

HIV Eradication:

Is it feasible?

COMMISSIONED BY:

aids2031 Science and Technology Working Group

AUTHORED BY:

STEPHEN G. DEEKS, MD
University of California, San Francisco

Disclaimer: The views expressed in this paper are those of the author(s) and do not necessarily reflect the official policy, position, or opinions of the wider aids2031 initiative or partner organizations

Despite the dramatic improvements that have resulted from combination antiretroviral treatment, long-term efficacy, toxicity, cost, and the requirements for life-long adherence remain as formidable challenges. Also, there is emerging consensus that persistent HIV-associated disease occurs, even among patients who have achieved an optimal virologic response to antiretroviral therapy. Hence, strategies aimed at achieving complete viral eradication may be needed in order to fully restore health among HIV infected individuals.

The development of an effective and scalable cure for HIV would also have obvious public health benefits. Given the challenges in developing an effective HIV vaccine, perhaps the only way to truly curtail the global epidemic is to cure for the disease. Even if complete eradication of all virus in an individual proves impossible—as most believe to be the case—a less rigorous but still desirable outcome might be achieving durable control of virus in the absence of therapy. This “functional” cure would likely dramatically reduce the risk of subsequent transmission.

The primary objective of this review is summarize for a diverse audience ongoing efforts for achieving what is clearly a daunting but perhaps not impossible task: the life-long eradication of replication competent HIV from an infected individual. Theoretical and practical barriers preventing progress in this area are highlighted.

How should HIV eradication be defined?

There is no consensus on how to define a cure in the context of HIV infection. Perhaps the most desirable outcome is a ***sterilizing cure***, in which all replication competent virus is removed from an infected person. The chance of an eventual recrudescence of viral replication in cured patient in this case would by definition be zero. As discussed below, the existence of long-lived latent reservoirs of HIV makes this outcome with any currently proposed approaches unlikely. Even if such an outcome was achieved, it may prove impossible to prove it with existing technologies. There is no way to prove the lack of residual virus given the multiple possible reservoirs, many of which are difficult in humans to access.[1, 2]

A far more likely outcome is a ***functional cure***, generally defined as the lack of detectable viral replication in the absence of ongoing antiviral therapy. This can be defined in several ways. One possibility is to use the cancer model, in which a functional cure might be defined as the lack of viral replication (and disease) for at least five years in the absence on any ongoing therapeutic intervention. From a practical perspective, the primary outcome for any future eradication study will be the lack of viral recrudescence in absence of therapy for a pre-defined period of time.

Do functional or sterilizing “cures” ever occur naturally?

There is no known case of an HIV seropositive individual who subsequently seroreverted in the absence of therapy (there are, however, rare cases of patients treated during early HIV infection whose HIV antibodies subsequently declined).[3] It is hence unlikely that a sterilizing cure—in which there is no residual antigen or replication—occurs naturally. It should be noted, however, that some investigators have found evidence of very low levels of HIV DNA in exposed, serogenative individuals.[4-6] No one has been able to document the containment and eventual eradication of replication competent virus in these individuals, but it would be exceedingly difficult prove transient infections even if it were a common event.

If a functional cure is defined as the lack of detectable viremia in absence of therapy for several years, then a functional cure clearly happens naturally, albeit rarely. A small subset of HIV seropositive individuals (probably less than 1%) are able to spontaneously control replication competent virus and maintain undetectable plasma HIV RNA levels for years to decades (“elite” controllers). As essentially all elite controllers have some evidence of persistent viremia, it is unlikely that any have truly been cured, at least as defined by the more rigorous definitions.[7-10] Replication competent virus is often easy to detect in these individuals. [11-17].

Several groups have recently been able to identify sufficient numbers of these individuals to allow careful and detailed investigation. To date, convincing evidence has pointed to several potential virus and host factors.[18] Approximately 50% have clear evidence of strong HIV-specific CD8 responses, as defined by the presence of certain class I alleles (e.g., HLA B5701) or presence of high numbers of HIV-specific CD8+ T cells.[19-24] Other host related factors associated with control include NK cells [25], low CCR5 expression [26], skewed HLA-C expression [27] and perhaps low immunoregulatory responses.[24]

Another potential naturally occurring model for HIV eradication are those who have repeated high level exposures to HIV, yet do not seroconvert. Transient, occult infection may occur in such patients, as evidenced by the detection of exceedingly low levels of HIV and/or the presence of detectable HIV-specific T cell response.[4-6, 28] A compelling argument has recently been made that occult infection is not uncommon. Among at risk HIV uninfected individuals enrolled in the STEP trial, exposure to a T cell vaccine resulted in higher risk of acquiring HIV infection. Since such a vaccine is more likely to enable the establishment of an infection once transmissions occurred (rather than increasing the risk of transmission), it seems reasonable to postulate that occult transmission events

are non uncommon.[29] The mechanisms that account for eradication of HIV at the earliest stages may have implications for eradicating established infection.

What would a functional cure look like?

The study of elite controllers is important not only because they might provide insights into potential mechanism for a “functional cure” but because they might be informative regarding the overall prognosis once durable control is achieved. The vast majority of controllers do well clinically and most meet the standard definition of “long-term non-progression” (i.e., they maintain a normal CD4+ T cell count and fail to develop AIDS for more than 10 years). A significant subset, however, exhibit clear evidence of immunologic progression, and up to 10% may progress to AIDS even as the viral load remains undetectable. Heightened immune activation—which may be related in part to a potent HIV-specific immune response—is associated with disease progression among elite controllers (as it is among non-controllers).[30, 31] There are also emerging data suggesting that the heightened inflammatory response which is observed in some elite controllers may result in premature atherosclerosis and perhaps early heart disease.[32] These observations suggest that persistent disease may occur in patients who achieve a “functional” cure, particularly if that cure is associated with persistent inflammatory responses.

Viral dynamics: how long does HIV persist in absence of replication?

Antiretroviral therapy prevents *de novo* infection of target cells. Theoretically, once full suppression is achieved, eradication will occur once all pre-existing cellular reservoirs decay to the point that either no virus is present, or the level of residual virus is too small to ignite subsequent rounds of viral replication.[33] Although controversial (see below), most evidence suggests that current HAART regimens are fully suppressive, or that the degree of residual viral replication during HAART is not sufficient to maintain life-long infection. The effectiveness of HAART has therefore allowed careful characterization of the HIV cellular reservoirs.

Viral dynamics during HAART Most of our understanding regarding the nature of where and how HIV replicates comes from careful measurements of viral dynamics during HAART. These studies showed that there are at least two and as much as four distinct phases of viral decay. Plasma HIV RNA levels decline by 100 fold in the first two weeks of HAART (first phase). This decline most likely reflects the rapid loss without replacement of the proliferating short-lived activated CD4+ T cells. Plasma HIV RNA levels then decline at a much slower rate (second phase). Although never proven experimentally, this second phase decay likely reflects the progressive loss of longer lived T cells, macrophage/monocytes and perhaps other cells. The original characterization of this second phase

decay suggested that it might be possible to clear all pre-existing viral reservoirs in two to three years.[33] The first well publicized “cure” paradigm was based in large part on these observations.

With the development of more sensitive assays, it soon became clear that a third and much longer phase of decay exists. Replication competent virus can be readily found in resting memory cells even after years of otherwise effective HAART.[34-36] The rate at which this virus population declines has been estimated to be approximately 44 months, suggesting that it would take many decades of fully suppressive HAART to eradicate the virus.[37]

As recently argued by Palmer, Coffin and colleagues, there may even be a fourth phase of decay.[38] Using an ultrasensitive quantitative viral load assay, it was observed in a cohort of protease inhibitor-treated patients that (1) the majority of patients had detectable plasma HIV RNA levels (≥ 1 copy RNA/mL), even after 7 years of HAART, (2) the level of residual viremia decays in a non-linear manner after the first year (suggesting a third and possibly a fourth phase of decay), (3) the putative third phase decay likely reflects the gradual loss of infected resting memory CD4+ T cells, given that the half-life of this phase was similar to that observed for resting memory T cells by Siliciano and colleagues[37], and (4) the fourth phase of decay (if it exists) has a half-life that may be infinite (i.e., no decay), or very long, thus arguing that eradication with standard HAART regimens is impossible.

Tissue versus blood sampling. The vast majority of HIV is thought to reside in tissues, with the colonic mucosa perhaps the single largest reservoir. Cross-sectional studies have consistently shown that the amount of virus in gut tissue is much higher than in blood (as defined by proportion of CD4+T cells that harbor HIV).[2, 39] Given logistical challenges in obtaining tissue over time, there have been few studies which have attempted to quantify the rate of viral decay in mucosal tissues. In one 15 patient study involving serial rectosigmoid biopsies, the decay of cellular HIV DNA levels was negligible (estimated half-life of over two years) and not statistically significant from zero.[40]

It has been argued that residual virus in gut likely reflects ongoing replication, because the proportion of cells which are “resting” and hence latently infected is thought to be very small.[40, 41] If true, then the dynamics of HIV decay may be very different in mucosal tissues than in blood. The high level microbial translocation that persists during HAART almost certainly causes elevated local mucosal inflammation; this could provide an environment that could support persistent viral replication.[42, 43] For these reasons, ongoing and future efforts at eradicating HIV will likely require intensive, longitudinal sampling of the gut mucosa, which is expensive, technically challenging and potentially risky to the study participant.

Acute versus chronic HIV infection. The vast majority of studies addressing viral dynamics during HAART focused on patients who initiated HAART after many years of untreated HIV infection. The amount of HIV in reservoirs—as defined by proviral DNA and presence of replication competent HIV—is higher in patients who initiate HAART during chronic infection compared to those who initiate HAART during acute/early HIV infection.[44, 45] Given that the major barrier to eradication may be the size and characteristics of this reservoir, many have argued that if a cure is to be achieved, it will be in patients who started therapy during acute infection.

The strongest data supporting early HAART comes from the work of Chun et al. Seven individuals were treated with HAART post-seroconversion but within the first 6 months of their infection. The latent reservoir—as measured by the frequency of resting CD4+ T cells carrying replication competent virus—decayed rapidly, with a half-life of 4.6 months. The time to complete eradication was estimated to be approximately seven years, assuming that all ongoing viral replication was inhibited and that the rate of decay remains constant.[46] The observation that residual virus can often be found among acutely infected patients who subsequently receiving long-term HAART argues against this optimistic prediction [43, 45], as do data suggesting biphasic rates of decay in this population.[44, 47]

Are current HAART regimens fully suppressive?

The preceding discussion regarding viral dynamics during HAART have shaped ongoing strategies aimed at HIV eradication. It should be emphasized, however, that these each of these studies assumed that HAART was fully suppressive. It is also possible that the residual virus that is often observed during long-term HAART reflects a new steady-state in which low amounts of viral replication continue to replenish the reservoir.

This raises a central and highly debated question: is all virus replication effectively prevented by HAART, or are there residual rounds of replication? Strong arguments have been made for both sides. Observations supporting the thesis that HAART is indeed fully effective include: (1) HAART works indefinitely, with limited if any evidence that drug resistance evolves over time in patients with detectable plasma HIV RNA levels [48, 49], (2) viral sequences obtained over time generally fail to exhibit any evidence of ongoing viral evolution [50], although this is controversial[51-54], and (3) the inherent potency of a regimen does not have a clear effect on steady-state HIV RNA levels (assuming a level of < 50 copies RNA/mL is achieved), arguing that the primary determinant of the steady-state is the size of the reservoir, rather than the degree of incompletely suppressed viral replication.[55] Collectively, these observations suggest that residual viremia during HAART likely reflects the gradual release of viral particles (and hence viral RNA) from stable cellular reservoirs, without subsequent

productive infection of new targets. If true, the current regimens are unlikely to result in viral eradication during the typical life-span of an infected adult.

Observations arguing that HAART is only partially suppressive include: (1) plasma HIV RNA levels are stable over years, suggesting that new sources of viral production need to be generated to replace the loss of older sources, (2) inflammation levels remain well above normal levels during long-term HAART[56] and may decline when antiviral drugs are added to a stable regimen[57], (3) HIV cDNA episomes—which are likely labile and reflect recent infection events—can be found in long-term HAART treated patients[58], (4) the proportion of cells harboring replication competent virus persists at high levels in gut tissue during otherwise effective HAART.[2, 59, 60] and (5) short-lived activated T cells harbor more virus than resting T cells during long-term HAART, with some evidence of ongoing cross-infection between these two cell populations.[61] These observations—none of which are definitive—argue that with more effective HAART, the rate of viral decay may be accelerated, leaving open the possibility that more potent antiretroviral regimens may in fact result in eventual eradication.

Perhaps the optimal manner to determine the effectiveness of HAART is to intensify a stable regimen with an additional agent, and to carefully measure residual HIV RNA levels before and after intensification. Again, the data from these types of experiments are controversial and inconsistent. In the first study, Havlir and colleagues added abacavir to a stable regimen, and observed a rapid decline in virus (of note, patients in this study were taking an unusual regimen and had a high median pre-intensification viral load). More recently, two independent groups (Maldarelli and colleagues at NCI; Siliciano and colleagues at Johns Hopkins University) performed similar single arm intensification studies in which either a protease inhibitor or NNRTI was added to a stable regimen. There was no clear change in plasma HIV RNA levels in these studies, leading the investigators to conclude that current regimens are indeed fully suppressive. Each of these studies was limited by (1) a small sample size, (2) the lack of a control arm, (3) the reliance of blood sampling (most residual virus replication is in tissues), (4) the relatively short term follow-up, and (5) the use of agents that may not have sufficient intrinsic potency to see the desired effect.

Several groups are now performing larger and more definitive intensification studies, using potent drugs with novel mechanisms of action (e.g., maraviroc and/or raltegravir). Most groups are also now routinely sampling the gastrointestinal tract, where residual viral replication is likely to be most readily detected. Most ongoing and planned studies are randomized. It is likely that a consensus to this important question will emerge within the year. If these studies confirm that prior regimens were likely fully suppressive, then our current understanding regarding the dynamics of viral persistence will remain unchanged. If, however, these studies reveal that viral replication is ongoing, and that this

replicating virus population can be inhibited with newer strategies, then our estimates regarding how long it would take to eradicate HIV by HAART alone will need to be revised.

Latent reservoirs and barriers to eradication

Assuming that current or future HAART regimens can effectively suppress all viral replication, then the primary barrier to eradication becomes the poorly defined but extensively studied “latent” reservoir. The key questions now facing the field pertain to the nature of this reservoir, and include: (1) what cells contribute to this reservoir?, (2) where are these cells located? (3) how long do these cells persist?, and (4) what can be done therapeutically to accelerate the decay of these cells?. There are no clear answers to these questions, despite years of intensive research.[62]

All investigators seeking a cure to HIV recognize that the existence of a transcriptionally silent form of stably integrated HIV DNA in resting memory T cells represents the major barrier to eradicating the virus. In peripheral blood, approximately 1 in 10^6 resting memory CD4+ T cells harbor replication competent virus[35]; these levels are likely much higher in certain tissues[2].

The exact life-span of activated memory CD4+ T cells is short (days), while the life-span of resting memory CD4+ T cells is unknown, but is likely many months to years. Careful measurements regarding the proportion of purified resting memory cells that harbor replication competent virus over time suggests a possible decay rate on the order of decades (the most cited work suggests that it will take over six decades for this reservoir to fully decay, assuming that HIV replication can be prevented indefinitely).[37]

Non-T cell Reservoirs. The primary source of HIV during untreated disease is undoubtedly activated CD4+ T cells.[63] These cells are also an important reservoir in long-term treated infection.[61] A small proportion of infected activated T cells revert to a resting state, and continue to harbor replication competent virus for years to decades, as described above.

Macrophages and perhaps monocytes express the requisite cell surface receptors for HIV, and appear to be primary non-T cell reservoir for HIV.[33, 35, 64-66] It has been suggested that these cells are the primary source for ongoing viral replication after the population of infected short-lived activated CD4+ T cells wanes, although experimental data for this is lacking.[33, 67] It has also been argued that activated monocyte/macrophages are an important source of residual virus production during long-term HAART, and that the decay of this cellular reservoir during HAART may be slower

than that observed for the much better characterized resting CD4⁺ T cell population.[54] As macrophages/monocytes are difficult to isolate from peripheral blood, the number of studies aimed at characterizing these cells *in vivo* are limited.

There is even less evidence supporting the role of other putative cellular reservoirs for HIV. In untreated HIV infection, dendritic cells, T regulatory cells, NK T cells, CD8⁺ T cells and perhaps even epithelial cells may either be infected with HIV, or carry on their cell surfaces intact virions that can later be delivered to other target cells.[63] [68, 69] The role of these cells as a barrier to HIV eradication is not known but of high interest, particularly if their decay rates prove to be longer than observed with resting memory T cells.

Can the latent reservoir be “purged” therapeutically?

As outlined above, resting memory CD4⁺ T cells are designed to persist for prolonged periods, perhaps decades. When HIV infected memory cells are activated by their cognate antigen, HIV transcription occurs, with the production of high numbers of new virions.[70] This process likely results in the death of the infected cells, either as a consequence of the virus’s cytopathic effect or as consequence of natural activation-induced apoptosis. Memory cells can also proliferate in response to homeostatic signals. Theoretically, homeostatic proliferation may result in the death of HIV infected cells (assuming virus is produced) or the production of daughter cells carrying integrated HIV. This latter scenario raises the possibility that the reservoir of HIV will be continually replaced even as all pre-existing HIV infected cells die off.

Molecular biology of HIV latency After infection of a susceptible target cell (i.e., an activated CD4⁺ T cell), reverse transcription occurs. The HIV DNA is then transferred to the nucleus and integrated into the host genome. The sites of integration is not entirely random as HIV seems to be preferentially found in the regions of transcriptionally active genes.[71, 72] Some activated cells return to resting state after HIV DNA integration but before transcription and the production of new virions occurs. These resting cells become transcriptionally silent and probably do not express any HIV proteins; they are hence invisible to the immune system and beyond the reach of current therapeutic strategies. How and why some activated cells are able to revert to this resting stage is not known.

The major promoter and enhancer elements regulating HIV transcription are located within the long-terminal repeats (LTR) found on the 5’ end of the integrated provirus. Proviral DNA transcription ensues when the critical host factors (e. g., NF-AT, NF- κ B, P-TEF β) and the HIV Tat protein interact with the relevant regions within the LTR and the nascent RNA templates.[73] In resting cells, the

regulatory regions within the LTR are protected by host nucleosomes, which attach to the proviral DNA and prevent access to transcription factors. These nucleosomes are stabilized by the presence of histone deacylases (e.g., HDAC1) and other enzymes. Upon stimulation by inflammatory stimuli, cells recruit enzymes that displace HDACs, which opens up the chromatin and allows transcription to occur. These observations point towards at least two potential mechanisms to reverse latency: activation of T cells and/or inhibition of HDAC.

Immune activation as a purging intervention Activation of T cells should reverse the blocks preventing transcription of the HIV genome, which in turn should result in the production of potentially cytopathic viruses and the preferential destruction of the cell harboring HIV. Even if HIV proves not be cytopathic to its host cells (a point of controversy), activated T cells have a short life span and should be cleared naturally. HAART in this scenario prevents the transmission of infectious particles to susceptible targets. A number of cytokines have been shown to be effective in enhancing HIV transcription *in vitro*, including interleukin-2, interleukin-6, interleukin-7, interferon- γ and TNF- α [74, 75]. There are at least two major theoretical barriers to this approach: the degree of inflammation necessary to purge all cells may result in too much collateral damage to the patient and/or the greatly expanded numbers of activated T cells will present result in HIV transcription. Despite these concerns, immune activation as a strategy to purge the latent reservoir has been attempted *in vivo* several times, with varying results, as outlined below.

Clinical approaches to eradication: what has been attempted and why did it fail?

Despite the many theoretical and practical barriers to performing a clinical trial aimed at curing HIV, a number of investigators have made the attempt.[76] All efforts to date have failed to show any meaningful benefit, and several have shown some evidence of harm. Each study is summarized below.

Interleukin-2 Recognizing that IL-2 may activate T cells *in vivo*, Chun and colleagues performed quantitative measurements of the latent reservoir in a cohort of patients receiving either HAART or HAART and IL-2, and found lower levels of replication competent virus among those receiving IL-2.[77] HIV rapidly emerged when therapy was stopped.[78, 79]

HAART plus IL-2 was also studied prospectively in the COSMIC study. Fifty-six HAART-treated were randomized to IL-2 or no IL-2. There was no clear effect of IL-2 on measures of residual HIV. Viral rebound was rapid in those eight IL-2 treated patients who chose to interrupt therapy.[80] The failure

of IL-2 in these studies may be due to relative resistance of non-T cell reservoirs to the effect of IL-2.[76]

IL2 and interferon- γ The Pentakine study examined treated 10 patients intensified HAART (five drugs), IL-2 and interferon- γ , a pro-inflammatory cytokine that may have direct effects on macrophages.[81] Both cytokines were administered during the first year of HAART. Peripheral blood proviral DNA and lymph node HIV RNA levels decreased during the study, but it was unclear if this was due to the HAART alone, or HAART plus the two cytokines. Viral rebound was rapid in the two patients interrupting therapy.

OKT3 and IL2 Perhaps the most aggressive attempts at clearing the latent reservoir to date involved the use of OKT3, a potent anti-CD3 murine monoclonal antibody that activates T cells via the TCR. Lange and colleagues administered IL-2 and OKT3 to three patients on a stable HAART regimen. T cell activation and T cell proliferation increased dramatically, as expected. OKT3/IL2 resulted in severe and prolonged CD4+ (but not CD8+) T cell depletion, while there was no evidence of any durable decrease in lymph node HIV RNA levels.[82]

Possible reasons for the failure of the OKT3/IL2 study to reduce viral burden include the presence of residual viral replication[83], the lack of concurrent immunosuppressant therapy (which is commonly co-administered with OKT3 when used in transplantation protocols)[84] and/or the use of repeated OKT3 doses[83]. Pomerantz and colleagues subsequently performed a pilot study in which three HAART treated patients received IL2 and OKT3. The HAART regimen was intensified with ddI and hydroxyurea, an immunosuppressant drug with some antiviral activity. Although the levels of plasma HIV RNA, two-LTR DNA circles and culturable replication competent virus may have declined during the study, viremia rebounded rapidly when HAART was subsequently interrupted.

Interleukin-7 Although IL-2 activates HIV transcription *in vitro* (and perhaps *in vivo*), it also likely causes a transient increase in the number of activated CD4+ T cells, and hence may actually increase the size of the reservoir. Interleukin-7 is involved in homeostatic regulation of the T cell pool and appears to cause T cell proliferation without T cell activation, making it a potential ideal cytokine for “flushing” the reservoir.[85] IL-7 may have greater ability to stimulate virus production from a more diverse cellular reservoir than IL-2, at least *in vitro*.[86] The drug is now undergoing phase I/II clinical testing.

Anti-HIV immunotoxins An ideal eradication intervention would specifically target and kill HIV infected cells. One possible approach that has not yet been tested in humans is to administer anti-

HIV immunoglobulins carrying a potent cytopathic toxin.[85, 87] For example, Berger and colleagues have manufactured anti-Env monoclonals that contain *Psuedomonas*-derived exotoxin. This antibody is active against clinical HIV samples *in vitro* and lacks clear hepatotoxicity in macaques. This approach would be an ideal complement to HAART, particularly if latently infected resting T cells express HIV envelop on their proteins (which is unlikely, but not yet conclusively ruled out). Human studies of this immunotoxin have been planned, while other less specific approaches have been considered.[88, 89]

Prostratin Prostratin is a naturally occurring non-tumor producing phorbol ester that was initially derived from the bark of certain trees in the South Pacific, but can now be made in the laboratory.[90] It has long been used by Samoan healers for the treatment of hepatitis and other diseases. Though poorly defined mechanisms, prostratin activates transcription of the pre-integrated and integrated proviral HIV DNA while preventing *de novo* infection of new target cells.[85, 91-93] Monkey studies and preliminary phase I human studies are ongoing.

HDAC inhibition Much of the more novel approaches now being considered involve disinhibition of HIV transcription. As outlined above, the histone deacetylase enzymes (HDACs) suppress gene transcription via its effect on chromatin structure. Inhibition of this enzyme results in increased HIV production from resting cells (at least *in vitro*).[94] The first study to address the potential role of HDAC inhibition in clearly HIV involved the use of valproic acid, a relatively weak but safe HDAC inhibitor. Four HAART treated patients underwent treatment intensification with enfuvirtide, followed by the addition of valproic acid. Three of four patients exhibited some evidence of a decline in the frequency of resting cells harboring replication competent HIV after the addition of valproic acid. [95] Subsequent studies failed to confirm these results.[96-100] Most investigators now feel that although the approach used here was valid, valproic acid is either too weak or targets the wrong HDAC. More potent (and potentially more toxic) HDAC inhibitors include suberoylanilide hydroxamic acid (SAHA), which is currently used for the treatment of cutaneous T cell lymphoma. A number of other HDAC inhibitors are being actively developed as anti-cancer agents.

Inhibitors of other chromatin modifying enzymes such as histone acetyltransferase (HAT), SIRT, and/or histone methyltransferase (HMT) may also have a role in reversing latency, and are being investigated *in vitro*. An alternative approach is the use of examethylbisacetamide (HMBA), which induces HIV expression in a Tat-independent manner.[101] Given their potential toxicities, it is unclear if regulatory agencies will allow the use of these drugs in otherwise healthy HIV infected patients.

Therapeutic vaccination Perhaps the ideal approach to eradicating HIV is the use of interventions that activate transcription of the proviral DNA (leading to expression of viral proteins) while enhancing the capacity of the immune system to rapidly clear infected cells once they begin to express HIV antigens. Since patients on long-term HAART do not have sufficient viral replication to stimulate strong HIV-specific T cell response, host mechanisms for viral clearance may be insufficient. It has therefore been proposed that therapeutic vaccination with an HIV vaccine may be an ideal complement to immune activating approaches. Most therapeutic vaccines studies to date have had limited effectiveness despite measurable immunogenicity.[102-111] Stronger and more effective vaccines are being developed for the prevention of HIV transmission; these in theory could also be used in eradication studies.

Gene therapy and bone marrow transplantation A number of gene therapy approaches for HIV eradication have either already been attempted, or are in the advanced stages of development.[112] Most may not be appropriate for eradication approaches as they are aimed at either protecting cellular infection or at controlling high level viral replication. A more optimal approach would be the development of approaches that specifically target and kill already infected cellular reservoirs. The largest clinical trial performed to date in which clearance of the latent reservoir was the primary outcome involved the *ex vivo* generation of autologous CD4+ and CD8+ T cells that were modified to express the CD4 receptor (which binds HIV envelop) connected to the CD3 T cell receptor zeta-chain (which activates the cell). An expanded population of HIV-specific effector cells was thus generated. Forty long-term HAART treated patients were randomized to repeated infusions of either gene modified T cells or unmodified T cells. Although there were no statistically significant differences in most outcome measures, there was a consistent decrease in tissue several measures of viral burden among those receiving gene modified cells.[113] The degree of residual viral replication in these well treated patients may have been insufficient to detect any clear effect.

There have been several other noteworthy attempts at using genetic approaches to reduce HIV replication. Most, however, will likely have limited utility in eradicating HIV because they target active replication, rather than the latent reservoir. As reviewed in detail elsewhere[112], one common approach involves gene modification of hematopoietic stem cells or mature T cells to express anti-HIV ribozymes (antisense RNAs that bind and degrade mRNA).[114] A similar approach now that has been extensively studied in the clinic is to use lentiviral vectors to deliver antisense RNA directed at the HIV envelop; successful long-term persistent of gene modified T cells has been achieved in humans[114], and there are emerging data suggesting a potential clinical benefit to this approach. More recent attention has focused on delivering small interfering RNAs molecules (siRNA), which bind

to and cause the degradation of specific RNA strands.[115] Both host genes (e.g. CCR5) and viral genes have been effectively targeted *in vitro*.

These and other approaches would likely not target latently infected cells *in vivo*; however, it might be possible to use myeloablative conditioning regimens to deplete those cells harboring latent HIV, followed by immune reconstitution with protected gene modified stem cells. The aforementioned use of siRNA targeting CCR5 utilized this approach.[115] A related approach is to use zinc-finger nucleases to specifically disrupt the CCR5 gene in autologous hematopoietic cells *ex vivo*, followed by myeloablative conditioning and stem cell transplantation. Another approach being considered is using gene therapy to express the simian version of TRIM5 α , which strongly protects T cells from HIV entry and is closely related to human TRIM5 α . In any of these examples, the gene modified cells should retain a competitive advantage over non-gene modified cells in presence of persistent viral replication, thus leading over time to the expansion of cells which naturally resist HIV. It is unclear if such approaches will ever be used in otherwise healthy patients, given the expense and high morbidity associated with myeloablation. Such approaches may be feasible in patients who require a bone marrow transplant for clinical reasons, as recently demonstrated by the successful allogeneic transplant of stem cells from a donor homozygous for the CCR5- δ 32 deletion (see below).[116]

There are many daunting challenges regarding the potential utility of gene therapy in HIV infection. The fact that HAART in general works well suggests that any potential intervention will need to be safe and well tolerated. Short-term and long-term toxicities associated with myeloablation might preclude the use of this approach in the vast majority of patients. Insertional oncogenesis is a major concern associated with any approach that utilizes retroviruses to deliver the relevant gene. Finally, and perhaps most importantly, the lack of “scalability” for these technically challenging and expensive approaches suggests that even if effective at viral eradication, their impact on the global epidemic will be limited.

Are there any examples of a therapeutic cure?

As outlined above, there have been several small clinical trials aimed at eradicating HIV from an infected person. All have failed to achieve any clear evidence of a cure, and many have shown some evidence of clinical harm. Only one published study has suggested that the accelerated clearance of the latent reservoir can be achieved therapeutically[95, 117, 118], although subsequent studies failed to confirm this outcome.[96-100]

There has been a recent case report that is generating growing enthusiasm, and may in fact prove to meet one a loose definition of “functional cure”.[116, 119] The case involved a 40 year old HIV positive man who developed acute myeloid leukemia (AML) while on long-term effective HAART. Since the standard treatment for AML involves transplantation of allogeneic stems cells, and since individuals who lack functional CCR5 appear to be highly protected against HIV infection, the clinical team aggressively searched for and found an HLA-matched homozygous CCR5- δ 32 donor. The patient subsequently underwent aggressive myeloablative conditioning regimen consisting fludarabine, Ara-C, and amsacrine, followed by 4 Gy of total body irradiation, antithymocyte globulin (ATG) and cyclophosphamide. The transplant was successfully performed.

The patient was known to have had chronic progressive HIV disease with high viral loads prior to the HAART. At the time of the transplant, the patient had undetectable plasma HIV RNA level (using conventional assays), a detectable proviral DNA level (> 15 copies DNA/ 10^5 PBMCs) and a peripheral CD4+ T cell count of x. For unclear reasons, HAART was interrupted on the day of the transplant and not resumed. The patient remained aviremic off HAART, at least through 285 days of observation. Repeated measures of proviral DNA (in both blood and rectal tissue) were negative (< 5 copies DNA/ 10^5 PBMCs). HIV antibody levels remained detectable.

There are many questions left to be addressed with this case, which some have argued may represent the first case of a functional cure.[119] Several factors likely contributed to the outcome: the aggressive myeloablative conditioning (which should reduce the level of the latent reservoir), the post-transplant use of immunosuppressants (which should greatly reduce the number of active T cells, which are the primary target for viral replication) and of course the use of CCR5- CCR5- δ 32 donor cells (which likely acts as life-long exposure to maraviroc “monotherapy”).

The primary problem with this approach is “scalability”; even if it becomes an accepted example of a therapeutic cure, it will not be feasible to transplant allogenic stem cells form homozygous CCR5- δ 32 donors in all but the rarest of circumstances. Also, this approach (or any related CCR5-focused intervention) will only work in that undefined proportion of patients lacking any CXCR4-utilizing variants.

What are the structural barriers to performing work on HIV eradication?

There are many theoretical barriers which make it unlikely that HIV will ever be “cured”. However, no one has argued that such an outcome is impossible. Given the unquestioned patient and societal

benefit that would be associated with an effective cure, work in this area is clearly desirable. Why then have there been very few hypothesis-driven clinical studies aimed at viral eradication?

Regulatory hurdles. As outlined above, several groups have attempted to accelerate the clearance of HIV from an infected person. Some of these interventions have involved complex and costly interventions (e.g., gene therapy) while others have had high potential for harm to the study volunteer. The number of these studies seems to be waning. Although this decline may reflect growing pessimism over the potential for a cure, it is likely that the increasing regulatory barriers to performing clinical research may have resulted in such a harsh environment that few groups have the time and resources to perform the necessary clinical trials.[120, 121] The number of groups involved in overseeing clinical trials continues to grow; since each group has its own agenda and regulatory needs, it can take years for a clinical researcher to gain the appropriate approvals to begin a study.

The growing perception that treatment is highly effective in preventing disease should make it even harder to perform high risk/high gain studies *in vivo*. For an agent to be tried *in vivo*, the FDA and other groups will understandably want to see clear evidence that the drug is safe and well tolerated. This might preclude the use of a number HDAC inhibitors, since these drugs often have significant toxicity (most are being developed for the treatment of cancer). This may also preclude attempts at using immune activating drugs. The “cytokine storm” and its associated morbidity that was observed in a recent phase I study of TGN1412 (a potent anti-CD28 monoclonal antibody) raises a number of legitimate concerns for using pro-inflammatory drugs in otherwise healthy subjects.[122] Since eradication studies generally require patients to have fully suppressed virus, and since such patients generally have a good prognosis, the risk/benefit ratio for potential study volunteer may make it difficult to justify anything but the safest interventions.

Clinical trial design. Given the difficulty in performing these types of studies, most have abandoned the more rigorous and methodical approach of studying one unique intervention at time in a controlled manner. The more typical approach is to go for a “homerun”. For example, Pomerantz and colleagues—who pioneered much of the clinical trials work outlined here—recently advocated for a study of IL-2, low dose OKT3 (both to induce T cell activation), interferon- γ (to induce macrophages/monocytes), valproic acid (to inhibit HDAC) and intensified HAART using more recently developed antiretroviral drugs (e.g., raltegravir, maraviroc). It is unclear if these “homerun” approaches are the most informative.

Lack of acceptable biomarker/endpoint. Another major barrier is the lack of clear well-validated outcome measure. The most optimal method to test efficacy in any eradication protocol is to stop

therapy (an “analytic’ treatment interruption). Long-term interruption of therapy in patients with moderately advanced disease is unquestionably risky and no longer recommended.[123] Short-term interruptions with careful viral load monitoring may still be feasible, particularly among patients with early HIV disease, but the field is clearly moving away from treatment interruption studies. Most current and future studies will likely focus on measures of viral burden, but all have practical limitations. Many patients have too little plasma HIV RNA levels to detect a real change (even using very sensitive techniques). Proviral DNA levels are more readily detectable, but much of the cell based DNA is probably replication defective and hence of unclear significance. Co-culture techniques to quantify the level of replication competent HIV are readily interpretable, but require access to large volumes of blood and are technically challenging. Tissue based studies are also feasible, but are expensive, difficult to perform and risky.

Funding The past year has been disappointing as it pertains to HIV prevention. Despite promising preliminary data, the Merck adenovirus vector T cell vaccine not only failed to prevent HIV transmission it appeared to increase the risk of transmission, at least in those who with pre-existing immunity to the vector. There were also high profile failures reported on the role of acyclovir in preventing transmission. Based on these reports and others, the NIH has made a “mid-course correction” and will in the future focus on basic discovery. There is now a growing consensus that the biologic prevention of HIV transmission will only occur if by discovering a completely novel mechanism of protection. It is hoped that support for both basic and clinical science focused on HIV eradication will increase as a consequence of these evolving NIAID priorities.

Conclusions

After a prolonged period in which “curing” HIV was thought to a taboo topic, there is now an unquestioned growing interest in pursuing this area. This renewed interest is driven in part by the approval of two new antiretroviral drugs, one of which may be more potent than previous drugs (i.e., raltegravir) and one of which has a unique host-based mechanism of action (i.e., maraviroc).[124, 125] The recent publicity and controversy surrounding a small “proof-of-concept” clinical trial raised the possibility that the latent reservoir may be amenable to therapeutic interventions.[95] If nothing else, this study allowed the concept to be discussed more freely. Finally, the increasing recognition that disease persists during otherwise effective HAART argues that perhaps the only way for an HIV infected person to achieve normal health is through a cure. Similarly, given the many failures regarding the biologic prevention of HIV transmission, a safe and scalable cure may prove to be the only way to truly alter the course of the epidemic.

REFERENCES

1. Han Y, Wind-Rotolo M, Yang HC, Siliciano JD and Siliciano RF. Experimental approaches to the study of HIV-1 latency. *Nat Rev Microbiol* 2007;5:95-106
2. Chun TW, Nickle DC, Justement JS, et al. Persistence of HIV in gut-associated lymphoid tissue despite long-term antiretroviral therapy. *J Infect Dis* 2008;197:714-20
3. Hare CB, Pappalardo BL, Busch MP, et al. Seroreversion in subjects receiving antiretroviral therapy during acute/early HIV infection. *Clin Infect Dis* 2006;42:700-8
4. Koning FA, van der Vorst TJ and Schuitemaker H. Low levels of human immunodeficiency virus type 1 DNA in high-risk seronegative men. *J Virol* 2005;79:6551-3
5. Zhu T, Corey L, Hwangbo Y, et al. Persistence of extraordinarily low levels of genetically homogeneous human immunodeficiency virus type 1 in exposed seronegative individuals. *J Virol* 2003;77:6108-16
6. Shacklett BL. Understanding the "lucky few": the conundrum of HIV-exposed, seronegative individuals. *Curr HIV/AIDS Rep* 2006;3:26-31
7. Hatano H, Delwart EL, Norris PJ, et al. Evidence for Persistent Low-level Viremia in Individuals Who Control HIV in the Absence of Antiretroviral Therapy. *J Virol* 2008
8. Bailey JR, Williams TM, Siliciano RF and Blankson JN. Maintenance of viral suppression in HIV-1-infected HLA-B*57+ elite suppressors despite CTL escape mutations. *J Exp Med* 2006;203:1357-69
9. Palmer S, Weigand A, Miura T, et al. Low-Level Viremia in HIV-1-Infected Patients Classified as "Elite Controllers" Compared to Patients on Suppressive Antiretroviral Therapy. . In: Program and Abstracts of the 3rd International Workshop on HIV Persistence during Therapy; December 4-7, 2007, St. Maarten, West Indies (abstract).
10. Blankson JN, Bailey JR, Thayil S, et al. Isolation and characterization of replication-competent human immunodeficiency virus type 1 from a subset of elite suppressors. *J Virol* 2007;81:2508-18
11. Deacon NJ, Tsykin A, Solomon A, et al. Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients. *Science* 1995;270:988-91
12. Yamada T, Iwamoto A. Comparison of proviral accessory genes between long-term nonprogressors and progressors of human immunodeficiency virus type 1 infection. *Arch Virol* 2000;145:1021-7
13. Lum JJ, Cohen OJ, Nie Z, et al. Vpr R77Q is associated with long-term nonprogressive HIV infection and impaired induction of apoptosis. *J Clin Invest* 2003;111:1547-54
14. Alexander L, Weiskopf E, Greenough TC, et al. Unusual polymorphisms in human immunodeficiency virus type 1 associated with nonprogressive infection. *J Virol* 2000;74:4361-76.
15. Kirchhoff F, Greenough TC, Brettler DB, Sullivan JL and Desrosiers RC. Brief report: absence of intact nef sequences in a long-term survivor with nonprogressive HIV-1 infection. *N Engl J Med* 1995;332:228-32
16. Wang B, Ge YC, Palasanthiran P, et al. Gene defects clustered at the C-terminus of the vpr gene of HIV-1 in long-term nonprogressing mother and child pair: in vivo evolution of vpr quasispecies in blood and plasma. *Virology* 1996;223:224-32
17. Hassaine G, Agostini I, Candotti D, et al. Characterization of human immunodeficiency virus type 1 vif gene in long-term asymptomatic individuals. *Virology* 2000;276:169-80
18. Deeks SG, Walker BD. Human immunodeficiency virus controllers: mechanisms of durable virus control in the absence of antiretroviral therapy. *Immunity* 2007;27:406-16
19. Emu B, Sinclair E, Hatano H, et al. HLA class I-restricted T-cell responses may contribute to the control of human immunodeficiency virus infection, but such responses are not always necessary for long-term virus control. *J Virol* 2008;82:5398-407
20. Pereyra F, Addo MM, Kaufmann DE, et al. Genetic and Immunologic Heterogeneity among Persons Who Control HIV Infection in the Absence of Therapy. *J Infect Dis* 2008;197:563-571
21. Migueles SA, Laborico AC, Shupert WL, et al. HIV-specific CD8+ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors. *Nat Immunol* 2002;3:1061-8
22. Betts MR, Nason MC, West SM, et al. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. *Blood* 2006;107:4781-9
23. Zimmerli SC, Harari A, Cellerai C, Vallelian F, Bart PA and Pantaleo G. HIV-1-specific IFN-gamma/IL-2-secreting CD8 T cells support CD4-independent proliferation of HIV-1-specific CD8 T cells. *Proc Natl Acad Sci U S A* 2005;102:7239-44
24. Saez-Cirion A, Lacabaratz C, Lambotte O, et al. HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype. *Proc Natl Acad Sci U S A* 2007;104:6776-81
25. Martin MP, Qi Y, Gao X, et al. Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. *Nat Genet* 2007;39:733-40

26. Ahuja SK, Kulkarni H, Catano G, et al. CCL3L1-CCR5 genotype influences durability of immune recovery during antiretroviral therapy of HIV-1-infected individuals. *Nat Med* 2008;14:413-20
27. Fellay J, Shianna KV, Ge D, et al. A whole-genome association study of major determinants for host control of HIV-1. *Science* 2007;317:944-7
28. Kaul R, Dong T, Plummer FA, et al. CD8(+) lymphocytes respond to different HIV epitopes in seronegative and infected subjects. *J Clin Invest* 2001;107:1303-10.
29. Uberla K. HIV vaccine development in the aftermath of the STEP study: re-focus on occult HIV infection? *PLoS Pathog* 2008;4:e1000114
30. Hunt PW, Brenchley J, Sinclair E, et al. Relationship between T Cell Activation and CD4(+) T Cell Count in HIV-Seropositive Individuals with Undetectable Plasma HIV RNA Levels in the Absence of Therapy. *J Infect Dis* 2008;197:126-133
31. Andrade A, Bailey JR, Xu J, et al. CD4+ T cell depletion in an untreated HIV type 1-infected human leukocyte antigen-B*5801-positive patient with an undetectable viral load. *Clin Infect Dis* 2008;46:e78-82
32. Hsue PY, Hunt PW, Waters DD, et al. Role of Antiretroviral Therapy, Viral Replication, and HIV Infection in Atherosclerosis. In: Program and Abstracts of the 15th Conference on Retroviruses and Opportunistic Infections; February 3-6, 2008; Boston, MA (abstract)
33. Perelson AS, Essunger P, Cao Y, et al. Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature* 1997;387:188-91.
34. Wong JK, Hezareh M, Gunthard HF, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science* 1997;278:1291-5.
35. Chun TW, Carruth L, Finzi D, et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* 1997;387:183-8.
36. Finzi D, Hermankova M, Pierson T, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 1997;278:1295-300.
37. Siliciano JD, Kajdas J, Finzi D, et al. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. *Nat Med* 2003;9:727-8
38. Palmer S, Maldarelli F, Wiegand A, et al. Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. *Proc Natl Acad Sci U S A* 2008;105:3879-84
39. Anton PA, Mitsuyasu RT, Deeks SG, et al. Multiple measures of HIV burden in blood and tissue are correlated with each other but not with clinical parameters in aviremic subjects. *AIDS* 2003;17:53-63
40. Poles MA, Boscardin WJ, Elliott J, et al. Lack of decay of HIV-1 in gut-associated lymphoid tissue reservoirs in maximally suppressed individuals. *J Acquir Immune Defic Syndr* 2006;43:65-8
41. Anton PA, Elliott J, Poles MA, et al. Enhanced levels of functional HIV-1 co-receptors on human mucosal T cells demonstrated using intestinal biopsy tissue. *AIDS* 2000;14:1761-5
42. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 2006;12:1365-71
43. Mehandru S, Poles MA, Tenner-Racz K, et al. Lack of Mucosal Immune Reconstitution during Prolonged Treatment of Acute and Early HIV-1 Infection. *PLoS Med* 2006;3:e484
44. Strain MC, Little SJ, Daar ES, et al. Effect of Treatment, during Primary Infection, on Establishment and Clearance of Cellular Reservoirs of HIV-1. *J Infect Dis* 2005;191:1410-8
45. Guadalupe M, Sankaran S, George MD, et al. Viral Suppression and Immune Restoration in the Gastrointestinal Mucosa of Human Immunodeficiency Virus Type 1-Infected Patients Initiating Therapy during Primary or Chronic Infection. *J Virol* 2006;80:8236-47
46. Chun TW, Justement JS, Moir S, et al. Decay of the HIV Reservoir in Patients Receiving Antiretroviral Therapy for Extended Periods: Implications for Eradication of Virus. *J Infect Dis* 2007;195:1762-4
47. Blankson JN, Finzi D, Pierson TC, et al. Biphasic decay of latently infected CD4+ T cells in acute human immunodeficiency virus type 1 infection. *J Infect Dis* 2000;182:1636-42
48. Hermankova M, Ray SC, Ruff C, et al. HIV-1 drug resistance profiles in children and adults with viral load of <50 copies/ml receiving combination therapy. *JAMA* 2001;286:196-207.
49. Kieffer TL, Finucane MM, Nettles RE, et al. Genotypic analysis of HIV-1 drug resistance at the limit of detection: virus production without evolution in treated adults with undetectable HIV loads. *J Infect Dis* 2004;189:1452-65
50. Bailey JR, Sedaghat AR, Kieffer T, et al. Residual Human Immunodeficiency Virus Type 1 Viremia in Some Patients on Antiretroviral Therapy Is Dominated by a Small Number of Invariant Clones Rarely Found in Circulating CD4+ T Cells. *J Virol* 2006;80:6441-57
51. Tobin NH, Learn GH, Holte SE, et al. Evidence that low-level viremias during effective highly active antiretroviral therapy result from two processes: expression of archival virus and replication of virus. *J Virol* 2005;79:9625-34

52. Zhang L, Ramratnam B, Tenner-Racz K, et al. Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy. *N Engl J Med* 1999;340:1605-13.
53. Martinez-Picado J, DePasquale MP, Kartsonis N, et al. Antiretroviral resistance during successful therapy of HIV type 1 infection. *Proc Natl Acad Sci U S A* 2000;97:10948-53.
54. Zhu T, Muthui D, Holte S, et al. Evidence for human immunodeficiency virus type 1 replication in vivo in CD14(+) monocytes and its potential role as a source of virus in patients on highly active antiretroviral therapy. *J Virol* 2002;76:707-16.
55. Maldarelli F, Palmer S, King MS, et al. ART suppresses plasma HIV-1 RNA to a stable set point predicted by pretherapy viremia. *PLoS Pathog* 2007;3:e46
56. Hunt PW, Martin JN, Sinclair E, et al. T Cell Activation Is Associated with Lower CD4+ T Cell Gains in Human Immunodeficiency Virus-Infected Patients with Sustained Viral Suppression during Antiretroviral Therapy. *J Infect Dis* 2003;187:1534-43
57. Kolber MA, Saenz MO, Tanner TJ, Arheart KL, Pahwa S and Liu H. Intensification of a suppressive HAART regimen increases CD4 counts and decreases CD8+ T-cell activation. *Clin Immunol* 2008;126:315-321
58. Sharkey ME, Teo I, Greenough T, et al. Persistence of episomal HIV-1 infection intermediates in patients on highly active anti-retroviral therapy. *Nat Med* 2000;6:76-81
59. Schacker T, Stevenson M, Brenchley JM, Douek DC and Haase AT. Evidence for Persistent Viral Replication in Lymph Node and GALT of ARV-treated Persons. In: Program and Abstracts of the 15th Conference on Retroviruses and Opportunistic Infections; February 3-6, 2008; Boston, MA (abstract 120).
60. Yukl S, Wong JK. Blood and guts and HIV: preferential HIV persistence in GI mucosa. *J Infect Dis* 2008;197:640-2
61. Chun TW, Nickle DC, Justement JS, et al. HIV-infected individuals receiving effective antiviral therapy for extended periods of time continually replenish their viral reservoir. *J Clin Invest* 2005;115:3250-3255
62. Stevenson M. HIV-1 pathogenesis. *Nat Med* 2003;9:853-60
63. Stebbing J, Gazzard B and Douek DC. Where does HIV live? *N Engl J Med* 2004;350:1872-80
64. Swingler S, Mann A, Jacque J, et al. HIV-1 Nef mediates lymphocyte chemotaxis and activation by infected macrophages. *Nat Med* 1999;5:997-103.
65. Jayakumar P, Berger I, Autschbach F, et al. Tissue-resident macrophages are productively infected ex vivo by primary X4 isolates of human immunodeficiency virus type 1. *J Virol* 2005;79:5220-6
66. Lambotte O, Taoufik Y, de Goer MG, Wallon C, Goujard C and Delfraissy JF. Detection of infectious HIV in circulating monocytes from patients on prolonged highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2000;23:114-9
67. Igarashi T, Brown CR, Endo Y, et al. Macrophage are the principal reservoir and sustain high virus loads in rhesus macaques after the depletion of CD4+ T cells by a highly pathogenic simian immunodeficiency virus/HIV type 1 chimera (SHIV): Implications for HIV-1 infections of humans. *Proc Natl Acad Sci U S A* 2001;98:658-63
68. Kimmel PL, Barisoni L and Kopp JB. Pathogenesis and treatment of HIV-associated renal diseases: lessons from clinical and animal studies, molecular pathologic correlations, and genetic investigations. *Ann Intern Med* 2003;139:214-26
69. Bruggeman LA, Ross MD, Tanji N, et al. Renal epithelium is a previously unrecognized site of HIV-1 infection. *J Am Soc Nephrol* 2000;11:2079-87
70. Chen HY, Di Mascio M, Perelson AS, Ho DD and Zhang L. Determination of virus burst size in vivo using a single-cycle SIV in rhesus macaques. *Proc Natl Acad Sci U S A* 2007;104:19079-84
71. Mitchell RS, Beitzel BF, Schroder AR, et al. Retroviral DNA integration: ASLV, HIV, and MLV show distinct target site preferences. *PLoS Biol* 2004;2:E234
72. Schroder AR, Shinn P, Chen H, Berry C, Ecker JR and Bushman F. HIV-1 integration in the human genome favors active genes and local hotspots. *Cell* 2002;110:521-9
73. Brooks DG, Arlen PA, Gao L, Kitchen CM and Zack JA. Identification of T cell-signaling pathways that stimulate latent HIV in primary cells. *Proc Natl Acad Sci U S A* 2003;100:12955-60
74. Chun TW, Engel D, Mizell SB, Ehler LA and Fauci AS. Induction of HIV-1 replication in latently infected CD4+ T cells using a combination of cytokines. *J Exp Med* 1998;188:83-91.
75. Scripture-Adams DD, Brooks DG, Korin YD and Zack JA. Interleukin-7 induces expression of latent human immunodeficiency virus type 1 with minimal effects on T-cell phenotype. *J Virol* 2002;76:13077-82
76. Geeraert L, Kraus G and Pomerantz RJ. Hide-and-seek: the challenge of viral persistence in HIV-1 infection. *Annu Rev Med* 2008;59:487-501
77. Chun TW, Engel D, Mizell SB, et al. Effect of interleukin-2 on the pool of latently infected, resting CD4+ T cells in HIV-1-infected patients receiving highly active anti- retroviral therapy. *Nat Med* 1999;5:651-5.
78. Chun TW, Davey RT, Jr., Engel D, Lane HC and Fauci AS. Re-emergence of HIV after stopping therapy. *Nature* 1999;401:874-5.

79. Davey RT, Jr., Bhat N, Yoder C, et al. HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. *Proc Natl Acad Sci U S A* 1999;96:15109-14.
80. Stellbrink HJ, van Lunzen J, Westby M, et al. Effects of interleukin-2 plus highly active antiretroviral therapy on HIV-1 replication and proviral DNA (COSMIC trial). *Aids* 2002;16:1479-87
81. Lafeuillade A, Poggi C, Chadapaud S, et al. Pilot study of a combination of highly active antiretroviral therapy and cytokines to induce HIV-1 remission. *J Acquir Immune Defic Syndr* 2001;26:44-55
82. Prins JM, Jurriaans S, van Praag RM, et al. Immuno-activation with anti-CD3 and recombinant human IL-2 in HIV-1-infected patients on potent antiretroviral therapy. *AIDS* 1999;13:2405-10
83. Kulkosky J, Nunnari G, Otero M, et al. Intensification and stimulation therapy for human immunodeficiency virus type 1 reservoirs in infected persons receiving virally suppressive highly active antiretroviral therapy. *J Infect Dis* 2002;186:1403-11
84. van Praag RM, Prins JM, Roos MT, et al. OKT3 and IL-2 treatment for purging of the latent HIV-1 reservoir in vivo results in selective long-lasting CD4+ T cell depletion. *J Clin Immunol* 2001;21:218-26.
85. Brooks DG, Hamer DH, Arlen PA, et al. Molecular characterization, reactivation, and depletion of latent HIV. *Immunity* 2003;19:413-23
86. Wang FX, Xu Y, Sullivan J, et al. IL-7 is a potent and proviral strain-specific inducer of latent HIV-1 cellular reservoirs of infected individuals on virally suppressive HAART. *J Clin Invest* 2005;115:128-37
87. Chaudhary VK, Mizukami T, Fuerst TR, et al. Selective killing of HIV-infected cells by recombinant human CD4-Pseudomonas exotoxin hybrid protein. *Nature* 1988;335:369-72
88. Saavedra-Lozano J, McCoig C, Xu J, et al. An anti-CD45RO immunotoxin kills latently infected human immunodeficiency virus (HIV) CD4 T cells in the blood of HIV-positive persons. *J Infect Dis* 2002;185:306-14
89. McCoig C, Van Dyke G, Chou CS, Picker LJ, Ramilo O and Vitetta ES. An anti-CD45RO immunotoxin eliminates T cells latently infected with HIV-1 in vitro. *Proc Natl Acad Sci U S A* 1999;96:11482-5
90. Wender PA, Kee JM and Warrington JM. Practical synthesis of prostratin, DPP, and their analogs, adjuvant leads against latent HIV. *Science* 2008;320:649-52
91. Kulkosky J, Culnan DM, Roman J, et al. Prostratin: activation of latent HIV-1 expression suggests a potential inductive adjuvant therapy for HAART. *Blood* 2001;98:3006-15
92. Korin YD, Brooks DG, Brown S, Korotzer A and Zack JA. Effects of prostratin on T-cell activation and human immunodeficiency virus latency. *J Virol* 2002;76:8118-23
93. Biancotto A, Grivel JC, Gondois-Rey F, et al. Dual role of prostratin in inhibition of infection and reactivation of human immunodeficiency virus from latency in primary blood lymphocytes and lymphoid tissue. *J Virol* 2004;78:10507-15
94. Ylisastigui L, Archin NM, Lehrman G, Bosch RJ and Margolis DM. Coaxing HIV-1 from resting CD4 T cells: histone deacetylase inhibition allows latent viral expression. *AIDS* 2004;18:1101-8
95. Lehrman G, Hogue IB, Palmer S, et al. Depletion of latent HIV-1 infection in vivo: a proof-of-concept study. *Lancet* 2005;366:549-55
96. Siliciano JD, Lai J, Callender M, et al. Stability of the latent reservoir for HIV-1 in patients receiving valproic acid. *J Infect Dis* 2007;195:833-6
97. Archin NM, Eron JJ, Palmer S, et al. Valproic acid without intensified antiviral therapy has limited impact on persistent HIV infection of resting CD4+ T cells. *Aids* 2008;22:1131-5
98. Sagot-Lerolle N, Lamine A, Chaix ML, et al. Prolonged valproic acid treatment does not reduce the size of latent HIV reservoir. *Aids* 2008;22:1125-9
99. Steel A, Clark S, Teo I, et al. No change to HIV-1 latency with valproate therapy. *Aids* 2006;20:1681-2
100. Schooley RT, Mellors JW. No cure yet for HIV-1, but therapeutic research presses on. *J Infect Dis* 2007;195:770-2
101. Choudhary SK, Archin NM and Margolis DM. Hexamethylbisacetamide and disruption of human immunodeficiency virus type 1 latency in CD4(+) T cells. *J Infect Dis* 2008;197:1162-70
102. Autran B, Murphy RL, Costagliola D, et al. Greater viral rebound and reduced time to resume antiretroviral therapy after therapeutic immunization with the ALVAC-HIV vaccine (vCP1452). *AIDS* 2008;22:1313-22
103. Goujard C, Marcellin F, Hendel-Chavez H, et al. Interruption of antiretroviral therapy initiated during primary HIV-1 infection: impact of a therapeutic vaccination strategy combined with interleukin (IL)-2 compared with IL-2 alone in the ANRS 095 Randomized Study. *AIDS Res Hum Retroviruses* 2007;23:1105-13
104. Levy Y, Durier C, Lascaux AS, et al. Sustained control of viremia following therapeutic immunization in chronically HIV-1-infected individuals. *Aids* 2006;20:405-13
105. Valor L, Navarro J, Carbone J, et al. Immunization with an HIV-1 immunogen induces CD4+ and CD8+ HIV-1-specific polyfunctional responses in patients with chronic HIV-1 infection receiving antiretroviral therapy. *Vaccine* 2008;26:2738-45

106. Vieillard V, Le Grand R, Dausset J and Debre P. A vaccine strategy against AIDS: an HIV gp41 peptide immunization prevents NKp44L expression and CD4+ T cell depletion in SHIV-infected macaques. *Proc Natl Acad Sci U S A* 2008;105:2100-4
107. Autran B, Carcelain G, Combadiere B and Debre P. Therapeutic vaccines for chronic infections. *Science* 2004;305:205-8
108. Hel Z, Venzon D, Poudyal M, et al. Viremia control following antiretroviral treatment and therapeutic immunization during primary SIV251 infection of macaques. *Nat Med* 2000;6:1140-6
109. Lisziewicz J, Trocio J, Xu J, et al. Control of viral rebound through therapeutic immunization with DermaVir. *Aids* 2005;19:35-43
110. Markowitz M, Jin X, Hurley A, et al. Discontinuation of Antiretroviral Therapy Commenced Early during the Course of Human Immunodeficiency Virus Type 1 Infection, with or without Adjunctive Vaccination. *J Infect Dis* 2002;186:634-643
111. Kilby JM, Bucy RP, Mildvan D, et al. A randomized, partially blinded phase 2 trial of antiretroviral therapy, HIV-specific immunizations, and interleukin-2 cycles to promote efficient control of viral replication (ACTG A5024). *J Infect Dis* 2006;194:1672-6
112. Rossi JJ, June CH and Kohn DB. Genetic therapies against HIV. *Nat Biotechnol* 2007;25:1444-54
113. Deeks SG, Wagner B, Anton PA, et al. A Phase II Randomized Study of HIV-Specific T-Cell Gene Therapy in Subjects with Undetectable Plasma Viremia on Combination Antiretroviral Therapy. *Mol Ther* 2002;5:788-97
114. Levine BL, Humeau LM, Boyer J, et al. Gene transfer in humans using a conditionally replicating lentiviral vector. *Proc Natl Acad Sci U S A* 2006;103:17372-7
115. An DS, Donahue RE, Kamata M, et al. Stable reduction of CCR5 by RNAi through hematopoietic stem cell transplant in non-human primates. *Proc Natl Acad Sci U S A* 2007;104:13110-5
116. Hutter G, Nowak D, Mossner M, et al. Treatment of HIV-1 Infection by Allogeneic CCR5-D32/D32 Stem Cell Transplantation: A Promising Approach. In: Program and Abstracts of the 15th Conference on Retroviruses and Opportunistic Infections; February 3-6, 2008; Boston, MA (abstract 719).
117. Cohen J. HIV/AIDS. Report of novel treatment aimed at latent HIV raises the 'c word'. *Science* 2005;309:999-1000
118. Routy JP. Valproic acid: a potential role in treating latent HIV infection. *Lancet* 2005;366:523-4
119. Laurence J. Seeking a cure for AIDS. *AIDS Read* 2008;18:228, 234
120. Nathan DG. Careers in translational clinical research-historical perspectives, future challenges. *JAMA* 2002;287:2424-7
121. Fontanarosa PB, DeAngelis CD. Translational medical research. *JAMA* 2003;289:2133
122. Suntharalingam G, Perry MR, Ward S, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med* 2006;355:1018-28
123. El-Sadr WM, Lundgren JD, Neaton JD, et al. CD4+ count-guided interruption of antiretroviral treatment. *N Engl J Med* 2006;355:2283-96
124. Steigbigel RT, Cooper DA, Kumar PN, et al. Raltegravir with optimized background therapy for resistant HIV-1 infection. *N Engl J Med* 2008;359:339-54
125. Gulick RM, Lalezari J, Goodrich J, et al. Maraviroc for previously treated patients with R5 HIV-1 infection. *N Engl J Med* 2008;359:1429-41