

# Challenges and Promise of Human Immunodeficiency Virus Remission

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Antiretroviral therapy effectively controls human immunodeficiency virus (HIV) replication but it is unable to fully eradicate the HIV reservoir and treatment must be life-long. Progress toward a strategy for HIV remission will require overcoming key hurdles to fill gaps in our understanding of HIV persistence, but the identification of individuals who have attained sterilizing or functional HIV cure show that such a goal is achievable. In this review, we first outline challenges in targeting the HIV reservoir, including difficulties identifying HIV-infected cells, ongoing work elucidating the complex intracellular environment that contribute to HIV latency, and barriers to reactivating and clearing the HIV reservoir. We then review reported cases of HIV sterilizing cure and explore natural models of HIV remission and the promise that such HIV spontaneous and posttreatment controllers may hold in our search for a broadly-applicable strategy for the millions of patients living with HIV.

**Keywords.** HIV reservoir; latency; post-treatment controller; provirus; spontaneous controller.

The introduction of combination antiretroviral therapy (ART) has turned human immunodeficiency virus (HIV) infection into a chronic, manageable disease. People with HIV (PWH) can enjoy a near-normal life expectancy if treated at an earlier stage with life-long ART and durable viral suppression [1]. However, ART is not curative and once interrupted, viral rebound occurs in nearly all patients [2, 3]. This is largely due to the establishment of a latent HIV reservoir in multiple anatomic compartments [4]. Unfortunately, this reservoir is not effectively eliminated by either ART or the immune response. For example, despite long-term suppressive ART, HIV decay can be divided by different phases, and the rate of decay has been found to level off in the setting of prolonged ART [5, 6].

Attempts to alter the decay of the HIV reservoir by ART intensification have largely been disappointing [7, 8], and together, these results raise a series of fundamental questions that remain unanswered about HIV persistence, including what fuels HIV persistence despite durable HIV viral suppression and how the HIV reservoir evades eradication by the host immune response. Understanding these questions will be paramount to our efforts to accelerate the development of novel strategies for HIV remission. In this review, we will first discuss the challenges of HIV persistence despite ART, with a focus on recent literature about virological and immunological mechanisms, and how they interact to promote HIV persistence. Then we will review the promise of HIV remission based on a number of reported cases.

## PART I: THE CHALLENGE OF IDENTIFYING AND ERADICATING HIV-INFECTED CELLS

### Rarity and Inaccessibility of HIV-Infected Cells

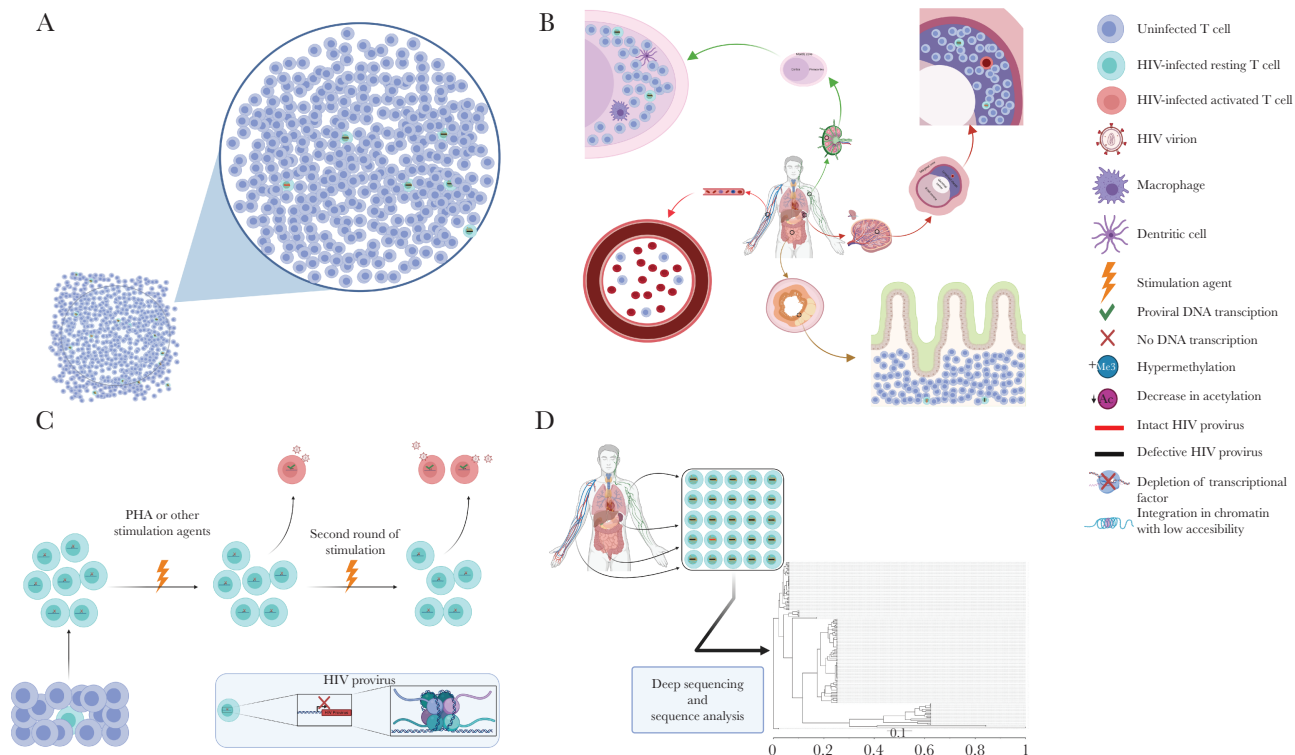
The identification and study of HIV-infected cells is challenging. First, CD4<sup>+</sup> T cells harboring integrated intact proviruses are rare. Early studies have shown that only 0.03–3 infectious unit per million of HIV latent reservoir can be isolated from chronically infected participants receiving suppressive ART, measured by quantitative viral outgrowth assay (QVOA), which has historically been regarded as the reference standard for identifying the frequency of the inducible HIV reservoir [4, 9]. Using polymerase chain reaction–based methods, however, the pool of CD4<sup>+</sup> T cells harboring HIV DNA is far greater, ranging from an estimate of 2 to >1000 cells per 1 million CD4<sup>+</sup> T cells [10]. This discrepancy was partially reconciled by the discovery that the vast majority of HIV proviruses are defective [11]. In PWH who initiated treatment either during the early or chronic phases of infection, >90% of HIV proviral genomes are defective, including deletions, inversions, hypermutations, and other defects (Figure 1A) [11].

A second hurdle to identifying HIV-infected cells is that the HIV proviral genome is often transcriptionally quiescent. Using a single-cell analysis technique, Wiegand and colleagues [12] found that only an average of 7% of HIV proviruses expressed HIV RNA, with a similar level between defective and intact provirus. In lymph nodes (LNs) and intestinal lymphoid tissue (LT), which is estimated to harbor >90% of the HIV-infected CD4<sup>+</sup> T cells in tissue (Figure 1B), only an estimated 0.2% of HIV DNA<sup>+</sup> CD4<sup>+</sup> cells expressed HIV RNA [13]. This, combined with the limited sensitivity of QVOA, has led to the estimate that the amount of proviruses that are genomically intact and capable of making replication-competent virus is likely to be ≥60-fold higher than estimates based on QVOA [14].

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**Figure 1.** Challenge of identifying and reactivating human immunodeficiency virus (HIV)–infected cells. *A*, CD4<sup>+</sup> T cells harboring HIV proviral DNA are rare among the total CD4<sup>+</sup> T-cell population; most of the proviral DNA is defective, and only <10% is intact. *B*, HIV-infected cells are mostly located in difficult-to-study anatomic sites, including lymph nodes, intestinal lymphoid tissue, and spleen. *C*, Multiple potential mechanisms contribute to HIV latency, including epigenetic modifications, depletion of transcriptional factors, and integration in dense regions of the chromosome. Even the most potent CD4<sup>+</sup> T-cell–activating agents (eg, phytohemagglutinin [PHA]) are only able to activate a small proportion of CD4<sup>+</sup> T cells harboring intact HIV proviruses, and the underlying mechanisms remain elusive. *D*, HIV achieves sequence diversity very early during infection; sequences obtained from different cell and anatomic compartments demonstrate substantial diversity as shown in this example phylogenetic analysis of intact HIV proviral sequences from 1 participant. Figures were generated with [BioRender.com](https://www.biorender.com).

A third hurdle to the study of the HIV reservoir is that almost all of the HIV reservoir is located in difficult-to-sample anatomic regions, hindering our ability to fully quantify and characterize the anatomic compartments that harbor HIV-infected cells. While most HIV reservoir studies use peripheral blood-derived samples, there have been conflicting data on whether there is compartmentalization or evolution of HIV within certain tissues that is not reflected within the peripheral blood [15–17]. In a nonhuman primate model, Estes [13] and colleagues used *in situ* hybridization to show that >99% of cells expressing simian immunodeficiency virus or simian-human immunodeficiency virus viral RNA (vRNA) reside in LNs and LTs, including within the intestines, spleen, and lungs. Despite suppressive ART, the frequency of vRNA<sup>+</sup> cells decreased only ≥2-fold in intestine or spleen compared with a 2-log<sub>10</sub> reduction in LNs; correspondingly, intracellular ART levels in these LTs are lower than peripheral blood mononuclear cell ART levels. Similarly, in LNs and intestinal LTs samples from chronically HIV-infected participants, >2–6 years of suppressive ART was associated with an almost 3-log<sub>10</sub> reduction in viral DNA (vDNA)<sup>+</sup> cells in LNs but no significant decrease in vDNA<sup>+</sup> cells in the intestinal LTs [13].

These findings from nonhuman primates and PWH indicate that ART alone cannot fully eradicate vRNA<sup>+</sup> and vDNA<sup>+</sup> cells in LTs. The importance of tissue reservoirs has also been demonstrated in treatment interruption studies, which have demonstrated the presence of multifocal origins of viral reactivation within tissue compartments after ART discontinuation [18] and shown that rebounding virus in plasma may originate from multiple tissue compartments [19].

#### Determining the Mechanisms Underlying HIV Latency and Reactivation

Understanding the pathways surrounding HIV latency and reactivation could accelerate progress for HIV latency reversal. There is evidence that HIV latency is established through a complex set of pathways [20], including epigenetic modifications that reduce histone acetylation [21], enhance histone/promoter methylation [22], and deplete transcriptional factors [23]. There is also evidence that the host chromosomal location of HIV integration may confer a deeper state of proviral latency [24] and that blocks in HIV transcriptional elongation and completion may also play a role in viral latency [25].

Assays like the QVOA use compounds including phytohemagglutinin to reverse proviral latency by inducing CD4<sup>+</sup> T-cell

activation and subsequently HIV provirus transcription [26]. However, even with maximal cellular activation in vitro, only a subset of proviruses can be induced during each round of immune stimulation (Figure 1C) [27]. This is despite the fact that intact noninduced HIV proviruses have the potential to be induced in vivo, because they harbor no lethal mutations, and most of them are integrated in transcriptionally active area with little cytosines followed by guanine residues (CpG) methylation [14]. With further modeling, these findings suggest that the “true” latent reservoir might be approximately 60-fold larger than what phytohemagglutinin stimulation QVOA can estimate [14]. Additional studies are needed to explore these findings and to determine the optimal latency pathways to target in the design of novel therapeutics.

Reactivating latent HIV provirus is challenging, and HIV-expressing cells may not be adequately cleared by the host immune response. There has been significant interest in ways to reactivate latent provirus [28, 29], potentially coupled with strategies to induce immune-mediated killing of reactivated cells in a strategy known as “kick and kill” [30, 31]. However, several early phase clinical trials with only administration of latency reversal agents (LRAs) and suppressive ART in the hope that preexisting HIV-specific immunity performs the killing have only reported moderate effects of “kick” and no clear evidence of reservoir elimination [32–37].

How can we explain the discrepancy between the in vivo and ex vivo studies? First, LRA administration alone is not sufficient to eliminate HIV reservoir. Huang and colleagues [38] used an ex vivo resting CD4<sup>+</sup> T-cell model to show that cells harboring replication-competent HIV may be more resistant to CD8-mediated killing after exposure to LRAs. It is possible that HIV-specific cytotoxic T lymphocytes (CTLs) are diverted by CD4<sup>+</sup> T cells harboring defective provirus; these CD4<sup>+</sup> T cells with defective proviruses represent the majority of HIV-infected CD4<sup>+</sup> T-cell populations [11], can still express HIV RNA, and can be recognized by HIV-specific CTLs [39].

In addition, different CD4<sup>+</sup> T-cell subpopulations respond to LRAs differently. Noticeably, CD4<sup>+</sup> T memory stem cells, a rare cell type that harbors a disproportionately high level of inducible HIV proviruses [40], is very resistant to LRA reactivation [41], which could contribute to the persistence of replication-competent reservoir despite “kick and kill.” Furthermore, several classes of currently studied LRA can potentially inhibit natural killer cells [42] and HIV-specific CTL function [43], thus jeopardizing the effector cells’ ability to eliminate the reactivated HIV reservoir. Thus, improved strategies are needed for both HIV latency reversal and the elimination of these reactivated cells. New classes of LRAs (eg, programmed cell death 1 protein blockade [44] and noncanonical NF- $\kappa$ B activation [45]) and reservoir clearance strategies (eg, convertible chimeric antigen receptor T cells [46]) are on the horizon, but further in vitro and clinical data are needed to evaluate their efficacy and safety profiles.

## HIV Sequence Diversity and Immune Escape as Barriers to Achieving HIV Remission

The rapidity of HIV diversification and adaptation to the host immune response represent another challenge to viral eradication (Figure 1D) [47–49]. Strategies to stimulate the host immune response is a cornerstone of current strategies toward HIV remission. However, Deng and colleagues [50] have shown that unless ART is started early, the vast majority of HIV proviruses already harbor CTL escape mutations that is likely to adversely affect the host immune clearance of HIV-infected cells even after successful viral reactivation. In addition, HIV proviral sequence diversity could represent significant barriers to other HIV gene or protein-targeted interventions.

A 2019 study using long-acting slow-effective release antiviral therapy and clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR associated protein 9 (Cas9) targeting multiple segments of HIV genome resulted in depletion of the HIV reservoir and HIV remission in a subset of animals after ART interruption [51]. However, there was no assessment of proviral diversity in this study, which was likely limited as the humanized mice were treated with ART during acute infection. Although the target specificity of CRISPR-mediated DNA modification limits off-target effects, this may also hinder its ability to excise a diverse proviral reservoir, especially in individuals receiving long-term treatment and with relatively diverse reservoirs [52].

As shown in prior studies, intrapatient sequence diversity creates a barrier to efficient CRISPR-Cas9 induced cleavage [53]. Furthermore, previous in vitro studies demonstrated that HIV was capable of escaping CRISPR-Cas9 induced excision [54, 55]. Similarly, HIV sequence diversity also hampers antibody-based therapy. VRC01, a broadly neutralizing antibody, was shown to delay viral rebound after analytical treatment interruption; however, preexisting resistance mutations against VRC01 were detected and contributed to early viral rebound [56]. To overcome this barrier, triple, or even quadruple broadly neutralizing antibody combinations are likely needed [57].

## PART II: THE PROMISE OF HIV REMISSION

### Defining the Characteristics of an HIV Cure

There are 2 fundamental approaches to an HIV cure: sterilizing and functional cures. *Sterilizing cure* is defined as the state in which no replication-competent virus can be detected after treatment interruption. The cases of sterilizing cures represent some of the greatest success stories in the field but are challenging to replicate and to develop into a broadly applicable therapeutic option. *Functional cure*, also known as sustained HIV remission, refers to the ability of the patient to maintain viral control despite potentially low levels of detectable virus in the blood and tissues. In the development of broadly applicable therapeutics, the induction of sustained HIV remission represents a more realistic goal, as has already been described

for HIV spontaneous (or elite) controllers and posttreatment controllers.

### Success and Failures of Sterilizing Cure Approaches

The field has already identified at least 2 cases of apparent sterilizing cures. These include the “Berlin patient” [58] and the “London patient” [59, 60]. Both individuals underwent hematopoietic stem cell transplantation (HSCT) with donor cells harboring homozygous CCR5Δ32/Δ32 deletions. Apparent sterilizing cures were achieved despite important differences between the 2 patients, including the type of cancer and varying intensity of the conditioning regimens. Furthermore, at the 2019 Conference on Retroviruses and Opportunistic Infections, Björn-Erik et al [61] reported on a third possible case of sterilizing cure. The “Dusseldorf patient” is a 49-year-old man who received underwent HSCT, receiving cells from a CCR5Δ32/Δ32 donor in February 2013 because of acute myeloid leukemia. Repeated laboratory testing has not detected any remaining HIV reservoir, and ART was stopped in November 2018 with no evidence of HIV rebound to date [61].

These cases have elicited a great deal of hope for the HIV community and provided momentum to this scientific field. However, such an approach cannot be broadly applied to the general population of PWH, given the morbidity and mortality risk associated with stem cell transplants [62–65]. Furthermore, the identification of suitable CCR5Δ32/Δ32 donors can be challenging but appears to be a vital component to achieving a sterilizing cure. This is demonstrated by the report of the 2 “Boston patients,” both of whom underwent HSCT with donor cells harboring wild-type, functional CCR5. HSCT was associated with dramatic reductions in the size of the HIV reservoir, but eventual viral rebound occurred 12 and 32 weeks after discontinuation of ART [66]. There have also been reports of incomplete viral eradication and viral escape in the setting of HSCT with homozygous CCR5Δ32 donor cells [66–70].

### Spontaneous Controllers as Possible Goal of HIV Remission?

Spontaneous controllers (SCs) are relatively rare patients with HIV who can maintain low or even undetectable levels of HIV RNA without needing to start ART. There is a wide range of

definitions for this group of participants [71–73], but they are often classified as either elite controllers, if they can maintain viral loads below the limit of detection for commercial viral load assays, or viremic controllers, if they have low levels of detectable viremia. Host immunity appears to play a decisive and prime role in controlling HIV viral replication. They have been found to harbor a robust and polyfunctional HIV-specific T-cell response both in the peripheral blood [74, 75] and within tissue [76]. The importance of CTL-mediated viral suppression is supported by the enrichment of certain protective HLA alleles, such as HLA-B\*27 and B\*57 [77, 78]. It has been found that CTL targeting of highly networked epitopes can identify SCs, even for those without the usual protective HLA alleles [79].

While the studies of SCs have revealed much about effective natural immunity against HIV replication and disease progression, whether they represent the ideal end point for HIV curative strategies remains unclear. First, viral replication and viral evolution can be detected in SCs [80, 81] and loss of viral control and HIV disease progression occurs in a subset of them [72]. SCs have also been found to have increased levels of immune activation and chronic inflammation [73, 82], which may play a role in the reports that SCs may have a higher risk of cardiovascular disease or hospitalization in comparison to ART-treated HIV patients [83–85]. The initiation of ART in SCs is associated with further suppression of viral replication and reduction in immune activation and systemic inflammation [71, 86]. Although the discovery of treatment strategies that could induce spontaneous HIV control would undoubtedly represent a transformative advance, questions remain about whether this represents only an intermediate step toward a strategy that is not associated with higher risk of disease progression or adverse outcomes.

### Posttreatment Controllers as Promising Model of HIV Remission

For most PWH, HIV plasma viral load rebounds within a few weeks after treatment interruption [3, 87]. However, a rare group of patients, termed *posttreatment controllers* (PTCs), are capable of suppressing the virus to low levels for ≥6 months after stopping ART [88, 89]. In 1999, Lisiewicz et al [90] described an

**Table 1. Comparison of Spontaneous and Posttreatment Controllers**

Finding	Description of Evidence	
	SCs	PTCs
Protective HLA alleles [78, 89]	Strong	Negative
CD4 <sup>+</sup> and CD8 <sup>+</sup> T-cell-mediated immunity [76, 79, 89, 102, 103]	Strong	Unclear or absent
Innate immune cells, eg, natural killer cells, involvement [104–106]	Modest	Modest
Immune activation [89, 107–109]	Modest	Unclear or absent
Antibody-mediated immunity [110–112]	Modest	Unclear or absent
High levels of defective provirus [75, 113]	Modest	Modest
Association of HIV proviral integration site [114]	Strong	Unclear or absent
Enhanced with early HIV treatment [88]	NA	Strong

Abbreviation: HIV, human immunodeficiency virus; NA not applicable; PTCs, posttreatment controllers; SCs, spontaneous controllers.



HIV-infected individual who was able to control the infection after multiple treatment interruptions. Since then, several observational studies and clinical trials have been done on these individuals to define and determine their characteristics [8, 88, 91–101]. One of the first in-depth studies of PTCs was the VISCONTI study of 14 early-treated PTCs, with median of 89 months of HIV suppression after treatment interruption [89]. The largest study to date is the Control of HIV after Antiretroviral Medication Pause (CHAMP) study of 67 PTCs, aggregated from 14 clinical studies from AIDS Clinical Trials Group and other North American cohorts [88].

A number of factors seem to differentiate PTCs from SCs (Table 1). Unlike SCs, PTCs do not appear to be enriched for protective HLA alleles [89] and, intriguingly, they may be able to control viremia without increased immune activation [115]. Not only may early ART initiation increase the chances of HIV remission in adults [88], there have also been several reports of children who started ART during infancy and have subsequently exhibited delayed HIV rebound and posttreatment control [116, 117]. However, even among those treated during the earliest phases of HIV infection, posttreatment control can still be challenging to achieve, as was demonstrated in a Thai study of 8 participants who started ART at Fiebig stage I. Despite extremely low HIV reservoirs, none were able to achieve HIV remission, and all experienced viral rebound within 7 weeks after ART interruption [118]. Overall, PTCs seem to represent a promising model for HIV remission, but additional studies are needed to define the mechanisms of HIV control in these patients.

## CONCLUSIONS

To achieve an HIV cure or ART-free remission, it is crucial to understand the temporospatial distribution of HIV reservoir and the virologic and immunologic mechanisms that sustain HIV persistence. As we summarized in this review, there are still knowledge gaps in our understanding of the nature of HIV reservoir and mechanisms behind HIV persistence. Future research will need to focus on addressing HIV reservoir in tissue compartments and in the characterization of HIV-infected cells. Elucidating the mechanisms underlying HIV latency and reactivation will be crucial in the development new therapies for either the silencing or reduction of the viral reservoir. While the Berlin and London patients have provided proof that an HIV cure is possible, the identification of HIV PTCs offers a more feasible path toward sustained HIV remission. Understanding the mechanisms behind their ability to achieve posttreatment control underscores not only the challenges that remain, but also the promise that a successful strategy for HIV remission would have for the millions of PWH worldwide.

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