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In Vivo Dynamics of the Latent Reservoir for HIV-1: New Insights and Implications for Cure

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Abstract

Although antiretroviral therapy (ART) can reduce viremia to below the limit of detection and allow persons living with HIV-1 (PLWH) to lead relatively normal lives, viremia rebounds when treatment is interrupted. Rebound reflects viral persistence in a stable latent reservoir in resting $CD4^+$ T cells. This reservoir is now recognized as the major barrier to cure and is the focus of intense international research efforts. Strategies to cure HIV-1 infection include interventions to eliminate this reservoir, to prevent viral rebound from the reservoir, or to enhance immune responses such that viral replication is effectively controlled. Here we consider recent developments in understanding the composition of the reservoir and how it can be measured in clinical studies. We also discuss exciting new insights into the in vivo dynamics of the reservoir and the reasons for its remarkable stability. Finally we discuss recent discoveries on the complex processes that govern viral rebound.

INTRODUCTION

In the midst of the global SARS-CoV-2 pandemic, it is easy to forget that 38 million people are currently living with HIV-1 infection and that without lifelong antiretroviral therapy (ART) most of them will die from AIDS. Tremendous advances in ART have given rise to simple regimens with few side effects (reviewed in 1). These regimens reduce plasma HIV-1 RNA levels to below the limit of detection of current clinical assays (20 copies of HIV-1 RNA/mL of plasma), halt and reverse disease progression, and give people living with HIV-1 (PLWH) near-normal life expectancy. However, ART is not curative, and viremia generally rebounds within weeks of treatment interruption (2–5). A vast amount of data suggests that HIV-1 persistence is due to a stable latent reservoir for the virus in resting $CD4^+$ T cells (6–10) rather than failure of ART to adequately arrest viral replication. As a result of the error-prone nature of HIV-1 reverse transcriptase (RT), viral evolution inevitably accompanies ongoing viral replication cycles (11, 12), and the consensus of many studies is that HIV-1 evolution is halted by modern ART (13–22). Further evidence that de novo infection of susceptible cells is blocked by ART comes from the recent finding that most cells in the latent reservoir are actually generated by proliferation of previously infected cells, and not by new infection events (23–25). Additionally, pharmacodynamic studies demonstrating unexpected cooperativity in dose-response curves for antiretroviral drugs and unique synergies between drug classes have provided a fundamental explanation for the remarkable efficacy of ART (26–28). A dramatic illustration of the efficacy of ART comes from recent clinical trials showing that ART regimens can be simplified from three drugs to two drugs without loss of viral suppression (1). Thus, the major problem in the treatment of HIV-1 infection is not inadequate suppression of viral replication by ART but rather the persistence of a stable latent form of the virus that is unaffected by ART.

The latent reservoir was initially identified in resting $CD4^+$ T cells, which are not normally permissive for active HIV-1 replication. Most resting $CD4^+$ T cells lack expression of the HIV-1 coreceptor CCR5, which is critical for entry of the R5 tropic viruses that constitute the latent reservoir in most PLWH (29, 30). Following entry, the viral genomic RNA must be reverse transcribed into DNA, but in resting $CD4^+$ T cells, levels of the requisite deoxynucleoside triphosphates (dNTPs) are very low as a result of the host dNTPase SAMHD1 (31–33). Even if reverse transcription can be completed and integration of the viral genome into host cell DNA occurs, viral gene expression in resting $CD4^+$ T cells is limited by the absence of active forms of the critical host factors NFκB and NFAT, which are needed for the initiation of HIV-1 transcription (34–36), and PTEFb, which is essential for efficient elongation of HIV-1 transcripts (37–40).

Given the blocks to HIV-1 infection of resting $CD4^+$ T cells, a plausible mechanism for the establishment of the latent reservoir in these cells involves infection of activated $CD4^+$ T cells that are transitioning back to the resting memory state (6, 30). During a narrow time window (approximately 6–9 days after activation), *CCR5* upregulation has occurred and there are adequate levels of dNTPs for completion of reverse transcription. However, active nuclear forms of NFκB are declining. This may limit viral gene expression and protect cells from the viral cytopathic effects and host effector mechanisms that normally limit the life span of productively infected $CD4^+$ T lymphoblasts to only 1 day (41, 42). Further silencing of the provirus can be mediated by a complex set of epigenetic modifications (43–46). The end result is a stably integrated but transcriptionally silent provirus in a long-lived memory T cell (6). This is a perfect recipe for persistence. It allows the virus to persist essentially as information, in the form of ~10,000 base pairs of DNA integrated somewhere in the 3 billion base pairs of human DNA. In this form, the virus is unaffected by host immune responses and ART. The normal mechanisms that ensure lifelong immunologic memory to previously encountered antigens (47, 48) then guarantee viral

persistence. Subsequent activation of latently infected $CD4^+$ T cells can reverse latency, and, if ART is interrupted, rebound occurs.

Although this model of reservoir establishment explains almost all of the known aspects of reservoir biology, other mechanisms for the establishment of the latent reservoir have been proposed. These mechanisms generally involve direct infection of resting $CD4^+$ T cells and are based on in vitro experiments, often with X4 tropic viruses (49, 50). Whether latency can result from direct infection of resting cells with the commonly transmitted R5 viruses is less clear. Importantly, infection of resting cells may be facilitated by stimulation with particular chemokines or tissue-derived factors (51).

Although uncertainty remains over the precise mechanism by which the reservoir is established, it is clear that a latent reservoir is established in all infected individuals. Even when ART is initiated within days of virus exposure, a sufficient number of latently infected cells are generated to ensure viral rebound when treatment is stopped (52–55). Thus, eliminating the reservoir has become a major goal of HIV-1 research. The most widely discussed approach to cure has been termed shock and kill (56–64). It involves the pharmacologic induction of viral genes with latency reversing agents (LRAs) (reviewed in 65). This would be done in the setting of ART to prevent new infection of susceptible cells. Infected cells in which latency was reversed could then be eliminated by natural or therapeutically enhanced immune mechanisms.

The development of effective shock-and-kill strategies requires an understanding of the molecular mechanism of HIV-1 latency. The low frequency of latently infected cells in vivo ($<1/10{,}000$) and the absence of any way to purify them have hindered efforts to understand mechanisms of latency, although much has been learned from various transformed and primary cell models of latency (46, 66–69). Rapidly accumulating information on molecular factors involved in HIV-1 latency has been summarized in several excellent reviews (70, 71), and only general principles are considered here. A guiding principle is that HIV-1 latency is associated with the resting state in $CD4^+$ T cells. The first evidence for latent HIV-1 infection in vivo came from studies in which latency was reversed by global T cell activation to allow viral outgrowth (6, 7), and this concept is the basis of the quantitative viral outgrowth assay (QVOA) used to measure the frequency of latently infected cells (8, 72, 73). Early studies showed that highly purified resting $CD4^+$ T cells from infected individuals had very few if any detectable spliced or unspliced HIV-1 transcripts (74). In vitro activation of resting $CD4^+$ T cells with a protein kinase C agonist and a calcium ionophore, a combination that mimics key signaling events in T cell activation, can routinely induce a 100-fold increase in HIV-1 RNA levels within 24 h (75, 76). Thus, the nonpermissive transcriptional environment in resting $CD4^+$ T cells is a key factor in HIV-1 latency. Initial shock-and-kill approaches involved agents that induce global T cell activation (2, 77), but the associated toxicities necessitated a search for LRAs that do not induce global T activation. Interestingly, T cell activation does not always reverse latency (24, 78–80), and it is likely that multiple barriers to HIV-1 gene expression must be overcome to induce all latent proviruses.

In this review, we focus on the in vivo dynamics of the reservoir, how it can be measured, where it is found in the body, the mechanisms underlying its stability, and how viral rebound from the reservoir occurs. Of note, we have chosen to focus on the best-understood HIV-1 reservoir, that is, the one found in resting $CD4^+$ T cells. To date, this is the only cell population that has been consistently shown to harbor replication-competent HIV-1 on a timescale of years in optimally treated PLWH. HIV-1 proviruses can be found in activated $CD4^+$ T cells, but these cells are likely short-lived effector cells generated by the activation of latently infected resting cells and thus are not a distinct reservoir (7). Interesting recent work on other potential reservoirs, particularly in macrophages (81–83), is not considered here due to length constraints, and we refer readers to insightful reviews (84, 85).

MEASUREMENT OF THE LATENT RESERVOIR

The clinical importance of the latent reservoir is that it provides a source for viral rebound upon interruption of ART. Currently, the clinical management of HIV-1 infection does not require reservoir measurements (1). However, almost 100 clinical trials of HIV-1 cure strategies targeting the reservoir are currently underway (**https://www.treatmentactiongroup.org/cure/trials/**). Evaluation of the efficacy of these interventions will require accurate measurement of the latent reservoir. A large number of reservoir assays have been described (for reviews see 86–88). The choice of an optimal reservoir assay requires understanding of the fundamental principles underlying reservoir measurement.

The first principle is that accurate reservoir measurement can only be carried out when active viral replication has been suppressed. In PLWH who are viremic, reservoir analysis is complicated by the presence of labile unintegrated HIV-1 DNA in recently infected cells (89–93) and labile populations of infected cells that decay rapidly during the first months of ART (94). These labile populations are quantitatively dominant but do not become part of the stable latent reservoir. Thus, accurate assessment of the stable latent reservoir can only be performed in PLWH who have been on suppressive ART for >6–12 months (20).

A second major principle is that the population of proviruses persisting in the setting of ART is dominated by highly defective proviruses that are unable to cause viral rebound. Evaluation of cure strategies requires accurate measurement of the subset of proviruses with the potential to cause rebound; thus, defective proviruses must be excluded from reservoir measurements. Early evidence for defective HIV-1 proviruses came from studies detecting large internal deletions (95) or APOBEC3G-mediated hypermutation (96) in HIV-1 proviruses and from comparative studies (7, 97) showing that proviral frequencies measured by polymerase chain reaction (PCR) were much higher than frequencies measured with the virus culture methods initially used to define the reservoir. Among the experimental approaches used in early reservoir studies, the QVOA became the gold standard assay because it provided a definitive minimal estimate of the frequency of cells carrying replication-competent proviruses. In this limiting dilution assay, latency is reversed with T cell activation to allow viral outgrowth and estimation of the frequency of latently infected cells by Poisson statistics (8, 73, 98–100). This assay was used to establish that the frequency of cells carrying readily inducible, replication-competent proviruses is low ($\sim 1/10^6$ resting $CD4^+$ T cells) and that the decay rate of the latent reservoir is so slow ($t_{1/2}$ = 44 months) that lifetime persistence of the virus was guaranteed even with optimal ART (101, 102). These findings have been confirmed in PLWH on modern regimens that include integrase inhibitors (72). Although it has proven extremely informative, the QVOA requires large blood volumes and 2–3 weeks of tissue culture in a BSL3 laboratory. Thus, many investigators have used simple DNA PCR assays for proviral DNA. These assays give infected cell frequencies that are 2–3 logs higher than and poorly correlated with QVOA results (97).

Studies in which near full-length sequences of individual proviruses were obtained from treated individuals provided a simple explanation for the discrepancy between PCR and QVOA results (78, 103, 104). These studies showed that most proviruses (>90%) have obvious fatal defects that fall into two major categories. Many proviruses have large internal deletions arising through a template switching event during reverse transcription analogous to that responsible for recombination of HIV-1 genomes (105). On average, these deletions eliminate one-half of the viral genome, rendering deleted proviruses completely noninfectious (78, 103, 104, 106, 107). A second major category of defects involves G→A hypermutation introduced by the host enzymes APOBEC3G and APOBEC3F (96, 108, 109). The preferred plus strand consensus sequence for APOBEC3G-mediated hypermutation is the tryptophan codon TGG, for which G→A

mutations always generate a stop codon (TGA, TAG, or TAA). Thus, most hypermutated proviruses have multiple stop codons in most open-reading frames and are replication defective (106). Given the profound nature of these defects, it is important to exclude proviruses with deletions and/or hypermutation from reservoir measurements because these proviruses cannot give rise to viral rebound.

Defective and intact proviruses can be distinguished by near full-length single genome sequencing. DNA from infected cells is diluted such that amplification reactions are unlikely to contain more than one proviral template. A near full-length outer PCR of ~9 kb is then run (full-length PCR cannot be done because of the 634-base-pair long terminal repeats (LTRs) at each end of the viral genome). Subsequent nested PCR reactions allow reconstruction of most of the proviral sequence. Since the original description of this approach (78), several studies using this approach have confirmed that the proviral landscape in treated PLWH is dominated by defective sequences (103, 104, 110–112). The frequency of defective viral genomes has also been studied in rhesus macaques infected with simian immunodeficiency virus (SIV) or simian-human immunodeficiency virus (SHIV). Following treatment with ART, the proviral landscapes in these animal models are also dominated by defective sequences, but not to the same extent as in humans with HIV-1 infection (107). In the case of humans with HIV-2 infection who are treated with ART, defective proviruses are also present at frequencies similar to those observed in individuals with HIV-1 infection (107).

Although defective and intact proviruses can be distinguished by near full-length sequencing of individual proviruses, this approach is time and labor intensive. In addition, all such methods depend on a 9-kb outer PCR at limit dilution. This reaction is inherently inefficient and nonquantitative (J.A. White et al., in preparation). Fortunately, because the deletions are very large (average size of 5 kb) and the hypermutation is extensive, it is possible to distinguish intact and defective proviruses using multiplex PCR reactions carried out on individual proviruses in a digital droplet PCR reaction (**Figure 1**). The intact proviral DNA assay (IPDA) based on this principle provides a scalable approach to distinguishing intact from defective proviruses (106, 113, 114). The digital nature of the assay with short, highly efficient amplicons allows separate direct counting of proviruses with 5′ deletions, proviruses with 3′ deletions and/or hypermutation, and proviruses lacking these defects, approximately 70% of which are intact at the primary sequence level. This degree of discrimination between intact and overtly defective proviruses is much greater than that provided by standard, single amplicon PCR (106). Future refinements of the IPDA are likely to provide even greater discrimination between intact and defective proviruses and coverage of diverse HIV-1 variants including non-clade B viruses (113, 115, 116).

The development of a scalable assay that allows for better discrimination between intact and defective proviruses has provided significant new insights into the biology of the latent reservoir. Several recent longitudinal studies with the IPDA have shown that the decay rate of the population of cells with intact proviruses is faster than that of cells with defective proviruses, which show no decay (106, 114, 117). This finding means that the presence of a provirus has an effect on the fate of a $CD4^+$ T cell that can be measured with a precise assay in longitudinal studies over a sufficiently long time interval. Most defective proviruses are defective in most viral proteins, including the Tat protein, which is required for efficient elongation of HIV-1 transcripts (106). Therefore, even following cellular activation, many cells with defective proviruses will not express high levels of viral RNA or protein and are less likely to experience viral cytopathic effects or clearance by cytolytic host effector mechanisms. For these cells, the provirus is essentially a harmless barcode that allows tracking of the cells over time, providing an unprecedented view of the normal dynamics of human $CD4^+$ T cells. The remarkable stability of cells with defective proviruses is consistent

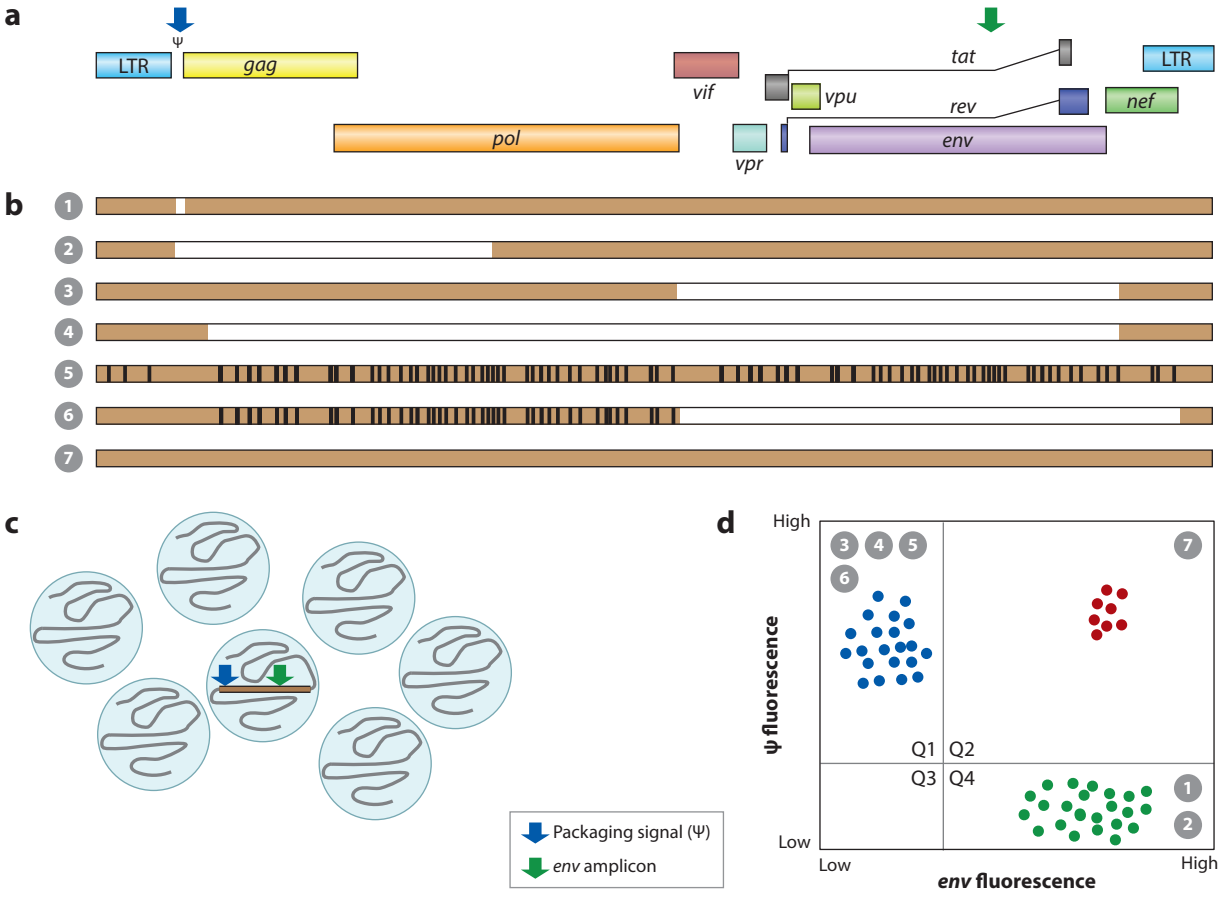

Figure 1

Accurate measurement of the latent reservoir requires excluding proviruses with overt fatal defects. (*a*) Map of the HIV-1 genome. Blue and green arrows indicate positions of the packaging signal (ψ) and *env* amplicons used to distinguish intact and defective proviruses (106, 113). (*b*) Major classes of HIV-1 proviruses found in treated PLWH. More than 90% of proviruses have overt defects including deletions (*white*) and G→A hypermutation (*black lines*). The major classes of proviruses include proviruses with small deletions in ψ (❶), proviruses with large 5′ deletions (❷), proviruses with large 3′ deletions (❸), proviruses with very large internal deletions (❹), hypermutated proviruses (❺), proviruses with deletions and hypermutation (❻), and intact proviruses lacking overt fatal defects (❼). (*c*) Digital droplet PCR used to distinguish intact and defective proviruses in the IPDA. DNA from infected cells is suspended into nanoliter-sized droplets such that no droplet contains more than a single provirus. Multiplex PCR reactions occurring in each droplet allow simultaneous evaluation of the ψ and *env* regions to distinguish intact and defective proviruses. The probe for the *env* amplicon is in a region that is very frequently affected by hypermutation such that hypermutated proviruses fail to give a signal for the *env* amplicon. (*d*) Schematic of IPDA output with fluorescence from each droplet plotted on a two-dimensional plot. The x-axis is fluorescence from the *env* amplicon and the y-axis is fluorescence from the ψ amplicon. Circled numbers indicate the expected quadrants (Q1–Q4) for the classes of proviruses indicated in panel *b*. Intact proviruses can be directly counted as individual droplets in Q2. The blue and green dots represent single positive events detected with the primer sets indicated with the blue and green arrows. The red dots are double positive events detected with both primer sets. Abbreviations: IPDA, intact proviral DNA assay; LTR, long terminal repeat; PCR, polymerase chain reaction; PLWH, persons living with HIV-1.

with studies demonstrating long-term stability of memory CD4$^+$ T cell responses in uninfected individuals (47, 48).

Although the population of cells with defective proviruses shows no decay over time, slow decay of cells with intact proviruses can be detected with precise assays such as the IPDA (106, 114, 118). The half-life of this population of cells, as measured by the IPDA, is similar to the half-life of cells with readily inducible replication-competent proviruses ($t_{1/2}$ = 44 months) (72, 101, 102). The discovery that there is decay over time in the population of cells with intact proviruses but not in the population of cells with defective proviruses suggests that following latency reversal in vivo, some cells with intact proviruses are vulnerable to elimination, presumably by viral cytopathic effects or host cytolytic mechanisms. This phenomenon may also be evident in subtle shifts in the proviral landscape favoring cells with integration sites in chromosomal positions less favorable for transcription (119). Unfortunately, this decay is far too slow to allow eradication of HIV-1 infection with ART alone, and viral rebound generally occurs rapidly after treatment interruption, even in PLWH who start ART during acute infection (55). A detailed study of proviral populations in treated PLWH over long time intervals did not show dramatic evidence for selective loss of cells with intact proviruses (120). In fact, changes in reservoir composition were actually dominated by proliferation of infected cells (see below).

Interestingly, in elite controllers (ECs), rare PLWH who control viral replication without ART through strong antiviral immune responses, there appears to be selection against cells with intact proviruses integrated into active genomic regions and an enrichment of cells with intact proviruses integrated into chromosomal regions that are relatively nonpermissive for transcription (121). This pattern was not seen for cells with defective proviruses. The implication is that in ECs, strong antiviral immune responses gradually eliminate cells with readily inducible intact proviruses. This is an extremely hopeful and encouraging finding that may reflect an accelerated version of the same process that leads to the slow decay of cells with intact proviruses in treated PLWH.

LOCATION OF THE VIRAL RESERVOIR

Initial studies demonstrated that latently infected CD4$^+$ T cells were present in blood and lymph node at roughly similar frequencies (7). Since that time, there has been intense interest in whether latently infected cells are present in particular anatomic locations or CD4$^+$ T cell subpopulations. This topic is the subject of an excellent recent review (122) and is not covered exhaustively here. Instead, we focus on issues relevant to targeting the reservoir. Because latently infected cells do not express viral antigens, they cannot be directly targeted by HIV-1-specific cytolytic T lymphocytes or through antibody-dependent cell-mediated cytotoxicity. Therefore, there has been considerable interest in using host cell markers to target subpopulations of CD4$^+$ T cells that are enriched in latently infected cells. An extreme example of this approach involved a report that CD32a, an Fcγ receptor not normally expressed on T lymphocytes, was upregulated on resting CD4$^+$ T cells carrying latent HIV-1 genomes (123). Unfortunately, subsequent studies failed to confirm this finding and attributed the result to technical issues associated with analysis of extremely rare subpopulations (124–126). Other studies have attempted to identify phenotypic markers for latently infected cells by activating CD4$^+$ T cells from PLWH to induce viral gene expression and then sorting for cells that express HIV-1 Env protein (127), Gag protein (128), or HIV-1 RNA (129), with subsequent phenotypic and transcriptomic analysis. Although a number of intriguing genes have been identified in these studies, the activation step changes the transcriptional state of the cells, complicating the identification of phenotypic markers specific for latently infected cells. Roan and colleagues (130) have recently attempted to overcome this problem by using high-dimensional phenotypic analysis of activated Gag$^+$ cells to infer a phenotype for

latently infected cells in the preactivation state. The authors suggest that there are sets of markers that can be used in combination to enrich for latently infected cells, although there was significant individual variation in the markers identified. An additional problem is that subsets defined by the overlapping expression of multiple markers will be very hard to selectively target in vivo. Overall, it appears unlikely there will be any simple and unique phenotype that can be used to eliminate latently infected cells in all PLWH (130).

Although it may not be possible to target latently infected cells on the basis of a unique surface phenotype, there has been interest in whether latently infected cells are contained preferentially in any of the defined subpopulations of $CD4^+$ T cells. The $CD4^+$ T cell population has been divided into subpopulations on the basis of the history of antigen exposure (naive and memory cell subsets), the types of cytokines produced following antigen exposure (Th1, Th2, Th17, Tfh, or Treg), and the activation state (resting or activated). Studies from many laboratories have examined the distribution of latently infected cells in various subpopulations of $CD4^+$ T lymphocytes (30, 80, 110, 128, 131–140; reviewed in 122). Unfortunately, no consensus has emerged. Latently infected cells have been detected in all $CD4^+$ T cell subpopulations examined, with substantial variation between individuals within a single study and between studies. The differences between studies may reflect differences in methods used to measure the reservoir, differences in markers used to define subpopulations, and plasticity in subpopulations (80). At a more fundamental level, there is considerable uncertainty regarding the best way to subdivide $CD4^+$ T cells into discrete subpopulations (reviewed in 141). The most consistent finding is a higher frequency of latently infected cells among memory cells compared with naive cells. This difference was noted in the earliest studies of the HIV-1 reservoir (7) and is consistent with theories of reservoir establishment (6, 30).

With respect to anatomical location, it is likely that latently infected cells are present in all tissue sites where mature $CD4^+$ T cells are found. Recent studies comparing the size and composition of infected cell populations in blood and lymph node have confirmed that the frequencies of latently infected cells are similar (7, 142, 143) and that the same viral variants are common to blood and lymph node (142–144), consistent with the well-known patterns of lymphocyte trafficking (145). Less is known about the frequencies of infected $CD4^+$ T cells in other locations, but higher frequencies have been reported in the gut-associated lymphoid tissue (146, 147), and wide distribution of infected cells has been documented with advanced in situ methods (147, 148). An elegant recent study compared rebound sequences observed following treatment interruption with viral sequences in different tissues and $CD4^+$ T cell subpopulations prior to treatment interruption (149). The sources of rebound were shown to be diverse and variable between individuals. Therefore, it is safe to assume that latently infected cells are widely distributed in tissue sites and $CD4^+$ T cell subpopulations and that eradication strategies focused on specific anatomic sites or $CD4^+$ T cell subpopulations are unlikely to be useful.

PROLIFERATION OF RESERVOIR CELLS

Although the latent reservoir is clearly associated with the resting state of $CD4^+$ T cells, it has recently become clear that most cells in the reservoir have some history of previous proliferation after infection. To some extent, this is unexpected since T cell activation can reverse latency, generating productively infected cells that typically have a short half-life (41, 42). Proliferation of infected cells copies the integrated provirus into each of the progeny cells with minimal error. In contrast, de novo infection events in untreated PLWH allow extensive viral diversification over time due to the error rate of RT (21, 150). Thus, in PLWH who start ART during chronic infection, the independent detection of identical proviruses provides evidence for proliferation.

The first evidence for infected cell proliferation came from studies of residual viremia (RV), the trace level of free virus that can be detected in treated PLWH. Although ART reduces viremia to below the limit of detection of approved clinical assays (typically 20 copies of HIV-1 RNA/mL of plasma), trace levels of HIV-1 virions can be detected in plasma using ultrasensitive assays (151–153). This RV is typically on the order of one to three copies of HIV-1 RNA/mL of plasma. Importantly, RV cannot be reduced by treatment intensification, indicating that it is derived from cells infected prior to initiation of ART or their progeny (154, 155). Consistent with this conclusion are results of sequencing studies showing that RV is composed of viruses that are archival, nonevolving, and sensitive to the current ART regimen (13, 14). Thus, RV cannot be attributed to ongoing cycles of replication not fully suppressed by ART. The most interesting feature of RV is that despite extensive viral diversification occurring prior to ART, RV is often dominated by identical sequences captured in multiple independent limiting dilution PCRs over a period of months to years (14, 156, 157). It was proposed that this feature reflects the in vivo proliferation of infected cells.

Definitive evidence for the proliferation of infected cells came from studies of HIV-1 integration sites. In each newly infected cell, the reverse transcribed HIV-1 genome integrates somewhere in the 3 billion base pairs of human genomic DNA, typically into accessible genomic regions such as actively transcribed genes (158, 159). The presence of multiple cells with the identical proviral integration sites can only be explained by infected cell proliferation. Several groups used integration site analysis to demonstrate that multiple infected cells within a single blood sample have an identical integration site, indicative of extensive in vivo clonal expansion (160–162). Interestingly, some of the expanded cellular clones had integrations in specific introns of genes associated with cell survival or proliferation such as *BACH2*, *MKL2*, and *STAT5B*. This was not due to an integration site preference, but rather to selection events following integration. In such cases, proviral integration may have caused a promoter-insertion type of mutagenesis that altered expression of the relevant host gene such that survival and/or proliferation of the infected cells was favored (163).

One problem with standard methods of integration site analysis is that only the ends of the proviral genome are captured. Thus, it is likely that most of the expanded clones captured in these studies carried defective proviruses. However, Simonetti et al. (164) described a very large $CD4^+$ T cell clone carrying replication-competent provirus in a patient with a malignancy, and several studies used the QVOA to show that in a given blood sample, 50–60% of independent viral isolates have an identical proviral sequence in the same sample, consistent with massive clone expansion of cells carrying inducible, replication-competent proviruses (23–25). Analysis with ecological methods suggests that most if not all cells in the reservoir have some history of proliferation (165). Some of these expanded $CD4^+$ T clones persist over a timescale of months to years, while others wax and wane (157). Thus, the latent reservoir is dynamic and constantly changing in composition while the total size remains relatively constant, decaying with a half-life of 44 months (72, 102). The process of clonal expansion can start very early after individuals are infected with HIV-1 (166), but it may take several years for clones to expand to the point where they dominate the reservoir. For example, in SIV-infected rhesus macaques treated with ART for one year, sets of identical proviral sequences could be detected but represented only a small fraction of the total number of proviral sequences (107).

There are at least three mechanisms that could drive the infected cell proliferation. First, as mentioned above, HIV-1 integration into particular positions in certain host genes could allow a promoter-insertion mutagenesis that drives aberrant expression of the host gene, resulting in a cell-autonomous proliferative stimulus (160–163). However, many expanded clones have proviral integration sites in genes not associated with proliferation or not expressed in $CD4^+$ T cells

(164, 167). In addition, the finding that infected cell clones wax and wane is inconsistent with a continuous, cell-autonomous proliferative stimulus as the sole driver of infected cell proliferation (157). A second potential mechanism involves cytokines such as IL-7 and IL-15, which are important for homeostasis of naive and memory T cell populations (168). These cytokines may drive proliferation of infected $CD4^+$ T cells in a nonspecific manner (131). However, it is unclear whether this mechanism can explain the observed patterns in which a very small number of extremely large clones dominate the reservoir (23–25).

A third mechanism for infected cell proliferation involves direct stimulation by antigen. Henrich and colleagues (169) showed that following cytoreductive chemotherapy for malignancies, a high fraction of HIV-1 DNA was found in $CD4^+$ T cells responsive to cytomegalovirus (CMV) or Epstein-Barr virus antigens. They suggested that this could reflect clonal expansion of infected $CD4^+$ T cells in response to these viruses, which frequently reactivate during chemotherapy. In PLWH who did not have malignancies, $CD4^+$ T cells responding to CMV have been shown to include cells with identical proviruses (170). T cell receptor (TCR) sequencing has also been used to demonstrate the presence of expanded cellular clones among $CD4^+$ T cells that can be induced to express HIV-1 p24 antigens following nonspecific T cell activation (171). A more complete picture of the role of antigens in driving infected cell proliferation has been provided by Simonetti et al. (167). They identified large clones of infected cells with identical proviruses among $CD4^+$ T cells responding to CMV or HIV-1 antigens. Clonality was confirmed by integration site analysis. Some of these large clones had integration into genes such as *BACH2* or *STAT5B*, which had been previously associated with clonal expansion. However, other large clones had proviruses integrated in chromosomal regions with no connection to cell proliferation. This study also determined the TCR β chain sequence for individual clones. This allowed an analysis of the fraction of cells within an antigen-responsive $CD4^+$ T cell clone that carried a given provirus (167). In some cases, all cells of the clone carried the provirus, indicating that infection occurred early during the initial response to the antigen, with subsequent proliferation of infected cells. In other cases, only some cells of the clone carried the provirus, indicating that cells proliferated in response to the antigen before one of the cells became infected. Subsequently, both infected and uninfected cells proliferated. Together, these results suggest that during the course of normal immune responses, $CD4^+$ T cells can become infected and subsequently proliferate, generating very large clones carrying the same provirus.

Because clonal expansion is a cardinal feature of normal T cell responses to antigens, it is difficult to block the proliferation of infected cells in a nonspecific way without inducing immunosuppression. Nevertheless, modeling studies suggest that interventions causing a modest reduction in the T cell proliferation rates could lead to reservoir elimination (172), and a clinical trial of the antiproliferative agent mycophenolate mofetil is underway (**https://www.treatmentactiongroup.org/cure/trials/**). Targeting proliferation in an antigen-specific way is unlikely to be useful since the antigen specificity of most reservoir cells is unknown, and even for dominant antigens such as CMV, responding cells constitute only a minute fraction of the reservoir (167).

The discovery that the latent reservoir is dominated by large clones of $CD4^+$ T cells that have expanded in response to antigens raises a number of challenging problems and important questions: Are all cells in the reservoir capable of enormous clonal expansion? Is proliferation accompanied by latency reversal? Are all members of the clone equally inducible? What fraction of the cells in a clone are induced at any given time? At the present time, we have only limited information on these important topics. One particularly important question is whether the stimuli driving proliferation also induce viral gene expression. If so, then proliferating cells would be

vulnerable to viral cytopathic effects and host cytolytic effector mechanisms. The issue of proviral inducibility is discussed in the next section.

PROVIRAL INDUCIBILITY

The presence of a latent reservoir in resting $CD4^+$ T cells was initially demonstrated through experiments in which latency was reversed by global T cell activation, thereby allowing viral outgrowth (6–10). However, it is becoming increasingly clear that not all intact proviruses are induced by maximum T cell activation. Ho et al. (78) used near full-genome proviral sequencing to show that the frequency of cells with genetically intact proviruses is much greater than the frequency of latently infected cells that can be induced to produce virus following a single round of T cell activation in the QVOA. Current estimates suggest that the ratio of intact to induced proviruses is about 40:1 (113). The intact, noninduced proviruses have functional, nonmethylated LTRs and are integrated into active chromosomal regions (78). Furthermore, when reconstructed, these proviruses give rise to virus that replicates normally in vitro (78). At least some of these intact, noninduced viruses can be induced by additional rounds of T cell activation (24, 78). Others may have minor defects affecting viral fitness or may not produce a sufficient amount of virus to initiate a spreading infection (173). One study suggested that proviral inducibility might be increased by differentiation of cells from a central memory state, characterized by recirculation through secondary lymphoid organs, to an effector memory state, characterized by the potential for rapid acquisition of effector function and migration to inflamed tissues (174). However, another study of carefully sorted subpopulations of resting $CD4^+$ T cells found that proviral inducibility was low in all $CD4^+$ T cell subsets even with full T cell activation (80). Together, these results suggest that a single round of T cell activation does not induce all latent proviruses that have the potential to be induced.

Low inducibility of latent HIV-1 proviruses presents a significant problem for HIV-1 cure strategies. Given the toxicity associated with global T cell activation (77), shock-and-kill strategies for curing HIV-1 utilize LRAs that do not induce global T cell activation (61). Unfortunately, most of the LRAs used in these strategies are much less efficient at inducing latent HIV-1 than global T cell activation (75).

Low inducibility may also explain how latently infected cells are able to undergo clonal expansion without succumbing to viral cytopathic effects or host cytolytic effector mechanisms. Bosque and colleagues (175) used an in vitro model of HIV-1 latency to show that latently infected cells could proliferate in response to homeostatic cytokine signals without induction of viral gene expression. However, stimuli mimicking antigen-driven activation (anti-CD3+anti-CD28) did induce viral gene expression in this system. In contrast, ex vivo studies using cells from treated PLWH have shown that $CD4^+$ T cells carrying replication-competent proviruses can proliferate in response to mitogen stimulation without releasing infectious virus and initiating a spreading infection (24, 78). It is thus possible that $CD4^+$ T cells can be driven to proliferate by encounters with antigens without induction of viral gene expression. A recent analysis of expanded cellular clones in an infected individual with a malignancy has shown that most cells comprising a given clone do not have detectable viral RNA (79). This result suggests that in most cells comprising the clone, the viral genome remains latent either due to failure to encounter inducing stimuli such as cognate antigens or failure of proviral induction following encounters with inducing stimuli. In this study, the relationship between proliferation and viral gene expression was not directly addressed. Another recent study has described treated PLWH who had RV in the detectable range that could not be suppressed by treatment intensification (176). This nonsuppressible viremia

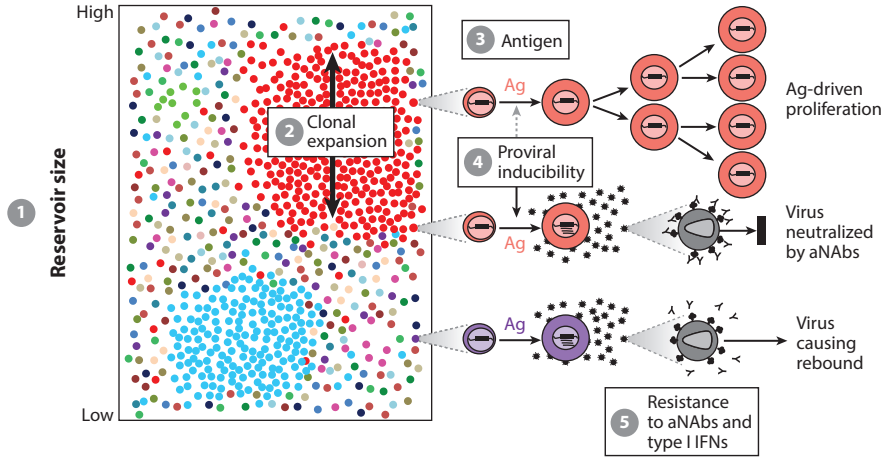

Figure 2

Factors affecting time to viral rebound. Numerous factors affect the time required for viral rebound following interruption of antiretroviral therapy. Reservoir size (❶) is an important major factor. Colors represent distinct clonal lineages of infected $CD4^+$ T cells specific for particular antigens (Ag) and carrying replication-competent proviruses. Long delays in rebound require large multilog reductions in reservoir size (180). The composition of the reservoir is also a factor. The reservoir is often dominated by large clones of infected $CD4^+$ T cells (❷) that have proliferated in response to Ag (❸) (167). Exposure to Ag or other activating stimuli is required to induce viral gene expression from latently infected cells. However, proviral inducibility (❹) is low, and not every encounter with Ag results in proviral induction. In some cases, infected cells proliferate without expressing viral genes (80). Following induction of viral gene expression, the viruses released from an infected cell can only cause rebound if they are resistant to neutralization by autologous neutralizing antibodies (aNAbs) (198) and other immune mechanisms including those induced by type I interferons (IFNs) (❺) (199). Some expanded clones carry viruses that are readily neutralized and that cannot cause rebound.

was due to continuous virus release by some cells belonging to very large expanded $CD4^+$ T cell clones. However, the precise relationship between proliferation and viral gene expression remains unclear.

What is the mechanism underlying the low inducibility of latent proviruses? If we assume that activation of T cells through the TCR induces nuclear translocation of key transcription factors needed for HIV-1 transcription, then the failure of induction may reflect epigenetic changes that interfere with access of the transcriptional machinery to the provirus (reviewed in 70, 71). Alternatively, it has been suggested that induction is a stochastic process heavily dependent on a positive feedback loop involving the Tat protein (177). Given that most HIV-1 proviruses are integrated within actively transcribed host genes in resting $CD4^+$ T cells (159), it is possible that transcriptional interference from the host gene prevents the correct initiation of HIV-1 transcription (178, 179) and production of sufficient levels of Tat to start the positive feedback loop. In this case, there is only a finite probability that each encounter with an activating stimulus would lead to virus production. Such a model could explain how large $CD4^+$ T cell clones carrying replication-competent proviruses could arise (**Figure 2**). Only a fraction of cells encountering antigens may upregulate HIV-1 gene expression following TCR stimulation, while the remaining cells proliferate and increase clone size. Of course, the clone would also be subject to normal cell death mechanisms that are responsible for the contraction phase of the immune response.

DETERMINANTS OF VIRAL REBOUND

From a clinical perspective, the most important goal of reservoir studies is to find a way to allow PLWH to stop ART without viral rebound. It is important to point out that treatment interruption does not cause latency reversal. Rather, it allows outgrowth of viruses that arise from the daily activation of a small fraction of reservoir cells that encounter antigens or other activating stimuli. The viruses released cannot infect new cells in the setting of ART but can be detected as RV with sensitive assays. Interruption of ART allows outgrowth of these viruses. Normally, multiple infected cells are induced every day, and rebound viremia is typically rapid and polyclonal (2, 3, 5, 180). Except for a rare subset of PLWH who are able to control viral replication after treatment interruption (posttreatment controllers) (reviewed in 181), rebound occurs within weeks of treatment interruption (within 3 weeks in 50% of cases and within 8 weeks in 90% of cases) (reviewed in 5).

Factors affecting time to rebound have been reviewed (5). The size of the latent reservoir is clearly a major determinant as evidenced by the fact that interventions resulting in profound reductions in reservoir size are associated with long delays before rebound. Compelling examples include infected individuals who received hematopoietic stem cell transplantation (HSCT) to treat a concurrent malignancy (182). Host cells of hematopoietic origin, including latently infected $CD4^+$ T cells, can be largely eliminated by the graft versus host disease that is a feature of HSCT and by the lymphocyte-depleting effects of the chemotherapy and irradiation used in preparative regimens. Transplantation with donor cells homozygous for a deletion in *CCR5* has resulted in cure in at least two cases (183, 184). However, in two cases described by Henrich and colleagues (182), donors with wild-type *CCR5* alleles were used, and new infection of donor cells was blocked by continuation of ART throughout the transplant period. After host hematopoietic cells were largely replaced by donor cells and infected cells were no longer detected by QVOA or PCR assays, ART was interrupted. The two transplant recipients maintained suppression of viremia for 3 and 8 months before a sudden and dramatic rebound occurred, likely due to the activation of one of the small number of remaining infected cells. Delayed rebound has also been seen in cases where treatment within days of exposure limited the size of the reservoir (54, 185). Mathematical modeling suggests that reservoir reductions of ~3 logs may be needed to delay rebound for 1 year (180). Clinical trials of curative interventions can be evaluated with an analytical treatment interruption (ATI). Some delays in viral rebound have been observed in studies involving infusion of broadly neutralizing antibodies (186, 187), but these delays likely reflect the neutralizing activity of the antibodies that persist for weeks after infusion. Importantly, in SIV-infected macaques on ART, therapeutic vaccination and treatment with a TLR7 agonist produced a slight delay in viral rebound and better control of viral replication following ATI (62). What is still lacking in cure studies is a clear demonstration that an intervention has produced a significant decrease in the size of the reservoir that is evident in a subsequent delay in viral rebound following ATI.

Elegant studies from several laboratories have described biomarkers that could potentially be used to predict time to viral rebound (4, 188–190) or for early detection of imminent rebound following ATI (191, 192). However, no consensus has emerged, and to date the available data take the form of correlations rather than precise predictions. With the exception of virologic biomarkers, clear mechanisms are lacking.

Recent qualitative studies of rebound viruses have led to a new understanding of the timing and mechanism of viral rebound. Previously, two groups had reported that rebound viruses detected in the plasma following ATI were often phylogenetically distinct from viruses detected in the latent reservoir using the QVOA or near full-length sequencing prior to ATI (187, 193–195). This difference could not be attributed to sampling issues because in several of the individuals

studied, the reservoir was dominated by large clones that should have contributed to rebound if rebound was solely a function of infected cell frequency. Alternative explanations included the suggestion that rebound viruses were generated by recombination between two different viral variants present in the reservoir (194, 195). Recombination occurs frequently in the setting of ongoing HIV-1 replication (reviewed in 196), and it is likely that many viruses deposited in the reservoir are recombinants. These recombinants can be detected among the rebound viruses (194, 195), but it is not clear that the recombination between distinct viral variants occurred after ATI. A recent study by Liu et al. (197) examined rebound sequences in SIV-infected macaques following ATI. Recombination was not evident in the rebound sequences but could be detected after several weeks of ongoing viral replication. Thus, it appears less likely that a recombination event is directly involved in the process of viral rebound.

A much simpler explanation for the differences between rebound viruses and predominant viral clones in the reservoir has been provided with the encouraging discovery that only a subset of the replication-competent proviruses persisting in the reservoir may be capable of causing rebound on interruption of ART, with elements of the immune response suppressing outgrowth of many reservoir viruses (198, 199). Bertagnolli et al. (198) used the QVOA to show that low concentrations of autologous neutralizing antibodies could block outgrowth of a significant but variable fraction of reservoir viruses. In 6 of 15 participants studied, outgrowth of more than 80% of reservoir viruses was blocked by purified autologous IgG. A phylogenetic relationship between rebound viruses and viruses that were resistant to neutralization by autologous IgG was evident. The mechanism was direct neutralization. In some participants, expanded cellular clones harbored replication-competent viruses that were readily neutralized by autologous IgG and unlikely to cause rebound. These findings are entirely consistent with previous studies in untreated individuals showing that the antibody response to HIV-1 is continually evolving in response to rapid viral evolution. Antibodies present at a given time point frequently neutralize viral variants present at earlier times but not contemporaneous viruses (200, 201). Given that viral variants can be deposited in the reservoir whenever there is active viral replication, it is not surprising that some viruses in the reservoir should be suppressed by autologous antibodies. The reservoir is likely to be very dynamic during untreated infection, and a recent study suggests that many viruses found in the reservoir may have entered shortly before initiation of ART (202). These newer variants may be less likely to be neutralized by the more slowly evolving antibody response. Strategies targeting the subpopulation of reservoir viruses not neutralized by autologous antibodies might produce a durable remission.

Other elements of the immune response may also control outgrowth of some reservoir viruses. Blankson and colleagues (203) have documented expanded $CD4^+$ T cell clones carrying replication-competent virus in individuals who control viral replication without ART or following interruption of ART. This control appears to be mediated by $CD8^+$ T cells. Hahn and colleagues (204) have shown that innate immune responses involving type I interferons function as a sieve and limit initial infection following mucosal transmission to viral variants with interferon resistance. In a more recent study, they demonstrated that rebound viruses also exhibit a high level of interferon resistance relative to viral variants detected in the latent reservoir prior to ATI (199). Together, these studies suggest that among the diverse viral variants archived in the latent reservoir, only a subset may be capable of evading host innate and adaptive immune responses and establishing rebound viremia (**Figure 2**).

CONCLUSIONS

The goal of HIV-1 cure efforts is to allow PLWH to stop ART without experiencing viral rebound. Recent studies of the in vivo dynamics of the latent reservoir allow for a new view of the

process of viral rebound. Rebound is a function of the size and composition of the latent reservoir. The population of cells carrying intact proviruses decays very slowly over time, but underlying the apparent stability of the reservoir are complex dynamics in which clones of infected cells expand in a process driven at least in part by antigen exposure. The clones can also undergo contraction. Stimulation of latently infected $CD4^+$ T cells through the TCR invariably leads to proliferation but does not always induce viral gene expression. Nevertheless, a fraction of reservoir cells are induced every day, giving rise to RV, and if treatment is interrupted, to viral rebound. Thus, in addition to reservoir size and composition, rebound depends on the presence of activating stimuli. Finally, only a subset of the viruses released from the reservoir are resistant to neutralizing antibodies and type I interferons. These are the viruses that actually cause rebound. It is to be hoped that a better understanding of this process will allow the development of effective cure strategies targeting these viruses.

DISCLOSURE STATEMENT

R.F.S. is an inventor on a patent application for the intact proviral DNA assay filed by Johns Hopkins University and licensed by AccelevirDx. He holds no equity interest in AccelevirDx.

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LITERATURE CITED

1. Saag MS, Gandhi RT, Hoy JF, Landovitz RJ, Thompson MA, et al. 2020. Antiretroviral drugs for treatment and prevention of HIV infection in adults: 2020 recommendations of the International Antiviral Society–USA panel. *JAMA* 324:1651–69
2. Davey RT Jr., Bhat N, Yoder C, Chun TW, Metcalf JA, et al. 1999. HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. *PNAS* 96:15109–14
3. Rothenberger MK, Keele BF, Wietgrefe SW, Fletcher CV, Beilman GJ, et al. 2015. Large number of rebounding/founder HIV variants emerge from multifocal infection in lymphatic tissues after treatment interruption. *PNAS* 112:1126–34
4. Li JZ, Etemad B, Ahmed H, Aga E, Bosch RJ, et al. 2016. The size of the expressed HIV reservoir predicts timing of viral rebound after treatment interruption. *AIDS* 30:343–53
5. Wen Y, Bar KJ, Li JZ. 2018. Lessons learned from HIV antiretroviral treatment interruption trials. *Curr. Opin. HIV AIDS* 13:416–21
6. Chun TW, Finzi D, Margolick J, Chadwick K, Schwartz D, Siliciano RF. 1995. In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. *Nat. Med.* 1:1284–90
7. Chun TW, Carruth L, Finzi D, Shen X, DiGiuseppe JA, et al. 1997. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* 387:183–88
8. Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, et al. 1997. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 278:1295–300
9. Wong JK, Hezareh M, Gunthard HF, Havlir DV, Ignacio CC, et al. 1997. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science* 278:1291–95

10. Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican JA, et al. 1997. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *PNAS* 94:13193–97
11. Coffin JM. 1992. Genetic diversity and evolution of retroviruses. *Curr. Top. Microbiol. Immunol.* 176:143–64
12. Mansky LM, Temin HM. 1995. Lower in vivo mutation rate of human immunodeficiency virus type 1 than that predicted from the fidelity of purified reverse transcriptase. *J. Virol.* 69:5087–94
13. Kieffer TL, Finucane MM, Nettles RE, Quinn TC, Broman KW, et al. 2004. Genotypic analysis of HIV-1 drug resistance at the limit of detection: virus production without evolution in treated adults with undetectable HIV loads. *J. Infect. Dis.* 189:1452–65
14. Bailey JR, Sedaghat AR, Kieffer T, Brennan T, Lee PK, et al. 2006. 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.* 80:6441–57
15. Evering TH, Mehandru S, Racz P, Tenner-Racz K, Poles MA, et al. 2012. Absence of HIV-1 evolution in the gut-associated lymphoid tissue from patients on combination antiviral therapy initiated during primary infection. *PLOS Pathog.* 8:e1002506
16. Josefsson L, von Stockenstrom S, Faria NR, Sinclair E, Bacchetti P, et al. 2013. The HIV-1 reservoir in eight patients on long-term suppressive antiretroviral therapy is stable with few genetic changes over time. *PNAS* 110:4987–96
17. Kearney MF, Spindler J, Shao W, Yu S, Anderson EM, et al. 2014. Lack of detectable HIV-1 molecular evolution during suppressive antiretroviral therapy. *PLOS Pathog.* 10:e1004010
18. Kearney MF, Anderson EM, Coomer C, Smith L, Shao W, et al. 2015. Well-mixed plasma and tissue viral populations in RT-SHIV-infected macaques implies a lack of viral replication in the tissues during antiretroviral therapy. *Retrovirology* 12:93
19. Lorenzo-Redondo R, Fryer HR, Bedford T, Kim EY, Archer J, et al. 2016. Persistent HIV-1 replication maintains the tissue reservoir during therapy. *Nature* 530(7588):51–56
20. Rosenbloom DIS, Hill AL, Laskey SB, Siliciano RF. 2016. Re-evaluating evolution in the HIV reservoir. *Nature* 551(7681):E6–9
21. Brodin J, Zanini F, Thebo L, Lanz C, Bratt G, et al. 2016. Establishment and stability of the latent HIV-1 DNA reservoir. *eLife* 5:e18889
22. Van Zyl GU, Katusiime MG, Wiegand A, McManus WR, Bale MJ, et al. 2017. No evidence of HIV replication in children on antiretroviral therapy. *J. Clin. Investig.* 127:3827–34
23. Lorenzi JC, Cohen YZ, Cohn LB, Kreider EF, Barton JP, et al. 2016. Paired quantitative and qualitative assessment of the replication-competent HIV-1 reservoir and comparison with integrated proviral DNA. *PNAS* 113:E7908–16
24. Hosmane NN, Kwon KJ, Bruner KM, Capoferri AA, Beg S, et al. 2017. Proliferation of latently infected CD4$^+$ T cells carrying replication-competent HIV-1: potential role in latent reservoir dynamics. *J. Exp. Med.* 214:959–72
25. Bui JK, Sobolewski MD, Keele BF, Spindler J, Musick A, et al. 2017. Proviruses with identical sequences comprise a large fraction of the replication-competent HIV reservoir. *PLOS Pathog.* 13:e1006283
26. Shen L, Peterson S, Sedaghat AR, McMahon MA, Callender M, et al. 2008. Dose-response curve slope sets class-specific limits on inhibitory potential of anti-HIV drugs. *Nat. Med.* 14:762–66
27. Shen L, Rabi SA, Sedaghat AR, Shan L, Lai J, et al. 2011. A critical subset model provides a conceptual basis for the high antiviral activity of major HIV drugs. *Sci. Transl. Med.* 3:91ra63
28. Jilek BL, Zarr M, Sampah ME, Rabi SA, Bullen CK, et al. 2012. A quantitative basis for antiretroviral therapy for HIV-1 infection. *Nat. Med.* 18:446–51
29. Pierson T, Hoffman TL, Blankson J, Finzi D, Chadwick K, et al. 2000. Characterization of chemokine receptor utilization of viruses in the latent reservoir for human immunodeficiency virus type 1. *J. Virol.* 74:7824–33
30. Shan L, Deng K, Gao H, Xing S, Capoferri AA, et al. 2017. Transcriptional reprogramming during effector-to-memory transition renders CD4$^+$ T cells permissive for latent HIV-1 infection. *Immunity* 47:766–75.e3
31. Baldauf HM, Pan X, Erikson E, Schmidt S, Daddacha W, et al. 2012. SAMHD1 restricts HIV-1 infection in resting CD4$^+$ T cells. *Nat. Med.* 18:1682–87

32. Descours B, Cribier A, Chable-Bessia C, Ayinde D, Rice G, et al. 2012. SAMHD1 restricts HIV-1 reverse transcription in quiescent CD4+ T-cells. *Retrovirology* 9:87
33. Ruffin N, Brezar V, Ayinde D, Lefebvre C, Schulze Zur Wiesch J, et al. 2015. Low SAMHD1 expression following T-cell activation and proliferation renders CD4+ T cells susceptible to HIV-1. *AIDS* 29:519–30
34. Nabel G, Baltimore D. 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature* 326:711–13
35. Bohnlein E, Lowenthal JW, Siekevitz M, Ballard DW, Franza BR, Greene WC. 1988. The same inducible nuclear proteins regulates mitogen activation of both the interleukin-2 receptor-alpha gene and type 1 HIV. *Cell* 53:827–36
36. Duh EJ, Maury WJ, Folks TM, Fauci AS, Rabson AB. 1989. Tumor necrosis factor α activates human immunodeficiency virus type 1 through induction of nuclear factor binding to the NF-κB sites in the long terminal repeat. *PNAS* 86:5974–78
37. Kao SY, Calman AF, Luciw PA, Peterlin BM. 1987. Anti-termination of transcription within the long terminal repeat of HIV-1 by *tat* gene product. *Nature* 330:489–93
38. Herrmann CH, Rice AP. 1995. Lentivirus Tat proteins specifically associate with a cellular protein kinase, TAK, that hyperphosphorylates the carboxyl-terminal domain of the large subunit of RNA polymerase II: candidate for a Tat cofactor. *J. Virol.* 69:1612–20
39. Zhu Y, Pe'ery T, Peng J, Ramanathan Y, Marshall N, et al. 1997. Transcription elongation factor P-TEFb is required for HIV-1 Tat transactivation in vitro. *Genes Dev*. 11:2622–32
40. Liou LY, Herrmann CH, Rice AP. 2002. Transient induction of cyclin T1 during human macrophage differentiation regulates human immunodeficiency virus type 1 Tat transactivation function. *J. Virol.* 76:10579–87
41. Wei X, Ghosh SK, Taylor ME, Johnson VA, Emini EA, et al. 1995. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* 373:117–22
42. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 373:123–26
43. Van Lint C, Emiliani S, Ott M, Verdin E. 1996. Transcriptional activation and chromatin remodeling of the HIV-1 promoter in response to histone acetylation. *EMBO J.* 15:1112–20
44. He G, Ylisastigui L, Margolis DM. 2002. The regulation of HIV-1 gene expression: the emerging role of chromatin. *DNA Cell Biol*. 21:697–705
45. Pearson R, Kim YK, Hokello J, Lassen K, Friedman J, et al. 2008. 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.* 82:12291–303
46. Jordan A, Bisgrove D, Verdin E. 2003. HIV reproducibly establishes a latent infection after acute infection of T cells in vitro. *EMBO J.* 22:1868–77
47. Takaki A, Wiese M, Maertens G, Depla E, Seifert U, et al. 2000. Cellular immune responses persist and humoral responses decrease two decades after recovery from a single-source outbreak of hepatitis C. *Nat. Med.* 6:578–82
48. Hammarlund E, Lewis MW, Hansen SG, Strelow LI, Nelson JA, et al. 2003. Duration of antiviral immunity after smallpox vaccination. *Nat. Med.* 9:1131–37
49. Swiggard WJ, Baytop C, Yu JJ, Dai J, Li C, et al. 2005. Human immunodeficiency virus type 1 can establish latent infection in resting CD4+ T cells in the absence of activating stimuli. *J. Virol.* 79:14179–88
50. Chavez L, Calvanese V, Verdin E. 2015. HIV latency is established directly and early in both resting and activated primary CD4 T cells. *PLOS Pathog*. 11:e1004955
51. Saleh S, Solomon A, Wightman F, Xhilaga M, Cameron PU, Lewin SR. 2007. 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* 110:4161–64
52. Chun TW, Engel D, Berrey MM, Shea T, Corey L, Fauci AS. 1998. Early establishment of a pool of latently infected, resting CD4+ T cells during primary HIV-1 infection. *PNAS* 95:8869–73
53. Whitney JB, Hill AL, Sanisetty S, Penaloza-MacMaster P, Liu J, et al. 2014. Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. *Nature* 512:74–77

54. Luzuriaga K, Gay H, Ziemniak C, Sanborn KB, Somasundaran M, et al. 2015. Viremic relapse after HIV-1 remission in a perinatally infected child. *N. Engl. J. Med.* 372:786–88
55. Colby DJ, Trautmann L, Pinyakorn S, Leyre L, Pagliuzza A, et al. 2018. Rapid HIV RNA rebound after antiretroviral treatment interruption in persons durably suppressed in Fiebig I acute HIV infection. *Nat. Med.* 24:923–26
56. Korin YD, Brooks DG, Brown S, Korotzer A, Zack JA. 2002. Effects of prostratin on T-cell activation and human immunodeficiency virus latency. *J. Virol.* 76:8118–23
57. Scripture-Adams DD, Brooks DG, Korin YD, Zack JA. 2002. Interleukin-7 induces expression of latent human immunodeficiency virus type 1 with minimal effects on T-cell phenotype. *J. Virol.* 76:13077–82
58. Brooks DG, Hamer DH, Arlen PA, Gao L, Bristol G, et al. 2003. Molecular characterization, reactivation, and depletion of latent HIV. *Immunity* 19:413–23
59. Ylisastigui L, Archin NM, Lehrman G, Bosch RJ, Margolis DM. 2004. Coaxing HIV-1 from resting CD4 T cells: Histone deacetylase inhibition allows latent viral expression. *AIDS* 18:1101–8
60. Williams SA, Chen LF, Kwon H, Fenard D, Bisgrove D, et al. 2004. Prostratin antagonizes HIV latency by activating NF-κB. *J. Biol. Chem.* 279:42008–17
61. Archin NM, Liberty AL, Kashuba AD, Choudhary SK, Kuruc JD, et al. 2012. Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature* 487:482–85
62. Borducchi EN, Cabral C, Stephenson KE, Liu J, Abbink P, et al. 2016. Ad26/MVA therapeutic vaccination with TLR7 stimulation in SIV-infected rhesus monkeys. *Nature* 540:284–87
63. Borducchi EN, Liu J, Nkolola JP, Cadena AM, Yu WH, et al. 2018. Antibody and TLR7 agonist delay viral rebound in SHIV-infected monkeys. *Nature* 563:360–64
64. Nixon CC, Mavigner M, Sampey GC, Brooks AD, Spagnuolo RA, et al. 2020. Systemic HIV and SIV latency reversal via non-canonical NF-κB signalling in vivo. *Nature* 578:160–65
65. Spivak AM, Planelles V. 2016. HIV-1 eradication: early trials (and tribulations). *Trends Mol. Med.* 22:10–27
66. Folks T, Powell DM, Lightfoote MM, Benn S, Martin MA, Fauci AS. 1986. Induction of HTLV-III/LAV from a nonvirus-producing T-cell line: implications for latency. *Science* 231:600–2
67. Sahu GK, Lee K, Ji J, Braciale V, Baron S, Cloyd MW. 2006. A novel in vitro system to generate and study latently HIV-infected long-lived normal CD4+ T-lymphocytes. *Virology* 355:127–37
68. Yang HC, Xing S, Shan L, O'Connell K, Dinoso J, et al. 2009. Small-molecule screening using a human primary cell model of HIV latency identifies compounds that reverse latency without cellular activation. *J. Clin. Investig.* 119:3473–86
69. Tyagi M, Pearson RJ, Karn J. 2010. Establishment of HIV latency in primary $CD4^+$ cells is due to epigenetic transcriptional silencing and P-TEFb restriction. *J. Virol.* 84:6425–37
70. Mbonye U, Karn J. 2017. The molecular basis for human immunodeficiency virus latency. *Annu. Rev. Virol.* 4:261–85
71. Dutilleul A, Rodari A, Van Lint C. 2020. Depicting HIV-1 transcriptional mechanisms: a summary of what we know. *Viruses* 12:1385
72. Crooks AM, Bateson R, Cope AB, Dahl NP, Griggs MK, et al. 2015. Precise quantitation of the latent HIV-1 reservoir: implications for eradication strategies. *J. Infect. Dis.* 212:1361–65
73. Laird GM, Rosenbloom DI, Lai J, Siliciano RF, Siliciano JD. 2016. Measuring the frequency of latent HIV-1 in resting $CD4^+$ T cells using a limiting dilution coculture assay. *Methods Mol. Biol.* 1354:239–53
74. Hermankova M, Siliciano JD, Zhou Y, Monie D, Chadwick K, et al. 2003. Analysis of human immunodeficiency virus type 1 gene expression in latently infected resting $CD4^+$ T lymphocytes in vivo. *J. Virol.* 77:7383–92
75. Bullen CK, Laird GM, Durand CM, Siliciano JD, Siliciano RF. 2014. New ex vivo approaches distinguish effective and ineffective single agents for reversing HIV-1 latency in vivo. *Nat. Med.* 20:425–29
76. Laird GM, Bullen CK, Rosenbloom DI, Martin AR, Hill AL, et al. 2015. Ex vivo analysis identifies effective HIV-1 latency-reversing drug combinations. *J. Clin. Investig.* 125:1901–12
77. Prins JM, Jurriaans S, van Praag RM, Blaak H, van Rij R, et al. 1999. Immuno-activation with anti-CD3 and recombinant human IL-2 in HIV-1-infected patients on potent antiretroviral therapy. *AIDS* 13:2405–10

78. Ho YC, Shan L, Hosmane NN, Wang J, Laskey SB, et al. 2013. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell* 155:540–51
79. Musick A, Spindler J, Boritz E, Perez L, Crespo-Velez D, et al. 2019. HIV infected T cells can proliferate in vivo without inducing expression of the integrated provirus. *Front. Microbiol.* 10:2204
80. Kwon KJ, Timmons AE, Sengupta S, Simonetti FR, Zhang H, et al. 2020. Different human resting memory $CD4^+$ T cell subsets show similar low inducibility of latent HIV-1 proviruses. *Sci. Transl. Med.* 12:eaax6795
81. Honeycutt JB, Wahl A, Baker C, Spagnuolo RA, Foster J, et al. 2016. Macrophages sustain HIV replication in vivo independently of T cells. *J. Clin. Investig.* 126:1353–66
82. Avalos CR, Abreu CM, Queen SE, Li M, Price S, et al. 2017. Brain macrophages in simian immunodeficiency virus-infected, antiretroviral-suppressed macaques: a functional latent reservoir. *mBio* 8:e01186-17
83. Andrade VM, Mavian C, Babic D, Cordeiro T, Sharkey M, et al. 2020. A minor population of macrophage-tropic HIV-1 variants is identified in recrudescing viremia following analytic treatment interruption. *PNAS* 117:9981–90
84. Stevenson M. 2017. HIV persistence in macrophages. *Nat. Med.* 23:538–39
85. Veenhuis RT, Abreu CM, Shirk EN, Gama L, Clements JE. 2021. HIV replication and latency in monocytes and macrophages. *Semin. Immunol.* 51:101472
86. Bruner KM, Hosmane NN, Siliciano RF. 2015. Towards an HIV-1 cure: measuring the latent reservoir. *Trends Microbiol.* 23:192–203
87. Massanella M, Yek C, Lada SM, Nakazawa M, Shefa N, et al. 2018. Improved assays to measure and characterize the inducible HIV reservoir. *EBioMedicine* 36:113–21
88. Abdel-Mohsen M, Richman D, Siliciano RF, Nussenzweig MC, Howell BJ, et al. 2020. Recommendations for measuring HIV reservoir size in cure-directed clinical trials. *Nat. Med.* 26:1339–50
89. Zack JA, Arrigo SJ, Weitsman SR, Go AS, Haislip A, Chen IS. 1990. HIV-1 entry into quiescent primary lymphocytes: Molecular analysis reveals a labile, latent viral structure. *Cell* 61:213–22
90. Bukrinsky MI, Stanwick TL, Dempsey MP, Stevenson M. 1991. Quiescent T lymphocytes as an inducible virus reservoir in HIV-1 infection. *Science* 254:423–27
91. Pierson TC, Kieffer TL, Ruff CT, Buck C, Gange SJ, Siliciano RF. 2002. Intrinsic stability of episomal circles formed during human immunodeficiency virus type 1 replication. *J. Virol.* 76:4138–44
92. Pierson TC, Zhou Y, Kieffer TL, Ruff CT, Buck C, Siliciano RF. 2002. Molecular characterization of preintegration latency in human immunodeficiency virus type 1 infection. *J. Virol.* 76:8518–31
93. Zhou Y, Zhang H, Siliciano JD, Siliciano RF. 2005. Kinetics of human immunodeficiency virus type 1 decay following entry into resting $CD4^+$ T cells. *J. Virol.* 79:2199–210
94. Blankson JN, Finzi D, Pierson TC, Sabundayo BP, Chadwick K, et al. 2000. Biphasic decay of latently infected $CD4^+$ T cells in acute human immunodeficiency virus type 1 infection. *J. Infect. Dis.* 182:1636–42
95. Sanchez G, Xu X, Chermann JC, Hirsch I. 1997. Accumulation of defective viral genomes in peripheral blood mononuclear cells of human immunodeficiency virus type 1-infected individuals. *J. Virol.* 71:2233–40
96. Sheehy AM, Gaddis NC, Choi JD, Malim MH. 2002. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature* 418:646–50
97. Eriksson S, Graf EH, Dahl V, Strain MC, Yukl SA, et al. 2013. Comparative analysis of measures of viral reservoirs in HIV-1 eradication studies. *PLOS Pathog.* 9:e1003174
98. Siliciano JD, Siliciano RF. 2005. Enhanced culture assay for detection and quantitation of latently infected, resting $CD4^+$ T-cells carrying replication-competent virus in HIV-1-infected individuals. *Methods Mol. Biol.* 304:3–15
99. Laird GM, Eisele EE, Rabi SA, Lai J, Chioma S, et al. 2013. Rapid quantification of the latent reservoir for HIV-1 using a viral outgrowth assay. *PLOS Pathog.* 9:e1003398
100. Rosenbloom DI, Elliott O, Hill AL, Henrich TJ, Siliciano JM, Siliciano RF. 2015. Designing and interpreting limiting dilution assays: general principles and applications to the latent reservoir for human immunodeficiency virus-1. *Open Forum. Infect. Dis.* 2:ofv123

101. Finzi D, Blankson J, Siliciano JD, Margolick JB, Chadwick K, et al. 1999. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat. Med.* 5:512–17
102. Siliciano JD, Kajdas J, Finzi D, Quinn TC, Chadwick K, et al. 2003. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. *Nat. Med.* 9:727–28
103. Imamichi H, Dewar RL, Adelsberger JW, Rehm CA, O'Doherty U, et al. 2016. Defective HIV-1 proviruses produce novel protein-coding RNA species in HIV-infected patients on combination antiretroviral therapy. *PNAS* 113:8783–88
104. Bruner KM, Murray AJ, Pollack RA, Soliman MG, Laskey SB, et al. 2016. Defective proviruses rapidly accumulate during acute HIV-1 infection. *Nat. Med.* 22:1043–49
105. Delviks-Frankenberry K, Galli A, Nikolaitchik O, Mens H, Pathak VK, Hu WS. 2011. Mechanisms and factors that influence high frequency retroviral recombination. *Viruses* 3:1650–80
106. Bruner KM, Wang Z, Simonetti FR, Bender AM, Kwon KJ, et al. 2019. A quantitative approach for measuring the reservoir of latent HIV-1 proviruses. *Nature* 566:120–25
107. Bender AM, Simonetti FR, Kumar MR, Fray EJ, Bruner KM, et al. 2019. The landscape of persistent viral genomes in ART-treated SIV, SHIV, and HIV-2 infections. *Cell Host Microbe* 26:73–85.e4
108. Harris RS, Bishop KN, Sheehy AM, Craig HM, Petersen-Mahrt SK, et al. 2003. DNA deamination mediates innate immunity to retroviral infection. *Cell* 113:803–9
109. Wiegand HL, Doehle BP, Bogerd HP, Cullen BR. 2004. A second human antiretroviral factor, APOBEC3F, is suppressed by the HIV-1 and HIV-2 Vif proteins. *EMBO J.* 23:2451–58
110. Hiener B, Horsburgh BA, Eden JS, Barton K, Schlub TE, et al. 2017. Identification of genetically intact HIV-1 proviruses in specific CD4+ T cells from effectively treated participants. *Cell Rep.* 21:813–22
111. Sharaf R, Lee GQ, Sun X, Etemad B, Aboukhater LM, et al. 2018. HIV-1 proviral landscapes distinguish posttreatment controllers from noncontrollers. *J. Clin. Investig.* 128:4074–85
112. Cohn LB, Silva IT, Oliveira TY, Rosales RA, Parrish EH, et al. 2015. HIV-1 integration landscape during latent and active infection. *Cell* 160:420–32
113. Simonetti FR, White JA, Tumiotto C, Ritter KD, Cai M, et al. 2020. Intact proviral DNA assay analysis of large cohorts of people with HIV provides a benchmark for the frequency and composition of persistent proviral DNA. *PNAS* 117:18692–700
114. Peluso MJ, Bacchetti P, Ritter KD, Beg S, Lai J, et al. 2020. Differential decay of intact and defective proviral DNA in HIV-1-infected individuals on suppressive antiretroviral therapy. *JCI Insight*. 5:e132997
115. Kinloch NN, Ren Y, Conce Alberto WD, Dong W, Khadka P, et al. 2021. HIV-1 diversity considerations in the application of the intact proviral DNA assay (IPDA). *Nat. Commun.* 12:165
116. Gaebler C, Falcinelli SD, Stoffel E, Read J, Murtagh R, et al. 2021. Sequence evaluation and comparative analysis of novel assays for intact proviral HIV-1 DNA. *J. Virol.* 95:e01986-20
117. Gandhi RT, Cyktor JC, Bosch RJ, Mar H, Laird GM, et al. 2020. Selective decay of intact HIV-1 proviral DNA on antiretroviral therapy. *J. Infect. Dis.* 223:225–33
118. Gandhi R, Cyktor JC, Bosch R, Mar H, Laird G, et al. 2020. *Intact proviral DNA levels decline in people with HIV on antiretroviral therapy.* Paper presented at Conference on Retroviruses and Opportunistic Infections, Boston, MA, March 8–11. **https://www.natap.org/2020/CROI/croi_236.htm**
119. Einkauf KB, Lee GQ, Gao C, Sharaf R, Sun X, et al. 2019. Intact HIV-1 proviruses accumulate at distinct chromosomal positions during prolonged antiretroviral therapy. *J. Clin. Investig.* 129:988–98
120. Antar AA, Jenike KM, Jang S, Rigau DN, Reeves DB, et al. 2020. Longitudinal study reveals HIV-1-infected CD4+ T cell dynamics during long-term antiretroviral therapy. *J. Clin. Investig.* 130:3543–59
121. Jiang C, Lian X, Gao C, Sun X, Einkauf KB, et al. 2020. Distinct viral reservoirs in individuals with spontaneous control of HIV-1. *Nature* 585:261–67
122. Fromentin R, Chomont N. 2021. HIV persistence in subsets of CD4+ T cells: 50 shades of reservoirs. *Semin. Immunol.* 51:101438
123. Descours B, Petitjean G, Lopez-Zaragoza JL, Bruel T, Raffel R, et al. 2017. CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses. *Nature* 543:564–67
124. Bertagnolli LN, White JA, Simonetti FR, Beg SA, Lai J, et al. 2018. The role of CD32 during HIV-1 infection. *Nature* 561:E17–19

125. Perez L, Anderson J, Chipman J, Thorkelson A, Chun TW, et al. 2018. Conflicting evidence for HIV enrichment in CD32$^+$ CD4 T cells. *Nature* 561:E9–16
126. Osuna CE, Lim SY, Kublin JL, Apps R, Chen E, et al. 2018. Evidence that CD32a does not mark the HIV-1 latent reservoir. *Nature* 561:E20–28
127. Cohn LB, da Silva IT, Valieris R, Huang AS, Lorenzi JCC, et al. 2018. Clonal CD4$^+$ T cells in the HIV-1 latent reservoir display a distinct gene profile upon reactivation. *Nat. Med.* 24:604–9
128. Pardons M, Baxter AE, Massanella M, Pagliuzza A, Fromentin R, et al. 2019. Single-cell characterization and quantification of translation-competent viral reservoirs in treated and untreated HIV infection. *PLOS Pathog.* 15:e1007619
129. Liu R, Yeh YJ, Varabyou A, Collora JA, Sherrill-Mix S, et al. 2020. Single-cell transcriptional landscapes reveal HIV-1-driven aberrant host gene transcription as a potential therapeutic target. *Sci. Transl. Med.* 12:eaaz0802
130. Neidleman J, Luo X, Frouard J, Xie G, Hsiao F, et al. 2020. Phenotypic analysis of the unstimulated in vivo HIV CD4 T cell reservoir. *eLife* 9:e60933
131. Chomont N, El-Far M, Ancuta P, Trautmann L, Procopio FA, et al. 2009. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat. Med.* 15:893–900
132. Perreau M, Savoye AL, De Crignis E, Corpataux JM, Cubas R, et al. 2013. Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. *J. Exp. Med.* 210:143–56
133. Buzon MJ, Martin-Gayo E, Pereyra F, Ouyang Z, Sun H, et al. 2014. Long-term antiretroviral treatment initiated at primary HIV-1 infection affects the size, composition, and decay kinetics of the reservoir of HIV-1-infected CD4 T cells. *J. Virol.* 88:10056–65
134. Buzon MJ, Sun H, Li C, Shaw A, Seiss K, et al. 2014. HIV-1 persistence in CD4$^+$ T cells with stem cell-like properties. *Nat. Med.* 20:139–42
135. Soriano-Sarabia N, Bateson RE, Dahl NP, Crooks AM, Kuruc JD, et al. 2014. Quantitation of replication-competent HIV-1 in populations of resting CD4$^+$ T cells. *J. Virol.* 88:14070–77
136. Sun H, Kim D, Li X, Kiselinova M, Ouyang Z, et al. 2015. Th1/17 polarization of CD4 T cells supports HIV-1 persistence during antiretroviral therapy. *J. Virol.* 89:11284–93
137. Banga R, Procopio FA, Noto A, Pollakis G, Cavassini M, et al. 2016. PD-1 and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals. *Nat. Med.* 22:754–61
138. Baxter AE, Niessl J, Fromentin R, Richard J, Porichis F, et al. 2016. Single-cell characterization of viral translation-competent reservoirs in HIV-infected individuals. *Cell Host Microbe* 20:368–80
139. Lee GQ, Orlova-Fink N, Einkauf K, Chowdhury FZ, Sun X, et al. 2017. Clonal expansion of genome-intact HIV-1 in functionally polarized Th1 CD4$^+$ T cells. *J. Clin. Investig.* 127:2689–96
140. Kulpa DA, Talla A, Brehm JH, Ribeiro SP, Yuan S, et al. 2019. Differentiation into an effector memory phenotype potentiates HIV-1 latency reversal in CD4$^+$ T cells. *J. Virol.* 93:e00969-19
141. Ruterbusch M, Pruner KB, Shehata L, Pepper M. 2020. In vivo CD4$^+$ T cell differentiation and function: revisiting the Th1/Th2 paradigm. *Annu. Rev. Immunol.* 38:705–25
142. Vibholm LK, Lorenzi JCC, Pai JA, Cohen YZ, Oliveira TY, et al. 2019. Characterization of intact proviruses in blood and lymph node from HIV-infected individuals undergoing analytical treatment interruption. *J. Virol.* 93:e01920-18
143. Martin AR, Bender AM, Hackman J, Kwon KJ, Lynch BA, et al. 2020. Similar frequency and inducibility of intact HIV-1 proviruses in blood and lymph nodes. *J. Infect. Dis.* 224:258–68
144. Bozzi G, Simonetti FR, Watters SA, Anderson EM, Gouzoulis M, et al. 2019. No evidence of ongoing HIV replication or compartmentalization in tissues during combination antiretroviral therapy: implications for HIV eradication. *Sci. Adv.* 5:eaav2045
145. Lee M, Mandl JN, Germain RN, Yates AJ. 2012. The race for the prize: T-cell trafficking strategies for optimal surveillance. *Blood* 120:1432–38
146. Chun TW, Nickle DC, Justement JS, Meyers JH, Roby G, et al. 2008. Persistence of HIV in gut-associated lymphoid tissue despite long-term antiretroviral therapy. *J. Infect. Dis.* 197:714–20
147. Estes JD, Kityo C, Ssali F, Swainson L, Makamdop KN, et al. 2017. Defining total-body AIDS-virus burden with implications for curative strategies. *Nat. Med.* 23:1271–76

148. Cadena AM, Ventura JD, Abbink P, Borducchi EN, Tuyishime H, et al. 2021. Persistence of viral RNA in lymph nodes in ART-suppressed SIV/SHIV-infected rhesus macaques. *Nat. Commun.* 12:1474
149. De Scheerder MA, Vrancken B, Dellicour S, Schlub T, Lee E, et al. 2019. HIV rebound is predominantly fueled by genetically identical viral expansions from diverse reservoirs. *Cell Host Microbe* 26:347–58.e7
150. Shankarappa R, Margolick JB, Gange SJ, Rodrigo AG, Upchurch D, et al. 1999. Consistent viral evolutionary changes associated with the progression of human immunodeficiency virus type 1 infection. *J. Virol.* 73:10489–502
151. Dornadula G, Zhang H, VanUitert B, Stern J, Livornese L Jr., et al. 1999. Residual HIV-1 RNA in blood plasma of patients taking suppressive highly active antiretroviral therapy. *JAMA* 282:1627–32
152. Palmer S, Wiegand AP, Maldarelli F, Bazmi H, Mican JM, et al. 2003. New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. *J. Clin. Microbiol.* 41:4531–36
153. Maldarelli F, Palmer S, King MS, Wiegand A, Polis MA, et al. 2007. ART suppresses plasma HIV-1 RNA to a stable set point predicted by pretherapy viremia. *PLOS Pathog.* 3:e46
154. Dinoso JB, Kim SY, Wiegand AM, Palmer SE, Gange SJ, et al. 2009. Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. *PNAS* 106:9403–8
155. Gandhi RT, Zheng L, Bosch RJ, Chan ES, Margolis DM, et al. 2010. The effect of raltegravir intensification on low-level residual viremia in HIV-infected patients on antiretroviral therapy: a randomized controlled trial. *PLOS Med.* 7:e1000321
156. Tobin NH, Learn GH, Holte SE, Wang Y, Melvin AJ, et al. 2005. Evidence that low-level viremias during effective highly active antiretroviral therapy result from two processes: expression of archival virus and replication of virus. *J. Virol.* 79:9625–34
157. Wang Z, Gurule EE, Brennan TP, Gerold JM, Kwon KJ, et al. 2018. Expanded cellular clones carrying replication-competent HIV-1 persist, wax, and wane. *PNAS* 115:E2575–84
158. Schroder AR, Shinn P, Chen H, Berry C, Ecker JR, Bushman F. 2002. HIV-1 integration in the human genome favors active genes and local hotspots. *Cell* 110:521–29
159. Han Y, Lassen K, Monie D, Sedaghat AR, Shimoji S, et al. 2004. 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.* 78:6122–33
160. Maldarelli F, Wu X, Su L, Simonetti FR, Shao W, et al. 2014. Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. *Science* 345:179–83
161. Wagner TA, McLaughlin S, Garg K, Cheung CY, Larsen BB, et al. 2014. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. *Science* 345:570–73
162. Cesana D, Santoni de Sio FR, Rudilosso L, Gallina P, Calabria A, et al. 2017. HIV-1-mediated insertional activation of *STAT5B* and *BACH2* trigger viral reservoir in T regulatory cells. *Nat. Commun.* 8:498
163. Bushman FD. 2020. Retroviral insertional mutagenesis in humans: evidence for four genetic mechanisms promoting expansion of cell clones. *Mol. Ther.* 28:352–56
164. Simonetti FR, Sobolewski MD, Fyne E, Shao W, Spindler J, et al. 2016. Clonally expanded CD4$^+$ T cells can produce infectious HIV-1 in vivo. *PNAS* 113:1883–88
165. Reeves DB, Duke ER, Wagner TA, Palmer SE, Spivak AM, Schiffer JT. 2018. A majority of HIV persistence during antiretroviral therapy is due to infected cell proliferation. *Nat. Commun.* 9:4811–15
166. Coffin JM, Wells DW, Zerbato JM, Kuruc JD, Guo S, et al. 2019. Clones of infected cells arise early in HIV-infected individuals. *JCI Insight.* 4:e128432
167. Simonetti FR, Zhang H, Soroosh GP, Duan J, Rhodehouse K, et al. 2021. Antigen-driven clonal selection shapes the persistence of HIV-1-infected CD4$^+$ T cells in vivo. *J. Clin. Investig.* 131:e145254
168. Boyman O, Krieg C, Homann D, Sprent J. 2012. Homeostatic maintenance of T cells and natural killer cells. *Cell. Mol. Life Sci.* 69:1597–608
169. Henrich TJ, Hobbs KS, Hanhauser E, Scully E, Hogan LE, et al. 2017. Human immunodeficiency virus type 1 persistence following systemic chemotherapy for malignancy. *J. Infect. Dis.* 216:254–62
170. Mendoza P, Jackson JR, Oliveira TY, Gaebler C, Ramos V, et al. 2020. Antigen-responsive CD4$^+$ T cell clones contribute to the HIV-1 latent reservoir. *J. Exp. Med.* 217:e20200051

171. Gantner P, Pagliuzza A, Pardons M, Ramgopal M, Routy JP, et al. 2020. Single-cell TCR sequencing reveals phenotypically diverse clonally expanded cells harboring inducible HIV proviruses during ART. *Nat. Commun.* 11:4089–88
172. Reeves DB, Duke ER, Hughes SM, Prlic M, Hladik F, Schiffer JT. 2017. Anti-proliferative therapy for HIV cure: a compound interest approach. *Sci. Rep.* 7:4011
173. Hataye JM, Casazza JP, Best K, Liang CJ, Immonen TT, et al. 2019. Principles governing establishment versus collapse of HIV-1 cellular spread. *Cell Host Microbe* 26:748–63.e20
174. Kulpa DA, Talla A, Brehm JH, Ribeiro SP, Yuan S, et al. 2019. Differentiation into an effector memory phenotype potentiates HIV-1 latency reversal in $CD4^+$ T cells. *J. Virol.* 93:e00969-19
175. Bosque A, Famiglietti M, Weyrich AS, Goulston C, Planelles V. 2011. Homeostatic proliferation fails to efficiently reactivate HIV-1 latently infected central memory CD4+ T cells. *PLOS Pathog.* 7:e1002288
176. Halvas EK, Joseph KW, Brandt LD, Guo S, Sobolewski MD, et al. 2020. HIV-1 viremia not suppressible by antiretroviral therapy can originate from large T cell clones producing infectious virus. *J. Clin. Investig.* 130:5847–57
177. Weinberger LS, Burnett JC, Toettcher JE, Arkin AP, Schaffer DV. 2005. Stochastic gene expression in a lentiviral positive-feedback loop: HIV-1 Tat fluctuations drive phenotypic diversity. *Cell* 122:169–82
178. Lenasi T, Contreras X, Peterlin BM. 2008. Transcriptional interference antagonizes proviral gene expression to promote HIV latency. *Cell Host Microbe* 4:123–33
179. Shan L, Yang HC, Rabi SA, Bravo HC, Shroff NS, et al. 2011. Influence of host gene transcription level and orientation on HIV-1 latency in a primary-cell model. *J. Virol.* 85:5384–93
180. Hill AL, Rosenbloom DI, Fu F, Nowak MA, Siliciano RF. 2014. Predicting the outcomes of treatment to eradicate the latent reservoir for HIV-1. *PNAS* 111:13475–80
181. Cockerham LR, Hatano H, Deeks SG. 2016. Post-treatment controllers: role in HIV "cure" research. *Curr. HIV/AIDS Rep.* 13:1–9
182. Henrich TJ, Hanhauser E, Marty FM, Sirignano MN, Keating S, et al. 2014. Antiretroviral-free HIV-1 remission and viral rebound after allogeneic stem cell transplantation: report of 2 cases. *Ann. Intern. Med.* 161:319–27
183. Hutter G, Nowak D, Mossner M, Ganepola S, Mussig A, et al. 2009. Long-term control of HIV by *CCR5* Delta32/Delta32 stem-cell transplantation. *N. Engl. J. Med.* 360:692–98
184. Gupta RK, Abdul-Jawad S, McCoy LE, Mok HP, Peppa D, et al. 2019. HIV-1 remission following CCR5Δ32/Δ32 haematopoietic stem-cell transplantation. *Nature* 568:244–48
185. Henrich TJ, Hatano H, Bacon O, Hogan LE, Rutishauser R, et al. 2017. HIV-1 persistence following extremely early initiation of antiretroviral therapy (ART) during acute HIV-1 infection: an observational study. *PLOS Med.* 14:e1002417
186. Bar KJ, Sneller MC, Harrison LJ, Justement JS, Overton ET, et al. 2016. Effect of HIV antibody VRC01 on viral rebound after treatment interruption. *N. Engl. J. Med.* 375:2037–50
187. Mendoza P, Gruell H, Nogueira L, Pai JA, Butler AL, et al. 2018. Combination therapy with anti-HIV-1 antibodies maintains viral suppression. *Nature* 561:479–84
188. Giron LB, Papasavvas E, Azzoni L, Yin X, Anzurez A, et al. 2020. Plasma and antibody glycomic biomarkers of time to HIV rebound and viral setpoint. *AIDS* 34:681–86
189. Offersen R, Yu WH, Scully EP, Julg B, Euler Z, et al. 2020. HIV antibody Fc N-linked glycosylation is associated with viral rebound. *Cell Rep.* 33:108502
190. Pasternak AO, Grijsen ML, Wit FW, Bakker M, Jurriaans S, et al. 2020. Cell-associated HIV-1 RNA predicts viral rebound and disease progression after discontinuation of temporary early ART. *JCI Insight.* 5:e134196
191. De Scheerder MA, Van Hecke C, Zetterberg H, Fuchs D, De Langhe N, et al. 2020. Evaluating predictive markers for viral rebound and safety assessment in blood and lumbar fluid during HIV-1 treatment interruption. *J. Antimicrob. Chemother.* 75:1311–20
192. Prator CA, Thanh C, Kumar S, Pan T, Peluso MJ, et al. 2020. Circulating $CD30^+CD4^+$ T cells increase before human immunodeficiency virus rebound after analytical antiretroviral treatment interruption. *J. Infect. Dis.* 221:1146–55
193. Salantes DB, Zheng Y, Mampe F, Srivastava T, Beg S, et al. 2018. HIV-1 latent reservoir size and diversity are stable following brief treatment interruption. *J. Clin. Investig.* 128:3102–15

194. Lu CL, Pai JA, Nogueira L, Mendoza P, Gruell H, et al. 2018. Relationship between intact HIV-1 proviruses in circulating $CD4^+$ T cells and rebound viruses emerging during treatment interruption. *PNAS* 115:E11341–48
195. Cohen YZ, Lorenzi JCC, Krassnig L, Barton JP, Burke L, et al. 2018. Relationship between latent and rebound viruses in a clinical trial of anti-HIV-1 antibody 3BNC117. *J. Exp. Med.* 215:2311–24
196. Onafuwa-Nuga A, Telesnitsky A. 2009. The remarkable frequency of human immunodeficiency virus type 1 genetic recombination. *Microbiol. Mol. Biol. Rev.* 73:451–80
197. Liu PT, Keele BF, Abbink P, Mercado NB, Liu J, et al. 2020. Origin of rebound virus in chronically SIV-infected rhesus monkeys following treatment discontinuation. *Nat. Commun.* 11:5412
198. Bertagnolli LN, Varriale J, Sweet S, Brockhurst J, Simonetti FR, et al. 2020. Autologous IgG antibodies block outgrowth of a substantial but variable fraction of viruses in the latent reservoir for HIV-1. *PNAS* 117:32066–77
199. Gondim MVP, Sherrill-Mix S, Bibollet-Ruche F, Russell RM, Trimboli S, et al. 2021. Heightened resistance to host type 1 interferons characterizes HIV-1 at transmission and after antiretroviral therapy interruption. *Sci. Transl. Med.* 13:eabd8179
200. Wei X, Decker JM, Wang S, Hui H, Kappes JC, et al. 2003. Antibody neutralization and escape by HIV-1. *Nature* 422:307–12
201. Richman DD, Wrin T, Little SJ, Petropoulos CJ. 2003. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. *PNAS* 100:4144–49
202. Abrahams MR, Joseph SB, Garrett N, Tyers L, Moeser M, et al. 2019. The replication-competent HIV-1 latent reservoir is primarily established near the time of therapy initiation. *Sci. Transl. Med.* 11:eaaw5589
203. Veenhuis RT, Kwaa AK, Garliss CC, Latanich R, Salgado M, et al. 2018. Long-term remission despite clonal expansion of replication-competent HIV-1 isolates. *JCI Insight*. 3:e122795
204. Iyer SS, Bibollet-Ruche F, Sherrill-Mix S, Learn GH, Plenderleith L, et al. 2017. Resistance to type 1 interferons is a major determinant of HIV-1 transmission fitness. *PNAS* 114:E590–99

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Errata

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