Eradication Therapies for HIV Infection: Time to Begin Again

David M. Margolis

Abstract

Despite the success of antiretroviral therapy (ART) in decreasing mortality for HIV-1-infected patients, ART has not cured the disease. A persistent viral reservoir in the T cells of HIV patients receiving potent ART is a significant barrier preventing eradication of HIV infection. We will briefly review what is known about the mechanisms that establish and maintain persistent HIV infection despite ART, to create a framework in which to consider approaches to the clearance or eradication of infection (“cure”), or to allow clinical stability in the absence of ART (“functional cure”). With regard to eradication therapies, it could be said that as a field our position is analogous to that of ART early in the HIV pandemic. As then we must now simultaneously develop and optimize platforms and paradigms for the discovery and testing of eradication therapies, and begin to advance candidate therapies toward human testing.

Introduction

SUCCESSFUL ANTIRETROVIRAL THERAPY (ART) often results in clinical stability and plasma levels of HIV-1 RNA below the limits of detection in routine assays. Despite the success of ART, more people contract human immunodeficiency virus (HIV) infection daily than initiate ART. The difficulties of lifelong ART—particularly in the developing world—have recently stimulated a reevaluation of approaches to the eradication of HIV infection.

The inability of ART to eradicate HIV was first suggested by the demonstration of latent infection of resting CD4⁺ T cells,¹ and then demonstrated by the recovery of rare, integrated, replication-competent HIV from the resting CD4⁺ memory T cells of patients receiving potent ART.^{2–4} To date, this reservoir remains the most widely studied and best understood cause of viral persistence. Evidence suggests that the resting T cell reservoir is established early after infection and is extremely stable.^{5,6} Current ART does not eradicate HIV infection, as these latently infected cells remain persistently infected and unrecognized by the immune system, with minimal expression of HIV genes or proteins.⁷ It appears that the persistence of quiescent HIV infection, primarily within central memory T cells, is currently the major obstacle to eradication of HIV infection. This is a scientific hurdle that has yet to be overcome, as we completely lack the ability to therapeutically target proviral HIV genomes that express little or no HIV RNA or protein.

Furthermore, in a substantial proportion of treated patients very low levels of viral RNA can be detected by research assays.^{8–11} This low-level viremia does not appear to lead to drug resistance or failure of therapy, and appears to represent expression of viral particles without effective rounds of new replication,^{12,13} but is nevertheless a potential additional obstacle to viral eradication.

Finally, other reservoirs of persistent infection despite ART have been reported that could reignite HIV infection. These reservoirs have not been defined as well in patients on successful, suppressive ART. Naive T cells have been suggested to harbor persistent replication-competent HIV, but the frequency of these cells appears low.¹⁴ Macrophages have long been identified as another cell type capable of supporting persistent infection despite ART. Macrophages and monocytes are long-lived cells that may serve as potential sites of persistent viral expression, surviving with ongoing low levels of virus release in patients on ART.^{15,16} A minor subset of CD16⁺ monocytes has been shown to be more permissive to HIV-1 replication compared with the major CD14^{high}CD16[−] compartment, and HIV-1 was identified within the CD16⁺ monocytes of patients after full suppression on HAART.¹⁷ However, it has yet to be clearly documented that these cells carry quiescent provirus *in vivo* for many months, as can resting CD4⁺ T cells. This is an important distinction, as viral persistence in a cell that *is* expressing viral proteins or particles may be addressed by improvements in ART or the antiviral immune response. Recent reports have demonstrated the

Department of Medicine, Microbiology & Immunology, Epidemiology, Institute for Global Health and Infectious Diseases, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina.

recovery of replication-competent HIV immediately post-mortem from follicular dendritic cells in patients on ART¹⁸ and suggested that hematopoietic stem cells are a source of persistent HIV.¹⁹ However, these observations are controversial.^{20,21}

The clearance of a retroviral infection in patients on ART is therefore a herculean task. While much is known about HIV persistence despite ART, many puzzles remain. Of even greater significance, while the clinical development path for antiviral therapies is well trodden, and paradigms for studying preventive microbicides or vaccines are being developed, a validated framework for inventing and testing eradication therapies does not exist.

Mechanisms of Persistent HIV Infection

Over the past two decades, a wealth of knowledge has been uncovered to explain the mechanisms that drive the HIV infection and viral production, and that rarely allow latent proviral infection to develop and persist. For the purposes of this discussion, we define quiescent but replication-competent provirus as *proviral latency*. In our view, such latent infection is clearly defined and quantified in resting CD4⁺ T lymphocytes, and while it may persist in cells that are not CD4⁺ T cells, this has yet to be carefully and longitudinally quantified in effectively treated patients. Measures of viral DNA in various cell populations likely dramatically overestimate the frequency of viral genomes that are capable of replicating and reigniting infection. On the other hand, quantitation of replication-competent virus is laborious and may underestimate the true frequency of such genomes *in vivo*. As with so many things, the truth likely lies in between.

Nevertheless, it cannot be overemphasized that the persistence of HIV infection despite ART is not a unidimensional problem. While proviral latency is clearly a central problem, the origin of *persistent production* of HIV RNA that can be detected in plasma of HIV-infected patients on durably successful ART by research assays is yet to be fully explained.²² This expression of viral particles appears to occur without evidence of full rounds of replication,^{12,13} as this would inevitably select for ART resistance. It is incompletely understood how expression may persist on ART without the development of drug resistance. One explanation is that persistent production of HIV RNA originates exclusively in cells that were infected prior to ART initiation. Another explanation is that complete replication events occur below the frequency required for the emergence and subsequent selection of drug-resistant genomes. The first explanation begs the question of where all the cells capable of producing a steady but low level of plasma viremia for months or years are hiding. The second explanation seems increasingly unlikely in the face of an expanding number of patients who remain suppressed on therapy for years without the noticeable appearance of patients with spontaneous, unexplained drug failure.

Finally, low levels of cell-free or cell-associated HIV RNA have been measured or observed in tissue, such as the GALT (gut-associated lymphoid tissue) or central nervous system.^{23,24} It is not clear whether these observations represent (1) anatomic regions or cellular compartments that are sanctuaries in which complete cycles of ongoing replication are incompletely blocked by ART, or (2) transient bursts of viral

production originating from resident but previously latently infected cells, or from latently infected cells or virions in transit through this compartment that will fail to result in ongoing replication due to the presence of ART.

Mechanisms of Proviral Latency

The activity of the viral long terminal repeat (LTR) promoter of the HIV-1 is the stage on which the drama of the host-virus interaction is played out, leading to latency or production, as HIV co-opts numerous cellular factors to control the rate of viral transcript production.²⁵ In addition to cellular factors that bind LTR DNA sequences, acting as classical cis regulators, the viral transactivator Tat binds to TAR, the viral leader messenger RNA (mRNA) sequence that serves as a unique target in the regulation of LTR transcription. In most settings, HIV rapidly appropriates the resources of the activated CD4⁺ T cell to transform it into a factory for virus production.

However, should factors that assist in HIV transcription, the production of the viral Tat activator, and the subsequent action of Tat be even transiently deficient, this presents an opportunity for factors that antagonize HIV expression to exert their effect, dampening HIV expression. Over time, these factors may establish restrictions on HIV expression that are increasingly potent and durable. Here we highlight the basic mechanisms for the establishment and maintenance of viral reservoirs within resting CD4⁺ T cells, mechanisms that may be targets for therapeutics to disrupt latency and eliminate persistent infection.

It is the down-regulation of expression of the HIV-1 genome that plays a pivotal role in the establishment of the rare latent stage of the life cycle of this pathogenic retrovirus. Latency may be established (1) by direct albeit inefficient, infection of resting memory cells, (2) by the infection of T cells just prior to their natural reversion to a quiescent state, as with memory T cells, or (3) in the case of the naive T cell population, infection of cells that are undergoing differentiation during thymopoiesis. Given the potency of the viral transactivator Tat, and the responsiveness of the HIV promoter to many cellular activating signals, counterregulatory mechanisms that repress transcription appear to be required to allow HIV to establish or maintain a persistent, nonproductive infection.

Low nuclear levels of the coactivating factors nuclear factor (NF)- κ B and nuclear factor of activated T cells (NFAT) are a feature of resting CD4⁺ cells, and may set the stage for the establishment of latency.²³ Naturally low levels of the P-TEFb component CycT1 and sequestration of the P-TEFb complex by the HEXIM/7SK RNA complex in resting lymphocytes present an additional restriction to viral expression in this cellular milieu. Posttranscriptional mechanisms may also pose a barrier to expression of provirus in resting cells.^{26–28} Recent data have enhanced the argument that transcriptional interference mediated by nearby host gene promoters contributes to the quiescence of some HIV proviral genomes,^{29–31} a concept observed because most viral integrants reside in introns of actively transcribed genes.¹⁶ Finally, modeling studies suggest that stochastic, transient deficiencies in the availability of the viral Tat transactivator could allow the viral promoter to slip down into a kinetic well of repressed expression, where counterregulatory cellular influences enforce repression and silence the provirus.³²

Posttranslational histone modifications, previously referred to as the “histone code,” are distinct modifications occurring at particular sites on the histone tail that can direct protein complexes to interact with the histone–DNA multimer, directing gene activity.³³ These modifications may in general make chromatin more or less accessible to transcription factors, but also form biophysical marks on genes that signal the ordered recruitment of complexes of regulatory factors that up- or down-regulate gene expression. Such epigenetic histone markings appear to play a key contributory role in regulating HIV expression, and in particular in establishing LTR quiescence and latency. Furthermore, to add another layer of complexity, many of the host enzymes that modify histones also modify other cellular proteins, and so the regulatory network that may down-modulate HIV gene expression may be linked to other biochemical cellular events that enforce HIV latency.^{34,35}

Initial studies by Verdin demonstrated that a strictly positioned nucleosome (Nuc-1) was found at the viral RNA start site (+10 to +155), and increased accessibility of chromatin near Nuc-1 associated with transcriptional activation. Furthermore, histone deacetylase inhibitors were shown to up-regulate LTR expression.^{36,37} Later, recruitment and occupancy of histone deacetylase 1 (HDAC1) at the HIV LTR were shown to directly mediate transcriptional silencing.^{38–40} Further study of the role of HDACs in LTR regulation revealed multiple cellular DNA-binding protein complexes that could recruit HDACs to the integrated provirus.^{41–45}

Recently it has been found that the class I HDACs 1, 2, and 3 predominate at the HIV LTR in CD4⁺ T cell models, and that viral outgrowth could be induced from the resting CD4⁺ T cells of HIV-infected, aviremic, ART-treated patients by selective HDAC inhibitors (HDACis) targeting these same class I HDACs.^{46–52} Other epigenetic modifications, such as methylation of histones or of DNA itself, contribute to the regulation of proviral latency, and may be targets for therapy. CpG methylation of HIV promoter DNA has been shown to contribute to establish a durable, “locked” state that is difficult to reactivate. Histone methyltransferases such as EZH2 and SUV39H1 can regulate HIV-1 transcription by inducing histone H3 at lysine 9 (H3K9) methylation, and other repressive proteins can accumulate on transcriptionally inactive proviruses.^{53–56} Pearson and colleagues further corroborated those findings and showed that progressive iterative histone modifications drive a proviral promoter into latency in primary CD4⁺ T cells.⁵⁷

Further restrictions to proviral expression and the escape from latency may exist beyond the step of the production of the initial viral mRNA transcripts. Host enzymes may inhibit the activity Tat protein that is produced by any initial burst of viral expression, raising another obstacle to the escape from latency.^{58,59} HIV mRNA export may be impaired in resting T cells, posing another barrier to production of provirus.²⁸ Host miRNAs may also impede HIV mRNA expression or translation.^{26,27}

Overall, the view of the establishment and maintenance of latent proviral infection is one of a dynamic process in which latency is established by a series of or accumulation of infrequent events. However, once established, expression of the proviral promoter is then restricted on numerous levels. The first translational challenge is to develop approaches that are capable of safely and effectively overcoming the obstacles

to proviral production. This in and of itself may allow purging of the persistent proviral reservoir, or an alternative additional step may need to be taken to ensure the clearance of productively infected cells.

Source(s) of Persistent Production

Paradoxically, in the majority of patents on fully suppressive ART in whom latently infected resting CD4⁺ T cells can be routinely measured, the baffling phenomenon of persistent, low-level viremia can be observed in parallel. Initially, it was assumed that this viremia originated when latently infected resting memory cells were periodically activated, undergoing proliferation and high-level virion production. However, one study suggests that this chronic production of HIV RNA may not originate entirely from the resting CD4⁺ T cell reservoir,⁶⁰ and in some patients viral sequences found in circulating viral particles could not be found within resting CD4⁺ T cells. A follow-up study found that residual viremia was genetically distinct from proviruses in activated CD4⁺ T cells, monocytes, and unfractionated peripheral blood mononuclear cells.⁶¹ In another study phylogenetic analysis of low-level viremia in four of five patients showed that these viral species were genetically distinct from most species found in CD4⁺ cells.⁶² However, in the absence of the identification of the cellular source of residual viremia despite ART, these observations can only be suggestive rather than definitive. Alternatively, a subpopulation of CD4 cells that was not captured for analysis could be the source of residual viremia. In preliminary studies, single-genome analysis of circulating viral species in residual viremia in two patients suppressed on ART was identical to that found in resting CD4⁺ T cells, but found only when replication-competent viruses were recovered from millions of resting CD4⁺ cells.⁶³ If novel cell type(s) are the source(s) of persistent viremia, they have yet to be definitively identified.

Maintenance of the Latent Pool

A recent, alternative model of persistent infection, not exclusive of the mechanisms discussed above, implies that proviral infection is not completely stable, and that while infected resting cells leave the quiescent memory pool at a steady rate, the frequency of infection in this pool of cells is maintained by homeostatic proliferation of infected cells.⁶⁴ This is an important new concept, and is consistent with general concepts of immunological memory. Although the numbers of cells available in this first study were too limited to perform robust quantitation of replication-competent virus with memory cell populations, it was suggested that central (T_{CM}) and transitional memory (T_{TM}) CD4⁺ T cells are the major cellular reservoirs in which integrated HIV DNA persists. In this model in stable patients on ART, CD4⁺ depletion could drive interleukin (IL)-7-mediated homeostatic proliferation, allowing host-driven replication of proviral genomes without the death of these infected cells, ensuring the persistence of this reservoir. It was hypothesized that the relative burden of latent infection in these cell subpopulations may depend on the stage of disease in which ART was initiated. This model would require close matching of the rates of infected memory cell activation and of homeostatic proliferation, as in most studies the size of this pool of infected cells is stable, regardless of the stage of disease at which the patient

was treated. Clearly this issue requires further study, as therapies designed to target proviral genomes might perform differently in T_{CM} or T_{TM} cells.

Translating Mechanisms to Therapies: Creating a Development Pathway

As can readily be seen in the above discussion, a great deal is known about persistent HIV infection and proviral latency, specifically in various *in vitro* and *ex vivo* biological systems. However, it has been difficult to translate this progress into therapies that might eradicate established HIV infection. While there is unanimity in scientific opinion that there is no effective cure for HIV infection on the horizon, until recently little concerted effort has been directly devoted to this significant challenge. To create both the scientific and translational infrastructure to support a serious and sufficiently durable effort to eradicate HIV infection, an investment is needed in three critical areas: (1) a better understanding of the biological complexity of HIV and the factors that drive persistent infection; (2) improvements in the cell and animal model systems to provide a fuller representation of persistent HIV infection in the setting of ART; and (3) improved assays to ameliorate the challenges of translational studies seeking to deplete persistent infection.

Given the several mechanisms that drive proviral latency identified, the time would seem ripe for careful translational studies designed to target these mechanisms, perturb latency, and carefully measure the results of these translational approaches. HDACs are clearly a potentially useful target in this regard. Suberoylanilide hydroxamic acid (SAHA), an HDAC inhibitor with nanomolar potency selective for the crucial Class I HDACs, has been shown to induce the expression of latent HIV *ex vivo* from the resting CD4⁺ T cells of HIV-infected, ART-treated, aviremic patients at a concentration of protein-unbound drug that is achieved with clinical dosing.^{48–50} Our group has received FDA approval for a clinical experiment in humans to directly test the hypothesis that a clinically tolerable dose of SAHA can induce expression of HIV within resting CD4⁺ T cells *in vivo*, and to investigate the safety, tolerability, and spectrum of side effects of a limited exposure to SAHA in combination with ART. This proof-of-concept study may allow for optimization of advanced assays to measure effects on latent HIV infection, and provide evidence that HDACis can perturb persistent HIV infection.

However, SAHA is a mutagen *in vitro* in standard bacterial assays, and so prolonged exposure in a clinical trial is currently unacceptable. Furthermore, a direct demonstration of the desired antilatency effect of HDAC inhibitors *in vivo*—induction of HIV expression—may alone not be sufficient to result in the clearance of infected cells. Several reports have recently suggested combinatorial strategies to effectively and comprehensively purge the pool of replication-competent, integrated, persistent HIV.^{54,55,65–69} Concepts proposed include combining SAHA with the inhibition of histone and/or DNA methylation, or with various cellular signaling or activating pathways. For example, a novel protein kinase agonist, bryostatin, now under study in Alzheimer's disease, can reactivate latent HIV-1.^{68,69} Bryostatin was also synergistic when tested in combination with HDACis, and was found to downregulate the expression of the HIV-1 coreceptors CD4 and CXCR4 and prevented *de novo* HIV-1 infection in susceptible cells.

When comparing the potential of different compounds or different approaches to deplete persistent proviral infection, several caveats bear consideration:

1. *Are drugs or combinations that induce a higher level of HIV gene expression more desirable than those that induce less robust expression of latent provirus?* Clearly some threshold must be exceeded if an approach is to be sufficiently potent to be effective, but at what level will virion production lead to spreading infection despite the presence of ART?
2. *What concentrations of drug, alone or in combination, can be achieved *in vivo*?* Pharmacokinetic and pharmacodynamic information, and considerations of free and protein-bound drug *in vitro* and *in vivo*, are important.
3. *How long and at what level must these exposures be maintained to be effective?* It is difficult to model the temporal dynamics of drug activity *in vivo* in cell culture systems.
4. *What are the temporal effects of single exposures, and repeated exposure to reagents that perturb latency? If combinations of drugs are given, must they be given simultaneously?*
5. *How can we be sure that virus will be cleared once its proviral latency is interrupted?* This is a critical question that must be addressed, but perhaps can be addressed only once antilatency therapies are in place. If viral replication does not result in the clearance of infected cells, strategies to augment the immune response or the use of agents that induce the death of such productively infected cells may have to be developed and employed.

While combination approaches to perturbing latent infection may be mechanistically valid in experimental systems, given the clinical challenges of testing a single HDACi such as SAHA, combination approaches will be difficult to test in clinical trials. Given the success of current ART and the relative good health and significant life expectancy of patients with stably suppressed viremia, it is crucial that clinical approaches to eradication be safe and tolerable.

The fact that *in vitro* efficacy has not yet translated into success in the clinical setting highlights the need for the development of a larger and more complex substrate for latency studies than offered by *in vitro* models currently in use. The complexity of HIV-1 persistence and latency requires animal models for assessing and optimizing eradication therapies that activate latent virus and then evaluate potential toxicities to accelerate their use either singly or in combination in human clinical trials. Currently, the most advanced models available appear to be the SIV nonhuman primate (NHP) and humanized bone marrow-liver-thymus (BLT) mouse.^{70–77} Optimization of ART regimens may be required to ideally reflect current human therapy.

However, animal models provide several key advantages. While residual plasma viremia can be quantitated effectively in humans, measurement of residual viremia in tissues is impractical and in many tissues impossible; thus, residual viremia in lymphoid organs, central nervous system, and GALT may be evaluable only in animal models. Latency in resting CD4⁺ T cells has been well characterized in plasma and lymph nodes in HIV-infected humans. However, the level of latently infected CD4⁺ T cells in other tissues remains to be studied. In addition, the level of viral latency in monocytes in plasma and macrophages in tissues is largely unknown.

Latency in macrophages can be characterized in both the SIV macaque model and BLT mice. It will be essential in these animal models to induce activation of latent virus and then withdraw ART to examine whether and when virus reactivation occurs compared to ART-treated animals without virus activation. Obviously, such experiments cannot be performed in HIV-infected individuals who have controlled virus replication on ART. Finally, such systems permit the study of pharmacological questions.

The Road Ahead

Few scientific and medical challenges are as daunting and complex as curing HIV infection. As we renew efforts to further understand viral persistence, and bring these insights to bear toward the ultimate goal of HIV eradication, additional obstacles to clearance of HIV infection are likely to be uncovered. The progress made so far in gaining a detailed understanding of HIV biology and pathogenesis and the stunning achievements of ART should give us hope that we can overcome the recognized and the yet-to-be-discovered challenges of persistent HIV infection. It is time to begin again in earnest.

Acknowledgments

This work was supported by National Institutes of Health Grants AI082608, AI083158, MH085597, and DA030156 to D.M.M.

Author Disclosure Statement

DMM reports research funding and honoraria from Merck Research Laboratories, and a patent application for the use of HDAC inhibitors in HIV therapy.

References

- Chun T-W, Finzi D, Margolick J, Chadwick K, *et al.*: In vivo fate of HIV-1-infected T cells: Quantitative analysis of the transition to stable latency. *Nature Med* 1995;12:1284–1290.
- Chun TW, Stuyver L, Mizell SB, *et al.*: Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc Natl Acad Sci USA* 1997;94:13193–13197.
- Finzi D, Hermankova M, Pierson T, *et al.*: Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 1997;278:1295–1300.
- Wong JK, Hezareh M, Gunthard HF, *et al.*: Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science* 1997;278:1291–1295.
- 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;182:1636–1642.
- Chun TW, Engel D, Berrey MM, *et al.*: Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. *Proc Natl Acad Sci USA* 1998;95:8869–8873.
- Lassen K, Han Y, Zhou Y, *et al.*: The multifactorial nature of HIV-1 latency. *Trends Mol Med* 2004;10:525–531.
- Dornadula G, Zhang H, Van Uiter B, *et al.*: Residual HIV-1 RNA in blood plasma of patients taking suppressive highly active antiretroviral therapy. *JAMA* 1999;282:1627–1632.
- Gunthard HF, Havlir DV, Fiscus S, *et al.*: Residual human immunodeficiency virus (HIV) Type 1 RNA and DNA in lymph nodes and HIV RNA in genital secretions and in cerebrospinal fluid after suppression of viremia for 2 years. *J Infect Dis* 2001;183:1318–1327.
- 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;340:1605–1613.
- Palmer S, Wiegand AP, Maldarelli F, *et al.*: New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. *J Clin Microbiol* 2003;41:4531–4536.
- Dinosa JB, Kim SY, Wiegand AM, *et al.*: Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. *Proc Natl Acad Sci USA* 2009;106:9403–9408.
- Gandhi RT, Zheng L, Bosch RJ, *et al.*, on behalf of the AIDS Clinical Trials Group A5244 team: The effect of raltegravir intensification on low-level residual viremia in HIV-infected patients on antiretroviral therapy: A randomized controlled trial. *PLoS Med* 2010;7(8):e1000321.
- Wightman F, Solomon A, Khoury G, *et al.*: Both CD31(+) and CD31 naive CD4(+) T cells are persistent HIV type 1-infected reservoirs in individuals receiving antiretroviral therapy. *J Infect Dis* 2010;202:1738–1748.
- Collman R, Hassan NF, Walker R, *et al.*: Infection of monocyte-derived macrophages with human immunodeficiency virus type 1 (HIV-1). Monocyte-tropic and lymphocyte-tropic strains of HIV-1 show distinctive patterns of replication in a panel of cell types. *J Exp Med* 1989;170:1149–1163.
- Orenstein JM, Fox C, and Wahl SM: Macrophages as a source of HIV during opportunistic infections. *Science* 1997;276:1857–1861.
- Ellery PJ, Tippet E, Chiu YL, *et al.*: The CD16⁺ monocyte subset is more permissive to infection and preferentially harbors HIV-1 in vivo. *J Immunol* 2007;178:6581–6589.
- Keele BF, Tazi L, Gartner S, *et al.*: Characterization of the follicular dendritic cell reservoir of human immunodeficiency virus type 1. *J Virol* 2008;82:5548–5561.
- Carter C, Onafuwa-Nuga A, McNamara L, *et al.*: HIV-1 infects multipotent progenitor cells causing cell death and establishing latent cellular reservoirs. *Nat Med* 2010;16:446–445.
- Zhang J and Crumpacker CS: Hematopoietic stem and progenitor cells in HIV/AIDS and immune reconstitution. *Cell Res* 2010;20:745–747.
- Otero M, Nunnari G, Leto D, *et al.*: Peripheral blood dendritic cells are not a major reservoir for HIV type 1 in infected individuals on virally suppressive HAART. *AIDS Res Hum Retroviruses* 2003;19:1097–1103.
- 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 USA* 2008;105:3879–3884.
- Lambotte O, Chaix ML, Gasnault J, *et al.*: Persistence of replication-competent HIV in the central nervous system despite long-term effective highly active antiretroviral therapy. *AIDS* 2005;19:217–218.
- Yukl SA, Shergill AK, McQuaid K, *et al.*: Effect of raltegravir-containing intensification on HIV burden and T-cell activation in multiple gut sites of HIV-positive adults on suppressive antiretroviral therapy. *AIDS* 2010;24:2451–2460.
- Williams SA and Greene WC: Regulation of HIV-1 latency by T-cell activation. *Cytokine* 2007;39:63–74.
- Huang J, Wang F, Argyris E, *et al.*: Cellular microRNAs contribute to HIV-1 latency in resting primary CD4⁺ T lymphocytes. *Nat Med* 2007;13:1241–1247.

27. Klase Z, Kale P, Winograd R, *et al.*: HIV-1 TAR element is processed by Dicer to yield a viral micro-RNA involved in chromatin remodeling of the viral LTR. *BMC Mol Biol* 2007; 8:63.
28. Lassen KG, Ramyar KX, Bailey JR, *et al.*: Nuclear retention of multiply spliced HIV-1 RNA in resting CD4+ T cells. *PLoS Pathog* 2006;2:e68.
29. Han Y, Lassen K, Monie D, *et al.*: Resting CD4+ T cells from human immunodeficiency virus type 1 (HIV-1)-infected individuals carry integrated HIV-1 genomes within actively transcribed host genes. *J Virol* 2004;78:6122–6133.
30. Han Y, Lin YB, An W, *et al.*: Orientation-dependent regulation of integrated HIV-1 expression by host gene transcriptional readthrough. *Cell Host Microbe* 2008;4:134–146.
31. Lenasi T, Contreras X, and Peterlin BM: Transcriptional interference antagonizes proviral gene expression to promote HIV latency. *Cell Host Microbe* 2008;4:123–133.
32. Weinberger LS, Burnett JC, Toettcher JE, *et al.*: Stochastic gene expression in a lentiviral positive-feedback loop: HIV-1 Tat fluctuations drive phenotypic diversity. *Cell* 2005;122: 169–182.
33. Jenuwein T and Allis CD: Translating the histone code. *Science* 2001;293:1074–1080.
34. Smith KT and Workman JL: Introducing the acetylome. *Nat Biotechnol* 2009;27:917–919.
35. Smith E and Shilatifard A: The chromatin signaling pathway: Diverse mechanisms of recruitment of histone-modifying enzymes and varied biological outcomes. *Mol Cell* 2010;4: 689–701.
36. Van Lint C, Emiliani S, Ott M, and Verdin E: Transcriptional activation and chromatin remodeling of the HIV-1 promoter in response to histone acetylation. *EMBO J* 1996;15:1112–1120.
37. Verdin E, Paras P Jr, and Van Lint C: Chromatin disruption in the promoter of human immunodeficiency virus type 1 during transcriptional activation. *EMBO J* 1993;12: 3249–3259.
38. Coull J, Romerio F, Sun JM, *et al.*: The human factors YY1 and LSF repress the human immunodeficiency virus type-1 long terminal repeat via recruitment of histone deacetylase 1. *J Virol* 2000;74:6790–6799.
39. Ylisastigui L, Coull JJ, Rucker V, *et al.*: Polyamides reveal a role for repression in viral latency within HIV-infected donors' resting CD4+ T cells. *J Infect Dis* 2004;190:1429–1437.
40. Ylisastigui L, Archin NM, Lehrmann G, *et al.*: Coaxing HIV-1 from resting CD4 T cells: Histone deacetylase inhibition allows latent viral expression. *AIDS* 2004;18:1101–1108.
41. Imai K and Okamoto T: Transcriptional repression of human immunodeficiency virus type 1 by AP-4. *J Biol Chem* 2006; 281(18):12495–12505.
42. Jiang G, Espeseth A, Hazuda DJ, and Margolis DM: c-Myc and Sp1 contribute to proviral latency by recruiting histone deacetylase 1 to the human immunodeficiency virus type 1 promoter. *J Virol* 2007;81:10914–10923.
43. Marban C, Suzanne S, Dequiedt F, *et al.*: Recruitment of chromatin-modifying enzymes by CTIP2 promotes HIV-1 transcriptional silencing. *EMBO J* 2007;26:412–423.
44. Tyagi M and Karn J: CBF-1 promotes transcriptional silencing during the establishment of HIV-1 latency. *EMBO J* 2007;26:4985–4995.
45. Williams SA, Chen LF, Kwon H, *et al.*: NF-kappaB p50 promotes HIV latency through HDAC recruitment and repression of transcriptional initiation. *EMBO J* 2006;25: 139–149.
46. Archin NM, Keedy KS, Espeseth A, *et al.*: Expression of latent human immunodeficiency virus type 1 is induced by novel and selective histone deacetylase inhibitors. *AIDS* 2009;23:1799–1806.
47. Keedy KS, Archin NM, Gates AT, *et al.*: A limited group of class I histone deacetylases acts to repress human immunodeficiency virus type 1 expression. *J Virol* 2009;83:4749–4756.
48. Archin NM, Espeseth A, Parker D, *et al.*: Expression of latent HIV induced by the potent HDAC inhibitor suberoylanilide hydroxamic acid. *AIDS Res Hum Retroviruses* 2009;25: 207–212.
49. Contreras X, Schweneker M, Chen CS, *et al.*: Suberoylanilide hydroxamic acid reactivates HIV from latently infected cells. *J Biol Chem* 2009;284:6782–6789.
50. Edelstein LC, Micheva-Viteva S, Phelan BD, and Dougherty JP: Activation of latent HIV type 1 gene expression by suberoylanilide hydroxamic acid (SAHA), an HDAC inhibitor approved for use to treat cutaneous T cell lymphoma. *AIDS Res Hum Retroviruses* 2009;25:883–887.
51. Matalon S, Palmer BE, Nold MF, *et al.*: The histone deacetylase inhibitor ITF2357 decreases surface CXCR4 and CCR5 expression on CD4(+) T-cells and monocytes and is superior to valproic acid for latent HIV-1 expression in vitro. *J Acquir Immune Defic Syndr* 2010;54:1–9.
52. Ying H, Zhang Y, Lin S, *et al.*: Histone deacetylase inhibitor Scriptaid reactivates latent HIV-1 promoter by inducing histone modification in in vitro latency cell lines. *Int J Mol Med* 2010;26:265–272.
53. du Chene I, Basyuk E, Lin YL, *et al.*: Suv39H1 and HP1gamma are responsible for chromatin-mediated V-1 transcriptional silencing and post-integration latency. *EMBO J* 2007;26:424–435.
54. Blazkova J, Trejbalova K, Gondo-Rey F, *et al.*: CpG methylation controls reactivation of HIV from latency. *PLoS Pathog* 2009;5:e1000554.
55. Kauder SE, Bosque A, Lindqvist A, *et al.*: Epigenetic regulation of HIV-1 latency by cytosine methylation. *PLoS Pathog* 2009;5:e1000495.
56. Friedman J, Pearson R, and Karn J: Direct evidence that histone trimethylation is required for HIV proviral silencing during the establishment of latency. *Conf. Retrovir. Oppor. Infect.*, 2009. Abstract 264.
57. Pearson R, Kim YK, Hokello J, *et al.*: Epigenetic silencing of human immunodeficiency virus (HIV) transcription by formation of restrictive chromatin structures at the viral long terminal repeat drives the progressive entry of HIV into latency. *J Virol* 2008;82:12291–12303.
58. Pagans S, Kauder SE, Kaehlcke K, *et al.*: The cellular lysine methyltransferase Set7/9-KMT7 binds HIV-1 TAR RNA, monomethylates the viral transactivator Tat, and enhances HIV transcription. *Cell Host Microbe* 2010;7:234–244.
59. Pagans S, Pedal A, North BJ, *et al.*: SIRT1 regulates HIV transcription via Tat deacetylation. *PLoS Biol* 2005;3:e41.
60. 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;80:6441–6457.
61. Brennan TP, Woods JO, Sedaghat AR, *et al.*: Analysis of human immunodeficiency virus type 1 viremia and provirus in resting CD4+ T cells reveals a novel source of residual viremia in patients on antiretroviral therapy. *J Virol* 2009; 83:8470–8481.

62. Sahu GK, Paar D, Frost SD, *et al.*: Low-level plasma HIVs in patients on prolonged suppressive highly active antiretroviral therapy are produced mostly by cells other than CD4 T-cells. *J Med Virol* 2009;81:9–15.
63. Archin NM, Anderson J, Keedy KS, *et al.*: Persistent low-level viremia despite clinically successful antiretroviral therapy appears correlated with more frequent resting CD4+ T cell infection. XVIII International AIDS Conference, 2010. Abstract WEPDA101.
64. Chomont N, El-Far M, Ancuta P, *et al.*: HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med* 2009;15:893–900.
65. Burnett JC, Lim KI, Calafi A, *et al.*: Combinatorial latency reactivation for HIV-1 subtypes and variants. *J Virol* 2010;84:5958–5974.
66. Reuse S, Calao M, Kabeya K, *et al.*: Synergistic activation of HIV-1 expression by deacetylase inhibitors and prostratin: Implications for treatment of latent infection. *PLoS One* 2009;4:e6093.
67. Savarino A, Mai A, Norelli S, *et al.*: "Shock and kill" effects of class I-selective histone deacetylase inhibitors in combination with the glutathione synthesis inhibitor buthionine sulfoximine in cell line models for HIV-1 quiescence. *Retirovirology* 2009;6:52.
68. Mehla R, Bivalkar-Mehla S, Zhang R, *et al.*: Bryostatins modulates latent HIV-1 infection via PKC and AMPK signaling but inhibits acute infection in a receptor independent manner. *PLoS One* 2010;5:e11160.
69. Perez M, de Vinuesa AG, Sanchez-Duffhues G, *et al.*: Bryostatins synergizes with histone deacetylase inhibitors to reactivate HIV-1 from latency. *Curr HIV Res* 2010;8:418–429.
70. Denton PW, Estes JD, Sun Z, *et al.*: Antiretroviral pre-exposure prophylaxis prevents vaginal transmission of HIV-1 in humanized BLT mice. *PLoS Med* 2008;5:e16.
71. Denton PW, Krisko JF, Powell DA, *et al.*: Systemic administration of antiretrovirals prior to exposure prevents rectal and intravenous HIV-1 transmission in humanized BLT mice. *PLoS One* 2010;5:e8829.
72. Choudhary SK, Rezk NL, Ince WL, *et al.*: Suppression of human immunodeficiency virus type 1 (HIV-1) viremia with reverse transcriptase and integrase inhibitors, CD4+ T-cell recovery, and viral rebound upon interruption of therapy in a new model for HIV treatment in the humanized Rag2-/- γ c-/- mouse. *J Virol* 2009;83:8254–8258.
73. Shen, A, Zink MC, Mankowski JL, *et al.*: Resting CD4+ T lymphocytes but not thymocytes provide a latent viral reservoir in a simian immunodeficiency virus–Macaca nemestrina model of human immunodeficiency virus type 1-infected patients on highly active antiretroviral therapy. *J Virol* 2003;77:4938–4949.
74. Dinoso JB, Rabi SA, Blankson JN, *et al.*: A simian immunodeficiency virus-infected macaque model to study viral reservoirs that persist during highly active antiretroviral therapy. *J Virol* 2009;83:9247–9257.
75. Zink MC, Brice AK, Kelly KM, *et al.*: Simian immunodeficiency virus-infected macaques treated with highly active antiretroviral therapy have reduced central nervous system viral replication and inflammation but persistence of viral DNA. *J Infect Dis* 2010;202:161–170.
76. Clements JE, Babas T, Mankowski JL, *et al.*: The central nervous system as a reservoir for simian immunodeficiency virus (SIV): Steady-state levels of SIV DNA in brain from acute through asymptomatic infection. *J Infect Dis* 2002;186:905–9013.
77. North TW, Higgins J, Deere JD, *et al.*: Viral sanctuaries during highly active antiretroviral therapy in a nonhuman primate model for AIDS. *J Virol* 2010;84:2913–2922.

Address correspondence to:

David M. Margolis

University of North Carolina at Chapel Hill

2060 Genetic Medicine Bldg., CB#7042

Chapel Hill, North Carolina 27599-7042

E-mail: dmargo@med.unc.edu