



HHS Public Access

Author manuscript

Annu Rev Med. Author manuscript; available in PMC 2015 April 01.

Published in final edited form as:

Annu Rev Med. 2015 ; 66: 407–421. doi:10.1146/annurev-med-092112-152941.

Understanding HIV Latency: The Road to an HIV Cure

Matthew Dahabieh, Emilie Battivelli, and Eric Verdin

Gladstone Institute of Virology and Immunology, University of California, San Francisco, California 94941

Eric Verdin: everdin@gladstone.ucsf.edu

Abstract

Treatment with antiretroviral therapy dramatically increases the survival of HIV-infected individuals. However, treatment has to be continued for life because it does not lead to the full eradication of infection. HIV persists in resting CD4⁺ T cells, and possibly other cell types, and can reemerge from these cells when therapy is interrupted. Here, we review molecular mechanisms that have been proposed to contribute to HIV latency, as well as the relative roles of *cis*- and *trans*-acting mechanisms. We also discuss existing and future therapeutic opportunities regarding HIV latency that might lead to a future cure for HIV infection.

Keywords

Transcriptional regulation; latency-reversing therapeutics; immunological mechanisms; cellular mechanisms; viral mechanisms

INTRODUCTION

HIV (type 1) is an incurable infection that causes AIDS. Although antiretroviral therapy (ART) suppresses HIV to undetectable levels, interrupting ART causes the virus to rapidly rebound to pretreatment levels. Thus, HIV-infected individuals must commit to lifelong ART to keep HIV replication suppressed. Critically however, ART does not fully prevent pathology or restore a normal lifespan in HIV-infected patients (1). These limitations are due to drug side effects (reviewed in 2) and/or incomplete viral suppression, particularly in viral anatomical sanctuaries (3). Moreover, with continuous and expanded usage of ART, HIV is becoming progressively more drug resistant. This erodes the efficacy of ART, especially in settings with limited resources or inadequate access to therapy. Thus, lifelong ART is not a sustainable solution to treating HIV/AIDS at either an individual or global scale, and curative therapies are urgently needed.

To develop curative treatment for HIV, we must understand the site of HIV persistence under ART. An important source of viral rebound is a reservoir of long-lived, quiescent,

Copyright © 2015 by Annual Reviews. All rights reserved

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

memory CD4⁺ T cells that harbor integrated latent HIV proviruses. There, the HIV provirus lies transcriptionally silent and immunologically inert. Under circumstances that are not entirely clear, but that may include reactivation of the T cell by its cognate antigen, the T cell adopts a cellular state that promotes and sustains productive HIV transcription, and virus production resumes.

Several therapeutic approaches are being considered to control or eliminate the HIV latent reservoir. These involve either a complete elimination of all persistent HIV (sterilizing cure) or the immunological control of persistent HIV (functional cure). The “shock and kill” approach is the main focus of current research efforts for a sterilizing cure. In this approach, small molecules that activate HIV transcription would be used to force the reactivation of latent HIV in memory CD4⁺ T cells under the cover of ART. Subsequently, reactivation of HIV expression would induce viral cytopathic effects, immune clearance, and cell death, thereby purging latently infected cells while uninfected cells are protected by ART (4). Challenges to this approach involve the heterogeneity of latent HIV and the lack of evidence that lymphocytes, in which HIV is reactivated, are eliminated from the latent pool (reviewed in 5–7). Recent studies have also illustrated that different latent viruses might become differentially reactivated in response to different drugs (8–10), and that HIV-latency reactivation might behave in a stochastic rather than deterministic manner when challenged by small molecules (10). Additionally, many of the non-reactivated proviruses appear to be replication competent, indicating that the latent reservoir may be up to 60-fold larger than previously estimated (10).

The complex mechanisms of HIV transcriptional regulation, as well as HIV latency, have been recently summarized in a number of excellent reviews (5–7, 11). In this review, we highlight the core principles that regulate HIV transcription and then focus on two broad mechanisms that control HIV latency: (a) *cis*-acting mechanisms, dependent on the site of integration of the virus into the genome and the local chromatin environment at that site, and (b) *trans*-acting mechanisms, including basal and activated transcription factors, their regulation by the state of activation of T cells, and the environmental cues that these cells receive.

HETEROGENEOUS CELLULAR AND VIRAL MECHANISMS REGULATE HIV-1 LATENCY

HIV transcription is regulated by a variable combination of cellular and viral processes that include different integration sites and different levels of critical transcription factors (TFs) present in different lymphocytes. This heterogeneity yields a number of possible distinct basal transcriptional states for the HIV promoter soon after infection. Importantly, if and when basal HIV transcription reaches a certain threshold, the HIV-encoded Tat protein becomes synthesized and accumulates within the infected cell. Once Tat has accumulated to a critical level, it further activates HIV transcription by binding to an RNA stem loop called the trans-activation response element (TAR) present at the 5' extremity of all viral transcripts and by recruiting the positive transcription elongation factor-b (pTEFb), which dramatically activates HIV transcription rate. This leads to further Tat expression and the creation of a feed-forward loop. This modular organization of HIV transcription is

responsible for the ability of HIV to adopt two different transcriptional functional states, i.e., latent and productive, and implies that the factors that control basal HIV transcription and/or Tat stability are critical in determining the entry of HIV into a productive versus latent state.

In the following section, we focus on the *cis*-acting cellular and viral components that heterogeneously regulate HIV-1 transcription. These components include (a) viral integration site and proviral orientation, (b) genomic architecture, and (c) stochastic gene expression.

Viral Integration Site and Proviral Orientation

Because the eukaryotic chromatin environment is highly heterogeneous in terms of transcriptional activity and histone posttranscriptional modifications, it has long been suspected that integration of HIV into certain unique chromatin environments might lead to HIV transcriptional repression and latency, whereas integration into other sites might lead to active transcription and productive infection (12, 13). However, HIV integrates into the host cell genome in a nonrandom manner—the HIV integrase protein interacts with the cellular coactivator lens epithelium-derived growth factor (LEDGF/p75) (14) and, to a lesser extent, histidine-rich protein (HRP-2) (15). This interaction directs the HIV preintegration complex to the introns of actively transcribing genes. There, HIV preferentially integrates into the outward-facing major groove of nucleosomal (AT-rich) DNA.

However, the interaction between integrase and LEDGF is not an absolute requirement for HIV integration and, in a fraction of integration events, HIV integrates in a more random manner into the genome. These random events can lead to integration within a repressive chromatin environment such as heterochromatin, thereby promoting HIV latency. In Jurkat cell J-Lat model of HIV-1 latency, the site of viral integration into the genome appears to be dominant in determining whether a provirus becomes latent, as integration at different genomic sites leads to different levels of basal HIV promoter activity (16). In a small fraction of latently infected cell lines, latent HIV-1 was found integrated into alphoid centromeric repeats, a clear and well-documented example of a heterochromatin transcriptionally repressive environment (17, 18). Moreover, investigators found that across a small number of genomic loci, the location of integrated provirus determined the variability (noise) in HIV-1 gene expression (19). Dar et al. (20) extended this finding by monitoring the proviral transcriptional activity of an HIV-derived lentiviral vector at multiple integration sites using time-lapse fluorescence microscopy. Here, the authors showed significant variability in transcriptional noise (both burst size and frequency) across ~8,000 unique genomic loci. Taken together, these studies demonstrate that HIV integration site variability can result in substantial differences in proviral transcriptional output, which may play an important role in driving HIV latency.

Another source of heterogeneity in HIV-1 transcription is the orientation of the provirus relative to the host gene. Generally, parallel orientations increase transcriptional interference from the RNA polymerase in the host gene and antiparallel orientations decrease this interference (21). However, the conclusive role of proviral orientation in HIV transcription remains unclear. Studies in J-Lat clones, primary CD4⁺ T cells, and other *in vitro* models suggest that interfering with transcription represses HIV (18, 22–24). Conversely, a study in

latently infected CD4⁺ T cells derived from patients did not reveal a bias in orientation (25). Thus, the orientation in which the provirus integrates may significantly affect HIV-1 transcription, however more work is needed to fully understand this phenomenon.

To investigate the relationship between HIV integration and transcription in a more systematic way, Sherrill-Mix et al. (8) recently performed a meta-analysis of five well-characterized models of HIV latency so as to determine the overlap between the site of integration and known transcriptional regulatory mechanisms. In their analysis, they correlated latency with 135 genomic and epigenetic features in CD4⁺ T cells (e.g., gene density and expression, histone posttranslational modifications, chromatin accessibility). Strikingly, the authors found that these features did not correlate with latency, either individually or in combination. Another study using a double-labeled latency model showed that features of integration sites and/or provirus orientation did not distinguish productively and latently infected cells (26). Thus, these conflicting findings imply that the relationship between HIV-1 integration site and transcription is not easily defined, and that a more complex type of correlation—which integrates other properties—likely exists.

Although genomic features on their own may not predict HIV latency, integration of genomic factors and the availability of TFs may provide insight into this process. For example, chromatin accessibility at HIV integration sites predicted the cellular threshold of nuclear factor (NF)- κ B RelA/p65 needed to activate HIV gene expression (27). This study suggests that multiple factors (i.e., chromatin accessibility and NF- κ B levels) work together to control HIV transcription, but further work is needed to link this process to establishing HIV latency. The studies mentioned in this section support the hypothesis that the site and orientation of integrated provirus may influence HIV transcription, but these effects are not universally observed across HIV latency models.

Genomic Architecture

The eukaryotic nucleus is structurally and spatially heterogeneous. The nuclear core contains transcriptionally active and structurally relaxed euchromatin, whereas the periphery contains transcriptionally inactive and structurally condensed heterochromatin. At the boundaries of the nuclear lamina, a number of highly transcribed genes are associated with the nuclear pore complex (reviewed in 28, 29). Additionally, the nucleus contains many types of nuclear bodies—distinct subdomains that are classified by their function and composition. For example, transcription factories and splicing speckles are located near machinery that facilitates high levels of gene transcription. In contrast, promyelocytic leukemia (PML) and polycomb bodies are composed of PML protein (TRIM19) and polycomb machinery, respectively, and repress gene transcription (reviewed in 30).

The nuclear localization of HIV provirus may dynamically influence its transcription and thereby latency. For instance, using confocal microscopy and fluorescently labeled HIV preintegration complexes, researchers observed that HIV selectively targets decondensed chromatin in the nuclear periphery (31). This finding is consistent with evidence that HIV integrates into actively transcribing genes. Specifically, within the nuclear periphery of latent J-Lat clone A1 cells (integrated in ChrXp21.1), the HIV provirus interacts in *trans*

with the alphoid pericentromeric region of chromosome 12, an interaction that was lost in cells treated with *phorbol*-12-myristate-13-acetate (PMA) (32).

Another method by which nuclear architecture may dynamically regulate HIV transcription involves PML bodies. Using 3D-ImmunoFISH in Jurkat and primary CD4⁺ T cell models, Lusic et al. (33) showed that latent provirus interacts with PML bodies, an interaction not seen in activated cells. This interaction causes PML to recruit histone methyl transferase G9a to potentially inactivate the HIV LTR (33). Interestingly, other work suggests that PML can sequester CyclinT1 (part of the P-TEFb elongation complex), which recruits catalytically inactive, acetylated CDK9 (the kinase component of the P-TEFb elongation complex) into PML bodies, preventing elongation in HIV transcription (34). This finding supports that PML bodies inactivate the P-TEFb complex and thereby repress HIV transcription.

Although we still have much to learn, these results suggest that the site of integration of HIV might influence its transcription not only via *cis* effects (i.e., the gene in which HIV integrates) but also via *trans* effects by the local chromatin environment composed of nearby genes that might not be contiguous on the chromosome but are in close proximity.

Stochastic Gene Expression and HIV Latency

HIV provirus senses the T cell environment via binding sites for common immune-responsive activators of transcription that are downstream of T cell receptor (TCR) activation [e.g., NF- κ B, c-Jun, c-Fos, nuclear factor of activated T cells (NFAT)]. Thus, in resting CD4⁺ T cells, expression of immune-responsive genes and their cognate transcription factors is depleted and HIV transcription might be suppressed as a consequence. In contrast, in activated T cells, transcription factors specific for the activation state lead to efficient HIV transcription and a productive infection.

Based on this model, it has been proposed that HIV latency is established when an activated T cell (necessary for HIV infection) returns to the resting state. However, there is little evidence for this type of deterministic model. Instead, there is growing evidence that HIV transcription might have a significant stochastic component that contributes to the two states of HIV transcription, productive versus latent (35). Indeed, within presumably homogeneous activated T cell populations, both productive and latent infections can result (17, 18, 36–44). This evidence also includes recent observations using dual-fluorescence viruses to monitor HIV transcriptional state. Using these new tools, researchers revealed high levels of both productive and latent infections (26, 45, 46). Finally, in multiple models of HIV latency, treatment with agents that reactivate latent HIV fails to uniformly reactivate all cells within the population. This is observed not only in cell lines such as J-Lat (17) but also in latently infected CD4⁺ T cells derived from patients. When initially treated with phytohaemagglutinin (PHA) a proportion of cells remained latent, yet could be subsequently reactivated by repeated PHA stimulation (~25% of latent replication-competent proviruses) (10). Thus, latent HIV-1 provirus stochastically responds to full T cell activation, suggesting that a similar stochastic process may also establish latency. However, one should note that the assumption is made that cell populations in these experimental systems are truly homogeneous. This has not yet been proven and is actually unlikely in the case of CD4⁺ T

cells isolated from humans. There is therefore a possibility that HIV transcription behaves in a deterministic manner in cells that behave probabilistically in terms of their T cell activation status. Resolving this issue will require the simultaneous single-cell analysis of HIV and cellular transcriptomics.

What is the Best Experimental System to Study HIV Latency?

A key question in the latency field is which experimental system best reflects the state of HIV latency in CD4⁺ T cells from patients infected with HIV and on ART. Various latency models have been developed, based either in transformed lymphocytic cell lines or in primary CD4⁺ T cells. A recent study reported on the activity of 13 stimuli, known to reactivate HIV by defined mechanisms of action, in five primary T cell models, four J-Lat cell models, and a model obtained via a viral outgrowth assay using patient-derived infected cells (9). Interestingly, no single *in vitro* cell model alone was able to capture accurately the *ex vivo* response characteristics of latently infected T cells from patients (as defined by the outgrowth model). Protein kinase C (PKC) agonists and PHA reactivated latent HIV uniformly across models, although drugs in most other classes did not (9). The diversity of the responses of each model to different stimuli may reflect the heterogeneity of the mechanisms driving the establishment and maintenance of latency in each system.

Thus, we currently do not know whether latency *in vivo* is similarly heterogeneous or whether latency as we see it in primary CD4⁺ T cells isolated from patients is the relevant model system. The key unanswered question is the cellular source of the virus that persists under ART and is able to reseed the infection upon cessation of therapy.

IMMUNOLOGICAL AND ENVIRONMENTAL VARIABILITY IN HIV LATENCY

The mechanisms that establish and maintain HIV latency are also likely to involve many *cis*-acting immunological and environmental factors (e.g., T cell subsets, anatomical sanctuaries, the cytokine environment, and immunological synapses). As discussed below, these mechanisms further support the idea that HIV latency is a heterogeneous process.

Cellular and Anatomical HIV Reservoirs

HIV tropism is governed by expression of the entry receptor complex of CD4 and CXCR4 and/or CCR5 on the surface of target cells. Other chemokine receptors, such as CCR3 and CCR8, are also HIV coreceptors—a fact that expands the pool of cells and tissues that HIV targets. HIV predominantly targets CD4⁺ T lymphocytes and monocytes/macrophages, although additional cell populations can also be infected. For example, dendritic cells (DCs), natural killer (NK) cells, and other specialized cell types derived from various tissue reservoirs of HIV (e.g., renal, mucosal, and cervical epithelial cells; mastocytes, astrocytes, and microglia in the central nervous system; skin fibroblasts; and bone marrow stem cells) may contribute to the viral reservoir.

The best-characterized cellular reservoir of HIV comprises resting CD4⁺ T cells. The members of this highly heterogeneous population are distinguished by their differentiation state (reviewed in 47): naïve T cells (T_N), memory stem cells (T_{SCM}), central memory T cells (T_{CM}), transitional memory T cells (T_{TM}), and effector memory T cells (T_{EM}). T_N cells

are poorly susceptible to HIV infection. T_{CM} cells survive and proliferate after activation and migrate to secondary lymphoid organs. Conversely, T_{EM} cells display immediate effector functions after antigen stimulation and migrate to peripheral tissues. T_{CM} cells differentiate into T_{EM} cells either after TCR crosslinking or, to a lesser extent, in response to homeostatic cytokines. The functional and transcriptional characteristics of T_{TM} cells are intermediate between those of T_{CM} and T_{EM} cells (47). Chomont et al. (48) showed that T_{CM} and T_{TM} cells comprise most of the latent CD4 T cell reservoir, whereas T_N and effector T cells only marginally contribute to this pool. More recently, investigators identified another subset of memory T cells—T_{SCM}—endowed with self-renewal, high proliferative potential, and homeostatic proliferation (49). Buzon et al. (50) compared T_{SCM} cells to other memory compartments and demonstrated that they have higher levels of HIV DNA per cell, slower decay, and identical HIV sequences after four to eight years, suggesting that this pool may also contribute to the long-term HIV reservoir.

HIV targets a range of tissues—including the gut-associated lymphoid tissues, genital tract, lymph nodes, central nervous system, spleen, liver, kidney, and lungs—and the HIV species targeting these sites may be biologically distinct from those in the systemic circulation. In addition, each anatomical compartment's T cell subset has unique functional, phenotypic, and survival properties. This diversity may have a substantial impact on the long-term *in vivo* persistence of latent HIV infection through various mechanisms, which raises the possibility that latency is driven by different mechanisms in different anatomical compartments. This possibility has not been explored yet because most primary CD4⁺ T cell models rely on the use of activated peripheral CD4⁺ T cells since accessing these other sites is more challenging.

Immunological Synapses

Cell-mediated HIV infection can occur via virological or infectious synapses that form between productively infected cells and uninfected target cells, or between uninfected antigen-presenting cells (APCs) harboring HIV and T cells, respectively. Infectious synapses occur with or without cognate antigens, a distinction that affects T cell survival rates or regenerative capacity. Independent of antigen interactions, synapse formation requires coreceptors and adhesion molecules, and weakens processes that maintain homeostasis of naïve cells (e.g., proliferation, T cell activation, and long-term survival) (2, 51, 52). One example of infectious synapses involves DCs, a particular type of APC. These cells constantly scavenge for pathogens, including HIV, in peripheral tissue before transitioning to lymphoid tissue, where they interact with immune cells. When DCs capture HIV, they confine the infectious virus in a non-degrading compartment (without becoming infectious) and then transmit the virus to target cells through infectious synapses (3, 53, 54). Thus, DCs disseminate virus, through a *trans*-infection process, to sites and cells that contribute to HIV latency (53, 55–57).

Importantly, these synapses may change the biology of HIV-infected cells to keep HIV silent. Indeed, a recent study shows that co-culturing productively infected T cells with DCs increases HIV latency rates in T cells (4, 56). However, this is independent of DC-to-T cells transfer of HIV, suggesting that DCs can transform productive infection into latently

infection, regardless of T cell activation, through signaling during contact with CD4⁺ T cells contact.

Cytokine Environments

HIV infection causes a potent inflammatory response that produces excess proinflammatory cytokines, including interferons, interleukins, chemokines, and tumor necrosis factor alpha (TNF α). Several of these cytokines regulate viral replication, and innate and adaptive immune responses, and may also be involved in establishing and maintaining a viral reservoir.

When HIV transitions from the acute to the chronic phase, an anti-inflammatory response produces immunosuppressive cytokines such as interleukin (IL)-10 and transforming growth factor (TGF)- β . IL-10 reduces Th1 type responses and antigen presentation by downregulating major histocompatibility complex (MHC) class II at the surface of APCs (58). IL-10 also inhibits activation of CD4⁺ T cells by directly upregulating suppressors of T cell activation, such as programmed cell death protein (PD)-1, discussed below (59). Similarly, TGF- β reduces T cell activation by upregulating suppressors of cytotoxic T lymphocyte antigen (CTLA)-4, also discussed below, and inhibiting IL-2 production (60, 61). By modulating T cell activation, these cytokines may therefore regulate the entry of HIV into latency and its persistence.

There is also evidence for a role of specific chemokines (CCL19, CCL20, CCL21, CXCL9, CXCL10) and gamma-chain (γ c) cytokines (IL-2, IL-4, IL-7, IL-15) in latency. These cytokines may allow resting CD4 T cells, which are poorly permissive to HIV infection (62), to become infected by HIV in vitro without T cell activation (63–66).

Once a viral reservoir is established, cytokines may also regulate the maintenance of the reservoir. First, during HIV progression, cytokines that reactivate latent HIV may promote the replenishment of the HIV reservoir. For example, the combination of TNF, IL-2, and IL-6 reactivates viral production in resting CD4⁺ T cells isolated from virally suppressed patients (67). Additionally, IL-18, which exists at high levels in the serum of chronically infected patients, increases HIV replication in infected CD4⁺ T cells (68). Second, homeostatic proliferation of memory T cells, the major pool of latently infected cells, may also contribute to the maintenance of the viral reservoir. For example, IL-7 promotes the survival and basal homeostatic proliferation of memory T cells via the AKT/FOXO3a pathway (69, 70) and stabilizes the size and genetic diversity of the HIV reservoir (48).

Negative Regulatory Molecules

During chronic HIV infection, the persistence of the virus and cytokines induces a state of chronic activation of the immune system. As a result, T cells become exhausted, gradually lose their ability to proliferate, and become hyporesponsive to antigen-specific stimulation. T cell exhaustion is associated with the upregulation of negative costimulatory molecules on the cell surface, such as PD-1, CTLA-4, T cell immunoglobulin and mucin protein (TIM)-3, lymphocyte activation gene (LAG)-3, TNF-related apoptosis-inducing ligand (TRAIL), and others (reviewed in 71). These inhibitory receptors regulate distinct cell functions that establish and maintain peripheral tolerance and abortive T cell responses.

PD-1 is associated with T cell exhaustion in HIV infection (72), and its upregulation correlates with the size of the HIV cellular reservoir (73). Engagement of PD-1 by its ligand PD-L1/2 (broadly expressed on APCs and non-APCs) inhibits HIV production in primary CD4⁺ T cells isolated from viremic and virally suppressed patients (74). Interestingly, PD-1 binds to its ligands only when it is engaged at the same time as TCR. Binding of PD-1 to its ligands induces the phosphorylation of its cytoplasmic domain, which then recruits SH2 domain-containing tyrosine phosphatase (SHP)-1 and -2. SHP-1 and -2 then dephosphorylate molecules involved in TCR proximal signaling, such as zeta-chain-associated protein kinase 70 (ZAP70), protein kinase C (PKC)- θ , and CD3 ζ . This modification reduces phosphatidylinositol 3-kinase (PI3K) activity, Akt phosphorylation, and glucose metabolism and inhibits T cell activation (75). Similarly, CTLA-4 expression negatively regulates T cell activation and IL-2 production by interacting with its ligands, CD80 (B7-1) and CD86 (B7-2), on APCs (76). In HIV-infected patients, CTLA-4 expression negatively correlates with CD4⁺ T cell counts, becomes upregulated on CD4⁺ T cells, and may inhibit HIV replication and promote latency (77). Both PD-1 and CTLA-4 inhibit signaling through different molecular mechanisms that involve Akt, but PD-1 more effectively suppresses T cell-specific transcription than does CTLA-4 (75).

Other studies have also indicated that exhausted T cells represent a unique state of T cell differentiation (78) characterized by changes in the expression of unique transcription factors such as basic leucine zipper transcription factor *ATF*-like (BATF), B lymphocyte-induced maturation protein 1 (Blimp-1), T-box expressed in T cells (T-bet) protein, and many others (reviewed in 71). BATF, for example, is induced by PD-1 ligation and forms dimers with the TF c-Jun. This displaces the TF c-Fos, thereby inhibiting canonical transcription mediated by AP-1 (79). AP-1 is a potential key factor in establishing and maintaining HIV-1 latency (80).

Thus, co-expression of negative regulatory molecules is associated with T cell exhaustion and rapid HIV disease progression, making them potential targets of therapeutics in HIV infection. Blocking inhibitory molecules would favor antigen-specific TCR activation and downstream signals that are needed for proliferation, effector function and HIV replication, therefore reactivating latent provirus.

T Cell Quiescence

Human T lymphocytes, both naïve and memory, remain quiescent over long periods of time. This state is actively maintained by specific transcription factors and is characterized by decreased cell metabolism, RNA synthesis, and cell size. Because quiescent T cells comprise most of the HIV reservoir, we need to understand the mechanisms that drive and maintain T cells in this state. Many of these mechanisms are still under investigation, but several transcription factors have already been shown to regulate this process. These include FOXO3a, signal transducer and activator of transcription 5A (STAT5A); Lung Kruppel-like factor (KLF2); and Tob (81) (reviewed in 82). It is likely that some of these factors participate actively in maintaining HIV latency. In particular, FOXO3a regulates cell cycle, differentiation, apoptosis, stress resistance, and metabolism of resting T cells. Recent studies suggest that FOXO3 promotes the survival of T_{CM} cells during HIV infection. Specifically,

T_{CM} cells express high levels of inactive phosphorylated forms of FOXO3, which repress expression of proapoptotic genes, such as Fas ligand (FasL) and Bim (83), and, consequently, favor their persistence over time (69).

THERAPEUTIC OUTLOOK

Three recent isolated instances of cure for HIV infection—the “Berlin patient” Timothy Brown (84), the French VISCONTI cohort (85), and the Mississippi and California babies (86)—have fueled the notion that a cure for HIV is possible with important notes of caution. Indeed, rebounded HIV has been recently observed in the Mississippi and California infants previously thought to have been cured.

Recent efforts towards widespread curative solutions for HIV infection have focused on the shock-and-kill approach based on the early discovery that HIV can be reactivated from latency by treatment with histone deacetylase (HDAC) inhibitors (87). Although initial efforts to reduce the size of the latent reservoir were unimpressive (88–90), recent studies with more potent compounds are promising. For example, a small clinical trial demonstrated that a single dose of the HDAC inhibitor vorinostat, also known as suberanilohydroxamic acid (SAHA), induced cell-associated HIV-1 Gag RNA in some patients (91). Drug discovery and clinical efforts have focused on compounds that do not activate T cells, such as HDAC inhibitors, PKC agonists, and bromodomain inhibitors (reviewed in 92). Surprisingly, a recent study of popular latency-reactivating agents in a novel viral outgrowth assay suggested that only drugs that activate T cells (PMA + Ionomycin) actually induce *de novo* viral transcription and synthesis (9, 93). Non-T cell-activating compounds (vorinostat, romidepsin, panobinostat, disulfiram, JQ1, bryostatin-1) failed to induce viral outgrowth. Furthermore, as mentioned, vorinostat induced cell-associated HIV-1 Gag RNA, was shown to be the result of host-gene readthrough transcription rather than bona fide HIV-1 transcription (93). These results were also independently confirmed by another recent study that observed SAHA-induced reactivation of only 0.079% of proviruses in patient-derived resting CD4⁺ T cells, despite anti-CD3/CD28-induced reactivation of 1.5% of proviruses in the same cells (94). The study also noted a significant positive correlation between unspliced cellular HIV-1 RNA and virion synthesis following anti-CD3/CD28 treatment but not SAHA treatment (94). These studies suggest that non-T cell-activating compounds inefficiently reactivate latent HIV-1, and that alternative compounds must be discovered.

A number of significant hurdles must be cleared to find a successful shock-and-kill therapy: (a) the need for non-T cell-activating compounds that induce viral outgrowth in patient cells (93, 94), (b) a latent reservoir that may be 60-fold larger than previously thought (10), (c) stochastic latency reactivation despite maximal T cell activation (10), and (d) the lack of effective small molecules that induce the killing of cells in which HIV has reactivated (95). Many of the aspects of the heterogeneity of HIV latency discussed in this review further complicate these hurdles. Indeed, all of these issues exemplify the complexity of HIV-1 latency and of our attempts to eradicate HIV-1.

It is important to note however, that we still have only a rudimentary understanding of the molecular mechanisms that drive HIV latency, and that much more work needs to be done to

identify the factors that contribute to HIV latency and its reactivation. More specifically, we need to study latency with systems-level approaches rather than evaluating specific mechanisms individually. The studies highlighted in this review indicate that many redundant factors, both cellular and viral, contribute to establishing, maintaining, and reactivating latent HIV. The emerging picture is that HIV latency is multifactorial, a circumstance likely to complicate the identification of small molecules that reactivate all latent HIV.

Permanent HIV Suppression

Endogenous retroviruses make up ~8% of the human genome and may be the benign legacy of exogenous retroviruses that were once pathogenic (reviewed in 96). The nonpathogenic nature of permanently suppressed, endogenous retroviruses may provide an alternative strategy for HIV therapy. Given the mounting barriers to a successful shock-and-kill approach, this strategy is becoming increasingly attractive. Thus, a viable alternative to latency-purging strategies may involve therapies designed to mimic a transcriptionally silent endogenized state, or, instead, actually accelerate HIV-1 endogenization. Although the idea of permanently suppressing latency is not novel, little research has been published in this area.

A number of possible targets for permanent HIV-1 suppression have been identified. They include p-TEFb (CDK9/Cyclin T1), whose activity affects HIV-1 transcriptional elongation (97, 98); PIM-1, a “gatekeeper” kinase needed to reactivate latency (99); and Hsp90, a heat-shock protein that activates the NF- κ B pathway on HIV-1 transcription in response to T cell activation (100). Of these targets, the p-TEFb inhibitors 5,6-di-chloro-1-b-D-ribofuranosyl-benzimidazole (DRB), flavopiridol, and seliciclib, as well as the Hsp90 inhibitor AUY922, have already been evaluated in clinical trials as cancer chemotherapeutics. Their clinical effectiveness in HIV-1 suppression remains unknown. Supplementary to these efforts are suppression/silencing strategies based on DNA editing. Recently, both CRISPR and TALEN genome editing technologies have been used to ‘cut out’, or otherwise inactivate/ permanently suppress, latent proviruses. Although these studies are only proof of concept results, they offer an important new approach to solving HIV latency.

CONCLUSION

During the past 30 years, highly effective ART has saved the lives of millions of people worldwide, yet HIV latency prevents eradication of the virus in most infected patients. Much remains to be learned about HIV latency, and especially the multifactorial and probabilistic nature of establishing, maintaining, and reactivating HIV-1 latency. Systems-level studies will be needed to design and test model systems that fully recapitulate the dynamics and complexity of HIV-1 latency *in vivo*. Moreover, it seems prudent for the field to move beyond an almost exclusive focus on shock-and-kill strategies and explore alternative therapeutic approaches, e.g., permanent HIV suppression, that may be more feasible and efficacious.

Acknowledgments

We thank Gary Howard for editorial assistance and Veronica Fonseca for administrative assistance. Eric Verdin is supported by the NIDA Avant-Garde-1DP1 DA031126, 1R01 DA030216-01, and is a member of the CARE collaboratory (UNC/NIH-Federal-5-31532). Matthew Dahabieh is supported by the Canadian Institutes for Health Research (CIHR – 201311MFE-321128-179658). Emilie Battivelli is supported by the California HIV/AIDS Research Program (CHRP – F13-G1-305).

LITERATURE CITED

1. Lohse N, Hansen AB. Survival of persons with and without HIV infection in Denmark. *Ann Intern Med.* 2007; 146(2):87–95. [PubMed: 17227932]
2. Torres RA, Lewis W. Aging and HIV: AIDS: pathogenetic role of therapeutic side effects. *Lab Invest.* 2013; 94(2):120–28. [PubMed: 24336070]
3. Cory TJ, Schacker TW, Stevenson M, et al. Overcoming pharmacologic sanctuaries. *Curr Opin HIV AIDS.* 2013; 8(3):190–95. [PubMed: 23454865]
4. Deeks SG. HIV: shock and kill. *Nature.* 2012; 487(7408):439–40. [PubMed: 22836995]
5. Donahue DA, Wainberg MA. Cellular and molecular mechanisms involved in the establishment of HIV-1 latency. *Retrovirology.* 2013; 10:11. [PubMed: 23375003]
6. Ruelas DS, Greene WC. An integrated overview of HIV-1 latency. *Cell.* 2013; 155(3):519–29. [PubMed: 24243012]
7. Van Lint C, Bouchat S, Marcello A. HIV-1 transcription and latency: an update. *Retrovirology.* 2013; 10:67. [PubMed: 23803414]
8. Sherrill-Mix S, Lewinski MK, Famiglietti M, et al. HIV latency and integration site placement in five cell-based models. *Retrovirology.* 2013; 10:90. [PubMed: 23953889]
9. Spina CA, Anderson J, Archin NM, et al. An in-depth comparison of latent HIV-1 reactivation in multiple cell model systems and resting CD4+ T cells from aviremic patients. *PLoS Pathog.* 2013; 9(12):e1003834. [PubMed: 24385908]
10. Ho Y-C, Shan L, Hosmane NN, et al. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell.* 2013; 155(3):540–51. [PubMed: 24243014]
11. Hakre S, Chavez L, Shirakawa K, et al. HIV latency: experimental systems and molecular models. *FEMS Microbiol Rev.* 2012; 36:706–16. [PubMed: 22372374]
12. Wang GP, Ciuffi A, Leipzig J, et al. HIV integration site selection: analysis by massively parallel pyrosequencing reveals association with epigenetic modifications. *Genome Res.* 2007; 17(8):1186–94. [PubMed: 17545577]
13. Brady T, Agosto LM, Malani N, et al. HIV integration site distributions in resting and activated CD4+ T cells infected in culture. *AIDS.* 2009; 23(12):1461–71. [PubMed: 19550285]
14. Ciuffi A, Llano M, Poeschla E, et al. A role for LEDGF· p75 in targeting HIV DNA integration. *Nat Med.* 2005; 11(12):1287–89. [PubMed: 16311605]
15. Schrijvers R, De Rijck J, Demeulemeester J, et al. LEDGF· p75-independent HIV-1 replication demonstrates a role for HRP-2 and remains sensitive to inhibition by LEDGINs. *PLoS Pathog.* 2012; 8(3):e1002558. [PubMed: 22396646]
16. Jordan A, Defechereux P, Verdin E. The site of HIV-1 integration in the human genome determines basal transcriptional activity and response to Tat transactivation. *EMBO J.* 2001; 20(7):1726–38. [PubMed: 11285236]
17. Jordan A, Bisgrove D, Verdin E. HIV reproducibly establishes a latent infection after acute infection of T cells in vitro. *EMBO J.* 2003; 22(8):1868–77. [PubMed: 12682019]
18. Lewinski MK, Bisgrove D, Shinn P, et al. Genome-wide analysis of chromosomal features repressing human immunodeficiency virus transcription. *J Virol.* 2005; 79(11):6610–19. [PubMed: 15890899]
19. Singh A, Razoooky B, Cox CD, et al. Transcriptional bursting from the HIV-1 promoter is a significant source of stochastic noise in HIV-1 gene expression. *Biophys J.* 2010; 98(8):L32–L34. [PubMed: 20409455]

20. Dar RD, Razoooky BS, Singh A, et al. Transcriptional burst frequency and burst size are equally modulated across the human genome. *Proc Natl Acad Sci USA*. 2012; 109(43):17454–59. [PubMed: 23064634]
21. 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(2):134–46. [PubMed: 18692773]
22. Shan L, Yang HC, Rabi SA, et al. Influence of host gene transcription level and orientation on HIV-1 latency in a primary-cell model. *J Virol*. 2011; 85(11):5384–93. [PubMed: 21430059]
23. Lenasi T, Contreras X, Peterlin BM. Transcriptional interference antagonizes proviral gene expression to promote HIV latency. *Cell Host Microbe*. 2008; 4(2):123–33. [PubMed: 18692772]
24. Gallastegui E, Millan-Zambrano G, Terme JM, et al. Chromatin reassembly factors are involved in transcriptional interference promoting HIV latency. *J Virol*. 2011; 85(7):3187–202. [PubMed: 21270164]
25. 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(12):6122–33. [PubMed: 15163705]
26. Dahabieh MS, Ooms M, Brumme C, et al. Direct non-productive HIV-1 infection in a T-cell line is driven by cellular activation state and NF κ B. *Retrovirology*. 2014; 11(1):1–17.
27. Miller-Jensen K, Dey SS, Pham N, et al. Chromatin accessibility at the HIV LTR promoter sets a threshold for NF- κ B mediated viral gene expression. *Integr Biol Quant Biosci Nano Macro*. 2012; 4(6):661–71.
28. Politz JCR, Scalzo D, Groudine M. Something silent this way forms: the functional organization of the repressive nuclear compartment. *Annu Rev Cell Dev Biol*. 2013; 29(1):241–70. [PubMed: 23834025]
29. Luperchio TR, Wong X, Reddy KL. ScienceDirectGenome regulation at the peripheral zone: lamina associated domains in development and disease. *Curr Opin Genet Dev*. 2014; 25:50–61. [PubMed: 24556270]
30. Morimoto M, Boerkoel C. The role of nuclear bodies in gene expression and disease. *Biology*. 2013; 2(3):976–1033. [PubMed: 24040563]
31. Albanese A, Arosio D, Terreni M, et al. HIV-1 pre-integration complexes selectively target decondensed chromatin in the nuclear periphery. *PLoS ONE*. 2008; 3(6):e2413. [PubMed: 18545681]
32. Dieudonne M, Maiuri P, Biancotto C, et al. Transcriptional competence of the integrated HIV-1 provirus at the nuclear periphery. *EMBO J*. 2009; 28(15):2231–2243. [PubMed: 19478796]
33. Lusic M, Marini B, Ali H, et al. Proximity to PML nuclear bodies regulates HIV-1 latency in CD4+ T cells. *Cell Host Microbe*. 2013; 13(6):665–77. [PubMed: 23768491]
34. Marcello A, Ferrari A, Pellegrini V, et al. Recruitment of human cyclin T1 to nuclear bodies through direct interaction with the PML protein. *EMBO J*. 2003; 22:2156–66. [PubMed: 12727882]
35. 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(2):169–82. [PubMed: 16051143]
36. Kutsch O, Benveniste ENN, Shaw GMM, et al. Direct and quantitative single-cell analysis of human immunodeficiency virus type 1 reactivation from latency. *J Virol*. 2002; 76(17):8776–8786. [PubMed: 12163598]
37. Sahu GK, Lee K, Ji J, et al. A novel in vitro system to generate and study latently HIV-infected long-lived normal CD4+ T-lymphocytes. *Virology*. 2006; 355(2):127–37. [PubMed: 16919704]
38. Tyagi M, Pearson RJ, Karn J. Establishment of HIV latency in primary CD4+ cells is due to epigenetic transcriptional silencing and P-TEFb restriction. *J Virol*. 2010; 84(13):6425–37. [PubMed: 20410271]
39. Marini A, Harper JM, Romerio F. An in vitro system to model the establishment and reactivation of HIV-1 latency. *J Immunol*. 2008; 181(11):7713–20. [PubMed: 19017960]
40. Bosque A, Planelles V. Induction of HIV-1 latency and reactivation in primary memory CD4+ T cells. *Blood*. 2009; 113(1):58–65. [PubMed: 18849485]

41. Yang H-CC, Xing S, Shan L, et al. Small-molecule screening using a human primary cell model of HIV latency identifies compounds that reverse latency without cellular activation. *J Clin Invest*. 2009; 119(11):3473–86. [PubMed: 19805909]
42. Lassen KG, Hebbeler AM, Bhattacharyya D, et al. A flexible model of HIV-1 latency permitting evaluation of many primary CD4 T-cell reservoirs. *PLoS ONE*. 2012; 7(1):e30176. [PubMed: 22291913]
43. van der Sluis RM, van Montfort T, Pollakis G, et al. Dendritic cell-induced activation of latent HIV-1 provirus in actively proliferating primary T lymphocytes. *PLoS Pathog*. 2013; 9(3):e1003259. [PubMed: 23555263]
44. van der Sluis RM, Derking R, Breidel S, et al. The interplay between the viral Tat protein and the c-Jun transcription factor in controlling the activity of the LTR promoter in the different human immunodeficiency virus type I subtypes. *J Gen Virol*. 2014; 5(4):968–979. [PubMed: 24447950]
45. Dahabieh MS, Ooms M, Simon V, et al. A doubly fluorescent HIV-1 reporter shows that the majority of integrated HIV-1 is latent shortly after infection. *J Virol*. 2013; 87(8):4716–27. [PubMed: 23408629]
46. Calvanese V, Chavez L, Laurent T, et al. Dual-color HIV reporters trace a population of latently infected cells and enable their purification. *Virology*. 2013; 446(1–2):283–92. [PubMed: 24074592]
47. Farber DL, Yudanin NA, Restifo NP. Human memory T cells: generation, compartmentalization and homeostasis. *Nat Rev Immunol*. 2014; 14(1):24–35. [PubMed: 24336101]
48. 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(8):893–900. [PubMed: 19543283]
49. Gattinoni L, Lugli E, Ji Y, et al. A human memory T cell subset with stem cell–like properties. *Nat Med*. 2011; 17(10):1290–97. [PubMed: 21926977]
50. Buzon MJ, Sun H, Li C, et al. HIV-1 persistence in CD4+ T cells with stem cell–like properties. *Nat Med*. 2014; 20(2):139–42. [PubMed: 24412925]
51. Delon J, Bercovici N, Raposo G, et al. Antigen-dependent and -independent Ca²⁺ responses triggered in T cells by dendritic cells compared with B cells. *J Exp Med*. 1998; 188(8):1473–84. [PubMed: 9782124]
52. Revy P, Sospedra M, Barbour B, et al. Functional antigen-independent synapses formed between T cells and dendritic cells. *Nat Immunol*. 2001; 2(10):925–31. [PubMed: 11561183]
53. Geijtenbeek TB, Kwon DS, Torensma R, et al. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell*. 2000; 100(5):587–97. [PubMed: 10721995]
54. Yu HJ, Reuter MA, McDonald D. HIV traffics through a specialized, surface-accessible intracellular compartment during trans-infection of T cells by mature dendritic cells. *PLoS Pathog*. 2008; 4(8):e1000134. [PubMed: 18725936]
55. Stadeli KM, Richman DD. Rates of emergence of HIV drug resistance in resource-limited settings: a systematic review. *Antivir Ther*. 2012; 18(1):115–23. [PubMed: 23052978]
56. Evans VA, Kumar N, Filali A, et al. Myeloid dendritic cells induce HIV-1 latency in non-proliferating CD4+ T cells. *PLoS Pathog*. 2013; 9(12):e1003799. [PubMed: 24339779]
57. Rodriguez-Plata MT, Puigdomenech I, Izquierdo-Useros N, et al. The infectious synapse formed between mature dendritic cells and CD4⁺ T cells is independent of the presence of the HIV-1 envelope glycoprotein. *Retrovirology*. 2013; 10:42. [PubMed: 23590845]
58. Kwon DS, Angin M, Hongo T, et al. CD4+ CD25+ regulatory T cells impair HIV-1-specific CD4 T cell responses by upregulating interleukin-10 production in monocytes. *J Virol*. 2012; 86(12):6586–94. [PubMed: 22496237]
59. Said EA, Dupuy FP, Trautmann L, et al. Programmed death-1-induced interleukin-10 production by monocytes impairs CD4+ T cell activation during HIV infection. *Nat Med*. 2010; 16(4):452–59. [PubMed: 20208540]
60. Elrefaei M, Burke CM, Baker CA, et al. HIV-specific TGF-beta-positive CD4+ T cells do not express regulatory surface markers and are regulated by CTLA-4. *AIDS Res Hum Retrovir*. 2010; 26(3):329–37. [PubMed: 20433405]

61. Li MO, Sanjabi S, Flavell RA. Transforming growth factor-beta controls development, homeostasis, and tolerance of T cells by regulatory T cell-dependent and -independent mechanisms. *Immunity*. 2006; 25(3):455–71. [PubMed: 16973386]
62. Baldauf HM, Pan X, Erikson E, et al. SAMHD1 restricts HIV-1 infection in resting CD4⁺ T cells. *Nat Med*. 2012; 18(11):1682–87. [PubMed: 22972397]
63. Cameron PU, Saleh S, Sallmann G, et al. Establishment of HIV-1 latency in resting CD4⁺ T cells depends on chemokine-induced changes in the actin cytoskeleton. *Proc Natl Acad Sci USA*. 2010; 107(39):16934–39. [PubMed: 20837531]
64. Saleh S, Solomon A, Wightman F, et al. CCR7 ligands CCL19 and CCL21 increase permissiveness of resting memory CD4⁺ T cells to HIV-1 infection: a novel model of HIV-1 latency. *Blood*. 2007; 110(13):4161–64. [PubMed: 17881634]
65. Ducrey-Rundquist O, Guyader M, Trono D. Modalities of interleukin-7-induced human immunodeficiency virus permissiveness in quiescent T lymphocytes. *J Virol*. 2002; 76(18):9103–9111. [PubMed: 12186894]
66. Unutmaz D, Kewalramani VN, Marmon S, et al. Cytokine signals are sufficient for HIV-1 infection of resting human T lymphocytes. *J Exp Med*. 1999; 189(11):1735–1746. [PubMed: 10359577]
67. Chun TW, Engel D, Mizell SB, et al. Induction of HIV-1 replication in latently infected CD4⁺ T cells using a combination of cytokines. *J Exp Med*. 1998; 188(1):83–91. [PubMed: 9653086]
68. Iannello A, Boulassel MR, Samarani S, et al. HIV-1 causes an imbalance in the production of interleukin-18 and its natural antagonist in HIV-infected individuals: implications for enhanced viral replication. *J Infect Dis*. 2010; 201(4):608–17. [PubMed: 20078197]
69. Riou C, Yassine-Diab B, Van Grevenynghe J, et al. Convergence of TCR and cytokine signaling leads to FOXO3a phosphorylation and drives the survival of CD4⁺ central memory T cells. *J Exp Med*. 2007; 204(1):79–91. [PubMed: 17190839]
70. Chetoui N, Boisvert M, Gendron S, et al. Interleukin-7 promotes the survival of human CD4⁺ effector- memory T cells by up-regulating Bcl-2 proteins and activating the JAK- STAT signalling pathway. *Immunology*. 2010; 130(3):418–26. [PubMed: 20465565]
71. Larsson M, Shankar EM, Che KF, et al. Molecular signatures of T-cell inhibition in HIV-1 infection. *Retrovirology*. 2013; 10:31. [PubMed: 23514593]
72. Day CL, Kaufmann DE, Kiepiela P, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature*. 2006; 443(7109):350–54. [PubMed: 16921384]
73. Hatano H. Immune activation and HIV persistence: considerations for novel therapeutic interventions. *Curr Opin HIV AIDS*. 2013; 8(3):211–16. [PubMed: 23454864]
74. DaFonseca S, Chomont N, El Far M, et al. Purging the HIV-1 reservoir through the disruption of the PD-1 pathway. *J Int AIDS Soc*. 2010; 13(Suppl 3):O15.
75. Parry RV, Chemnitz JM, Frauwrith KA, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. *Mol Cell Biol*. 2005; 25(21):9543–53. [PubMed: 16227604]
76. Kaufmann DE, Walker BD. PD-1 and CTLA-4 inhibitory cosignaling pathways in HIV infection and the potential for therapeutic intervention. *J Immunol*. 2009; 182(10):5891–97. [PubMed: 19414738]
77. Leng Q, Bentwich Z, Magen E, et al. CTLA-4 upregulation during HIV infection: association with anergy and possible target for therapeutic intervention. *AIDS*. 2002; 16(4):519–29. [PubMed: 11872994]
78. Wherry EJ, Ha SJ, Kaech SM, et al. Molecular signature of CD8⁺ T cell exhaustion during chronic viral infection. *Immunity*. 2007; 27(4):670–84. [PubMed: 17950003]
79. Williams KL, Nanda I, Lyons GE, et al. Characterization of murine BATF: a negative regulator of activator protein-1 activity in the thymus. *Eur J Immunol*. 2001; 31(5):1620–27. [PubMed: 11466704]
80. Duverger A, Wolschendorf F, Zhang M, et al. An AP-1 binding site in the enhancer- core element of the HIV-1 promoter controls the ability of HIV-1 to establish latent infection. *J Virol*. 2013; 87(4):2264–77. [PubMed: 23236059]

81. Juffroy O, Bugault F, Lambotte O, et al. Dual mechanism of impairment of interleukin-7 (IL-7) responses in human immunodeficiency virus infection: decreased IL-7 binding and abnormal activation of the JAK·STAT5 pathway. *J Virol*. 2010; 84(1):96–108. [PubMed: 19864382]
82. Tzachanis D, Lafuente EM, Li L, et al. Intrinsic and extrinsic regulation of T lymphocyte quiescence. *Leuk Lymphoma*. 2004; 45(10):1959–67. [PubMed: 15370239]
83. Van Der Heide LP, Hoekman MF, Smidt MP. The ins and outs of FoxO shuttling: mechanisms of FoxO translocation and transcriptional regulation. *Biochem J*. 2004; 380(Pt. 2):297–309. [PubMed: 15005655]
84. Swiggard WJ, Baytop C, Yu JJ, et al. Human immunodeficiency virus type 1 can establish latent infection in resting CD4+ T cells in the absence of activating stimuli. *J Virol*. 2005; 79(22):14179–88. [PubMed: 16254353]
85. Shalek AK, Satija R, Adiconis X, et al. Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. *Nature*. 2013; 498(7453):236–240. [PubMed: 23685454]
86. Feinerman O, Jentsch G, Tkach KE, et al. Single-cell quantification of IL-2 response by effector and regulatory T cells reveals critical plasticity in immune response. *Mol Syst Biol*. 2010; 6:1–16.
87. Van Lint C, Emiliani S, Ott M, et al. Transcriptional activation and chromatin remodeling of the HIV-1 promoter in response to histone acetylation. *EMBO J*. 1996; 15(5):1112–20. [PubMed: 8605881]
88. 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; 195:833–36. [PubMed: 17299713]
89. Sagot-Lerolle N, Lamine A, Chaix ML, et al. Prolonged valproic acid treatment does not reduce the size of latent HIV reservoir. *AIDS*. 2008; 22:1125–29. [PubMed: 18525257]
90. 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; 22:1131–35. [PubMed: 18525258]
91. Archin NM, Liberty AL, Kashuba AD, et al. Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature*. 2012; 487(7408):482–85. [PubMed: 22837004]
92. Xing S, Siliciano RF. Targeting HIV latency: pharmacologic strategies toward eradication. *Drug Discov Today*. 2013; 18(11–12):541–51. [PubMed: 23270785]
93. Bullen CK, Laird GM, Durand CM, et al. New ex vivo approaches distinguish effective and ineffective single agents for reversing HIV-1 latency in vivo. *Nat Med*. 2014; 20(4):425–429. [PubMed: 24658076]
94. Cillo AR, Sobolewski MD, Bosch RJ, et al. Quantification of HIV-1 latency reversal in resting CD4+ T cells from patients on suppressive antiretroviral therapy. *Proc Natl Acad Sci USA*. 2014; 111(19):7078–7083. [PubMed: 24706775]
95. Shan L, Deng K, Shroff NS, et al. Stimulation of HIV-1-specific cytolytic T lymphocytes facilitates elimination of latent viral reservoir after virus reactivation. *Immunity*. 2012; 36(3):491–501. [PubMed: 22406268]
96. Jern P, Coffin JM. Effects of retroviruses on host genome function. *Annu Rev Genet*. 2008; 42(1):709–732. [PubMed: 18694346]
97. Mancebo HS, Lee G, Flygare J, et al. P-TEFb kinase is required for HIV Tat transcriptional activation in vivo and in vitro. *Genes Dev*. 1997; 11(20):2633–44. [PubMed: 9334326]
98. Biglione S, Byers SA, Price JP, et al. Inhibition of HIV-1 replication by P-TEFb inhibitors DRB, seliciclib and flavopiridol correlates with release of free P-TEFb from the large, inactive form of the complex. *Retrovirology*. 2007; 4:47. [PubMed: 17625008]
99. Duverger A, Wolschendorf F, Anderson JC, et al. Kinase control of latent HIV-1 infection: PIM-1 kinase as a major contributor to HIV-1 reactivation. *J Virol*. 2013; 88(1):364–376. [PubMed: 24155393]
100. Anderson I, Low JS, Weston S, et al. Heat shock protein 90 controls HIV-1 reactivation from latency. *Proc Natl Acad Sci USA*. 2014; 111(15):1528–1537.