REVIEW

Human Immunodeficiency Virus 1 (HIV-1): Viral Latency, the Reservoir, and the Cure

John D. Ventura*

Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

An estimated 37 million people globally suffer from Human Immunodeficiency Virus-1 (HIV-1) infection with 1.7 million newly acquired infections occurring on average each year. Although crucial advances in combined antiretroviral therapy (ART) over the last two decades have transformed an HIV-1 diagnosis into a tolerable and controlled condition, enabling over 20 million people living with HIV-1 to enjoy healthy and productive lives, no cure or vaccine yet exists. Developing a successful cure strategy will require a firm understanding of how viral latency is established and how a persistent and long-lived latent is generated. The latent reservoir remains the primary obstacle for cure development and most putative cure strategies proposed fundamentally address its eradication or permanent suppression.

INTRODUCTION

Human Immunodeficiency Virus-1 (HIV-1) remains a clinical burden after more than three decades following its discovery as the cause of acquired immunodeficiency syndrome (AIDS). Until the introduction of highly effective combined antiretroviral therapy (ART) in 1995, a positive HIV-1 diagnosis was generally accepted as a death sentence. Today, with more effective daily ART regimens available every year, HIV-1 has become a manageable condition during which people living with HIV-1 lead productive and otherwise healthy lives. It is apparent, however, that ART is not a cure and cannot completely clear the infection because any protracted interruption in the daily ART schedule will lead to rebound of viral replication and clinical symptoms. ART functions to inhibit viral replication of a new generation of target cells but it cannot eradicate a small but robust long-lived cellular reservoir of infected cells that have archived replication-competent viral DNA. The cellular latent reservoir is generated by the capacity of HIV-1 to integrate a DNA copy of its genome, reverse transcribed from two RNA

*To whom all correspondence should be addressed: Dr. John D. Ventura, Email: jdventur@bidmc.harvard.edu. ORCID iD: 0000-0002-4373-3242.

genomic templates, into the genome of infected host cells. Reservoir persistence is maintained despite daily ART treatment and as a result, there remains no cure, full or functional, for HIV-1 infection.

This review provides a comprehensive overview of our current understanding of the HIV-1 viral latency and the latent reservoir with a focus on current cure strategies designed to prevent viral rebound and promote viral remission in patients undergoing therapy interruption. A brief examination on promising cure strategies will follow, concentrating on how the reservoir can be targeted, eradicated, or permanently suppressed.

HIV-1 VIRAL ENTRY AND INTEGRATION

Viral entry begins upon engagement of the viral envelope glycoprotein (Env) with surface CD4 on the plasma membrane of the target cell. HIV-1 Env is structured as a trimer of heterodimers consisting of two non-covalently bound subunits, gp120 and gp41, originally cleaved from a gp160 precursor. HIV-1 Env functions as a Type I membrane fusion machine with gp120 positioned on the trimer apex and the transmembrane gp41 protein embedded within the viral membrane anchoring the trimer to the viral particle [1-7]. The CD4 binding site of gp120 engages one or more CD4 molecules on the host cell, inducing a conformational change that reveals an additional co-receptor binding site. HIV-1 has evolved to exploit two main co-receptors for entry, C-C chemokine receptor 5 (CCR5) and C-X-C chemokine receptor type 4 (CXCR4) [8-10]. Once the co-receptor binds the gp120 co-receptor binding site, it activates the gp41 for fusion. Fusion peptide insertion into the target cell membrane purportedly induces gp41 to undergo an additional conformational change into a "six-helix bundle," releasing enough free energy to overcome the repulsion between the negatively charged membranes to fuse viral and cellular membranes, delivering the viral capsid into the target cell cytosol [1-11].

The viral capsid engages with several host proteins to facilitate uncoating and reverse transcription of two RNA genome strands into a complimentary DNA (cDNA) copy and the formation of an integration-competent pre-integration complex (PIC) for entry though the nuclear pore. The capsid travels along the host cell's microtubule network to traffic to the nuclear pores during which time reverse transcriptase within the viral capsid core begins to generate a double-stranded cDNA molecule. Successful reverse transcription and uncoating requires the binding of the host protein Cyclophilin A (CypA) to the CA subunit of the viral capsid, suggesting that uncoating is a sequential process that may require some cytosolic remodeling of the capsid core prior to full disassembly [12-16]. In addition, successful import of the completed PIC

into the nucleus requires the binding of an additional host protein, Cleavage and poly-adenylation specificity factor 6 (CPSF6) to the capsid core, further enhancing core stability during cytoplasmic trafficking [17-20].

The PIC consists of a large nucleoprotein complex comprised of host proteins as well as a processed cDNA molecule associated with HIV-1 Integrase, which catalyzes the endonuclease-mediated priming of the 5' and 3' ends of the cDNA for full integration into the host genome [21]. HIV-1 primarily integrates into transcriptionally active chromatin regions exhibiting hallmark histone modifications associated with active transcriptional sites, *e.g.* H3K4me1 and me2, H3K27me1, and H3K36me3 [22-26]. The host protein LEDGF/p75 functions to greatly enhance integration efficiency while mediating HIV integration into actively transcribed genes, mainly within introns [27,28]. Once integrated, the HIV-1 cDNA is termed a *provirus* and can now be accessed by the host cell transcriptional machinery to produce viral protein. Overall, integration of an HIV-1 provirus into the host cell genome can lead to three general outcomes: 1. the triggering of apoptosis from double-strand DNA breakage caused by the integration event, 2. active transcription of the provirus, 3. and the transcriptional repression of the proviral sequence, the critical hallmark of latent HIV-1 infection [29]. Such latently infected cells constitute a persistent reservoir of HIV-1 genetic information that, when ART suppression is lifted, will give rise to recrudescent viremia.

THE HIV-1 LATENT RESERVOIR: CELLULAR COMPOSITION, MAINTENANCE, AND PERSISTENCE

A cellular reservoir of HIV-1 latently infected cells was discovered in the years following the advent of ART [30-33]. Groundbreaking studies conducted between 1996 and 1997 determined that over the course of treated infection, HIV-1 plasma viremia exhibited a multiphasic decay pattern, in which the majority of plasma viremia declined rapidly with a half-life of approximately one day with a subsequent phase calculated to have a half-life of about 20 days [34-38]. These phases were quickly associated with the depletion of productively infected cells and the gradual depletion of longer-lived target cells, respectively. In addition, if ART was optimal, HIV-1 viremia could be brought to below the limit of detection of PCR-based assays used at the time. These results were encouraging and it was believed that ART could fully clear HIV-1 infection in patients after only 2-3 years of interrupted treatment, if indeed the decay kinetics measured truly represented the total HIV-1 infected cell compartment in infected patients [38]. However, this notion was soon discredited as clinical findings revealed that virtually all

patients experienced full rebounding viremia if ART was interrupted, usually within 2 weeks following therapy interruption [39,40]. Moreover, the length of ART adherence had no effect on whether this rebound would occur. It soon became evident that earlier measurements were incomplete and additional latently infected cell compartments persisted in ART treated patients. Subsequent studies showed that the main cellular reservoir consisted of resting memory CD4+ T cells due to the fact that these populations could readily produce replication-competent virus upon *in vitro* induction in ART treated patients [31,33,41]. Afterwards, the existence of a latent reservoir comprised predominately of resting memory CD4+ T cells was widely accepted.

Memory T cells can be categorized into five general groups, central memory cells (Tcm), transitional memory (Ttm), effector memory cells (Tem), tissue-resident memory (Trm), and stem cell memory (Tscm) [42]. Each group is defined phenotypically by the surface expression of specific chemokine and homing receptors. The differential expression of these select surface markers determine the trafficking behavior and functionality of each group [43]. Latent HIV-1 has been detected in each memory cell subset described, yet the reservoir appears to be comprised primarily of Tcm and Ttm cells, which together preferentially contain the majority of integrated HIV-1 DNA in infected patients [44-46]. Tem reservoirs have also been claimed to make up a large fraction of the total latent reservoir in patients and Tscm cells have been identified as a self-renewing reservoir in patents during long-term ART [47,48].

In order to design rational strategies against the latent reservoir, it is important to understand where the reservoir resides within the body and how the reservoir persists over the course of decades while patients continue to maintain daily ART. From the discussion above, it can be assumed that resting memory CD4+ T cells are present throughout the body, both in circulation and within tissues. Initial reports surveying the distribution of latently infected resting memory cells in humans and in SIV-infected rhesus macaques demonstrated that such cells could be found in about equal frequency in blood and tissue compartments [31,49-52]. Preliminary reports also provide evidence that latently infected cells are harbored within multiple secondary lymphoid tissue systems. These reports claim that active transcription occurs in lymphoid tissues *in vivo* during ART suppression, and that viral recrudescence emerges in multiple lymph node tissues in parallel during ART interruption, both suggesting a reservoir that is, in part, tissue localized [47,53].

Since its discovery, the stability of the latent reservoir was believed to be due to the natural longevity of different subsets of resting memory CD4+ T cells [31,54]. Indeed, IL-7 and IL-15 mediated homeostatic prolifera-

tion of memory T cell subsets has been proposed to explain reservoir stability over time as well as a mechanism to increase the level of infected cells *in vivo* [44,55]. Infection of a memory T cell population discovered to have self-renewing stem-like properties has also been put forward to explain some degree of reservoir persistence, as described earlier [48]. New findings have also revealed that latent HIV-1 infection can be established in resting memory cells displaying a T follicular helper cell (Tfh) phenotype, a crucial helper T cell population that provides necessary signals to B cells undergoing proliferation and somatic hypermutation in germinal centers [56-59]. Tfh cells can enter germinal centers and purportedly evade CD8+ T cell mediated cytotoxicity. As a result, germinal centers are increasingly described as a site of "immune privilege" in which latently infected Tfh cells can persist for long periods while avoiding the adaptive immune response. When transitioning to a resting memory state, infected Tfh cells harboring a latent provirus can comprise a significant fraction of the latent reservoir [57,58,60]. Identifying the contribution made by Tfh cells, both circulating in the peripheral blood and resident in secondary lymphoid tissues, to HIV-1 persistence during active and suppressed infection has currently become a stimulating area of research.

Residual viral replication in tissues during suppressive ART may indirectly maintain the latent reservoir and act as a means of HIV-1 persistence. In theory, low-grade inflammation induced by trace levels of viral replication could promote reservoir replenishment to a small extent. Although one report suggested that low-level viral replication occurs in secondary lymphoid tissues under ART, these findings have been met with skepticism and the concept remains highly controversial [61]. However, additional findings from another report support the claim of low-level active viral replication in elite controllers in the absence of ART [47]. The authors found genetic signatures of active viral replication in Tfh and non-Tfh memory cells in lymphoid tissues and also revealed that latently infected cells found in the blood compartment contained inducible provirus closely related to less diversified virus present during early infection. Between the tissue and blood compartments were circulating memory cells that contained more diversified virus, presumably of recent origin. Taken together, these data suggest that active viral replication in Tfh cells protected in privileged immune sites may facilitate reservoir persistence in unsuppressed individuals. Whether these events are also present in in non-elite controllers during ART suppression is still unknown.

Important work in recent years has identified that the clonal expansion of resting memory CD4+ T cell populations can contribute up to 50-60% of the entire reservoir at any given time during infection [62-66]. Some studies

have demonstrated that clonal propagation resulting from the recognition of cognate antigen by a memory cell clonal population, the so-called memory response mounted against a previously encountered antigen, can contribute to reservoir persistence [67]. In support of this concept, co-infections with Epstein-Barr Virus (EBV) and cyto-megalovirus (CMV) have also been shown to promote antigen-associated proliferation of latently infected memory T cell clones specific against EBV and CMV antigens [68].

Clonal expansion was convincingly demonstrated by parallel reports that took advantage of next-generation genome sequencing of the junction between integrated HIV-1 proviruses and adjacent host sequences to determine the exact chromosomal location of each integration event [62,69]. Together, these reports concluded that the proliferation of rare CD4+ T cell clones may be a major factor involved in maintaining the latent reservoir over time. A follow-up study revealed that the vast majority of expanded clones harbored defective proviruses, unable to produce mature infectious viral particles, and expanded clones carrying fully infectious replication-competent provirus were extremely rare [70]. To explain this observation, this report suggested the paucity of replication-competent clones was the result of HIV-1 induced apoptosis following activation from a resting state. This model implies that most clonally expanded latently infected cells would then carry defective proviruses that would not lead to apoptosis upon reactivation, which is indeed what the group observed in their patient data [70]. Other groups argue that T cell activation and HIV-1 transcription are separate and decoupled processes, and they claim rare expanded clones carrying replication-competent proviruses do persist under normal infection conditions [63,65-67]. Moreover, up to about 11% of proviral sequences in the reservoir persisting in any given patient may be replication-competent [71]. Interestingly, subsequent reports have even suggested that some open reading frames in defective proviruses were indeed transcriptionally intact, providing a mechanism to "distract" CD8+ T cell responses from the replication-competent reservoir via antigen presentation by latently infected cells that cannot produce infectious virus [72]. Although clonal expansion has been well documented in multiple patient samples, whether this process is a primary driver of latent reservoir persistence in a general sense is still a matter of debate.

MOLECULAR BIOLOGY OF HIV-1 LATENCY

In order to interrupt the development of the latent reservoir, it is crucial to understand the molecular mechanisms underlying the promotion and maintenance of HIV-1 latency. HIV-1 integration occurs primarily within the introns of transcriptionally active genes found in euchromatic regions of the host cell genome [22-26]. This is mainly the result of the host factor LEDGF/p75, which binds to the HIV-1 preinitiation complex (PIC) following nuclear entry for recruitment to sites of open gene transcription [27,28]. A recent report claims that HIV-1 integrates preferentially in a subset of transcriptionally active regions and that integration most often occurs in chromatin loops in close proximity to the nuclear pores [73]. In contrast, HIV-1 integration is strongly disfavored in transcriptionally active regions located in the nuclear core, suggesting that chromatin topography may play a critical role in determining integration sites [73]. Integration also has been shown to occur frequently in close proximity to highly repetitive *Alu* elements, which are enriched in HIV-1 integration hotspots [23,70].

The integrated provirus possesses identical 5' and 3' long terminal repeat (LTR) sequences, a consequence of reverse transcription, and the 5'-LTR acts as a transcriptional promoter, containing binding sites for host transcription factors found in high concentrations in activated CD4+ T cells, *e.g.* NF-κB, AP1, and NFAT [74-77]. Although these transcription factors alone can promote low-level HIV-1 gene expression via recruitment of coactivators p300 and CREB and the RNA Polymerase II (Pol II) transcriptional machinery, transcriptional activation at the 5'-LTR is induced predominantly via the binding of the HIV-1 accessory protein Trans-activator of transcription (Tat) to two RNA stemloop structures formed from nascent HIV-1 RNA transcripts (*i.e.* TAR sites) [78]. Cooperative binding of Tat to the host coactivator pTEF-b (a protein complex consisting of cyclin T1 and CDK9) mediates binding to TAR sequences within the 5'-LTR and allows pTEF-b to phosphorylate Pol II resting on the 5'LTR promoter [79-82]. Pol II phosphorylation is required for continuous RNA transcription and precludes premature pausing on the DNA template [83-85].

Epigenetic modification is also required for successful proviral gene expression. The transcriptionally repressive nucleosomes nuc-0 and nuc-1 preferentially assemble at the 5'-LTR of integrated proviruses and can preclude the elongation of HIV-1 RNA transcripts [86]. Histone hypoacetylation is required to maintain nuc-1 at sites of transcriptional repression, suggesting that the proper positioning of nucleosomes along the HIV-1 provirus, facilitating access to the host transcriptional machinery, is mediated in part by endogenous levels of histone deacetylases (HDACs) [87,88]. Notably, methylation of the LTR, a common epigenetic form of gene repression, is not prevalent on integrated proviral LTRs, even though some reports have shown that the histone methyltransferases EZH2, G9a, and SUV39H1 can contribute to HIV-1 latency [89,90].

Why and how latent infection occurs during HIV-1 infection is unclear. HIV-1 replicates continuously throughout untreated infection and exhibits remarkable immune evasion capacity, avoiding immunological control by both humoral and CD8+ cytotoxic T cell responses without the need to reside in a quiescent state. One model has been proposed to explain the generation and the low frequency of the latently infected cell pool during *in vivo* infection [91]. This model suggests that latent infection is the result of a small population of activated CD4+ T cells infected during the transition to or reversion back to a memory cell. The global intracellular environment of resting memory cells, as well as the genomic and chromatin landscape surrounding the integrated provirus, fosters latent over productive infection. In support of this model, resting memory cells maintain a repressive chromatin environment proximal to integrated proviral DNA, and crucial transcription factors are sequestered within the cytosol and are unavailable to bind the 5'-LTR [88,92]. Additionally, cofactors such as pTEF-b, and other essential cellular cofactors for HIV-1 proviral transcription such as CycK/CDK13 and CycL/CDK11 [93], are found primarily in an inactive non-phosphorylated state, unavailable for proviral transcriptional initiation at the 5'-LTR promoter [94]. It has been postulated that so-called effector-to-memory transitioning (EMT) cells, which exhibit high levels of CCR5+ coreceptor, sufficient levels of dNTPs due to low level expression of SAMHD1, and also sequester critical transcription factors in the cytosol, provide a suitable an intracellular environment that encourages latent infection [91]. Since very few activated CD4+ T cells survive the contraction phase of a natural immune response and progress to EMT cells during memory cell transition, rare infection of EMT cells can also explain the low frequency of latent cells (between 0.03-3 cells per million resting CD4+ T cells) comprising the reservoir [95]. In summary, according to this model, HIV-1 latent infection is primarily the result of both the availability of critical transcription factors and cellular cofactors and the overall chromatin landscape surrounding the integrated provirus. As a result, latent infection may be induced when target cells are infected while transitioning to a resting cellular state with low intracellular levels of required transcription factors, cofactors, and a higher frequency of chromatin modifications that lead to repressed gene transcription.

A cell-state dependent model for latency, although intuitive, does not explain some critical features of latent infection, in particular, recent findings suggesting that latency reversal and HIV-1 transcription is not always directly coupled to cellular reactivation and the availability of activation-associated transcription factors [71,96]. An alternative argument can be made that viral latency may be more directly associated with the expression and overall function of the viral accessory protein Tat, which may act as the primary driver of HIV-1 latency and proviral transcriptional elongation. Interestingly, *tat* is one of the earliest gene transcripts generated, and sustained *tat* expression can promote a powerful feed-forward loop of proviral transcriptional elongation, which results in the enhancement of total HIV-1 gene expression via a virally intrinsic latency program. Some studies have suggested that tat-dependent transcription is primarily a stochastic process in which cell-to-cell variability of available host transcription factors and cofactors establish a "threshold level" for successful HIV-1 transcription [71,97,98]. If the concentration of available Tat rises above this level, tat expression can amplify HIV-1 gene transcription and initiate the feed-forward loop described above. This model suggests that proviral gene expression is regulated mainly at the level of gene transcription with the proviral element persisting categorically in either an ON and OFF state, dependent on the successful recruitment of RNA Polymerase II to the 5'-LTR promoter. Studies showing that small molecule drugs that can increase transcriptional noise, due to the longer occupancy of RNA Polymerase II at the 5'-LTR and therefore greater frequency of sustained transcriptional bursts, increase the probability of the proviral ON state, providing support for this model [99]. Furthermore, additional studies have provided evidence that a Tat-mediated intrinsic latency program can actually provide an evolutionary benefit to HIV-1 and that latency is ultimately an adaptation derived from natural selection [100]. According to this view, HIV-1 latency enhances overall viral transmission between hosts by maintaining low infection levels at barrier mucosal tissues during the onset of infection and allowing for a higher probability of subsequent systemic spread [100]. Whether HIV-1 latency is the result of host cellular functional states at the moment of infection, is controlled via a virally intrinsic program, or is perhaps the consequence of both, remains to be determined.

CURE STRATEGIES TARGETING THE LATENT RESERVOIR

Eradication or the permanent suppression of the latent reservoir are the primary objectives for HIV-1 cure strategies. Current efforts concentrate on target reactivation of the CD4+ T cell pool in an infected patient in order to drive the clearance of all latently infected cells via host innate and adaptive cellular immune responses, termed "shock-and-kill." Extensive progress has been made on this front, particularly with small molecules and broadly neutralizing antibody immunotherapies.

Initial studies to reactivate cellular latent reservoirs employed IL-2 therapy and T cell receptor (TCR) agonists to mimic native CD4+ T cell activation [101,102].

Unfortunately, lymphocyte activation was not specific to cells latently infected with HIV-1, and these treatments resulted in severe complications without clear evidence for reservoir eradication. It became apparent that global reactivation of lymphocyte populations would not constitute a viable cure option. Small molecules targeting the epigenetic regulation of HIV-1 proviral transcription were screened in the hopes of discovering a reagent that could reverse latency without undesired immune toxicity. As described previously, nucleosomes nuc-0 and nuc-1 are recruited to integrated HIV-1 proviral DNA and inhibit host transcriptional machinery [86]. Acetylation of nuc-0 and nuc-1 remodels these nucleosomes and releases transcriptional inhibition of the provirus. Consequently, inhibition of histone deacetylases (HDACs) could potentially maintain replication-competent proviruses in a transcriptionally active state. As a result, the characterization of novel HDAC inhibitors as well as other small molecules targeting epigenetic regulation of HIV-1 transcription, collectively called latency reversing agents (LRAs), has become a top research priority. Other categories of LRAs include methylation inhibitors, bromodomain and extra-terminal domain (BET) inhibitors, and Protein Kinase C (PKC) agonists [103-110]. Pharmacological evaluation of candidate LRAs has been challenging, however, due to the fact that many drug candidates that reactivate latently infected cell lines *in vitro* fail to demonstrate a strong capacity to reactivate HIV-1 infected primary cells extracted from infected patients [110]. One report demonstrated that the only effective single-compound treatment in HIV-1 positive patient CD4+ T cells was the PKC agonist bryostatin-1, which unfortunately has a high clinical toxicity [110]. Additionally, clinical trials using the HDAC inhibitor valproic acid, also failed to show significant reduction of the latent reservoir in treated patients [111,112].

Strategies to reactivate the latent reservoir without inducing CD4+ T cell activation itself have also been attempted [96,99,113]. Interestingly, administration of 5-hydroxynaphthalene-1,4-dione (5HN) has been shown to reactivate latent HIV-1 without functionally activating the CD4+ T cell global activating NFAT and Protein Kinase C (PKC) signaling networks. Moreover, delivery of tat-loaded exosomes to resting CD4+ T cells reactivated latent HIV-1 gene expression without cotreatment of T cell activation agents or other LRAs [113]. These data suggest that HIV-1 latency reversal is possible without global T cell activation, albeit at lower overall levels, and further support the model that HIV-1 latency is a hard-wired feature of HIV-1 infection that relies more on the stochastic nature of gene transcription than the overall physiological state of the infected host cell.

Alternative approaches for reservoir reactivation target innate immune recognition pathways and exploit prior observations that a large fraction of HIV-1 infected CD4+ T cells are also specific for HIV-1 antigen. Toll-Like Receptor 7 (TLR7) activation by the TLR7 agonist GS-9620 has been demonstrated to promote profound reactivation of CD4+ T cells via increased levels of type II interferon (*i.e.* IFN-γ) [114]. Remarkably, in a SIV infected non-human primate (NHP) model in combination with an recombinant adenovirus serotype 26 prime vaccine (Ad26) prior to a modified vaccine Ankara (MVA) boost, GS-9620 stimulation promoted a broad immune response against SIV that led to a dramatic delay in viral rebound following ART interruption [115]. Furthermore, a vaccine cocktail that delivers an almost full representation of possible HIV-1 quasispecies could potentially reactivate HIV-1-specific CD4+ T cells within the reservoir leading to their ultimate elimination [116,117]. This method could reduce the reservoir to levels that could be managed in average patient lifetimes considering that HIV-1-specific CD4+ T cells comprise a large subset of HIV-1 infected cells *in vivo* [118,119].

Reactivating HIV-1 latent cells exposes them to attack by the host immune system, but native responses may need to be enhanced in order to achieve full eradication of the reservoir. CD8+ CTLs are largely responsible for establishing a viral set point during untreated chronic infection, underlying their importance in eliminating HIV-1 infected cells [120-124]. However, many challenges will have to be overcome in order to direct an efficient CTL response. HIV-1 quasispecies evolve rapidly during infection and CTL escape mutants ultimately emerge due to persistent positive selection exerted by the immune system and these escape mutants can become archived in the reservoir [125]. In addition, as previously discussed, defective proviruses can express HIV-1 protein antigens that can modulate the CTL response against the reservoir by acting as decoys [71,72]. Also, CD8+ CTLs undergo immune "exhaustion" during chronic viral infection such as HIV-1 due to persistent exposure to viral antigen, characterized by reduced responses to activation signals, reduced levels of cytotoxicity, and expression of exhaustion surface markers such as PD-1 [126,127].

To address these challenges, various strategies have been implemented to enhance CTL recognition of reactivated latently infected cells, maintain efficient CTL responses in the presence of escape mutants, and circumvent CTL exhaustion that normally occurs during prolonged responses against chronic viral infections. One strategy aims to stimulate a subset of CD8+ CTL clones that recognize predominating HIV-1 antigens presented following latency reactivation with potent IL-15 super-agonists [128]. Promisingly, a CMV-based vaccine vector developed from the CMV strain 68.1 has been used in SIV infected NHPs, resulting in a broader and more robust CTL response elicited from other viral vector

systems such as adenoviral vectors [129,130]. Amazingly, CTL responses generated from rhesus CMV vectors (RhCMV) were major histocompatibility class II (MHC Class II) and Human leukocyte antigen-E (HLA-E) restricted and therefore capable of recognizing almost three times as many HIV-1 epitopes as conventional class I restricted CD8+ CTLs [131]. Also, antigen presentation was unperturbed by Nef downregulation of MHC class I molecules due to the fact that HLA-E is protected from Nef-mediated degradation [132]. Overall, the enhanced CTL response caused by RhCMV vaccination led to complete immune control of about half of the NHPs used in the study as well as viral clearance for 4 years [129,130]. In addition, the use of PD-1 immunotherapy in humanized mice and NHPs has been shown to suppress viral loads, suggesting that common immunotherapies used in cancer treatment could be retooled to stimulate exhausted CTLs in junction with LRAs to reduce the size of the latent reservoir [124,133,134].

Curative strategies have also explored how antibodies and antibody effector functions, such as antibody-dependent cell cytotoxicity (ADCC) may contribute to reservoir eradication and a cure. Monotherapy of bNABs 3BNC117, VRC01, or 10-1074 have been shown to reduce viremia in patients during active infection and be safely tolerated [135-138]. Immunotherapy trials in humanized mice and macaques using monotherapy or combined therapy with antibodies specific for non-overlapping HIV-1 Env epitopes have also successfully controlled viremia during active infection and could protect against new infection [139-142]. In two humanized mouse studies, administration of bNAbs led to a delay in viral rebound following ART interruption and to accelerated clearance of HIV-1 infected cells [143,144]. These and subsequent studies were able to link this effect to Fc-receptor engagement to the bNAb Fc domain, suggesting that ADCC can be harnessed to eliminate cells comprising the latent reservoir [145]. Delayed viral rebound has also been observed in patients using 3BNC117 and VRC01 monotherapy [64,144,146]. Also, single doses of 3BNC117 also enhanced humoral immune responses in patients via the accelerated generation of neutralizing tier-2 antibodies, *i.e.* antibodies that recognize epitopes expressed by HIV-1 Env trimers in a closed confirmation [147].

Studies showing that bNAb immunotherapy can delay viral rebound and reduce the latent reservoir introduced bNAbs when ART was discontinued. It was unclear whether bNAbs would be able to effectively target and deplete the latent reservoir during ART suppression. To address this issue, a recent study administered the bNAb PGT121, as well as the potent TLR7 agonist GS-9620, described above, to SIV infected NHPs during ART suppression [148]. Viral rebound was significantly de-layed in each monkey treated with both PGT121 and GS-9620 and roughly half of dual-treated monkeys exhibits no viral rebound within six months of ART cessation. The authors demonstrated that GS-9620 activated multiple immune cells, including CD8+ CTLs and NK cells. The authors speculate that global immune activation, mediated by GS-9620, activated latently infected cells. Activated HIV-1 infected cells would express cell-surface Env which would bind to PGT121. PGT121 binding to activated HIV-1 infected cells would in turn facilitate cell clearance by ADCC via NK cell and CTL Fc receptor engagement [148].

The studies described above provide clear evidence that reactivation of resting memory CD4+ T cells that may promote latency reversal combined with a means to recruit both functional innate and adaptive cellular immune responses constitute a possible therapeutic cure for HIV-1 infection. In addition, these studies underscore the need for tractable animal models to test individual components of HIV-1 cure strategies, due to the fact that successful combination therapies using bNAbs have been shown to be effective in humanized mouse models before direct testing in NHPs and human patients.

CONCLUSION

Currently, two reports of individuals exhibiting long-term viremic remission have demonstrated that a functional cure is indeed possible, and a renewed interest in developing new HIV-1 eradication strategies has emerged as a result [149,150]. Consequently, there has been a robust research effort to characterize HIV-1 replication-competent cellular reservoirs and understand the mechanisms underlying reservoir persistence in peripheral and tissue compartments. Together with a growing repertoire of highly potent bNAbs, extensive efforts in LRA small molecule discovery, and combinations of different anti-viral and immunological regimens, one can remain optimistic about an eventual transition from potential cure strategy to effective clinical therapy. With continued funding, continuing clinical trials, and the courage to take risks, we can remain hopeful that the cure can be achieved.

REFERENCES

1. White JM, Delos SE, Brecher M, Schornberg K. Structures and Mechanisms of Viral Fusion Proteins. Crit Rev Biochem Mol Biol. 2008;43(3):189–219.
2. Wei X, Decker JM, Wang S, Hui H, Kappes JC, Wu X, et al. Antibody neutralization and escape by HIV-1. Nature. 2003;422(6929):307–12.
3. Julien JP, Cupo A, Sok D, Stanfield RL, Lyumkis D, Deller MC, et al. Crystal structure of a soluble cleaved HIV-1 envelope trimer. Science. 2013;342(6165):1477–83.

4. Lee JH, Ozorowski G, Ward AB. Cryo-EM structure of a native, fully glycosylated, cleaved HIV-1 envelope trimer. Science. 2016;351(6277):1043–8.

5. Lyumkis D, Julien JP, De Val N, Cupo A, Potter CS, Klasse PJ, et al. Cryo-EM structure of a fully glycosylated soluble cleaved HIV-1 envelope trimer. Science. 2013;342(6165):1484–90.

6. Liu J, Bartesaghi A, Borgnia MJ, Sapiro G, Subramaniam S. Molecular architecture of native HIV-1 gp120 trimers. Nature. 2008;455(7209):109–13.

7. Pancera M, Zhou T, Druz A, Georgiev IS, Soto C, Gorman J, et al. Structure and immune recognition of trimeric pre-fusion HIV-1 Env. Nature. 2014;514(7253):455–61.

8. Tran EEH, Borgnia MJ, Kuybeda O, Schauder DM, Bartesaghi A, Frank GA, et al. Structural mechanism of trimeric HIV-1 envelope glycoprotein activation. PLoS Pathog. 2012;8(7):37.

9. Pancera M, Majeed S, Ban Y-EA, Chen L, Huang C -c., Kong L, et al. Structure of HIV-1 gp120 with gp41-interactive region reveals layered envelope architecture and basis of conformational mobility. Proc Natl Acad Sci. 2010;107(3):1166–71.

10. Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA. Structure of an HIV gp 120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature. 1998;393:648–59.

11. Harrison SC. Viral membrane fusion. Virology. 2015;479-480:498–507.

12. Luban J, Bossolt KL, Franke EK, Kalpana G V., Goff SP. Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B. Cell. 1993;73(6):1067–78.

13. Thali M, Bukovsky A, Kondo E, Rosenwirth B, Walsh CT, Sodroski J, et al. Functional association of cyclophilin a with HIV-1 virions. Nature. 1994;372(6504):363–5.

14. De Iaco A, Luban J. Cyclophilin A promotes HIV-1 reverse transcription but its effect on transduction correlates best with its effect on nuclear entry of viral cDNA. Retrovirology. 2014;11(1).

15. Towers GJ, Hatziioannou T, Cowan S, Goff SP, Luban J, Bieniasz PD. Cyclophilin A modulates the sensitivity of HIV-1 to host restriction factors. Nat Med. 2003;9(9):1138–43.

16. Li Y, Kar AK, Sodroski J. Target Cell Type-Dependent Modulation of Human Immunodeficiency Virus Type 1 Capsid Disassembly by Cyclophilin A. J Virol. 2009;83(21):10951–62.

17. Price AJ, Fletcher AJ, Schaller T, Elliott T, Lee KE, KewalRamani VN, et al. CPSF6 Defines a Conserved Capsid Interface that Modulates HIV-1 Replication. PLoS Pathog. 2012;8(8).

18. Bhattacharya A, Alam SL, Fricke T, Zadrozny K, Sedzicki J, Taylor AB, et al. Structural basis of HIV-1 capsid recognition by PF74 and CPSF6. Proc Natl Acad Sci. 2014;111(52):18625–30.

19. Lee K, Mulky A, Yuen W, Martin TD, Meyerson NR, Choi L, et al. HIV-1 Capsid-Targeting Domain of Cleavage and Polyadenylation Specificity Factor 6. J Virol. 2012;86(7):3851–60.

20. Hori T, Takeuchi H, Saito H, Sakuma R, Inagaki Y, Yamaoka S. A Carboxy-Terminally Truncated Human CPSF6 Lacking Residues Encoded by Exon 6 Inhibits HIV-1 cDNA Synthesis and Promotes Capsid Disassembly. J Virol. 2013;87(13):7726–36.

21. Bowerman B, Brown PO, Bishop JM, Varmus HE. A nucleoprotein complex mediates the integration of retroviral DNA. Genes Dev. 1989;3(4):469–78.

22. Wang GP, Ciuffi A, Leipzig J, Berry CC, Bushman FD. HIV integration site selection: Analysis by massively parallel pyrosequencing reveals association with epigenetic modifications. Genome Res. 2007;17(8):1186–94.

23. Schröder ARW, Shinn P, Chen H, Berry C, Ecker JR, Bushman F. HIV-1 integration in the human genome favors active genes and local hotspots. Cell. 2002;110(4):521–9.

24. Brady T, Agosto LM, Malani N, Berry CC, O'Doherty U, Bushman F. HIV integration site distributions in resting and activated CD4+ T cells infected in culture. Aids. 2009;23(12):1461–71.

25. Sherrill-Mix S, Lewinski MK, Famiglietti M, Bosque A, Malani N, Ocwieja KE, et al. HIV latency and integration site placement in five cell-based models. Retrovirology. 2013;10(1).

26. Han Y, Lin YB, An W, Xu J, Yang HC, O'Connell K, et al. Orientation-Dependent Regulation of Integrated HIV-1 Expression by Host Gene Transcriptional Readthrough. Cell Host Microbe. 2008;4(2):134–46.

27. Meehan AM, Saenz DT, Morrison JH, Garcia-Rivera JA, Peretz M, Llano M, et al. LEDGF/p75 proteins with alternative chromatin tethers are functional HIV-1 cofactors. PLoS Pathog. 2009;5(7).

28. Ciuffi A, Llano M, Poeschla E, Hoffmann C, Leipzig J, Shinn P, et al. A role for LEDGF/p75 in targeting HIV DNA integration. Nat Med. 2005;11(12):1287–9.

29. Cooper A, García M, Petrovas C, Yamamoto T, Koup RA, Nabel GJ. HIV-1 causes CD4 cell death through DNA-dependent protein kinase during viral integration. Nature. 2013;498(7454):376–9.

30. Chun TW, Finzi D, Margolick J, Chadwick K, Schwartz D, Siliciano RF. In vivo fate of HIV-1-infected T cells: Quantitative analysis of the transition to stable latency. Nat Med. 1995;1(12):1284–90.

31. Chun T-W, Stuyver L, Mizell SB, Ehler LA, Mican JAM, Baseler M, et al. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc Natl Acad Sci. 1997;94(24):13193–7.

32. Chun T-W, Engel D, Berrey MM, Shea T, Corey L, Fauci AS. Early establishment of a pool of latently infected, resting CD4+ T cells during primary HIV-1 infection. Proc Natl Acad Sci. 1998;95(15):8869–73.

33. Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science. 1997;278(5341):1295–300.

34. Coffin J. HIV population dynamics in vivo: implications for genetic variation, pathogenesis, and therapy. Science. 1995 Jan 27;267(5197):483–9.

35. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature. 1995;373:123–6.

36. Wei X, Ghosh SK, Taylor ME, Johnson VA, Emini EA, Deutsch P, et al. Viral dynamics in human immunodeficien-

cy virus type 1 infection. Nature. 1995;373(6510):117–22.

37. Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho DD. HIV-1 Dynamics in Vivo: Virion Clearance Rate, Infected Cell Life-Span, and Viral Generation Time. Science. 1996;271(5255):1582–6.

38. Perelson AS, Essunger P, Cao Y, Vesanen M, Hurley A, Saksela K, et al. Decay characteristics of HIV-1-infected compartments during combination therapy. Nature. 1997 May;387(6629):188–91.

39. Davey RT, Bhat N, Yoder C, Chun T-W, Metcalf JA, Dewar R, et al. HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. Proc Natl Acad Sci. 1999;96(26):15109–14.

40. Chun T-W, Davey RT, Engel D, Lane HC, Fauci AS. Re-emergence of HIV after stopping therapy. Nature. 1999 Oct 28;401(6756):874–5.

41. Wong JK, Hezareh M, Günthard HF, Havlir D V., Ignacio CC, Spina CA, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science. 1997;278(5341):1291–5.

42. Mueller SN, Gebhardt T, Carbone FR, Heath WR. Memory T Cell Subsets, Migration Patterns, and Tissue Residence. Annu Rev Immunol. 2013;31(1):137–61.

43. Sallusto F, Lenlg D, Förster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature. 1999;401:708–12.

44. Chomont N, El-Far M, Ancuta P, Trautmann L, Procopio FA, Yassine-Diab B, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. Nat Med. 2009;15(8):893–900.

45. Lee GQ, Lichterfeld M. Diversity of HIV-1 reservoirs in CD4+ T-cell subpopulations. Curr Opin HIV AIDS. 2016;11(4):383–7.

46. Cantero-Pérez J, Grau-Expósito J, Serra-Peinado C, Rosero DA, Luque-Ballesteros L, Astorga-Gamaza A, et al. Resident memory T cells are a cellular reservoir for HIV in the cervical mucosa. Nat Commun. 2019 [cited 2019 Dec 17];10(1).

47. Boritz EA, Darko S, Swaszek L, Wolf G, Wells D, Wu X, et al. Multiple Origins of Virus Persistence during Natural Control of HIV Infection. Cell. 2016 Aug 11 [cited 2019 Apr 4];166(4):1004–15.

48. Buzon MJ, Sun H, Li C, Shaw A, Seiss K, Ouyang Z, et al. HIV-1 persistence in CD4+ T cells with stem cell–like properties. Nat Med. 2014;20(2):139–42.

49. Thome JJC, Yudanin N, Ohmura Y, Kubota M, Grinshpun B, Sathaliyawala T, et al. Spatial map of human t cell compartmentalization and maintenance over decades of life. Cell. 2014;159(4):814–28.

50. Reinhardt RL, Khoruts A, Merica R, Zell T, Jenkins MK. Visualizing the generation of memory CD4 T cells in the whole body. Nature. 2001;410(6824):101–5.

51. Shen A, Zink M, Mankowski J. Resting CD4 + T Lymphocytes but Not Thymocytes Provide a Latent Viral Reservoir in a Simian Immunodeficiency Virus- Macaca nemestrina Model of Human Immunodeficiency Virus Type 1-Infected Patients on Highly Active Antiretroviral Therapy. J Virol. 2003 Apr 15;77(8):4938–49.

52. Dinoso JB, Rabi SA, Blankson JN, Gama L, Mankowski JL, Siliciano RF, et al. A Simian Immunodeficiency Virus-Infected Macaque Model To Study Viral Reservoirs That Persist during Highly Active Antiretroviral Therapy. J Virol. 2009;83(18):9247–57.

53. Rothenberger MK, Keele BF, Wietgrefe SW, Fletcher C V., Beilman GJ, Chipman JG, et al. Large number of rebounding/founder HIV variants emerge from multifocal infection in lymphatic tissues after treatment interruption. Proc Natl Acad Sci. 2015;112(10):E1126–34.

54. Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, et al. CD4 + T Cell Depletion during all Stages of HIV Disease Occurs Predominantly in the Gastrointestinal Tract. J Exp Med. 2004;200(6):749–59.

55. Vandergeeten C, Fromentin R, DaFonseca S, Lawani MB, Sereti I, Lederman MM, et al. Interleukin-7 promotes HIV persistence during antiretroviral therapy. Blood. 2013;121(21):4321–9.

56. Hong JJ, Amancha PK, Rogers K, Ansari AA, Villinger F. Spatial Alterations between CD4+ T Follicular Helper, B, and CD8+ T Cells during Simian Immunodeficiency Virus Infection: T/B Cell Homeostasis, Activation, and Potential Mechanism for Viral Escape. J Immunol. 2012;188(7):3247–56.

57. Perreau M, Savoye A-L, De Crignis E, Corpataux J-M, Cubas R, Haddad EK, et al. Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. J Exp Med. 2013;210(1):143–56.

58. Banga R, Procopio FA, Noto A, Pollakis G, Cavassini M, Ohmiti K, et al. PD-1+and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals. Nat Med. 2016;22(7):754–61.

59. Pallikkuth S, Sharkey M, Babic DZ, Gupta S, Stone GW, Fischl MA, et al. Peripheral T Follicular Helper Cells Are the Major HIV Reservoir within Central Memory CD4 T Cells in Peripheral Blood from Chronically HIV-Infected Individuals on Combination Antiretroviral. J Virol. 2016;90(6):2718–28.

60. García M, Górgolas M, Cabello A, Estrada V, Ligos JM, Fernández-Guerrero M, et al. Peripheral T follicular helper Cells Make a Difference in HIV Reservoir Size between Elite Controllers and Patients on Successful cART. Sci Rep. 2017;7(1):1–7.

61. Lorenzo-Redondo R, Fryer HR, Bedford T, Kim E-Y, Archer J, Kosakovsky Pond SL, et al. Persistent HIV-1 replication maintains the tissue reservoir during therapy. Nature. 2016 Feb 27 [cited 2019 Jul 29];530(7588):51–6.

62. Maldarelli F, Wu X, Su L, Simonetti FR, Shao W, Hill S, et al. Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. Science. 2014;345(6193):179–83.

63. Bui JK, Sobolewski MD, Keele BF, Spindler J, Musick A, Wiegand A, et al. Proviruses with identical sequences comprise a large fraction of the replication-competent HIV reservoir. PLoS Pathog. 2017;

64. Scheid JF, Horwitz JA, Bar-On Y, Kreider EF, Lu CL, Lorenzi JCC, et al. HIV-1 antibody 3BNC117 suppresses viral rebound in humans during treatment interruption. Nature. 2016;535(7613):556–60.

65. Lorenzi JCC, Cohen YZ, Cohn LB, Kreider EF, Barton JP, Learn GH, et al. Paired quantitative and qualitative assessment of the replication-competent HIV-1 reservoir and comparison with integrated proviral DNA. Proc Natl Acad Sci U S A. 2016 Dec 6 [cited 2019 Jul 30];113(49):E7908–16.

66. Hosmane NN, Kwon KJ, Bruner KM, Capoferri AA, Beg S, Rosenbloom DIS, et al. Proliferation of latently infected CD4 + T cells carrying replication-competent HIV-1: Potential role in latent reservoir dynamics. J Exp Med. 2017 Apr 3;214(4):959–72.

67. Simonetti FR, Sobolewski MD, Fyne E, Shao W, Spindler J, Hattori J, et al. Clonally expanded CD4 + T cells can produce infectious HIV-1 in vivo. Proc Natl Acad Sci. 2016;

68. Henrich TJ, Hobbs KS, Hanhauser E, Scully E, Hogan LE, Robles YP, et al. Human immunodeficiency virus type 1 persistence following systemic chemotherapy for malignancy. J Infect Dis. 2017;216(2):254–62.

69. Wagner TA, Mclaughlin S, Garg K, Cheung CYK, Larsen BB, Styrchak S, et al. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. Science. 2014;345(6196):573.

70. Cohn LB, Silva IT, Oliveira TY, Rosales RA, Parrish EH, Learn GH, et al. HIV-1 integration landscape during latent and active infection. Cell. 2015;160(3):420–32.

71. Ho YC, Shan L, Hosmane NN, Wang J, Laskey SB, Rosenbloom DIS, et al. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. Cell. 2013;155(3):540–51.

72. Pollack RA, Jones RB, Pertea M, Bruner KM, Martin AR, Thomas AS, et al. Defective HIV-1 Proviruses Are Expressed and Can Be Recognized by Cytotoxic T Lymphocytes, which Shape the Proviral Landscape. Cell Host Microbe. 2017;21(4):494-506.e4.

73. Marini B, Kertesz-Farkas A, Ali H, Lucic B, Lisek K, Manganaro L, et al. Nuclear architecture dictates HIV-1 integration site selection. Nature. 2015;521(7551):227–31.

74. Nabel G, Baltimore D. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature. 1987;326(6114):711–3.

75. Siekevitz M, Josephs SF, Dukovich M, Peffer N, Wong-Staal F, Greene WC. Activation of the HlV-1 LTR by T cell mitogens and the trans-activator protein of HTLV-I. Science. 1987;238(4833):1575–8.

76. Böhnlein E, Lowenthal JW, Siekevitz M, Ballard DW, Franza BR, Greene WC. The same inducible nuclear proteins regulates mitogen activation of both the interleukin-2 receptor-alpha gene and type 1 HIV. Cell. 1988;53(5):827–36.

77. Kinoshita S, Chen BK, Kaneshima H, Nolan GP. Host control of HIV-1 parasitism in T cells by the nuclear factor of activated T cells. Cell. 1998;95(5):595–604.

78. Feng S, Holland EC. HIV-1 tat trans-activation requires the loop sequence within tar. Nature. 1988;334(6178):165–7.

79. Zhu Y, Pe'ery T, Peng J, Ramanathan Y, Marshall N, Marshall T, et al. Transcription elongation factor P-TEFb is required for HIV-1 Tat transactivation in vitro. Genes Dev. 1997;11(20):2622–32.

80. Mancebo HSY, Lee G, Flygare J, Tomassini J, Luu P, Zhu Y, et al. P-TEFb kinase is required for HIV Tat tran-

scriptional activation in vivo and in vitro. Genes Dev. 1997;11(20):2633–44.

81. Wei P, Garber ME, Fang SM, Fischer WH, Jones KA. A novel CDK9-associated C-type cyclin interacts directly with HIV-1 Tat and mediates its high-affinity, loop-specific binding to TAR RNA. Cell. 1998;92(4):451–62.

82. Peng J, Zhu Y, Milton JT, Price DH. Identification of multiple cyclin subunits of human P-TEFb. Genes Dev. 1998;12(5):755–62.

83. Parada CA, Roeder RG. Enhanced processivity of RNA polymerase II triggered by Tat-induced phosphorylation of its carboxy-terminal domain. Nature. 1996;384(6607):375–8.

84. Bieniasz PD, Grdina TA, Bogerd HP, Cullen BR. Recruitment of cyclin T1/P-TEFb to an HIV type 1 long terminal repeat promoter proximal RNA target is both necessary and sufficient for full activation of transcription. Proc Natl Acad Sci. 1999;96(14):7791–6.

85. Fujinaga K, Irwin D, Huang Y, Taube R, Kurosu T, Peterlin BM. Dynamics of Human Immunodeficiency Virus Transcription: P-TEFb Phosphorylates RD and Dissociates Negative Effectors from the Transactivation Response Element. Mol Cell Biol. 2004;24(2):787–95.

86. Verdin E, Paras P, Van Lint C. Chromatin disruption in the promoter of human immunodeficiency virus type 1 during transcriptional activation. EMBO J. 1993;12:3249–59.

87. Coull JJ, Romerio F, Sun J-M, Volker JL, Galvin KM, Davie JR, et al. The Human Factors YY1 and LSF Repress the Human Immunodeficiency Virus Type 1 Long Terminal Repeat via Recruitment of Histone Deacetylase 1. J Virol. 2000;74(15):6790-6799.

88. Van Lint C, Bouchat S, Marcello A. HIV-1 transcription and latency: An update. Retrovirology. 2013 Jun 26;10:67.

89. Chéné I Du, Basyuk E, Lin YL, Triboulet R, Knezevich A, Chable-Bessia C, et al. Suv39H1 and HP1γ are responsible for chromatin-mediated HIV-1 transcriptional silencing and post-integration latency. EMBO J. 2007;26(2):424–35.

90. Friedman J, Cho W-K, Chu CK, Keedy KS, Archin NM, Margolis DM, et al. Epigenetic Silencing of HIV-1 by the Histone H3 Lysine 27 Methyltransferase Enhancer of Zeste 2. J Virol. 2011;85(17):9078–89.

91. Shan L, Deng K, Gao H, Xing S, Capoferri AA, Durand CM, et al. Transcriptional Reprogramming during Effector-to-Memory Transition Renders CD4+ T Cells Permissive for Latent HIV-1 Infection. Immunity. 2017 Oct 17 [cited 2019 Jul 29];47(4):766-775.e3.

92. Coiras M, López-Huertas MR, Pérez-Olmeda M, Alcamí J. Understanding HIV-1 latency provides clues for the eradication of long-term reservoirs. Nat Rev Microbiol. 2009;7:798–812.

93. Yu W, Ramakrishnan R, Wang Y, Chiang K, Sung TL, Rice AP. Cyclin T1-dependent genes in activated CD4+ T and macrophage cell lines appear enriched in HIV-1 co-factors. PLoS One. 2008 Sep 5;3(9):e3146. [cited 2020 Jun 20].

94. Rice AP. The HIV-1 Tat Protein: Mechanism of Action and Target for HIV-1 Cure Strategies. Curr Pharm Des. 2017;23(28).

95. Siliciano JD, Kajdas J, Finzi D, Quinn TC, Chadwick K, Margolick JB, et al. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting

CD4+T cells. Nat Med. 2003;9(6):727–8.

96. Yang HC, Xing S, Shan L, O'Connell K, Dinoso J, Shen A, et al. Small-molecule screening using a human primary cell model of HIV latency identifies compounds that reverse latency without cellular activation. J Clin Invest. 2009 Nov 2 [cited 2020 Jun 20];119(11):3473–86.

97. Razooky BS, Pai A, Aull K, Rouzine IM, Weinberger LS. A hardwired HIV latency program. Cell. 2015;160(5):990–1001.

98. Weinberger LS, Burnett JC, Toettcher JE, Arkin AP, Schaffer D V. Stochastic gene expression in a lentiviral positive-feedback loop: HIV-1 Tat fluctuations drive phenotypic diversity. Cell. 2005;122(2):169–82.

99. Dar RD, Hosmane NN, Arkin MR, Siliciano RF, Weinberger LS. Screening for noise in gene expression identifies drug synergies. Science. 2014 Jun 20 [cited 2020 Jun 20];344(6190):1392–6.

100. Rouzine IM, Weinberger AD, Weinberger LS. An evolutionary role for HIV latency in enhancing viral transmission. Cell. 2015 Feb 26 [cited 2020 Jun 20];160(5):1002–12.

101. Prins JM, Jurriaans S, Van Praag RME, Blaak H, Van Rij R, Schellekens PTA, et al. Immune-activation with anti-CD3 and recombinant human IL-2 in HIV-1-infected patients on potent antiretroviral therapy. Aids. 1999;13(17):2405–10.

102. Kulkosky J, Nunnari G, Otero M, Calarota S, Dornadula G, Zhang H, et al. Intensification and stimulation therapy for human immunodeficiency virus type 1 reservoirs in infected persons receiving virally suppressive highly active antiretroviral therapy. J Infect Dis. 2002;186(10):1403–11.

103. Lehrman G, Hogue IB, Palmer S, Jennings C, Spina CA, Wiegand A, et al. Depletion of latent HIV-1 infection in vivo: A proof-of-concept study. Lancet. 2005;366(9485):549–55.

104. Archin NM, Liberty AL, Kashuba AD, Choudhary SK, Kuruc JD, Crooks AM, et al. Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. Nature. 2012;487(7408):482–5.

105. Elliott JH, Wightman F, Solomon A, Ghneim K, Ahlers J, Cameron MJ, et al. Activation of HIV Transcription with Short-Course Vorinostat in HIV-Infected Patients on Suppressive Antiretroviral Therapy. PLoS Pathog. 2014;10(11).

106. Rasmussen TA, Tolstrup M, Brinkmann CR, Olesen R, Erikstrup C, Solomon A, et al. Panobinostat, a histone deacetylase inhibitor, for latent virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: A phase 1/2, single group, clinical trial. Lancet HIV. 2014;1(1):e13–21.

107. Søgaard OS, Graversen ME, Leth S, Olesen R, Brinkmann CR, Nissen SK, et al. The Depsipeptide Romidepsin Reverses HIV-1 Latency In Vivo. PLoS Pathog. 2015;11(9):1–22.

108. Bouchat S, Delacourt N, Kula A, Darcis G, Van Driessche B, Corazza F, et al. Sequential treatment with 5-aza-2'-deoxycytidine and deacetylase inhibitors reactivates HIV-1. EMBO Mol Med. 2016;8(2):117–38.

109. Lu P, Shen Y, Yang H, Wang Y, Jiang Z, Yang X, et al. BET inhibitors RVX-208 and PFI-1 reactivate HIV-1 from latency. Sci Rep. 2017;7(1).

110. Bullen CK, Laird GM, Durand CM, Siliciano JD, Siliciano RF. New ex vivo approaches distinguish effective and ineffective single agents for reversing HIV-1 latency in vivo. Nat Med. 2014;20(4):425–9.

111. Siliciano JD, Lai J, Callender M, Pitt E, Zhang H, Margolick JB, et al. Stability of the Latent Reservoir for HIV-1 in Patients Receiving Valproic Acid. J Infect Dis. 2007;195(6):833–6.

112. Archin NM, Cheema M, Parker D, Wiegand A, Bosch RJ, Coffin JM, et al. Antiretroviral intensification and valproic acid lack sustained effect on residual HIV-1 viremia or resting CD4+ cell infection. PLoS One. 2010;5(2).

113. Tang X, Lu H, Dooner M, Chapman S, Quesenberry PJ, Ramratnam B. Exosomal Tat protein activates latent HIV-1 in primary, resting CD4+ T lymphocytes. JCI insight. 2018 Apr 5 [cited 2020 Jun 20];3(7).

114. Tsai A, Irrinki A, Kaur J, Cihlar T, Kukolj G, Sloan DD, et al. Toll-Like Receptor 7 Agonist GS-9620 Induces HIV Expression and HIV-Specific Immunity in Cells from HIV-Infected Individuals on Suppressive Antiretroviral Therapy. J Virol. 2017;91(8):e02166-16.

115. Borducchi EN, Cabral C, Stephenson KE, Liu J, Abbink P, Ng'ang'a D, et al. Ad26/MVA therapeutic vaccination with TLR7 stimulation in SIV-infected rhesus monkeys. Nature. 2016;540(7632):284–7.

116. Pankrac J, Klein K, Mann JFS. Eradication of HIV-1 latent reservoirs through therapeutic vaccination. AIDS Res Ther. 2017 Sep 12;14(1):45.

117. Fischer W, Perkins S, Theiler J, Bhattacharya T, Yusim K, Funkhouser R, et al. Polyvalent vaccines for optimal coverage of potential T-cell epitopes in global HIV-1 variants. Nat Med. 2007;13(1):100–6.

118. Demoustier A, Gubler B, Lambotte O, De Goër MG, Wallon C, Goujard C, et al. In patients on prolonged HAART, a significant pool of HIV infected CD4 T cells are HIV-specific. Aids. 2002;16(13):1749–54.

119. Douek DC, Brenchley JM, Betts MR, Ambrozak DR, Hill BJ, Okamoto Y, et al. HIV preferentially infects HIV-specific CD4+ T cells. Nature. 2002;417(6884):95–8.

120. Walker BD, Chakrabarti S, Moss B, Paradis TJ, Flynn T, Durno AG, et al. HIV-specific cytotoxic T lymphocytes in seropositive individuals. Nature. 1987;328(6128):345–8.

121. Koup RA, Safrit JT, Cao Y, Andrews CA, McLeod G, Borkowsky W, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J Virol. 1994;68:4650–5.

122. Borrow P, Lewicki H, Wei X, Horwitz MS, Peffer N, Meyers H, et al. Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. Nat Med. 1997;3(2):205–11.

123. Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA, et al. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science. 1999;283(5403):857–60.

124. Hersperger AR, Migueles SA, Betts MR, Connors M. Qualitative features of the HIV-specific CD8+ T-cell response associated with immunologic control. Curr Opin

HIV AIDS. 2011;6(3):169–73.

125. Deng K, Pertea M, Rongvaux A, Wang L, Durand CM, Ghiaur G, et al. Broad CTL response is required to clear latent HIV-1 due to dominance of escape mutations. Nature. 2015;517(7534):381–5.

126. Gallimore A, Glithero A, Godkin A, Tissot AC, Plückthun A, Elliott T, et al. Induction and Exhaustion of Lymphocytic Choriomeningitis Virus–specific Cytotoxic T Lymphocytes Visualized Using Soluble Tetrameric Major Histocompatibility Complex Class I–Peptide Complexes. J Exp Med. 1998;187(9):1383–93.

127. Zajac AJ, Blattman JN, Murali-Krishna K, Sourdive DJD, Suresh M, Altman JD, et al. Viral Immune Evasion Due to Persistence of Activated T Cells Without Effector Function. J Exp Med. 1998;188(12):2205–13.

128. Jones RB, Mueller S, O'Connor R, Rimpel K, Sloan DD, Karel D, et al. A Subset of Latency-Reversing Agents Expose HIV-Infected Resting CD4+T-Cells to Recognition by Cytotoxic T-Lymphocytes. PLoS Pathog. 2016;12(4):1–25.

129. Hansen SG, Ford JC, Lewis MS, Ventura AB, Hughes CM, Coyne-Johnson L, et al. Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. Nature. 2011;473(7348):523–7.

130. Hansen SG, Jr MP, Ventura AB, Hughes CM, Gilbride RM, Ford JC, et al. Immune clearance of highly pathogenic SIV infection. Nature. 2013;502(7469):100–4.

131. Hansen SG, Sacha JB, Hughes CM, Ford JC, Burwitz BJ, Scholz I, et al. Cytomegalovirus Vectors Violate CD8+ T Cell Epitope Recognition Paradigms. Science. 2013 May 24;340(6135):1237874–1237874.

132. Cohen GB, Gandhi RT, Davis DM, Mandelboim O, Chen BK, Strominger JL, et al. The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. Immunity. 1999;10(6):661–71.

133. Seung E, Dudek TE, Allen TM, Freeman GJ, Luster AD, Tager AM. PD-1 Blockade in Chronically HIV-1-Infected Humanized Mice Suppresses Viral Loads. PLoS One. 2013;8(10).

134. Velu V, Titanji K, Zhu B, Husain S, Pladevega A, Lai L, et al. Enhancing SIV-specific immunity in vivo by PD-1 blockade. Nature. 2009;458(7235):206–10.

135. Caskey M, Klein F, Lorenzi JCC, Seaman MS, West AP, Buckley N, et al. Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. Nature. 2015;522(7557):487–91.

136. Lynch RM, Boritz E, Coates EE, DeZure A, Madden P, Costner P, et al. Virologic effects of broadly neutralizing antibody VRC01 administration during chronic HIV-1 infection. Sci Transl Med. 2015;7(319).

137. Caskey M, Schoofs T, Gruell H, Settler A, Karagounis T, Kreider EF, et al. Antibody 10-1074 suppresses viremia in HIV-1-infected individuals. Nat Med. 2017;23(2):185–91.

138. Caskey M, Klein F, Nussenzweig MC. Broadly neutralizing anti-HIV-1 monoclonal antibodies in the clinic. Nat Med. 2019;25:547–53.

139. Klein F, Halper-Stromberg A, Horwitz JA, Gruell H, Scheid JF, Bournazos S, et al. HIV therapy by a combination of broadly neutralizing antibodies in humanized mice. Nature. 2012 Dec 24;492(7427):118–22.

140. Horwitz JA, Halper-Stromberg A, Mouquet H, Gitlin AD, Tretiakova A, Eisenreich TR, et al. HIV-1 suppression and durable control by combining single broadly neutralizing antibodies and antiretroviral drugs in humanized mice. Proc Natl Acad Sci. 2013;110(41):16538–43.

141. Barouch DH, Whitney JB, Moldt B, Klein F, Oliveira TY, Liu J, et al. Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. Nature. 2013;503(7475):224–8.

142. Shingai M, Nishimura Y, Klein F, Mouquet H, Donau OK, Plishka R, et al. Antibody-mediated immunotherapy of macaques chronically infected with SHIV suppresses viraemia. Nature. 2013;503(7475):277–80.

143. Halper-Stromberg A, Lu CL, Klein F, Horwitz JA, Bournazos S, Nogueira L, et al. Broadly neutralizing antibodies and viral inducers decrease rebound from HIV-1 latent reservoirs in humanized mice. Cell. 2014;158(5):989–99.

144. Lu CL, Murakowski DK, Bournazos S, Schoofs T, Sarkar D, Halper-Stromberg A, et al. Enhanced clearance of HIV-1-infected cells by broadly neutralizing antibodies against HIV-1 in vivo. Science. 2016;352(6288):1001–4.

145. Bournazos S, Klein F, Pietzsch J, Seaman MS, Nussenzweig MC, Ravetch JV. Broadly Neutralizing Anti-HIV-1 Antibodies Require Fc Effector Functions for In Vivo Activity. Cell. 2014;158(6):1243–53.

146. Bar KJ, Sneller MC, Harrison LJ, Justement JS, Overton ET, Petrone ME, et al. Effect of HIV Antibody VRC01 on Viral Rebound after Treatment Interruption. N Engl J Med. 2016;375(21):2037–50.

147. Schoofs T, Klein F, Braunschweig M, Kreider EF, Feldmann A, Nogueira L, et al. HIV-1 therapy with monoclonal antibody 3BNC117 elicits host immune responses against HIV-1. Science. 2016;352(6288):997–1001.

148. Borducchi EN, Liu J, Nkolola JP, Cadena AM, Yu WH, Fischinger S, et al. Antibody and TLR7 agonist delay viral rebound in SHIV-infected monkeys. Nature. 2018 Nov 15 [cited 2020 Jul 4];563(7731):360–4.

149. Hütter G, Nowak D, Mossner M, Ganepola S, Müßig A, Allers K, et al. Long-Term Control of HIV by *CCR5* Delta32/Delta32 Stem-Cell Transplantation. N Engl J Med. 2009;360(7):692–8.

150. Gupta RK, Abdul-Jawad S, McCoy LE, Mok HP, Peppa D, Salgado M, et al. HIV-1 remission following CCR5Δ32/Δ32 haematopoietic stem-cell transplantation. Nature. 2019;568:244–8.