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Barriers to a Cure: New concepts in targeting and eradicating HIV-1 reservoirs

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Abstract

Antiretroviral therapy for HIV infection requires life-long access and strict adherence to regimens that are both expensive and associated with toxicities. There is growing recognition that a curative intervention will be needed to fully stop the epidemic. The failure to eradicate HIV infection during long-term antiretroviral therapy reflects the intrinsic stability of the viral genome in latently infected $CD4^+$ T cells and other cells and perhaps ongoing low-level viral replication.

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Heterogeneity in latently infected cell populations and homeostatic proliferation of infected cells may influence the dynamics of virus production and persistence. Chronic immune activation, inflammation and immune dysfunction persist despite potent antiretroviral therapy and likely have important effects on the size and distribution of the viral reservoir. The inability of the immune system to recognize cells harboring latent virus and to eliminate cells actively producing virus represents the biggest challenge to finding a cure. In this perspective, we highlight new approaches toward unraveling the complex virus-host interactions that lead to persistent infection and latency and discuss the rationale for combining novel therapeutic strategies with current antiretroviral treatment options with the goal of curing HIV disease.

Keywords

Histone Deacetylases; Epigenetics; Gene Silencing; HIV-1 /immunology

Finding a cure for HIV: The magnitude of the problem

Antiretroviral therapy (ART) can be considered one of the major medical successes of the late 20th century. When used effectively, it results in indefinite viral suppression, restoration of immune function, an improved quality of life, the near normalization of expected life span and a reduction in viral transmission. Despite the inherent potency of ART to suppress virus replication, current therapeutic approaches have limitations. Antiretroviral therapy does not eliminate viral reservoirs, requiring life-long adherence to regimens that are expensive and which have potential short-term and long-term toxicities. There were over 34 million people estimated to be living with HIV in 2010, which represents an increase of 17% over the last ten years, and the numbers are expected to continue to increase. It is unclear if there will be sufficient resources on a global level to provide treatment and monitoring to all in need. Even for those with access to ART, significant challenges remain in terms of individual adherence. In addition, despite virus control, HIV-associated complications persist, including higher than normal risk for developing cardiovascular disease, cancer, osteoporosis and other end-organ diseases. This risk might be due to treatment toxicities and/or the consequences of persistent HIV-associated inflammation and immune dysfunction. Thus, there is a very strong need for novel therapeutic approaches that could eliminate persistent virus and would not require life-long adherence to expensive and potentially toxic antiretroviral drugs. For all these reasons, there is now widespread interest in potentially curative interventions.

Potential outcomes of the cure approach

There are two broadly defined categories of a cure for HIV infection. A *functional cure* can be defined as host-mediated control of HIV replication, in the absence of ART, during which there is (i) suppression of viral replication for a pre-defined period (*e.g.*, five years) in absence of therapy, (ii) restoration and stabilization of effective immune function, (iii) limited HIV induced inflammation that could lead to an increased risk for AIDS or non-AIDS morbidity, and (iv) limited risk of transmitting virus to others with stable low level plasma viral load. A *sterilizing cure* for HIV infection requires the complete elimination of replication-competent virus.

There are now examples for both types of cure. A functional cure is achieved spontaneously by a rare group of HIV-infected individuals who naturally control HIV replication in the absence of therapy (“elite controllers”) These patients are characterized by a favourable HLA profile and potent HIV-specific CD8+ T cell responses that are associated with a low viral DNA reservoir. A second group of patients was recently identified; they initiated ART

during acute infection and were found to control HIV for several years after interruption of ART[1]. These "post-treatment controllers" are exceedingly rare; in contrast to elite controllers, they do not exhibit strong HIV-specific CD8+ T cell responses or possess protective HLA alleles[2, 3].

A sterilizing cure was likely achieved following myeloablative chemotherapy, whole body irradiation, and subsequent successful transplantation of hematopoietic stems cells from a CCR5*Δ32* homozygous donor into an HIV infected individual who had developed acute myelogenous leukemia (the "Berlin Patient")[4, 5]. In a recent report from Boston, two antiretroviral-treated subjects with relapsed Hodgkin's lymphoma that received a $CCR5^{+/+}$ hematopoietic stem cell transplant, proviral DNA and replication competent HIV were undetectable 8–17 months after transplantation. These observations suggest that ablative conditioning, immunosuppressive therapy, and/or post-transplant graft-versus-host disease (GVHD) — all of which were common to the Berlin Patient and the Boston cases— may cause dramatic and perhaps curative reductions in the size of the reservoir[6]. Whether the Boston-based individuals were truly cured will require interruption of antiretroviral therapy.

Efforts to pursue both *functional* and *sterilizing* cures are on-going. It is possible that an effective cure will likely require combinatorial approaches. For example, attempts at eradicating the reservoir may not work unless the capacity of the immune system to clear and control the virus are enhanced.

HIV Reservoirs: Obstacles to a Cure

Establishment and maintenance of HIV latency

The HIV reservoir is established during primary infection. Administration of antiretroviral therapy in very early acute infection appears to result in a lower post-treatment total and integrated DNA and HIV-RNA levels, suggesting aggressive treatment can limit the size of the viral reservoir[1, 7–9]. Although early treatment can substantially reduce the total reservoir size, a stable population of latently infected CD4 cells exists that transits in to the long-lived latent reservoir and is relatively unaffected by early cART[10].

The vast majority of HIV proviral DNA is detected in $CD4^+$ T lymphocytesin lymphoid tissue[11, 12]. In blood, most HIV DNA can be found in central memory (TCM) and in transitional memory T cells (TTM); these cells maintain the reservoir because of their intrinsic capacity to persist through homeostatic proliferation and renewal[13]. Other cellular reservoirs may exist, including naïve $CD4^+$ T cells, monocytes/macrophages, astrocytes, and microglial cells[14]. During long-term effective antiretroviral therapy, a steady state low-level plasma HIV RNA, typically from less than one to three copies RNA/ml, is eventually achieved[15]. The source for this persistent HIV is not fully known. Chronic production of HIV from a stable reservoir of long-lived infected cells (the "latent reservoir") is likely the dominant source, but some have argued that persistent low-level replication is also involved particularly in tissues where it has been suggested that ongoing persistent viral replication despite ART is due to cell to cell spread and lack of 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 supposition that HIV can directly infect a subset of resting $CD4^+$ T cells[18, 19] while the post-activation latency model proposes that activated antigen-specific $CD4^+$memory T cells become preferentially infected yet avoid cell death and then revert to a resting state[20]. The frequency of resting $CD4^+$ T cells that become latently infected varies

widely between individuals and has been estimated to be as few as one per million $CD4^+$ T cells.

The molecular mechanisms contributing to HIV-DNA silencing are complex[21]. Infected cells containing replication-competent provirus are transcriptionally silenced by corepressor complexes containing histone deacetylases (HDAC), histone methyltransferases, and heterochromatin proteins; active methylation of the LTR may also be involved[22, 23]. Epigenetic silencing of provirus can be reversed by agents that mobilize chromatinre modeling complexes[24] to replace repressive complexes poised at the viral LTR[24]. Signals delivered through the T cell receptor (TCR/CD3 complex) and CD28 costimulation can drive productive transcription suggesting that physiologic 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. Multiple regulatory pathways are turned on that are designed to blunt the impact of cell activation. These pathways include upregulation of the "negative regulators of T cell activation" such as the cell-surface receptors PD-1, CTLA-4, TIM-3, LAG3, CD160, and 2B4. Theoretically, cells expressing these receptors may be preferentially latently infected with HIV. In a cross-sectional study of long-term treated individuals, PD-1 expressing cells were enriched for HIV DNA[13]. Agents aimed at interrupting the molecular pathways associated with these negative regulators might help clear the reservoir.

Novel strategies towards an HIV cure

ART treatment intensification

If persistent low-level replication of HIV (or *de* novo infection of new target cells) persists during therapy, then addition of potent agents ("intensification") to a stable regimen may help reduce reservoir size. To date, treatment intensification studies with new classes of drugs, such as the HIV integrase inhibitor raltegravir or the CCR5 inhibitor maraviroc, have had no effect in in reducing residual viremia[26]. A few reports have shown that intensification with an integrase inhibitor leads to transient accumulation of pre-integration episomal DNA (2-LTR circles) and simultaneous reductions in CD8 T cell activation markers, suggesting inhibition of low-levels of viral replication[26,27]. Another study showed that intensification with raltegravir reduced HIV RNA levels in ileum, which is rich in infected target cells, and may be an ideal environment to support cell-to-cell transfer of virus during ART; however, there was no evidence of a reduction in HIV-1 DNA or residual viremia[28]. Antiretroivral therapy may not be able to fully penetrate all tissues. Persistence of HIV replication in tissues justifies efforts to optimize drug delivery to enhance penetration into cells in tissues where current drugs fail to reach, including the central nervous system and possibly lymphoid tissues[29]. Measurements of virus activity in lymphoid tissue and other anatomical compartments such as the gut, reproductive organs, and the brain are needed to better understand the current limitations of ART intensification therapy to eradicate residual virus production.

The promise of gene therapy approaches

The "Berlin Patient" case has generated widespread interest in cell-based curative interventions. As performing allogenic transplants using stem cells from those very rare donors who are naturally resistant to HIV is not a feasible strategy, much of the interest is now focused on using gene therapy to delete virus from infected cells or to produce cells resistant to HIV infection[30]. Three classes of DNA-editing enzymes are currently being evaluated for safety and efficacy to target HIV coreceptors and proviral sequences:zinc finger nucleases (ZFNs), transcription activator-like (TAL) effector nucleases (TALENs),

and homing endonucleases (HEs)[31, 32]. The targeted gene therapy approach to block CCR5 (CCR5–ZFN knockout T cells) is currently being evaluated in phase I clinical trials of adoptive transfer of ex vivo expanded autologous T cells (Table 1)[33]. Phase I studies have shown the safety and feasibility of such approaches.

Other gene-based therapies that target HIV viral proteins using either an anti-HIV ribozyme or antisense RNA oligonucleotide constructs have demonstrated safety and feasibility in reducing viral load in phase I/IIa clinical trials[34], while CD4 aptamer-CCR5-siRNA chimer as have proven safe and efficacious in the humanized mouse model[35]. Interventions that interfere with pre-integration steps in the viral life cycle are promising and currently being evaluated in phase I trials[36]. The safety and, efficacy of gene delivery to specific cells and tissues, and ultimately access to these treatments are major hurdles for these approaches in eradicating HIV infection[37].

A Three Tiered Approach to Reverse Latency and Eliminate the Reservoir

Most investigators believe that a safe and scalable cure will require a three-tiered strategy: 1) reactivation of HIV proviral genomes to purge HIV from all latently infected cells, 2) suppression of residual (cryptic) viral replication, and 3) enhancement of host immune function to recognize virus-infected cells and eliminate rebound virus replication. Potential approaches to meet this goal are summarized below.

Step 1. Reactivation of latent infectious proviral genomes—The goal of HIV-1 reactivation therapy is to purge cellular reservoirs by activating the transcriptional activity of latent HIV-1 provirus in infected $CD4^+$ T cells resulting in the generation 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 expression of provirus in latently infected cells, one possible strategy is to trigger NF-κB activity, a major host transcription factor for HIV-1 replication[38, 39]. Initial attempts to reactivate virus in patients on ART using two known inducers of NFκB, IL-2 or anti-CD3 MAb (OKT3), were unsuccessful in reactivating virus and were associated with dose-dependent toxcity[40–42]. Other candidate NF-κB activating agents effective at inducing virus transcription in *in vitro* latency models include prostratin, TNF-α, and the transient NF-κB activating agent HIV-reactivating factor (HRF)[43]. Pharmacological agents that stimulate autophagy, protein kinase C, or the generation of reactive oxygen species (ROS) can also activate the NF-κB pathway to induce virus replication. Bryostatin 1 induce the PKC–NF-κB pathway and can synergize with other drugs to remodel chromatin transcriptional coactivator complexes in the cell, reactivate virus, and promote cellular apoptosis[44, 45]. Enthusiasm for these approaches is tempered by the recognition that generalized activation of CD4+ T cells has significant risk associated with systemic induction of proinflammatory cytokines.

Using small molecule drugs to more specifically reverse those pathways involved in chromatin silencing was advocated at about the time the concept of latency was first introduced[46]. There is now intense interest in inhibiting histone deacetylases (HDAC) as these molecules block HIV DNA transcription by preventing transcription factors from accessing the HIV promoter. Two candidate HDAC inhibitors (HDACi) have already entered clinical trial evaluation (Table I). Valproic acid, a relatively weak non-toxic HDACi, was the first drug studied. Despite initial promising results in a pilot study, subsequent studies failed to find any benefit and the field has moved on to more potent drugs[47–49]. Vorinostat (SAHA) has shown promise in reactivating virus in *in vitro* models of latency. In a recent pilot study, 11 of 16 antiretroviral-treated subjects exhibited potential susceptibility to vorinostat *ex vivo*[50]. Eight of these subjects were then administered a single dose of vorinostat and showed a significant increase in their expression of RNA levels compared to

baseline. Other clinical trials of vorinostat are ongoing (Table 1). A recent report showed that the HDAC inhibitor FK288 (romidepsin) was 1000 times more potent than vorinostat at inducing latent HIV and induced HIV RNA expression *ex vivo* in twelve of thirteen HIV infected subjects on cART[51]. Other HDACi, such as LBH589 (panobinostat), ITF2357 (givinostat), PXD101 (belinostat) also showed greater potency than vorinostatin reactivating virus in latently infected cell lines (Table 2)[52].

Coordinated action of multiple epigenetic modifications might be required to initiate viral transcription. Optimum reactivation of epigenetically silenced provirus DNA might require multi-drug combinations that include HDACi, PKC activators, and/or specific HMTase inhibitors. The combination of a HDACi with the H3K9 histone methyltransferase (HMT) inhibitor, BIX01294, reactivated latent virus better than either agent alone[53, 54]. The discovery of new molecules that are safe and can act alone or synergize with other drugs to reactivate virus transcription with high efficiency is a major priority. For example, three FDA-approved drugs (dactinomycin, aclacinomycin, and cytarabine) were recently described to act as priming agents when combined with other reactivating molecules to increase the frequency of cells producing virus[55]. Disulfiram, used for the treatment of alcoholism, was shown through its active metabolite diethyldithiocarbamate (DDTC) to reactivate latent virus in an *in vitro* latency model[56]. In a small pilot study, a single dose of disulfiram given daily for 2 weeks to cART treated subjects was well tolerated and led to a transient increase in plasma RNA in a subset of subjects[57] (Table 1).

There are other potential drug targets that maintain latency including the above described immune checkpoint blockers such as PD-1, which is expressed on latently infected $CD4^+$ cells in HIV infection. These molecules present a major barrier to T-cell activation by inhibiting signals transmitted through the TCR[58–60]. In ART treated patients, PD-1 expression remains elevated and correlates with the size of the latent reservoir and high levels of cell-associated RNA and proviral DNA[13, 61, 62]. High levels of PD-1 expression on CD4 memory T cells in HIV-1 infection prevents antigen-specific TCR activation and downstream signals that are needed for proliferation and effector function and HIV replication. PD-1 or PDL-1 blocking antibodies can relieve inhibitory signals on TCR activation and increase the sensitivity of the TCR to antigen. High levels of PD-1 expression found on antigen-specific CD8 T_{TM} and T_{EM} 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. Blocking PD-1 on antigen-specific CD8 T cells can prevent negative signals delivered through PDL-1 and PDL-2 ligand binding. Thus, blocking PD-1 or combinations of co-inhibitory molecules might serve a dual role in reactivating latent virus in CD4 cells, as well as relieving a functional block on virus-specific $CD8^+$ memory T cells by enhancing antigen-specific proliferation and effector cell differentiation[63, 64].

The cytokine IL-7 is required for homeostatic proliferation and survival of naïve and memory $CD4^+$ T cells that express the IL-7R[65]. In ART treated patients with low $CD4^+$ T cells, IL-7 was shown to reconstitute naïve and TCM cells[66, 67] and in one study induced transient viremia in 6 of 26 subjects[68]. Exogenous IL-7 could reactivate HIV replication in CD4 memory T cells from some HIV-infected donors *in vitro* when cells were stimulated in PBMC cocultures[69]. IL-7 induces the proliferation of CD4 memory T cells that carry integrated viral DNA including TCM and TTM, yet the ability to reactivate virus replication with IL-7 in ex vivo cultures is highly variable[70]. Based on these findings, IL-7 therapy as a reactivating intervention is currently tested in combination with ART intensification with raltegravir plus maraviroc to prevent proliferation or reseeding of the latent reservoir (ERAMUNE 01 study) (Table I).

Step 2. Targeting residual virus replication—Chronic inflammation is a major factor contributing to HIV immune activation and disease pathogenesis[71–73]. The inflammatory process appears 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 Tregs and production of TGF-β[74, 75]. Abundant amounts of HIV proteins (e.g. p24, p17, gp120) persist on the surface of the follicular dendritic cell network (FDC) despite suppressive ART and may contribute to chronic immune activation[41, 76–78]. Proinflammatory cytokines and IFN-α production results in a higher frequency of activated $CD4^+$ T cells that might contribute to persistent low level viremia and prevent healing damaged gut and lymph nodes[79, 80]. Chronic inflammation results in 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 aimed at preventing the negative aspects of inflammation on functional immune responses (*e.g.*, blocking PD-1-PDL interactions; blocking Type I Interferons and IL-6) and enabling the positive aspects (*e.g.,* enhancing innate and HIV-specific T-cell immunity) may prove to be an important combination for an effective cure strategy.

A number of anti-inflammatory drugs are now being advanced into hypothesis-testing phase I and phase II clinical trials to establish a role for attenuating chronic inflammatory responses in balance with immune responses needed for eradication of latent reservoirs. For example, a recent study found that the expression of CD38 and PD-1 on total $CD8^+$ and Gag-specific $CD8^+$ T cells was reduced in untreated HIV infected patients administered a COX-2 inhibitor[81, 82]. The Peroxisome Proliferator-Activated Receptor (PPAR-c) agonists represent a promising class of anti-inflammatory molecules. Among these, pioglitazone and leflunomide are relatively well tolerated and effective for treatment of chronic inflammation[83] and might reduce comorbidities (metabolic syndrome, dyslipidemias, and glucose intolerance) that have been associated with long-term ART in HIV infected patients[84]. Chloroquine or hydroxy chloroquine treatment can significantly reduce HLA-DR^+CD38^+ $CD8^+$ T cells, as well as Ki-67 expression in $CD4^+$ and $CD8^+$ T cells[85, 86], but failed to show efficacy in meeting these endpoints in HIV asymptomatic ART-naïve patients[87]. Other drugs currently being evaluated include methotrexate, mesalamine, which should reduce inflammation in the gut mucosa, where much of the virus resides, and anti-fibrotic agents such as ACE inhibitors (which may restore immune function) and a variety of drugs aimed at other microbial infections which may 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 demonstrated.

Step 3: Enhancing host-mediated clearance of residual virus—The importance of cellular immunity in controlling the size of the HIV reservoir has been suggested by several studies. HIV-1 Gag-specific CD8 T cells isolated from elite controllers but not subjects treated with ART were shown to effectively kill autologous resting CD4 T cells in which virus was reactivated with SAHA[88]. Moreover, functional anti-viral $CD8^+$ T cells are associated with limiting the size of the central memory $CD4^+$ T cell reservoir in patients controlling their virus in the absence of ART[89]. Indeed, high-avidity multifunctional $CD8^+$ CTL that target vulnerable regions in Gag are particularly important in limiting virus diversity and reservoirs in HIV-infected individuals possessing “protective” HLA-class I alleles[89–91]. Therapeutic vaccines have been proposed to restimulate CD8 CTL in order to prevent or control virus relapses and the reestablishment of latent infection in CD4 T cells after treatment interruptions[92–94]. A few therapeutic vaccine trials such as the Ad5-HIV-1 gag vaccine (ACTG A5197), or infusions of dendritic cells pulsed with inactivated HIV particles have shown transient viral suppression following treatment interruption[95, 96]. The Eramune-02 study is currently testing whether the VRC HIV-Gag, Pol, Nef, and Env

vaccine in a DNA prime-recombinant replication-defective adenovirus serotype-5 (rAd5) boost regimen can reduce the viral reservoir in subjects undergoing an ARV intensification regimen (Table 1).

The Type I IFN response is an essential innate mediator of the acute antiviral response and for the induction of effector $CD8^+$ CTL. A recent study found that pegylated interferon (IFN)-α-2A treatment could suppress virus replication in subjects whose immune function was partially restored by cART[97]. Adjuvants inducing the production of type I IFNs by conventional dendritic cells, plasmacytoid dendritic cells, and nonhematopoietic cells can potentially drive activation and differentiation of quiescent memory $CD4^+$ T cells and enhance *de novo* $CD8^+$ CTL responses and viral clearance. Push–Pull strategies combining synergistic combinations of molecular adjuvants such as Toll-like receptor (TLR) ligands and costimulatory molecules, while blocking of negative regulatory molecules (PD-1, CTLA-4, TIM-3, CD160, 2B4), immunosuppressive cytokines, and T regulatory function might further optimize induction of $CD8^+$T effector and effector memory cells[96, 98–100].

High dose IL-15 can skew differentiation of high levels of $CD8^+$ T effector memory cells[101]. IL-15 was also shown to be 4 fold more potent than IL-7 for inducing virus production in latently infected CD4 cells[70]. Therapeutic vaccines adjuvanted with IL-15 in combination with blockage of co-inhibitory molecules such as PD1 might enhance killing of infected cells and prevent reactivated virus from replenishing latent reservoirs.

The clinical challenges ahead: a “new look” at the design of clinical studies

To achieve a cure for HIV, the efforts of the global research community must be coordinated and transparent in sharing both positive as well as negative clinical results. Specifically we must:

1. Identify virus producing cells and the location of viral sanctuaries and determine the mechanisms by which virus establishes and maintains latent infection.
2. Develop standardized *in vitro* latency models in primary memory CD4 cells to facilitate the screening and prioritization of reactivating compounds and combinations.
3. Harmonizeon-going efforts of different laboratories to develop a high through put primary T-cell model of HIV latency to screen for novel anti-latency compounds.
4. Develop and implement robust monitoring tools to quantitate single copy cell-associated HIV RNA and HIV DNA as a measure of the HIV reservoir and the proportion of cells producing virus in response to treatment. Since the majority of proviral DNA is replication-incompetent, assays that can directly measure replication competent HIV DNA and quantitate HIV DNA/RNA double positive cells are needed.
5. Develop non-human primate (NHP) models of antiretroviral-treated SIV infection and conduct proof of concept studies testing the safety and efficacy of reactivation strategies and immune-based therapies
6. Develop humanized mouse models of HIV infection to assess novel eradication approaches and combinatorial strategies.
7. Develop non-comparative phase I/II clinical studies to rapidly screen multiple clinical strategies as in the oncology field to determine safety and efficacy and a regulatory framework that allows fast implementation of small scale (*e.g.* 10 to 20 subjects per arm) proof-of-concept hypothesis driven trials.

8. Develop guidelines for selection of patient populations according to the risk/benefit ratio associated with the primary objective of the study, *i.e.* sterilizing or functional cure.
9. Develop consensus protocols for defining time to primary endpoints and the follow-up period for secondary endpoints that quantify (i) reduction in the HIV reservoir in blood and tissues, (ii) levels of inflammatory biomarkers, and (iii) functional antigen-specific immune responses for advancing to phase II studies. A phase IIb/III approach will ultimately require analytical treatment interruption of ART in responders and evaluation of HIV reservoirs in blood and tissues after withdrawing therapy
10. Develop a universal web-based database for meta-analyses and hierarchical modelling of multi-center trials and protocols that can facilitate the identification of best approaches.
11. Involve the HIV community, funding agencies, and regulatory authorities prospectively to discuss the complex ethical considerations with special regard to the design and financial support of clinical studies in HIV cure research.
12. Prepare the public opinion for this new phase of research and discovery, preventing high expectations in a short-term and readdressing the emphasis on prevention as the main available strategy to fight the HIV/AIDS epidemic.

Conclusions

Given the burden of HIV infection throughout the world, the goal of finding a cure for HIV is one of the major medical challenges of our time. This goal has rallied all sectors of the biomedical community (public, private, academic and community groups). A consultation has led to elaboration of a document that describes a strategy that will lead to a cure[14]. There are still numerous scientific challenges ahead to identify combinatorial approaches that can effectively induce reactivation of the latent reservoir(s) and enhance specific immune responses to control virus replication and eliminate virus-infected cells. Ethical issues are also of great concern in strategies that reactivate virus infection in individuals that are being successfully treated on ART. Recent studies that immediate/early antiretroviral treatment following infection might minimize the size and complexity of the latent reservoir and could lead to post-treatment control provide us with the optimism for moving forward with therapeutic interventions aimed at eradicating HIV infection. We should all keep in mind that prevention and early initiation of ART is currently the best way to control the HIV pandemic.

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Table 1

CURE-related clinical pilot trials ongoing in 2012

Study/Principal investigator	Intervention	Study Population	Primary End -point	Clinicaltrials.gov
ERAMUNE 01C Katlama Objectif Recherche Vaccins SIDA (ORVACS) University PARIS VI	IL-7 plus ART intensification with RAL and MVC	29 chronically HIV infected patients with HIV RNA < 50 cp/ml on ART -HIVDNA :10–1000 cp/10^6 PBMC	Decrease of 05 $\log_{10}$ HIV DNA from baseline at 56 weeks	NCT01019551
ERAMUNE02 **R. Murphy** ORVACS Northwestern University	HIV rAD5 vaccine and ART intensification with RAL and MVC	28 chronically HIV infected patient with HIV RNA < 50 cp/ml on ART-HIV DNA 10+1000 cp/10^6 PBMC -Negative AD5 Ab	Decrease of −0. 5 $\log_{10}$ HIV DNA from baseline at 56 weeks	NCT00976404
Disulfiram **S. Deeks, A. Andrade** University of California, San Francisco. Johns Hopkins University	Disulfiram: 500mg/d x 14 days	20 chronically HIV-1 patients with HIV RNA < 50 cp/ml on ART	2 weeks frequency of replication, competent HIV-1 in resting CD4+ T cells	NCT01286259
Vorinostat D. **Margolis** University of North Carolina, Chapel Hill	Vorinostat (87) 200 mg to 600 mg	20 chronically HIV-1 patients with HIV RNA < 50 cp/ml	HIV expression in resting CD4+ T cells	NCT01319383
Vorinostat S. **Lewin** Monash University and The Alfred Hospital	Vorinostat 400 mg/d	20 chronically HIV-1 patients with HIV RNA < 50 cp/ml - CD4 >500/mm^3	HIV unspliced RNA in resting CD4+ T cells at 28 days	NCT01365065
Panobinostat T. Rasmussen Aarhus University Danemark	Panobinostat 20mg Three times a week Every other week for 8 weeks	16 chronic HIV+ patients with suppressed viremia and CD4>	HIV unspliced RNA in resting CD4+ T cells Change from baseline	NCT01680094).
CD4+ T cells modified at CCR5 by ZFN Winson TangSangamo Biosciences	Autologous CD4+ T cells modified at CCR5 gene by Zinc-finger nuclease SB-728-T	Chronic HIV+ patients with suppressed viremia and CD4 < 300/mm^3 or ART failing	Safety Persistence and activation of CCR5+ZFN modified autologous T cells	NCT01252641
CD4+ T cells modified at CCR5 by ZFN P. Tebas, D. Stein, C. June Pennsylvania University Jacobi Medical Center	Autologous CD4+ T cells modified at CCR5 gene by Zinc-finger nuclease	18 patients: 3 cohorts of HIV+ patients, either failing ART or with suppressed viremia	Safety	NCT00842634
Lentivirus vector rHIV7-shI-TARCCR5RZ-transduced hematopoietic progenitor cells A. Krishnan City of Hope Medical Center	Autologous CD34+ hematopoietic cells modified by lentivirus transduced nonfunctional CCR5RZ gene	Hematopoietic stem cell transplant AIDS related lymphoma	Safety and durability of transduced cells	NCT00569985
Interferonα2b F. Maldarelli National Cancer Institute (NCI)	INFα 2b intensification	Recruiting, Non-randonmized, single group assignment	Efficacy: viral RNA levels in blood and sequence diversification	NCT01295515

Table 2

Experimental Approaches For Reactivating Latent HIV infection

Approach	Mechanism of Action	Activation	Refs.
NF-kB Inducing Agents			
Anti-CD3/CD28	T-cell receptor activation	++++	102,103
TNF-α *,	TNFR	++	38
Prostratin PMA/ionomycin Bryostatin-1, Picolog	PKC activation	+++	44, 104
HDAC inhibitors	Histone and nonhistone protein acetylation		
SAHA (vorinostat)	Class I HDAC1, HDAC3 inhibitor; HDAC4 and HDAC6 Cell cycle arrest: Myc↓•p21 ↑ Reactive oxygen species– apoptosis↑ nonhomologous DSB repair↓ Angiogenesis: HIFα, VEGF↓ Innate immunity, proinflammatory cytokines ↓ STAT 5 hyperacetylation		
Depsipeptide/FK228 (romidepsin)	HDACs 1, 2, 4, and 6 inhibitor	+++	51
BH589 (panobinostat) ITF2357 (givinostat) PXD101 (belinostat)	Potency in latently infected cell lines: LBH589 >ITF2357 and PXD101 >SAHA >VPA	+++	105, 106
Sirtuin inhibitors	Acetylation of nonhistone proteins: HIV Tat, p65 RelA, p53, FOXO3a	untested	
HMT inhibitors			
BIX-01294	G9a (H3K9me2) inhibitor	++	54, 107
chaetocin	SUV39H (H3K9me3), inhibitor	++	108
Proapoptotic and cell differentiating molecules			
JQ1	Blocks TAT binding to P-TEFb Inhibits bromodomain reader BRD4 recruitment to H3K9ac, H4K16ac myc transcription↓ SIRT1 ↑	+ priming	109
Nutlin3	MDM2 blocker, p53 transcription, caspase activation ↑	untested	
Disulfram, aphidicolin	Oxidative stress, apoptosis ↑	++	56
HMBA Dactinomycin, aclacinomycin Cytarabine	P-TEFb release associated with RNAP II triggers transcriptional elongation	+	55
Wnt small molecule inhibitors, Notch (γ-secretase) inhibitors	myc, CtBP1, Cyclin D, cJun ↓ HES target genes ↓	untested	
Immune-modulators			
Anti-PD-1, CTLA-4, TIM-3 mAb	Block negative regulatory receptors	anti-PD-1 (ACTG 5301)	
Anti-PD1mAb + IL-15	STAT5, PI3K signaling, $CD4^+$ T cell effector memory differentiation	untested	
Therapeutic $CD4^+$ T cell vaccine + raltegravir	Reactivate virus in latently infected antigen-specific CD4 cells To limit reseeding viral reservoirs	Not determined	93, 110
Synergistic combinations			
SAHA + chateocin + prostratin	HDAC inhibitor + HMTI HDAC inhibitor + NFκB inducer	+++	108, 111

Approach	Mechanism of Action	Activation	Refs.
HDACi+ anti-PD-1	HDAC inhibitor + anti-PD-1mAb.	preclinical studies	
Anti-PD1mAb + IL-15 + Raltegravir, peptidomimetics	$CD4^{+}$T effector memory cell differentiation, Inhibit Integrase, LEDGF/p75	untested	112

* limited effect in primary cell models

phorbol ester 13-phorbol-12-myristate acetate (PMA), suberoylanilidehydroxamic acid (SAHA); Hexamethylenebisacetamide (HMBA); positive transcription elongation factor (p-TEFb)