to target HIV coreceptors and pro-viral sequences: zinc-finger nucleases, transcription activator-like effector nucleases, and homing endonucleases.[31,32] The targeted gene-therapy approach of blocking CCR5 (CCR5–zinc-finger nuclease knockout T cells) is under investigation in phase 1 clinical trials of adoptive transfer of ex-vivo expanded autologous T cells (table 1).[33] Phase 1 studies have shown that these approaches are safe and feasible. Other gene-based treatments that target HIV viral proteins with either an anti-HIV ribozyme or antisense RNA oligonucleotide constructs have been shown to safely and feasibly reduce viral load in phase 1 and phase 2a clinical trials.[34] Additionally, CD4 aptamer-CCR5-siRNA chimeras have proven to be safe and efficacious in the humanised mouse model.[35] Interventions that interfere with pre-integration steps in the viral-life cycle are promising, and are being assessed in phase 1 trials.[36] The safety and efficacy of gene delivery to specific cells and tissues, and access to these treatments, are major challenges for these approaches in the eradication of HIV infection.[37]

A three-tiered approach to reverse latency and eliminate reservoirs

Step one is the reactivation of latent infectious pro-viral genomes. The goal of HIV-1 reactivation treatment is to purge cellular reservoirs by stimulation of the transcriptional activity of latent HIV-1 pro-virus in infected CD4+ T cells. This purge results in the production of virus-producing cells that either die from the direct cytopathic effects of the virus or are cleared by host mechanisms. Although complex molecular mechanisms regulate the expression of pro-virus in latently infected cells, one possible strategy is to trigger NF-κB activity, the main host transcription factor for HIV-1 replication.[38,39] Initial attempts to reactivate virus in patients on ART with two known inducers of NF-κB, interleukin (IL) 2 or anti-CD3

	Intervention	Study population (patients chronically infected with HIV-1)		ClinicalTrials.gov
		Study population	Main endpoint	
ERAMUNE 01	Interleukin 7+ART intensification with raltegravir and miraviroc	29 patients with HIV RNA <50 copies per mL on ART and HIV DNA between 10 and 1000 copies per 10^6 PBMC	Decrease of 0·5 $\log_{10}$ HIV DNA from baseline at 56 weeks	NCT01019551
ERAMUNE 02	HIV rAD5 vaccine+ART intensification with raltegravir and miraviroc	28 patients with HIV RNA <50 copies per mL on ART and HIV DNA 10–1000 10^6 PBMC; negative AD5 antibody	Decrease of 0·5 $\log_{10}$ HIV DNA from baseline at 56 weeks	NCT00976404
Disulfiram	Disulfiram: 500 mg/day for 14 days	20 patients on ART with HIV RNA <50 copies per mL	2 weeks frequency of replication, competent HIV-1 in resting CD4 T cells	NCT01286259
Vorinostat	Vorinostat: 200–600 mg per day	30 patients with HIV RNA <30 copies per mL	HIV expression in resting CD4 T cells	NCT01319383
Vorinostat	Vorinostat: 400 mg/day	20 patients with HIV RNA <50 copies per mL and CD4 cell >500 per mL	HIV unspliced RNA in resting CD4 T cells at 28 days	NCT01365065
Panobinostat	Panobinostat: 20 mg three times a week, every other week for 8 weeks	16 patients with suppressed viraemia and CD4 cell count >500 per mL	HIV unspliced RNA in resting CD4 T cells change from baseline	NCT01680094
CD4 T cells modified at CCR5 by zinc-finger nuclease	Autologous CD4 T cells modified at *CCR5* gene by zinc-finger nuclease SB-728-T	30 patients with suppressed viraemia and CD4 cell count <300 per mL or failing ART	Safety; persistence and activation of CCR5+zinc-finger nuclease-modified autologous T cells	NCT01252641
CD4 T cells modified at CCR5 by zinc-finger nuclease	Autologous CD4 T cells modified at *CCR5* gene by zinc-finger nuclease	18 patients: three cohorts of HIV-positive patients, either failing ART or with suppressed viraemia	Safety	NCT00842634
Lentivirus vector rHIV7-shI-TAR-CCR5RZ-transduced haemopoietic progenitor cells	Autologous CD34+haemopoietic cells modified by lentivirus-transduced non-functional *CCR5RZ* gene	10 patients with AIDS-related lymphoma undergoing haemopoietic stem-cell transplantation	Safety and durability of transduced cells	NCT00569985
Interferon alfa-2b	Interferon alfa-2b intensification	Recruiting, non-randomised, one-group assignment	Efficacy: viral RNA levels in blood and sequence diversification	NCT01295515

ART=antiretroviral therapy. PBMC=peripheral blood mononuclear cells.

Table 1: **CURE-related clinical pilot trials in progress in 2012**

monoclonal antibody (OKT3), were unsuccessful, and were associated with dose-dependent toxic effects.[40-42] Other candidate NF-κB activating agents effective at inducing virus transcription in in-vitro latency models include prostratin, tumour necrosis factor-α, and the transient NF-κB-activating agent HIV-reactivating factor.[43]

	Mechanism of action	Activation
NF-κB-inducing agents		
Anti-CD3/CD28[52,53]	T-cell receptor activation	++++
TNFα*[38]	TNF receptor activation	++
Prostratin, PMA/ionomycin, bryostatin-1, Picolog[44,54]	Protein kinase C activation	+++
HDAC inhibitors	Histone and non-histone protein acetylation	
Vorinostat	Class I HDAC1, HDAC 3 inhibitor; HDAC4,and HDAC6; Cell cycle arrest: Myc ↓p21 ↑ Reactive oxygen species: apoptosis ↑ nonhomologous DSB repair ↓ Angiogenesis: HIF alpha, VEGF ↓ Innate immunity, proinflammatory cytokines ↓ STAT 5 hyperacetylation	++
Romidepsin (FK228)[51]	HDACs 1, 2, 4, and 6 inhibitor	+++
Panobinostat (BH589), givinostat (ITF2357), belinostat (PXD101)[55,56]	In order of most to least potent: LBH589, ITF2357, PXD101, SAHA, VPA	+++
Sirtuin inhibitors	Acetylation of non-histone proteins: HIV Tat, p65 RelA, p53, and FOXO3a	Not tested
HMT inhibitors		
BIX-01294[57,58]	G9a (H3K9me2) inhibitor	++
Chaetocin[59]	SUV39H (H3K9me3) inhibitor	++
Pro-apoptotic and cell differentiating molecules		
JQ1[60]	Blocks TAT binding to P-TEFb Inhibits bromodomain reader BRD4 recruitment to H3K9ac, H4K16ac myc transcription ↓ SIRT1 ↑	+ Priming
Nutlin3[61]	MDM2 blocker p53 transcription, caspase activation↑	Not tested
Disulfiram, aphidicolin[62]	Oxidative stress and apoptosis ↑	++
HMBA, dactinomycin, aclarubicin, cytarabine[63]	P-TEFb release associated with RNAP II triggers transcriptional elongation	+
Wnt small-molecule inhibitors, Notch (γ-secretase) inhibitors	Myc, CtBP1, Cyclin D, cJun ↓ HES target genes ↓	Not tested
Immune modulators		
Anti-PD-1, CTLA-4, TIM-3 monoclonal antibody	Block negative regulatory receptors	Anti-PD-1 (ACTG 5301)
Anti-PD1 monoclonal antibody and interleukin 15	STAT5, PI3K signalling, CD4+ T cell effector memory differentiation	Not tested
Therapeutic CD4+ T cell vaccine and raltegravir[64,65]	Reactivate virus in latently infected antigen-specific CD4 cells and limit reseeding viral reservoirs	Not recorded
Synergistic combinations		
Vorinostat and chateocin, Vorinostat and prostratin[59,66]	HDAC inhibitor and HMT inhibitor HDAC inhibitor and NF-κB inducer	+++
HDACi and anti-PD-1	HDAC inhibitor and anti-PD-1mAb	Preclinical studies
Anti-PD1 monoclonal antibody and interleukin 15, raltegravir, peptidomimetics[67]	CD4 T effector memory cell differentiation Inhibit integrase, LEDGF/p75	Not tested

*Small effect in primary cell models. TNF=tumour necrosis factor. PMA=phorbol ester 13-phorbol-12-myristate acetate; HDAC=histone deacetylase. HMT=histone methyltransferase. HMBA=hexamethylenebisacetamide. p-TEFb=positive transcription elongation factor. The ability to reactivate latent virus in primary CD4 T cells is shown on a relative scale of increasing strength from + to ++++. ↑ shows an increased activity or gene expression, ↓ shows decreased activity or expression.

Table 2: **Experimental approaches to reactivate latent HIV infection**

Drugs that stimulate protein kinase C, the generation of reactive oxygen species, or autophagy can also activate the NF-κB pathway to induce virus replication. Bryostatin 1 induces the PKC–NF-κB pathway and can work with other drugs to remodel chromatin transcriptional co-activator complexes in the cell, reactivate virus, and promote cellular apoptosis.[44,45] However, the generalised activation of CD4 T cells is associated with a substanital risk of systemic induction of proinflammatory cytokines. Advocacy for small-molecule drugs to specifically reverse the pathways involved in chromatin silencing coincided with the introduction of the idea of latency in the mid 1990s.[46] The inhibition of histone deacetylases is interesting because these molecules block HIV-DNA transcription by preventing transcription factors from accessing the HIV promoter. Two candidate histone deacetylase inhibitors have already entered clinical trials (table 1). Valproic acid, a weak non-toxic inhibitor, was the first drug to be studied. Despite promising results in a pilot study, subsequent studies failed to find any benefit, and investigators have moved on to study more potent drugs.[47–49] Vorinostat has shown promising results in the reactivation of virus in in-vitro models of latency.[50] In a 2012 pilot study, 11 of 16 patients treated with ART showed potential susceptibility to vorinostat ex vivo.[50] Eight of these patients were then administered one dose of vorinostat and showed a marked increase in their expression of RNA levels compared with baseline.

Other clinical trials of vorinostat are in progress (table 1). In 2012, the histone deacetylase inhibitor romidepsin (FK288) was shown to be 1000 times more potent than vorinostat in the induction of latent HIV, and induced HIV-RNA expression ex vivo in 12 of 13 patients with HIV on cART.[51] Panobinostat (LBH589), givinostat (ITF2357), and belinostat (PXD101) also showed greater potency than vorinostat in the reactivation of virus in latently infected cell lines (table 2).[68] Coordinated action of several epigenetic modifications might be needed to initiate viral transcription. Optimum reactivation of epigenetically silenced pro-virus DNA might need multidrug combinations including histone deacetylases, protein kinase C activators, and specific histone methyltransferase inhibitors. The combination of a histone deacetylase inhibitor with the H3K9 histone methyltransferase inhibitor BIX01294 reactivated latent virus better than either drug alone.[57,69] The main priority is to discover new molecules that are safe and can act alone or synergise with other drugs to reactivate virus transcription with high efficiency. For example, in 2012, three drugs approved by the US Food and Drug Administration (dactinomycin, aclarubicin, and cytarabine) were found to act as priming agents in combination with other reactivating molecules to increase the number of cells producing virus.[63]

Disulfiram, used for the treatment of alcoholism, reactivated latent virus in an in-vitro latency model through its active metabolite diethyldithiocarbamate (DDTC).[52] In a small pilot study, one dose of disulfiram given daily for 2 weeks to patients on cART was well tolerated and led to a transient increase in plasma virus RNA in a subset of patients (table 1).[70]

Other potential drug targets maintain latency, including the immune checkpoint blockers—eg, PD-1, which is expressed on latently infected CD4 cells during HIV infection. These molecules present a major barrier to T-cell activation by inhibiting signals transmitted through the T-cell receptor.[71–73] In patients given ART, PD-1 expression remains elevated and correlates with the size of the latent reservoir and high concentrations of cell-associated RNA and pro-viral DNA.[13,74,75] High expression of PD-1 on CD4 memory T cells in HIV-1 infection prevents antigen-specific activation of the T cell receptor and downstream signals that are needed for proliferation, effector function, and HIV replication. Antibodies that block PD-1 or PDL-1 can relieve inhibitory signals upon activation of the T-cell receptor, and can increase the sensitivity of the receptor to the antigen. High expression of PD-1 on antigen-specific CD8 transitional memory T cells and effector memory T cells in HIV infection—even in patients successfully treated with ART—could prevent recognition and killing of CD4 cells in which virus has been reactivated. The blocking of PD-1 on antigen-specific CD8 T cells can prevent negative signals delivered through ligand binding of PDL-1 and PDL-2. Therefore, blocking PD-1 or combinations of co-inhibitory molecules could serve a dual role in the reactivation of latent virus in CD4 cells, and relieve a functional block on virus-specific CD8 memory T cells by enhancement of antigen-specific proliferation and effector-cell differentiation.[76,77]

The cytokine IL7 is needed for homoeostatic proliferation and survival of naive and memory CD4 T cells expressing the IL-7 receptor.[78] In studies of patients on ART with low CD4 cell counts, IL7 reconstituted naive and central memory T cells,[79,80] and induced transient viraemia in six of 26 patients in one investigation.[81] In another study, exogenous IL7 reactivated HIV replication in CD4 memory T cells from some HIV-infected donors in vitro when cells were stimulated in cocultures of peripheral blood mononuclear cells.[82] IL7 induces the proliferation of CD4 memory T cells carrying integrated viral DNA (including central memory and transitional memory T cells); however, the ability to reactivate virus replication with IL7 in ex-vivo cultures is highly variable.[83] On the basis of these findings, IL7 as a reactivating intervention is being tested in combination with ART intensification with raltegravir plus maraviroc to prevent proliferation or reseeding of the latent reservoir (ERAMUNE 01 study; table 1).

Step two is to target residual virus replication. Chronic inflammation is a major contributory factor to HIV immune activation and disease pathogenesis.[84–86] The inflammatory process seems to be most destructive in lymphoid tissues, leading to collagen deposition, fibrosis, and irreversible damage to the lymphoid infrastructure as a result of early recruitment of regulatory T cells and production of transforming growth factor β.[87,88] Despite suppressive ART, abundant amounts of HIV proteins (eg, p24, p17, and gp120) persist on the surface of the follicular dendritic cell network, and possibly contribute to chronic immune activation.[41,89–91] Proinflammatory cytokines and interferon α production result in increased frequency of activated CD4 T cells, which could contribute to persistent low-level viraemia, and prevent the healing of damaged gut and lymph nodes.[92,93] Chronic inflammation causes the persistent upregulation of co-inhibitory receptors and impaired immune function on antigen-specific T cells, including those that are needed to clear HIV reservoirs. Drugs that prevent the negative effects of inflammation on functional immune responses (eg, inhibition of PD-1–PDL interactions, type I interferons, and IL6), and enable the positive effects (eg, increased innate and HIV-specific T-cell immunity) could prove to be important for an effective cure strategy.

Several anti-inflammatory drugs are in hypothesis-testing phase I and phase 2 clinical trials to find out whether they can attenuate chronic inflammatory responses along with immune responses needed to eradicate latent reservoirs. For example, expression of CD38 and PD-1 on total CD8 and Gag-specific CD8 T cells was reduced in untreated HIV-infected patients given a cyclo-oygenase-2 inhibitor.[94,95] The peroxisome proliferator-activated receptor (PPAR-c) agonists are a promising class of anti-inflammatory molecules that include pioglitazone and leflunomide. These two drugs are well tolerated, are effective in the treatment of chronic inflammation,[96] and might reduce comorbidities (metabolic syndrome, dyslipidaemias, and glucose intolerance) that have been associated with long-term ART in HIV-infected patients.[97] Although treatment with chloroquine or hydroxychloroquine sulphate can substantially reduce HLA-DR CD38 CD8 T cells, and Ki-67 expression in CD4 and CD8 T cells,[98,99] these drugs failed to show efficacy in meeting these endpoints in HIV asymptomatic ART-naive patients.[100] Other drugs are under investigation: methotrexate and mesalazine, which should reduce inflammation in the gut mucosa where much of the virus resides; anti-fibrotic agents such as ACE inhibitors, which may restore immune function; and several drugs aimed at other microbial infections, which can cause chronic immune activation including drugs aimed at reducing translocation of gut microbes across the damaged mucosal surfaces of the gut. Whether these anti-inflammatory agents will help decrease virus production and ultimately decrease viral reservoirs remains to be shown.

Step three is the enhancement of host-mediated clearance of residual virus. Findings of several studies

Panel: **A new look at the design of clinical studies**

- Identify virus-producing cells and the location of viral sanctuaries, and establish the mechanisms by which virus establishes and maintains latent infection.
- Develop standardised in-vitro latency models in primary memory CD4 cells to help screen and prioritise reactivating compounds and combinations.
- Harmonise continuing efforts of different laboratories to develop a high-throughput primary T-cell model of HIV latency to screen for novel antilatency compounds.
- Develop and implement robust monitoring methods to quantitate single-copy cell-associated HIV RNA and HIV DNA to measure HIV reservoir and the proportion of cells producing virus in response to treatment. Because most proviral DNA is replication incompetent, assays that can directly measure replication-competent HIV DNA and quantitate HIV DNA and RNA double-positive cells are needed.
- Develop non-human primate models of antiretroviral-treated simian immunodeficiency virus infection, and do proof-of-concept studies testing the safety and efficacy of reactivation strategies and immune-based treatments.
- Develop humanised mouse models of HIV infection to assess novel eradication approaches and combinatorial strategies.
- As in oncology, develop non-comparative phase 1 and phase 2 clinical studies to rapidly screen many clinical strategies to determine safety and efficacy, and introduce a regulatory framework that allows fast implementation of small-scale (eg, 10–20 patients per group) proof-of-concept hypothesis-driven trials.
- Develop guidelines to select patient populations on the basis of the risk–benefit ratio associated with the primary objective of the study—ie, sterilisation or functional cure.
- Develop consensus protocols to define time-to-primary-endpoints and the follow-up period for secondary endpoints that quantify reduction in the HIV reservoir in blood and tissues, levels of inflammatory biomarkers, and functional antigen-specific immune responses for advancement to phase 2 studies. A phase 2b or phase 3 approach will need analytical treatment interruption of antiretroviral therapy in responders, and the evaluation of HIV reservoirs in blood and tissues after treatment withdrawal.
- Develop a universal web-based database for meta-analyses and hierarchical modelling of multicentre trials and protocols that can help identify the best approaches.
- Prospectively involve the HIV community, funding agencies, and regulatory authorities to discuss the complex ethical considerations, with special regard to the design and financial support of clinical studies in HIV-cure research.
- Prepare public opinion for this new phase of research and discovery, and thereby prevent high expectations in the short-term, and re-address the emphasis on prevention as the main available strategy to fight the HIV/AIDS epidemic.

have shown the importance of cellular immunity in the control of HIV reservoir size. HIV-1 Gag-specific CD8 T cells isolated from elite controllers, but not from patients given ART, were shown to kill autologous resting CD4 T cells in which the virus was reactivated with vorinostat.[101] Moreover, functional anti-viral CD8 T cells are associated with reduced size of the central memory CD4 T cell reservoir in patients controlling their virus without ART.[102] High-avidity multifunctional CD8 cytotoxic T lymphocytes that target vulnerable regions in Gag are especially important in limiting virus diversity and reservoirs in individuals infected with HIV who have protective HLA class I alleles.[102–104] Therapeutic vaccines could re-stimulate CD8 CTL to prevent or control virus relapses and re-establish latent infection in CD4 T cells after treatment interruptions.[64,105,106] A few therapeutic vaccine trials such as the Ad5 HIV-1 gag vaccine (ACTG A5197 NCT00080106), and infusions of dendritic cells pulsed with inactivated HIV particles have shown transient viral suppression after treatment interruption.[107,108] Eramune-02 is testing whether a DNA prime, replication defective, recombinant adenovirus serotype-5 boost strategy, with the Vaccine Research Center's polyvalent HIV-Gag, Pol, Nef, and Env vaccine can reduce the viral reservoir in patients undergoing an antiretroviral-intensification regimen (table 1).

The type I interferon response is an essential innate mediator of the acute antiviral response and inducer of the CD8 CTL effector. A 2013 study found that pegylated interferon alfa-2A could suppress virus replication in patients whose immune function was partly restored by cART.[109] Adjuvants inducing the production of type 1 interferons by conventional dendritic cells, plasmacytoid dendritic cells, and nonhaemopoietic cells might drive the activation and differentiation of quiescent memory CD4 T cells to induce viral replication and to activate memory CD8 CTL antiviral responses. Push–pull strategies combining synergistic combinations of molecular adjuvants such as toll-like receptor ligands and co-stimulatory molecules while blocking negative regulatory molecules (eg, PD-1, CTLA-4, TIM-3, CD160, and 2B4), immunosuppressive cytokines, and T regulatory function might further optimise induction of CD8 T effector and effector memory cells.[108,110–112] High-dose IL15 can skew differentiation of high levels of CD8 T effector memory cells.[113] IL15 was also shown to be four times more potent than IL7 in the induction of virus production in latently infected CD4 cells.[83] Therapeutic vaccines with the adjuvant IL15, in combination with blockage of coinhibitory molecules such as PD-1, might enhance the killing of infected cells and prevent the replenishment of latent reservoirs by reactivated virus.

Conclusions

To achieve a cure for HIV, the efforts of the global research community need to be coordinated and transparent in sharing both positive and negative clinical results (panel). The discovery of a cure is one of the major medical challenges of our time. This goal has rallied all sectors of the biomedical community—ie, public, private, academic, and community groups, and consultation has led to the elaboration of a document that describes a strategy leading to a cure.[14] Many challenges are associated with the identification of combinatorial approaches that can effectively induce reactivation of the latent reservoirs and enhance specific immune responses to control virus replication and eliminate virus-infected cells. Additionally, ethical issues need to be addressed in strategies that reactivate virus infection in individuals who are successfully treated with ART. Findings of recent studies show that immediate or early treatment with ART after infection could minimise the size and complexity of the latent reservoir, and might lead to post-treatment control. These results should provide us with the optimism to move forward with therapeutic interventions aimed at the eradication of HIV infection. Presently, prevention and early initiation of ART is the best way to control the HIV pandemic.

Contributors

All authors contributed to the ideas and interpretation of data in this manuscript. CK, SGD, BA, JM-P, DDR, JA, and RPS were mainly responsible for the writing and editing of the manuscript and inclusion of data provided in the tables.

Conflicts of interest

MM is an employee of Merck Research Laboratories, which holds US Patent Nos. RE 38 506 E, 6 087 367 ZOLINZA ® (vorinostat).

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