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Review

Immune Selection and Reservoir Evolution Shape HIV-1 Persistence: Integrating Leukocyte Heterogeneity, Latency Mechanisms, and Emerging Cure Strategies

Randa A. Abdelnaser [1,*], Ahmed K. Hamdy [2,3], Mohammed A. S. Abourehab [4] and Stefan Bräse [5,*]

[1] Division of Infection & Hematopoiesis, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto 860-0811, Japan
[2] Collaborative Research Program with the Chemo-Sero-Therapeutic Research Institute for Anti-Viral Agents and Hematological Diseases, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto 862-0973, Japan; ahmededris@kumamoto-u.ac.jp
[3] Department of Medicinal Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt
[4] Department of Pharmaceutical Sciences, College of Pharmacy, Umm Al-Qura University, Makkah 21955, Saudi Arabia; maabourehab@uqu.edu.sa
[5] Institute of Biological and Chemical Systems—Functional Molecular Systems (IBCS-FMS), Karlsruhe Institute of Technology (KIT), 76131 Karlsruhe, Germany
* Correspondence: randaabdelnaser@kumamoto-u.ac.jp (R.A.A.); braese@kit.edu (S.B.)

Abstract

Despite sustained suppression of viral replication by antiretroviral therapy (ART), HIV-1 persists in long-lived and clonally expanding infected-cell populations across anatomical compartments. This persistence reflects interactions among proviral regulation, host-cell biology, immune selection, and tissue-specific microenvironments. Proviral heterogeneity in genetic integrity, transcriptional activity, inducibility, and antigen expression further influences immune recognition and susceptibility to cure interventions. This review examines the molecular mechanisms of latency, infected-cell survival and clonal maintenance, immune-driven reservoir selection, and anatomical compartmentalization, with particular emphasis on $CD4^+$ T-cell clonal dynamics and the potential contribution of macrophage-lineage populations, including brain microglia. It also distinguishes the detection of viral nucleic acids or intact proviruses from replication competence and demonstrated contributions to viral rebound. The central conceptual contribution of this review is a dynamic, multilayer reservoir-selection framework integrating four interdependent dimensions: proviral state, infected-cell fitness, immune-mediated selection, and tissue-specific context. Rather than treating these determinants as separate mechanisms, this framework conceptualizes HIV-1 persistence as an outcome of their reciprocal interactions, which collectively shape reservoir composition, stability, and therapeutic vulnerability during ART. Emerging single-cell, spatial, and multi-omic technologies offer opportunities to characterize this complexity, although functional validation remains essential to establish biological and clinical relevance. This integrated perspective highlights the need for complementary cure strategies targeting latency, infected-cell maintenance, immune-mediated clearance, and tissue-specific barriers. Achieving durable HIV-1 remission will require mechanistically informed interventions evaluated through functional reservoir measurements and clinically meaningful outcomes.

Keywords: HIV-1 latency; viral reservoirs; $CD4^+$ T cells; macrophages; microglia; immune selection; reservoir heterogeneity; HIV cure

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1. Introduction: HIV-1 Latency: A Regulated and Strategically Selected Viral State

Antiretroviral therapy (ART) has transformed HIV-1 infection into a manageable chronic disease by suppressing plasma viremia. However, ART does not eliminate integrated proviral DNA, and treatment interruption commonly leads to rapid viral rebound from long-lived infected cells. HIV-1 persistence reflects the interplay of proviral regulation, infected-cell survival, and clonal expansion, processes that allow infected cells to remain in the host despite sustained suppression of viral replication [1–3].

Persistent HIV-1 reservoirs comprise proviruses with different transcriptional activities, genetic integrity, and capacities for reactivation. Viral regulatory circuits, integration-site characteristics, chromatin organization, host transcriptional and epigenetic programs, cellular identity, immune pressure, and local tissue signals collectively influence proviral expression and the antigenic visibility of infected cells. These molecular features intersect with infected-cell phenotype and immune recognition to influence which proviruses remain transcriptionally restricted, which infected cells are eliminated, and which populations persist or expand during ART. Longitudinal sequencing shows that reservoir dynamics reflect both the loss of infected cells associated with viral expression and the persistence or expansion of infected-cell clones, revealing processes that latency alone cannot explain [2,4].

Although resting memory $CD4^+$ T cells remain the best-characterized reservoir, persistent HIV-1's cellular and anatomical distribution extends beyond circulating $CD4^+$ T cells. Experimental studies have identified replication-competent HIV-1 in urethral macrophages from individuals receiving suppressive ART and have demonstrated persistent HIV-1 in brain microglia, underscoring the importance of investigating tissue-specific reservoirs [5,6]. This cellular diversity adds another layer of reservoir regulation, in which lineage-specific properties and tissue context influence viral persistence. Consequently, reservoir biology cannot be fully defined by proviral latency or by analyses restricted to peripheral blood; cellular identity and anatomical context can modify reservoir survival, antigen visibility, and therapeutic accessibility.

Here, we develop a multilayer reservoir-selection framework that connects four interacting dimensions: proviral state, cellular fitness, immune selection, and tissue context. In this framework, proviral state shapes viral expression and antigenic visibility; cellular properties influence survival and clonal maintenance; immune pressure affects the relative persistence of infected-cell populations; and tissue environments modify these interactions. The central contribution of this framework is to connect mechanisms commonly studied separately and explain how their interaction can shape the composition and persistence of the HIV-1 reservoir during ART. This perspective also provides a rationale for evaluating cure strategies against distinct biological barriers, including restricted proviral expression, infected-cell maintenance, immune-mediated clearance, and tissue-specific drug access. It thereby links reservoir biology to a precision-treatment agenda in which latency modulation or sustained silencing is considered alongside interventions targeting infected-cell survival, immune recognition, and reservoir maintenance. This narrative review synthesizes current evidence on HIV-1 latency and reservoir persistence, with particular emphasis on proviral state, infected-cell fitness, immune selection, tissue compartmentalization, and emerging cure strategies. Relevant literature was identified through searches of PubMed/MEDLINE, Scopus, and Web of Science up to October 2026, using terms related to HIV-1 reservoirs, latency, immune selection, tissue-specific persistence, and HIV-1 cure strategies. Primary research articles were prioritized for mechanistic findings and clinical evidence, complemented by relevant reviews and reference-list screening. The findings were integrated through a narrative synthesis without formal quantitative meta-analysis.

2. Establishment of HIV-1 Latency

HIV-1 latency arises when an integrated provirus enters a transcriptionally restricted state, although integration alone does not determine whether a provirus becomes latent or persists long-term (Figure 1). The genomic context of integration provides an initial layer of proviral regulation. Schroder et al. demonstrated that HIV-1 integration preferentially occurs within transcriptionally active genes, establishing the genomic context for subsequent proviral regulation [7]. Shun et al. further established a role for lens epithelium-derived growth factor p75 (LEDGF/p75) in directing HIV-1 integration toward specific genomic regions [8]. However, integration-site selection alone cannot explain the establishment of latency, which also depends on the cellular environment and the regulatory mechanisms acting on the integrated provirus. Chavez et al. demonstrated that HIV-1 latency can be established early after infection in both resting and activated primary $CD4^+$ T cells, highlighting the diversity of pathways leading to transcriptional restriction [9]. At the molecular level, Tyagi et al. showed that epigenetic silencing and limited availability of positive transcription elongation factor b (P-TEFb) contribute to the establishment of HIV-1 latency in primary $CD4^+$ T-cell models [10]. Complementing these findings, Lindqvist et al. investigated the contribution of proviral chromatin organization to transcriptional regulation in resting $CD4^+$ T cells [11]. Collectively, these findings indicate that HIV-1 latency is not an inevitable consequence of integration but emerges from the interaction between integration-site context, cellular state, and post-integration transcriptional and epigenetic regulation. These processes establish heterogeneous patterns of proviral expression that influence the subsequent fate of infected cells and the potential formation of a persistent reservoir.

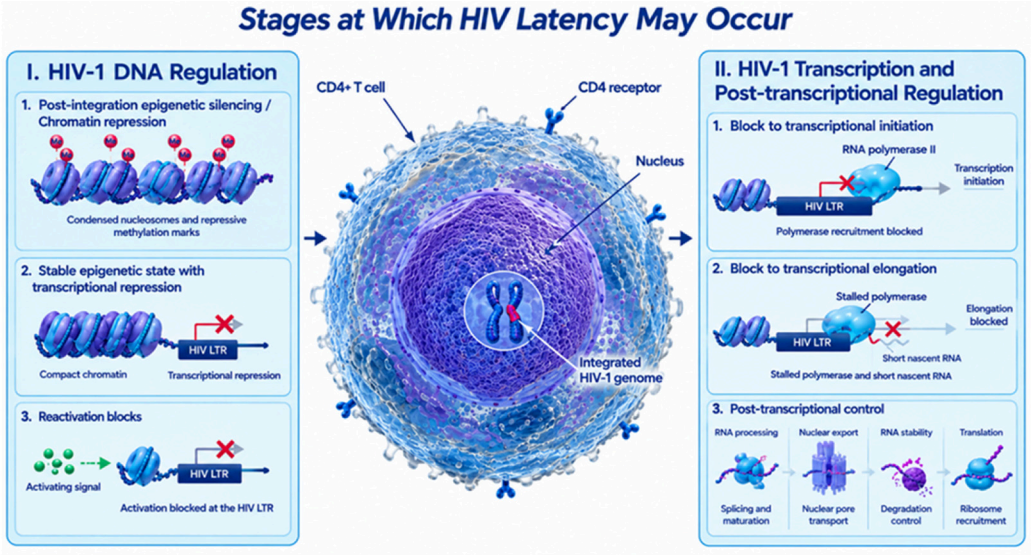


Figure 1. Mechanisms of Post-Integration Regulation of HIV-1 Latency. The figure illustrates two interconnected levels of post-integration regulation. (I) HIV-1 DNA regulation, encompassing epigenetic silencing, chromatin repression, and stable transcriptional restriction, which limit proviral expression and may impede reactivation. (II) HIV-1 transcriptional and post-transcriptional regulation, including blocks to transcriptional initiation and elongation, as well as regulation of RNA processing, nuclear export, RNA stability, and translation. These mechanisms restrict viral gene expression at distinct regulatory stages and contribute to the heterogeneous transcriptional states of HIV-1 proviruses. Created in BioRender. Abdelnaser, R. (2026) https://BioRender.com/7lvzhcm.

2.1. Direct Establishment of HIV-1 Latency

Experimental studies have challenged the view that HIV-1 latency arises only when productively infected $CD4^+$ T cells return to a resting state. Chavez et al. used the dual-reporter virus HIV Duo-Fluo I, flow cytometry, and fluorescence-activated cell sorting

(FACS) to distinguish latent from productive infection in primary human CD4$^+$ T cells. Their experiments showed that latency could be established following infection of both resting and activated cells; importantly, returning productively infected cells to a resting state did not produce substantial additional silencing of integrated HIV-1 [9]. Extending this work, Plasek Hegde et al. combined single-cell RNA sequencing with functional studies in primary CD4$^+$ T-cell models to investigate infection-associated cellular reprogramming. They identified activation of the p53 pathway and a KLF2-associated quiescence programme, providing experimental evidence that HIV infection can itself alter host-cell transcriptional states associated with entry into latency [12]. These findings support multiple routes to latency establishment while leaving open how individual transcriptionally restricted proviruses subsequently contribute to the long-lived reservoir. The latter question requires separate consideration of infected-cell survival, clonal expansion, and immune selection.

2.2. Early Reservoir Seeding During Acute Infection

The early HIV-1 reservoir is defined not only by the rapid appearance of infected cells but also by their anatomical distribution and capacity to persist after treatment begins. In a longitudinal study of people identified during acute HIV-1 infection, Leyre L et al. found that infected-cell frequencies were highest in gut-associated lymphoid tissue and lymph nodes as early as Fiebig stage II, before seroconversion; ART initiated during Fiebig stages I–III markedly reduced these populations, although rare infected cells remained preferentially detectable in lymphoid tissues [13].

Complementary evidence from SIV-infected rhesus macaques showed that reservoir seeding can precede detectable systemic viremia and that ART initiated as early as day 3 after infection did not reliably prevent viral rebound following treatment interruption [14]. These findings establish that the timing of treatment and the anatomical compartment sampled both influence estimates of early reservoir establishment. Crucially, reservoir persistence can begin to acquire a clonal dimension within the same early period: integration-site analysis by Coffin et al. detected expanded HIV-infected cell clones approximately four weeks after infection, with some clones persisting for years during suppressive ART [15]. Extending this observation, longitudinal analysis of individuals treated during acute infection by Struyve et al. combined integration-site sequencing, proviral characterization, and ex vivo viral reactivation to show that clonal expansion and changes in integration-site distribution remain detectable despite early ART, with proviruses in repressive genomic regions becoming more prominent over time [16]. Together, these studies suggest that early reservoir development is not a single seeding event followed by passive persistence. Rather, it involves an evolving interaction among anatomical compartmentalization, infected-cell survival and proliferation, and selection of proviral states. However, integration-site enrichment and clonal expansion do not, by themselves, demonstrate that immune selection is the causal mechanism; its contribution must be distinguished from other determinants of infected-cell survival and expansion.

2.3. Integration-Site Selection and Reservoir Landscape

HIV-1 integration is non-random at the genomic level. Genome-wide mapping by Schröder et al. demonstrated preferential integration into transcriptionally active genes, while LEDGF/p75 depletion experiments by Ciuffi et al. provided direct evidence that this host factor contributes to targeting HIV DNA to transcription units [7,17]. However, the integration landscape detected in persistent infection reflects both initial targeting and subsequent changes in the abundance of infected-cell clones. Using integration-site mapping in individuals receiving suppressive antiretroviral therapy (ART), Maldarelli et al. identified clonally expanded infected cells carrying proviruses at recurrent positions within genes

including BACH2 and MKL2, implicating particular integration events in the persistence or expansion of some infected-cell populations [2]. Longitudinal analysis by Kok et al. further showed that recurrent integration patterns associated with clonal expansion were already detectable during primary infection and occurred in both resting and activated $CD4^+$ T cells [18]. Importantly, these findings do not establish that all integrations in BACH2, MKL2, or other recurrent integration genes confer a selective advantage. Indeed, comparative analysis by Coffin JM et al. indicated that integration into oncogenes accounts for only a minority of the integration-site distribution observed in vivo [19]. Thus, the reservoir landscape is shaped by two distinct but interacting processes: host and viral determinants bias the initial distribution of integration sites, whereas infected-cell survival and proliferation subsequently alter the representation of individual clones. Integration-site mapping can reveal these dynamics, but integration location alone does not establish proviral intactness, replication competence, or the mechanism responsible for clonal expansion.

2.4. Latency as a Dynamic Viral–Host Equilibrium

HIV-1 latency reflects the interaction between viral regulatory sequences and the activation state of the host cell. The long terminal repeat (LTR) contains promoter and enhancer elements that integrate signals from cellular transcription factors. Using electrophoretic mobility shift assays, DNase footprinting, and transfection experiments, Perkins et al. demonstrated cooperative interactions between NF-κB and Sp1 during HIV-1 enhancer activation [20]. Kinoshita et al. further showed that NFAT can enhance LTR activity and viral gene expression in T cells, linking cellular activation signaling to the regulation of HIV-1 transcription [21]. These findings establish that transcriptional initiation depends not only on the presence of an integrated provirus but also on the regulatory signals available within the infected cell.

However, promoter activation does not necessarily result in sustained, productive viral gene expression. Regulatory checkpoints acting at subsequent stages can restrict transcriptional progression, alter RNA maturation, or limit the production of viral proteins. These mechanisms provide a molecular basis for variable proviral expression, which may affect antigen availability but does not, by itself, establish whether an infected cell will survive or be eliminated. Because reservoir establishment and persistence are assessed using assays that capture different biological properties, the following section summarizes the principal approaches used to measure and characterize the persistent HIV-1 reservoir.

2.5. Measuring the Persistent HIV-1 Reservoir

The persistent HIV-1 reservoir cannot be adequately characterized by a single assay because proviral abundance, genetic integrity, inducibility, and replication competence represent distinct biological properties. The great majority of integrated HIV-1 proviruses in individuals receiving suppressive antiretroviral therapy (ART) are defective and therefore unable to produce replication-competent virus. Consequently, quantification of total HIV-1 DNA estimates the burden of infected cells but includes both intact and defective proviruses, with only a minority potentially contributing to the replication-competent reservoir. Changes in total HIV-1 DNA therefore do not necessarily reflect equivalent changes in the reservoir capable of sustaining viral rebound after ART interruption. Studies comparing molecular and virological measurements have demonstrated that estimates of intact proviruses, inducible virus, and total proviral DNA can differ substantially because these methods measure distinct aspects of persistence [22,23].

The intact proviral DNA assay (IPDA) uses multiplex droplet digital PCR to quantify proviruses based on the integrity of selected genomic regions. Its principal advantage is the scalable estimation of putatively intact proviruses without requiring full-length sequenc-

ing of every detected genome. Bruner et al. established this approach and demonstrated its utility for quantifying intact and defective proviruses in ART-treated individuals [22]. However, IPDA does not directly establish replication competence, because defects outside the targeted regions may remain undetected. Moreover, sequence variation at primer or probe binding sites can impair amplification and affect classification. In a comparative study of 39 ART-suppressed individuals, Gaebler et al. found that IPDA and quadruplex PCR (Q4PCR) measurements were correlated but differed substantially in estimated intact-provirus frequencies, reflecting both assay-specific limitations and the detection of proviruses with defects outside the IPDA target regions [23].

The quantitative viral outgrowth assay (Q-VOA) addresses a different question by measuring replication-competent virus induced from infected cells under defined ex vivo stimulation conditions. Its functional readout provides evidence of recoverable infectious virus rather than inferring competence from selected DNA targets alone. Nevertheless, conventional Q-VOA can underestimate the replication-competent reservoir because a single round of stimulation does not induce every replication-competent provirus. Ho et al. demonstrated that some proviruses not induced during standard activation could nevertheless have intact genomes and produce replication-competent virus after reconstruction. Thus, Q-VOA estimates the fraction of the reservoir that can be induced under the specified assay conditions rather than necessarily capturing every replication-competent provirus. Results also depend on the number and type of cells tested, stimulation conditions, and viral outgrowth efficiency [23,24]. Accordingly, Q-VOA and IPDA measurements should not be interpreted as interchangeable estimates of reservoir size. Near-full-length or full-length proviral sequencing provides information about individual proviral structures, including large deletions, hypermutation, and other genetic defects that may compromise viral function. When combined with integration-site mapping, sequencing can also connect proviral structure to chromosomal location and help identify expanded infected-cell clones. Einkauf et al. developed the matched integration site and proviral sequencing (MIP-Seq) approach to examine proviral sequences alongside their corresponding integration sites in samples from three individuals receiving long-term ART [25]. They found that intact proviruses were enriched in particular genomic contexts, including non-genic locations and orientations opposite to host genes, consistent with the persistence of proviruses exhibiting features of deeper latency. These findings are observational and do not establish immune-mediated selection as the sole cause of the observed distribution. Sequencing-based estimates are also affected by template sampling, amplification efficiency, and the criteria used to classify proviruses as intact. White et al. demonstrated that long-distance PCR workflows used in near-full-length genome sequencing can substantially underrepresent full-length proviruses because of amplification failure, illustrating an important source of quantification bias [26].

These assays therefore provide complementary rather than equivalent information. IPDA estimates the frequency of proviruses meeting defined genetic-integrity criteria; Q-VOA measures inducible replication-competent virus under specified experimental conditions; and near-full-length sequencing characterizes proviral structure, with integration-site analysis providing additional information about genomic location and clonal expansion. Differences between estimates are not necessarily contradictory, because each method has a distinct biological target and technical limitations. Gaebler et al. directly demonstrated discrepancies between IPDA and Q4PCR measurements, while White et al. showed that amplification bias can affect sequencing-based estimates [23,26]. Longitudinal studies and therapeutic trials should therefore select assays according to the biological endpoint being investigated and, where feasible, combine complementary methods to distinguish changes in proviral abundance, genetic integrity, inducibility, and replication competence.

Interpretation of reservoir measurements also requires consideration of HIV-1 genetic diversity. Sequence-dependent assays may perform differently when primer or probe binding sites vary across viral subtypes. In their comparison of IPDA and Q4PCR, Gaebler et al. reported IPDA amplification failures in two of three participants with non-subtype-B infection, illustrating how an assay initially designed around subtype-B sequences may not perform equivalently across all viral backgrounds [23]. This small number of non-B infections does not establish the frequency of assay failure across global populations, but it demonstrates the need for subtype-aware validation. Studies comparing reservoir measurements across populations should report, where available, HIV-1 subtype, assay target regions, validation procedures, proviral classification criteria, sampling methods, and relevant ART history. Such standardization is important for distinguishing genuine biological differences from variation attributable to assay design or study populations.

The generalizability of HIV-1 reservoir studies is also limited by the global distribution of viral genetic diversity. Subtype B predominates in many cohorts from North America and Western Europe, whereas non-B subtypes and circulating recombinant forms account for a substantial proportion of infections worldwide. Recent global estimates indicate that subtype C remains the most prevalent HIV-1 subtype, emphasizing the importance of extending reservoir research beyond predominantly subtype-B populations [27,28]. Differences in viral sequence may affect the performance of molecular assays, while potential subtype-associated differences in viral biology should be distinguished from differences attributable to host characteristics, treatment history, and study design.

The timing of ART initiation is another important determinant of reservoir establishment and subsequent measurements. Initiation during primary or early infection is associated with reduced reservoir seeding and lower levels of cell-associated HIV-1 DNA during suppressive treatment compared with initiation during chronic infection. Longitudinal evidence further indicates that early ART can produce more rapid and pronounced HIV DNA decay, although persistent infected-cell populations remain detectable during long-term treatment [29,30]. The interpretation of reservoir size and decay should therefore account for the timing of treatment initiation, duration of suppressive ART, and the antiretroviral regimen received. Although regimen composition may influence viral suppression and the interpretation of longitudinal measurements, its independent contribution to reservoir size or decay should not be assumed without direct comparative evidence.

These considerations highlight the need for greater representation of non-B subtypes and diverse clinical populations in HIV-1 reservoir research. Comparisons across studies should account for viral subtype or recombinant form, timing of ART initiation, treatment duration, regimen composition, and assay validation. Such information is essential for determining whether observed differences reflect underlying reservoir biology, clinical history, or methodological variation, and for evaluating the applicability of reservoir-directed interventions across populations.

3. Molecular Mechanisms Governing HIV-1 Latency

Following integration, HIV-1 gene expression can be restricted at multiple regulatory stages. These restrictions are not interchangeable: chromatin organization can limit access to the proviral template, impaired transcriptional elongation can prevent efficient production of full-length RNA, and post-transcriptional mechanisms can alter RNA processing or localization. In addition, transcriptional activity can fluctuate over time rather than remain uniformly switched on or off. Distinguishing these mechanisms is essential for interpreting evidence of HIV-1 transcription and for understanding why detectable viral RNA does not necessarily correspond to productive viral gene expression.

3.1. Chromatin-Mediated Repression of the HIV-1 Provirus

The chromatin environment surrounding an integrated provirus can restrict transcription even when the cellular machinery required for gene expression is present. Nucleosome organization and histone modifications influence the accessibility of the proviral template and the recruitment or activity of transcriptional regulators. In primary resting $CD4^+$ T cells, Lindqvist et al. investigated the maturation of proviral chromatin and its relationship to HIV-1 transcriptional restriction, providing evidence that chromatin regulation is relevant to the establishment and maintenance of latency [11].

Additional experimental work has examined specific host factors involved in repressive chromatin regulation. Zhou et al. reported that origin recognition complex subunit 1 (ORC1) promotes repressive epigenetic modifications at the HIV-1 LTR, linking a host chromatin-associated factor to transcriptional restriction [31]. Such findings support a role for chromatin-associated mechanisms in controlling proviral expression. However, the presence of a repressive chromatin feature should not automatically be interpreted as proof of irreversible silencing; its functional significance depends on the experimental context and whether changes in viral transcription accompany changes in chromatin state.

3.2. Tat/P-TEFb-Dependent Transcriptional Elongation

Transcriptional initiation and productive elongation represent distinct regulatory steps. Even when transcription begins, the resulting RNA may not progress efficiently to full-length transcripts. HIV-1 Tat helps overcome this restriction through its interaction with the transactivation response (TAR) RNA element and positive transcription elongation factor b (P-TEFb), which contains CDK9 and cyclin T1 [32–34].

Wei et al. demonstrated that cyclin T1 interacts directly with Tat and supports its high-affinity, loop-specific binding to TAR RNA, establishing a molecular basis for Tat-dependent transcriptional elongation [35]. Budhiraja et al. subsequently showed that cyclin T1 and CDK9 T-loop phosphorylation are downregulated during the establishment of latency in primary resting memory $CD4^+$ T cells, implicating reduced P-TEFb activity in this process [36].

Evidence from patient-derived $CD4^+$ T cells further indicates that transcriptional restriction cannot be reduced to a single elongation block. Using assays that distinguish different HIV-1 RNA species, Yukl et al. identified blocks affecting transcriptional elongation, transcriptional completion, and RNA splicing [37]. These findings distinguish the initiation of transcription from the completion of the steps required to produce mature viral RNA. Accordingly, measurements of promoter activity or short HIV-1 transcripts alone may not reliably indicate the extent of productive viral gene expression.

3.3. Transcriptional Bursting and Nuclear Organization

HIV-1 transcription can vary over time, producing fluctuations in viral gene expression rather than a stable, uniform state. Weinberger et al. showed that fluctuations in Tat expression can drive phenotypic diversity through a stochastic positive-feedback circuit [38]. Razooky et al. further characterized HIV-1 latency as a hardwired regulatory programme shaped by the dynamics of viral gene expression [39]. Together, these studies provide experimental and modelling-based support for the contribution of transcriptional variability to different expression states.

Nuclear organization adds another layer to this regulation. Kizito et al. reported that structural rearrangements within the nucleus can localize latent HIV-1 proviruses to a perinucleolar compartment that supports reactivation [40]. This finding cautions against assuming that nuclear proximity to a particular structure invariably indicates transcriptional

repression. The functional outcome depends on the local nuclear environment and its relationship to proviral activity.

These mechanisms also require careful interpretation at the measurement level. A single-cell measurement captures expression at a particular time and may not distinguish persistent silencing from transiently low transcription. Conversely, variation in RNA abundance does not necessarily establish corresponding variation in viral protein production or antigen presentation. Those outcomes require separate experimental assessment.

3.4. Post-Transcriptional Regulation of HIV-1 RNA

HIV-1 expression can remain restricted after transcription has initiated and viral RNA has been produced. RNA processing, splicing, nuclear export, stability, and translation determine whether transcripts can contribute to viral protein production. These regulatory stages are particularly important when viral RNA is detectable despite limited evidence of productive infection [37]. Yukl et al. identified defects involving RNA splicing alongside blocks in transcriptional elongation and completion in HIV-1-infected cells, demonstrating that post-initiation restrictions can occur at more than one stage [37]. More recently, Dorman et al. identified nuclear retention of unspliced HIV-1 RNA as a reversible post-transcriptional restriction associated with latency [41]. Their findings support the distinction between RNA synthesis and the subsequent processing and localization required for productive viral gene expression.

The contribution of each regulatory step may differ according to cell type and experimental system. Findings obtained in $CD4^+$ T cells should therefore not automatically be generalized to macrophages or microglia, in which the cellular environment and viral RNA regulation may differ. Establishing the relative importance of a particular mechanism requires measurements suited to the relevant stage of gene expression and the specific cell population being studied.

Overall, HIV-1 latency is maintained through multiple regulatory checkpoints rather than a single universal silencing mechanism. Chromatin-associated repression, inefficient elongation, variable transcriptional activity, nuclear organization, and post-transcriptional restrictions can each limit different aspects of viral gene expression. Distinguishing these processes provides a mechanistic framework for interpreting latency measurements without assuming that RNA detection necessarily indicates protein production, antigen presentation, or productive viral replication.

4. Immune Selection and Reservoir Remodeling During HIV-1 Persistence

The persistence of HIV-1 during suppressive antiretroviral therapy (ART) cannot be explained by transcriptional latency alone. The long-term reservoir is shaped by interactions among proviral genetic integrity, transcriptional activity, infected-cell phenotype, immune recognition, and cellular proliferation. These processes influence different stages of persistence: whether an infected cell produces viral antigens, whether those antigens trigger immune recognition, whether recognition results in cell elimination, and whether surviving infected cells expand. Importantly, evidence that immune responses select particular viral sequences does not, by itself, demonstrate selection for transcriptionally silent or otherwise immune-invisible infected cells. These mechanisms must be distinguished to avoid conflating genetic immune escape with phenotypic persistence.

In this section, reservoir remodeling refers to changes in the composition, cellular distribution, transcriptional state, and clonal representation of HIV-1-infected cells during infection and ART. The term does not imply that all changes arise from ongoing viral genetic evolution. Longitudinal and single-provirus studies indicate that the reservoir is heterogeneous and dynamic, with different infected-cell populations experiencing distinct

selective pressures [42,43]. We therefore consider immune selection together with proviral activity and infected-cell proliferation to explain why some reservoir populations persist despite effective suppression of viral replication.

4.1. Proviral Activity and Immune Visibility Shape Reservoir Persistence

The potential for an HIV-1-infected cell to be recognized by immune effectors depends partly on its capacity to express viral proteins and present viral peptides. Transcriptionally silent proviruses may produce little or no viral antigen, reducing opportunities for immune recognition. However, transcriptional activity is not a binary property: infected cells can differ in the abundance, timing, and completeness of viral transcripts, and detectable transcription does not necessarily establish production of antigenic proteins or presentation of immunogenic peptides. Consequently, proviral transcription, antigen presentation, and immune-mediated killing should be treated as related but distinct biological outcomes. Single-provirus analyses provide direct evidence that transcriptional activity is associated with the genomic and epigenetic environment of individual proviruses. Using parallel profiling of proviral sequence, integration site, and transcriptional activity, Einkauf et al. found that transcriptionally active proviruses were subject to negative selection during ART, while some large transcriptionally active clones persisted despite this pressure [43]. These findings support a model in which viral expression can impose a disadvantage on infected cells without determining their fate independently of clonal expansion and other host-cell properties. They also demonstrate why the reservoir should not be described as uniformly transcriptionally silent: transcriptionally active and silent proviruses coexist, and their relative representation may change over time.

Longitudinal reservoir analyses further indicate that persistent proviruses occupy heterogeneous genomic and cellular contexts. Changes in integration-site distribution and the representation of infected-cell clones are consistent with differential survival and proliferation of infected cells during prolonged ART. Nevertheless, the observation that a provirus is located in a genomic environment associated with reduced transcription does not by itself prove that immune selection caused its persistence. Establishing that causal relationship requires measurements linking proviral activity to antigen expression, immune recognition, cell fate, and longitudinal changes in the same infected-cell population [44]. The distinction between proviral integrity and immune visibility is equally important. Bruner et al. developed the intact proviral DNA assay (IPDA) to distinguish proviruses classified as intact from those containing selected genetic defects [22]. This approach improved quantification of the genetically intact reservoir, but intactness is not equivalent to inducibility, expression, or demonstrated replication competence. Conversely, defective proviruses can sometimes produce viral transcripts or proteins and may therefore contribute to antigen exposure even when they cannot generate replication-competent virus. Thus, immune visibility cannot be inferred from proviral integrity alone, and the abundance of HIV DNA should not be interpreted as a direct measure of the population most relevant to rebound.

Together, these observations support a more precise interpretation of immune selection: proviral activity can influence the probability of immune recognition, but the eventual persistence of an infected cell also depends on the effectiveness of immune elimination and the capacity of that cell to survive or proliferate. The relative contribution of these processes remains an important question for longitudinal studies of reservoir composition.

4.2. Cytotoxic T Cells and Other Immune Effectors: Recognition Does Not Guarantee Elimination

HIV-1-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) can recognize viral peptides presented by major histocompatibility complex class I molecules and eliminate infected cells. Their activity has therefore motivated strategies that combine latency reversal with immune-mediated killing. However, the presence of HIV-specific CTLs, or evidence that an infected cell expresses viral antigen, does not establish that the cell will be eliminated. The outcome depends on antigen abundance and presentation, the functional properties of the effector cell, and the susceptibility of the infected target cell to killing [45].

Huang et al. provided a key experimental demonstration of this limitation. In ex vivo experiments using cells from ART-treated individuals, latency-reversing agents combined with autologous $CD8^+$ T cells reduced cell-associated HIV DNA but did not consistently reduce the replication-competent reservoir measured by quantitative viral outgrowth assay (QVOA) [46]. The $CD8^+$ T cells were capable of recognizing and eliminating newly infected target cells, suggesting that the persistence of reservoir cells could not be attributed solely to generalized CTL dysfunction or viral escape. The results instead supported the existence of barriers to eliminating cells harboring replication-competent HIV. This finding is particularly important because a reduction in total HIV DNA after an intervention does not necessarily demonstrate a corresponding reduction in the infectious reservoir.

These results should not, however, be interpreted as evidence that all intact proviruses are intrinsically resistant to immune killing. The degree of resistance may vary with proviral expression, target-cell state, immune-effector properties, and experimental conditions. The more defensible conclusion is that antigen induction and immune recognition are insufficient predictors of successful clearance and must be evaluated alongside direct measurements of infected-cell elimination and reservoir function.

Other immune mechanisms may also contribute to the differential survival of infected cells. Natural killer (NK) cells can recognize and kill susceptible targets through activating and inhibitory receptor pathways, while antibody-dependent cellular cytotoxicity (ADCC) can recruit Fc-receptor-bearing effector cells to antibody-coated targets. Experimental studies of antibody-based approaches, including bispecific molecules designed to redirect immune effectors toward infected cells, support the feasibility of enhancing immune recognition and killing. However, activity in experimental systems does not establish that these strategies can eliminate all persistent, replication-competent reservoir cells in vivo. Their effectiveness is likely to depend on antigen expression, antibody access to infected cells, effector-cell distribution and function, and the susceptibility of the target-cell population [44,47].

Accordingly, CTL, NK-cell, and antibody-mediated mechanisms should not be treated as interchangeable measures of immune pressure. Each depends on partly distinct recognition pathways and target-cell requirements. A reservoir population that is poorly controlled by one mechanism may remain susceptible to another, but this possibility requires direct experimental testing rather than inference from the presence of an immune response. For cure research, the critical endpoint is not simply increased immune activation or recognition; it is the measurable depletion of infected cells carrying intact, inducible, or replication-competent proviruses.

4.3. Genetic Immune Escape Versus Phenotypic Persistence

Immune-mediated reservoir persistence can arise through mechanisms that must be conceptually separated. Genetic immune escape occurs when viral sequence variation reduces recognition by a particular immune response, for example, through changes in an epitope or its processing. Phenotypic persistence, in contrast, describes the survival of an infected cell despite the absence of a necessary viral genetic escape mechanism.

Phenotypic persistence may reflect limited viral antigen production, inadequate peptide presentation, ineffective immune-cell access or function, or resistance of the target cell to immune-mediated elimination [48]. These mechanisms may coexist, but evidence for one cannot automatically be used to establish the other.

Studies of HIV-specific CTL responses have documented viral sequence changes associated with immune escape [45]. Such findings are evidence of genetic selection at the viral level; they do not by themselves show that the persistent reservoir is enriched for transcriptionally silent cells. Similarly, the detection of intact proviruses during prolonged ART does not establish that these proviruses escaped immune recognition through sequence variation [49]. Claims about phenotypic selection require evidence that connects the infected cell's expression or presentation state with its survival under immune pressure.

Experimental work has shown that defective proviruses can be transcriptionally active and may produce antigens recognized by CTLs, illustrating that genetic defects do not necessarily confer complete immunological invisibility [50]. Conversely, Huang et al. demonstrated that cells carrying replication-competent reservoir virus could resist $CD8^+$ T-cell-mediated elimination under the conditions tested, even when immune escape and generalized effector dysfunction were not sufficient explanations [46]. These findings indicate that viral sequence, proviral activity, and target-cell susceptibility represent distinct dimensions of persistence.

This distinction also affects the interpretation of latency-reversal studies. Induction of viral RNA or protein may increase the opportunity for immune recognition, but these readouts do not prove that sufficient antigen is processed and presented, nor that recognition results in target-cell death. Similarly, reductions in total cell-associated HIV DNA may reflect the loss of cells carrying defective proviruses without a measurable decrease in the replication-competent reservoir. Therefore, studies evaluating immune-mediated reservoir depletion should distinguish at least three outcomes: induction of viral expression, recognition of infected cells by immune effectors, and elimination of cells carrying replication-competent proviruses.

The central unresolved issue is whether particular combinations of proviral state and infected-cell phenotype predict survival under defined immune pressures. Addressing this question will require studies that measure viral sequence and integrity alongside transcription, antigen expression or presentation, effector-cell recognition, and target-cell fate. Such integrated measurements would help determine when reservoir persistence reflects genetic escape, restricted immune visibility, resistance to killing, or a combination of these processes.

4.4. Clonal Expansion and Antigen-Driven Selection Sustain the Reservoir

Reservoir persistence is not determined solely by the rate at which infected cells are eliminated. The proliferation of infected cells can maintain or expand reservoir populations even when new rounds of infection are effectively suppressed by ART. Homeostatic proliferation, antigen-driven responses, and integration-associated effects can all contribute to the persistence of infected-cell clones. Importantly, clonal expansion does not necessarily require ongoing productive infection, and an expanded clone may contain defective as well as intact proviruses [1,51,52].

Simonetti et al. demonstrated that clonally expanded $CD4^+$ T cells can harbor intact proviruses and produce infectious HIV-1 in vivo, establishing clonal expansion as a mechanism capable of maintaining replication-competent virus during antiretroviral therapy (ART) [52]. In a subsequent study, Simonetti et al. linked antigen responsiveness with T-cell receptor β-chain sequences, proviral sequences, and integration sites. Among ART-treated individuals, antigen-responsive $CD4^+$ T-cell populations contained large infected-

cell clones, including clones harboring replication-competent proviruses [51]. Notably, antigen-associated proliferation occurred even when proviruses were integrated at sites not previously associated with enhanced infected-cell proliferation. These findings provide direct evidence that antigen-driven clonal expansion contributes to reservoir maintenance and that persistence cannot be explained exclusively by transcriptional silence or integration-associated growth advantages [51,52].

These findings complicate a simple model in which infected cells with greater viral expression are consistently removed while silent cells persist. An infected cell may undergo proliferation in response to its cognate antigen or other growth signals, thereby increasing the number of cells carrying a provirus. Whether such a clone expands or contracts will depend on the balance between proliferation, cell death, immune recognition, and the proviral characteristics inherited by its daughter cells. In some circumstances, proliferation may offset negative selection against cells expressing viral genes. This interpretation is consistent with the observation by Einkauf et al. that large transcriptionally active proviral clones can persist despite evidence of negative selection against transcriptionally active proviruses overall [43].

Clonal expansion also has implications for how reservoir measurements are interpreted. A high frequency of a particular proviral sequence may reflect repeated proliferation of a single infected-cell clone rather than repeated independent infection events. Conversely, changes in the frequency of a clone over time may reflect differential expansion or survival rather than the acquisition of new viral mutations [53,54]. Accordingly, studies of reservoir remodeling should distinguish the number of infected cells, the number of unique proviral sequences, the number and size of infected-cell clones, and the proportion of proviruses that are intact or replication-competent. Together, these findings establish clonal expansion as a mechanism capable of sustaining reservoir populations under suppressive ART. They also identify an important interaction between immune selection and cellular proliferation: the long-term abundance of an infected-cell clone reflects not only whether its cells are recognized and eliminated, but also how rapidly the clone expands and whether its proviruses retain the capacity to generate infectious virus.

5. Tissue Compartmentalization Shapes Reservoir Heterogeneity

HIV-1 persistence extends beyond circulating $CD4^+$ T cells into anatomically distinct tissues that function as specialized niches for reservoir establishment and maintenance [55]. These compartments differ in cellular composition, immune surveillance, metabolic state, tissue architecture, and antiretroviral drug penetration, creating distinct selective environments that influence proviral activity, cellular survival, and therapeutic responsiveness (Figure 2, Table 1). HIV persistence should therefore be viewed not as a uniform systemic reservoir but as a multicompartment network of interacting niches, in which tissue-specific pressures continuously shape infected-cell states and reservoir stability.

However, the detection of HIV nucleic acids in a tissue does not, by itself, establish the presence of intact proviruses or replication-competent virus. The strength of evidence for a functional reservoir therefore varies among tissues and depends on the assays used to distinguish viral persistence from productive infection or residual viral material [56,57].

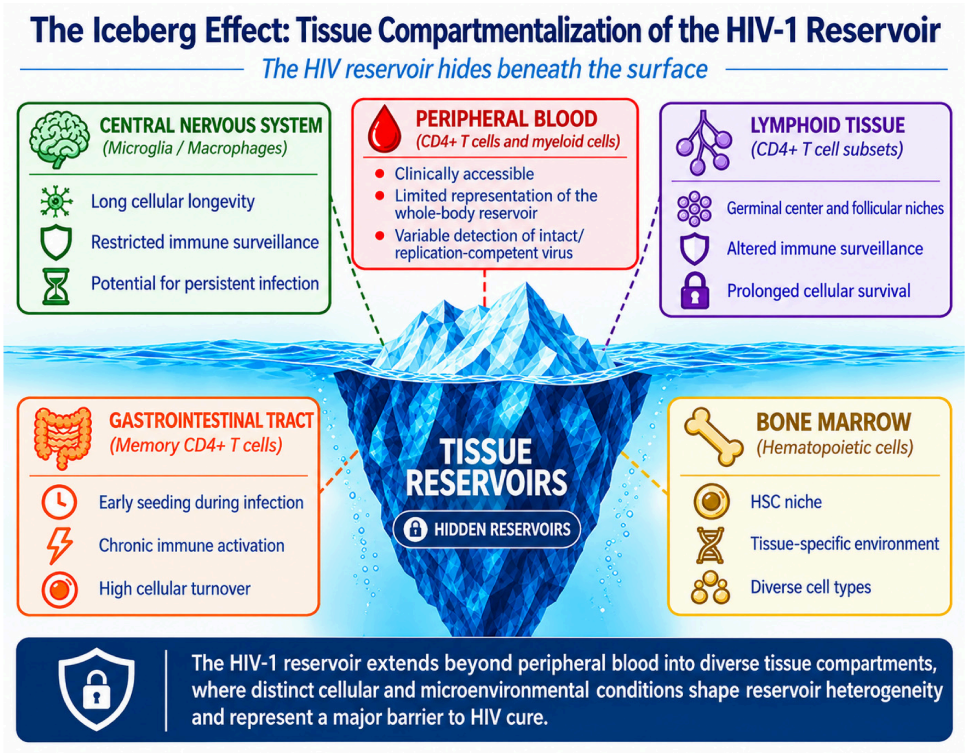


Figure 2. Tissue compartmentalization of the HIV-1 reservoir. Peripheral blood provides a clinically accessible but incomplete view of the persistent HIV-1 reservoir. Infected cells are distributed across tissue compartments, including lymphoid tissues, the gastrointestinal tract, the central nervous system, and bone marrow, where distinct cellular and microenvironmental conditions influence reservoir persistence. Differences in immune surveillance, antiretroviral drug exposure, cellular longevity, tissue architecture, and viral transcription may contribute to compartment-specific reservoir composition and infected-cell survival. The evidence for intact and replication-competent proviruses varies among tissues and study designs; detection of HIV nucleic acids alone does not establish the presence of a functional reservoir. These tissue-specific niches contribute to reservoir heterogeneity and may complicate HIV cure strategies. Created in BioRender. Abdelnaser, R. (2026) https://BioRender.com/f506rn7.

5.1. Lymphoid and Gastrointestinal Reservoirs

Lymphoid tissues and the gastrointestinal tract represent major sites of HIV-1 persistence during suppressive ART. Lymphoid architecture creates specialized cellular interactions and immune environments that can alter the accessibility and phenotype of infected cells. In germinal-center niches, follicular helper $CD4^+$ T cells can harbor HIV-1, but their contribution to the inducible reservoir should be distinguished from evidence of viral persistence in lymphoid tissue more broadly. In human lymph nodes, Connick et al. found that cytotoxic T lymphocytes (CTLs) were concentrated predominantly outside follicles, where HIV replication was observed, supporting the importance of spatial organization in determining immune access to infected cells [58]. Complementary tissue-model experiments identified $CD69^+CCR7^-$ follicular helper T cells as a major inducible reservoir population in tonsillar models, whereas $CD69^+CD49a^+$ tissue-resident memory T cells predominated in intestinal models [56]. Such anatomical organization can therefore shift the balance between viral visibility and cellular survival, favoring infected-cell states that remain difficult to eliminate despite systemic viral suppression.

The gastrointestinal tract is seeded early during infection and undergoes epithelial disruption, microbial translocation, and chronic immune activation, creating an inflammatory environment associated with persistent infection of memory CD4$^+$ T cells [59]. Estes et al. demonstrated extensive body-wide distribution of SIV-infected cells in macaques, illustrating that peripheral blood alone does not capture the full anatomical burden of infection; this animal-model evidence should not, however, be interpreted as a direct quantitative estimate of the human HIV-1 reservoir [60]. The gut therefore illustrates how local inflammation, tissue damage, immune dysregulation, and continuous cellular turnover can collectively generate a reservoir environment distinct from that of circulating blood. These observations support a model in which tissue-specific cellular composition and immune organization influence reservoir maintenance, although the relative contribution of each process to persistence during suppressive ART remains dependent on the tissue and experimental system studied.

5.2. Myeloid and CNS Reservoirs

Myeloid-lineage cells exhibit biological properties distinct from those of conventional CD4$^+$ T-cell reservoirs, including differences in cellular longevity, self-renewal, differentiation programs, and tissue-specific regulation [61,62]. These properties provide a distinct cellular context for HIV-1 persistence, particularly within anatomical compartments where local immune conditions and cellular organization influence viral expression and infected-cell survival [63]. The central nervous system (CNS) illustrates this complexity. Tang et al. demonstrated that brain microglia can harbor persistent, inducible, replication-competent HIV-1 during suppressive antiretroviral therapy (ART), including recovery of virus from microglia obtained from one person with HIV [6]. In a separate human postmortem study, Nühn et al. detected HIV DNA in microglia-enriched fractions from all five participants examined but identified intact proviruses in only one ART-treated participant [57]. Together, these findings support the existence of a potentially functional microglial reservoir while emphasizing that detection of HIV DNA does not establish proviral integrity or replication competence.

Experimental models provide complementary evidence that macrophages can support HIV-1 infection independently of T cells under defined conditions. Honeycutt et al. demonstrated productive infection in a myeloid-only humanized mouse model [64] and subsequently reported HIV persistence in tissue macrophages during ART, although evidence of persistence and replication competence varied among animals [65,66]. Consistent with these findings, Veenhuis et al. identified persistent latent HIV reservoirs in monocyte-derived macrophages [67], while Abreu et al. demonstrated contributions from both myeloid and CD4$^+$ T cells to the latent reservoir in ART-suppressed SIV-infected macaques [68]. These studies strengthen the evidence for myeloid-cell involvement in viral persistence, although they do not establish the relative contribution of these populations to HIV-1 rebound in humans. The principal biological and anatomical characteristics distinguishing CD4$^+$ T-cell and myeloid-lineage reservoirs are summarized in Table 1.

Anatomical compartmentalization further shapes reservoir persistence by exposing infected cells to distinct immune, metabolic, cellular, and pharmacological environments. Tissue-specific differentiation, restricted cellular trafficking, local self-renewal, and heterogeneous antiretroviral distribution can influence infected-cell accessibility and the biological state of persistent proviruses [55,66]. The local self-maintenance of tissue-resident macrophages and the distinct lifespan and turnover characteristics of human microglia provide additional biological context for persistence in these compartments [69,70]. At the molecular level, HIV-associated cellular reprogramming has been linked to quiescence and proviral latency in CD4$^+$ T cells [12,71], while type I interferon signaling has been

implicated in promoting HIV-1 latency in macrophages [72]. Consequently, the tissue environment is not merely the location in which HIV-1 persists; it can influence reservoir phenotype, immune visibility, cellular fitness, and susceptibility to therapeutic intervention. Strategies developed primarily around circulating $CD4^+$ T-cell reservoirs may therefore incompletely address tissue-associated populations.

Immune-mediated clearance may also be limited in infected macrophages. Clayton et al. demonstrated that HIV-infected macrophages can resist NK-cell-mediated killing while maintaining inflammatory cytokine responses [73]. Related findings showed resistance to $CD8^+$ T-lymphocyte-mediated killing, suggesting that macrophages may evade important antiviral effector mechanisms [74]. In addition, early ART has been shown to prevent or limit SIV reservoir seeding in rhesus macaques [75]. Potential approaches to modulating myeloid-associated latency include dual TLR2/TLR7 agonists [76] and targeting the bromodomain pathway, which has been implicated in HIV latency regulation in a macrophage-cell model [77]. These findings provide potential mechanistic and therapeutic directions, although their effectiveness against persistent human tissue reservoirs remains to be established.

The relative contributions of myeloid and $CD4^+$ T-cell reservoirs remain difficult to compare because the available evidence differs in anatomical sampling, experimental model, and demonstration of intact or replication-competent virus. The relationship between reservoir composition and viral rebound also warrants consideration. Tyers et al. reported that rebound HIV-1 consisted of a limited number of viral lineages that entered the reservoir near ART initiation [78]. Kincer et al. further demonstrated that the rebound virus detected in cerebrospinal fluid following treatment interruption was predominantly clonally amplified, R5 T-cell-tropic virus [79]. Although these findings highlight the importance of clonal dynamics and anatomical compartmentalization, they do not establish a major contribution of CNS-resident myeloid cells to systemic viral rebound. A central challenge is to establish which tissue-associated myeloid populations maintain replication-competent HIV-1 in vivo and whether they contribute meaningfully to systemic rebound. Addressing this question requires approaches that combine cell-type-resolved reservoir characterization with functional assessment of viral competence and evaluation of tissue-specific immune and pharmacological accessibility.

Table 1. Distinct Cellular and Anatomical Features of CD4$^+$ T-Cell and Myeloid HIV-1 Reservoirs.

Biological Feature	CD4$^+$ T-Cell Reservoir	Monocyte/Macrophage-Lineage Populations	Implication for Reservoir Persistence
Principal cellular populations	Memory CD4$^+$ T cells, including central-memory, transitional-memory, and tissue-resident subsets; follicular helper T cells in lymphoid tissues [1,2]	Circulating monocytes and tissue-associated macrophages, including CNS microglia [5,67]	Reservoir properties depend on cellular identity and differentiation state [68]
Tissue distribution	Peripheral blood, lymph nodes and other lymphoid tissues, gastrointestinal mucosa, and other tissues containing susceptible CD4$^+$ T-cell subsets [13,56]	Circulating blood monocytes and tissue-associated populations in selected organs; brain microglia are a distinct CNS-resident population [57,63]	Blood sampling does not fully capture the anatomical distribution of either reservoir. Tissue-specific evidence must be evaluated separately.
Principal persistence mechanisms	Clonal expansion, homeostatic proliferation, and prolonged survival of infected CD4$^+$ T-cell clones [3,15]	Potentially prolonged survival and local maintenance of selected tissue-associated populations; the contribution varies by macrophage subtype and experimental setting [67,69]	Cellular persistence can be maintained through different, potentially overlapping mechanisms.
Cellular turnover	Varies by CD4$^+$ T-cell subset and activation state; proliferation can maintain infected clones [16,53]	Varies among monocytes, differentiated macrophages, and tissue-resident populations; some macrophage populations have long lifespans or self-renewal capacity [69,70]	Turnover cannot be generalized across all cells within either lineage.
Evidence of productive or persistent infection	Intact and inducible proviruses and replication-competent virus have been demonstrated in CD4$^+$ T cells [22,25]	Persistent HIV DNA and, in selected studies, intact or inducible replication-competent virus have been demonstrated in macrophage-lineage populations [6,67]	Detecting viral DNA, intact proviruses, viral expression, and replication-competent virus represent distinct levels of evidence.
Transcriptional landscape	Ranges from transcriptionally silent proviruses to intermittently or actively expressed proviruses [10,12,71]	Heterogeneous; viral DNA may be present without detectable RNA, and transcriptional activity varies with cell type and experimental conditions [63,72]	Transcriptional state should be measured rather than inferred from cellular lineage alone.
Immune recognition	Recognition depends on antigen expression, antigen processing and presentation, CTL specificity, and effector function [44,50]	Recognition may also occur, but its efficiency depends on viral expression, antigen presentation, local immune conditions, and the myeloid subtype [73,74]	Neither lineage is uniformly visible or invisible to immune effectors
Evidence from tissue and animal models	Human tissue studies and experimental models identify tissue-specific CD4$^+$ T-cell reservoir populations [56,58,75]	Myeloid-only humanized mouse studies demonstrate that selected macrophage-tropic viruses can sustain infection independently of T cells; human CNS studies provide evidence of microglial persistence [57,65,68]	Model-specific findings establish biological plausibility but do not quantify each lineage's relative contribution to the human reservoir.
Response to latency reversal or reservoir-directed interventions	Responses vary according to proviral integrity, inducibility, transcriptional regulation, and immune-mediated clearance [50,76]	Evidence is more limited and depends on the cell subtype, model, and intervention examined [72,77]	Cell- and tissue-specific responses require direct experimental comparison; a universal difference in responsiveness should not be assumed.
Contribution to viral rebound	CD4$^+$ T cells are a well-established source of rebound-competent virus following ART interruption [78,79]	The extent to which tissue-associated myeloid reservoirs contribute to systemic rebound in people remains unresolved [67,68]	The potential importance of myeloid reservoirs should be distinguished from evidence that they are a major source of rebound in humans.

6. Therapeutic Implications for HIV Cure

Viewing HIV-1 persistence as a multicellular, immune-selected, and tissue-compartmentalized process has important therapeutic implications. Reservoir stability reflects not only transcriptional repression but also cellular longevity, clonal expansion, survival signaling, immune recognition, and anatomical accessibility. Accordingly, the therapeutic target is not a single latent provirus but a distributed reservoir system in which viral visibility, cellular fitness, immune pressure, and tissue context are mechanistically coupled [1–3,51]. This framework also helps explain why an intervention that changes one reservoir property—for example, HIV transcription—may not necessarily reduce the number of persistently infected cells or prevent rebound. Current HIV-1 cure strategies can therefore be organized according to the biological layer they perturb, ranging from viral transcriptional regulation and cellular maintenance to immune-mediated clearance and tissue accessibility, as summarized in Figure 3. The central question is whether a candidate intervention produces a measurable change in the relevant reservoir population, rather than only a change in an intermediate molecular or virological marker.

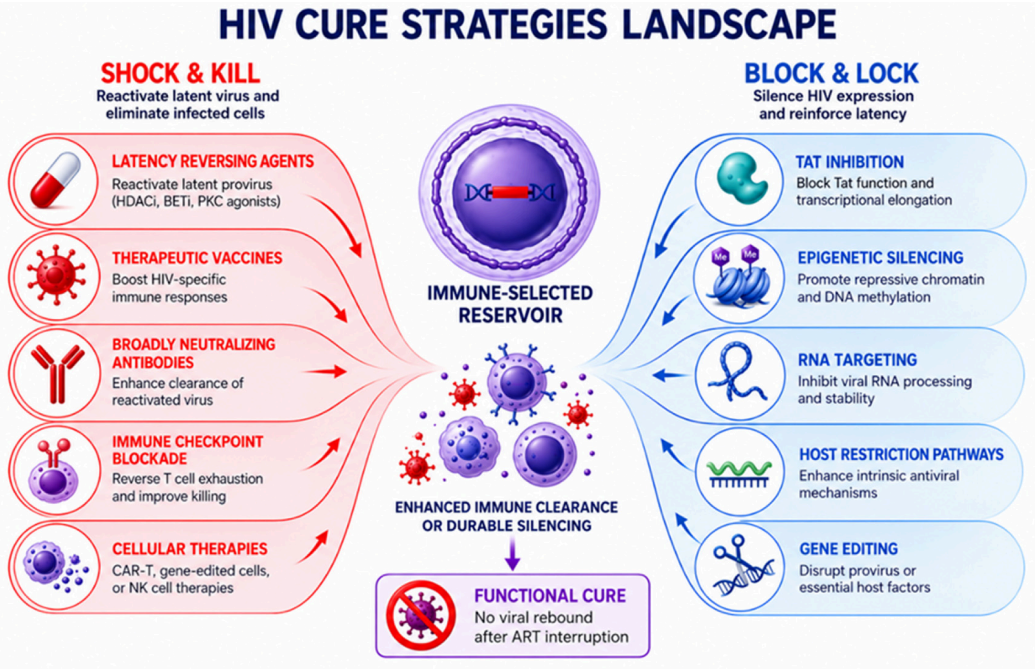


Figure 3. Therapeutic strategies targeting immune-selected HIV-1 reservoirs. Current HIV cure strategies aim either to eliminate persistent reservoir cells or to prevent their reactivation. Shock-and-kill combines latency-reversing agents with immune-based interventions to increase viral antigen expression and promote elimination of infected cells, whereas block-and-lock seeks durable proviral silencing through Tat inhibition, epigenetic regulation, RNA-targeting approaches, and gene-editing technologies. Complementary strategies targeting reservoir maintenance, cellular survival, and tissue accessibility may further address the cellular and anatomical determinants of persistence. Together, these approaches illustrate the need to simultaneously target molecular latency, immune recognition, reservoir maintenance, and tissue compartmentalization to achieve durable ART-free remission. Created in BioRender. Abdelnaser, R. (2026) https://BioRender.com/9woh1jc.

6.1. Shock-and-Kill: Opportunities and Limitations

Shock-and-kill strategies aim to reactivate latent proviruses using latency-reversing agents (LRAs), followed by immune-mediated elimination of the reactivated cells. Although LRAs can increase HIV-1 transcription, the magnitude of transcriptional induction does not necessarily predict reservoir clearance because viral reactivation, antigen production, immune recognition, and cellular elimination represent sequential but non-equivalent

biological checkpoints [50,80,81]. Studies of reservoir heterogeneity have demonstrated that many proviruses remain poorly inducible or produce insufficient antigen despite measurable transcription, providing a mechanistic explanation for the limited reservoir reduction achieved with LRA-based approaches. Consistent with this distinction, experimental studies have shown that latency reversal can increase HIV-1 transcription without proportionate increases in viral protein expression or effective $CD8^+$ T-cell recognition, highlighting the multiple barriers between transcriptional activation and infected-cell elimination [82–84]. This distinction is important when interpreting LRA studies: increased HIV RNA is evidence of transcriptional activity, but it is not, by itself, evidence of sufficient viral protein expression, effective immune-mediated killing, or a durable reduction in the replication-competent reservoir [82–84].

This limitation is particularly relevant to tissue-associated reservoirs, including TFH cells, gastrointestinal compartments, and the CNS, where restricted immune access, distinct cellular states, and heterogeneous drug exposure can uncouple viral reactivation from effective clearance. These barriers should not be assumed to operate identically across tissues: the relevant cellular populations, local immune environments, and evidence for persistent replication-competent virus differ by anatomical site [56,58,60]. More effective shock-and-kill approaches may therefore require coordinated enhancement of three linked processes: proviral activation, antigen presentation, and immune effector function, together with sufficient access to anatomically protected reservoir niches. Combination strategies should be evaluated against complementary endpoints—including proviral induction, viral protein or antigen expression, infected-cell elimination, and changes in intact or inducible reservoir measures—rather than relying on transcriptional induction alone.

6.2. Block-and-Lock

Block-and-lock strategies seek to establish durable HIV transcriptional suppression by targeting Tat activity, reinforcing restrictive chromatin states, or silencing the viral LTR. Rather than eliminating every infected cell, these approaches aim to reduce the probability that persistent proviruses transition from a restricted state to transcriptionally competent states capable of initiating viral rebound [85,86].

Mechanistic studies of Tat-dependent transcription and chromatin regulation support the concept that HIV latency can be stabilized by reinforcing multiple regulatory checkpoints rather than targeting transcription initiation alone. However, evidence that transcription can be suppressed in experimental models does not establish that durable silencing can be maintained across the heterogeneous cellular and anatomical reservoirs present in people receiving ART. Latency architecture varies according to cellular identity, integration-site context, chromatin organization, and tissue environment [37,85,87]. Moreover, transcriptional suppression during treatment does not establish that a provirus has been permanently silenced or that rebound cannot occur after the intervention is withdrawn [85,86]. Block-and-lock strategies may therefore be most effective when designed around the regulatory state of individual reservoir populations rather than assuming a uniform molecular mechanism of latency. Preclinical evaluation should consequently distinguish sustained suppression of HIV transcription from irreversible proviral silencing.

6.3. Targeting Reservoir Maintenance

Because reservoir persistence is strongly influenced by cellular survival, homeostatic proliferation, and clonal expansion, therapeutic strategies are increasingly exploring host pathways that support infected-cell maintenance [1–3]. Evidence that persistent HIV-infected clones can expand through cellular proliferation has shifted attention from the proviral genome toward the host-cell programs that determine whether an infected cell

survives, divides, or is eliminated. Modulation of survival signaling, inflammatory pathways, metabolic adaptation, or proliferation-associated mechanisms could therefore disrupt reservoir maintenance while complementing transcriptional or immune-based interventions [51,52,88].

This strategy reframes the reservoir from a collection of latent genomes into a population of infected cells whose persistence depends on host-cell fitness. The major challenge is achieving selective disruption of reservoir-supporting pathways while minimizing toxicity to uninfected cells and preserving essential tissue functions. Therapeutic selectivity will therefore depend on identifying vulnerabilities that distinguish infected reservoir cells from their uninfected counterparts, rather than broadly suppressing pathways required for normal cellular homeostasis. This requirement is especially important for pathways shared by infected cells and essential immune or tissue-resident cells, because broad inhibition could impair immune function or tissue maintenance without selectively depleting the reservoir. Accordingly, candidate host-directed interventions should be assessed for their effects on infected-cell survival and clonal maintenance alongside cytotoxicity, immune-cell function, and tissue-specific safety.

6.4. Translational Considerations for Cell- and Tissue-Specific Reservoir Targeting

The therapeutic potential of latency-reversing agents (LRAs) and latency-promoting agents (LPAs) depends not only on their molecular mechanisms but also on the biological characteristics and anatomical distribution of the reservoir populations they affect. A pharmacological response observed in circulating $CD4^+$ T cells cannot be assumed to predict an equivalent response in tissue-resident lymphocytes or macrophage-lineage cells. For example, ex vivo human tissue models have demonstrated differences in inducible HIV reservoirs across tonsillar and intestinal compartments [56], while studies in humanized mice and human brain tissue provide evidence that macrophages and microglia can harbor persistent HIV under particular experimental or clinical conditions [6,57,65].

The central translational challenge is to establish whether pharmacological effects translate into durable control of the replication-competent reservoir. Increased transcription following latency-reversing agent (LRA) treatment and reduced transcription following latency-promoting agent (LPA) treatment are intermediate pharmacodynamic outcomes, not evidence of reservoir elimination or irreversible silencing. Progress therefore depends on linking drug exposure and target engagement to changes in proviral integrity, inducibility, and infected-cell composition, with functional assessment of rebound potential and safety. This approach would prioritize interventions by their demonstrated effects on reservoir persistence rather than changes in viral expression.

6.5. Clinical Remission and Evaluation of ART-Free Control

The clinical relevance of HIV-1 cure strategies ultimately depends on whether they can achieve sustained remission or virological control without antiretroviral therapy (ART). Exceptional cases of prolonged HIV remission following allogeneic hematopoietic stem-cell transplantation (allo-HSCT) provide proof that long-term control without ART is possible. These cases include the Berlin patient [89], the London patient [90], and subsequent individuals reported after transplantation for hematological malignancies [91–93]. The Berlin patient, Timothy Ray Brown, achieved long-term remission following transplantation from a donor homozygous for the CCR5Δ32 mutation [89]. The London patient received a CCR5Δ32/Δ32 transplant for Hodgkin lymphoma [90], while the Düsseldorf patient achieved long-term remission following CCR5Δ32/Δ32 transplantation for acute myeloid leukemia [91]. The New York patient, a woman with HIV-1, achieved prolonged ART-free remission following a haplo-cord transplant incorporating CCR5Δ32/Δ32 cord-blood

cells [92]. However, these outcomes followed intensive interventions undertaken to treat life-threatening cancers and cannot be directly extrapolated to cure strategies intended for the broader population of people living with HIV. Their principal significance is to establish the biological possibility of durable remission while highlighting the need for safer and more widely applicable interventions. These cases also highlight the potential contribution of CCR5-mediated resistance to HIV-1 infection, although CCR5Δ32-associated protection does not necessarily prevent infection by viruses using alternative coreceptors.

ART-free control can also occur without transplantation. Post-treatment controllers are individuals who maintain low or undetectable plasma viraemia for a period after ART discontinuation, despite the persistence of HIV-1 infection. Their outcomes are heterogeneous: control may be transient or sustained for years in a smaller subset, and viral rebound may occur before subsequent control is established. In the Control of HIV After Antiretroviral Medication Pause (CHAMP) study, which analyzed participants from 14 clinical studies, post-treatment control was more frequently identified among individuals who initiated ART during early infection than among those who initiated treatment during chronic infection (13% versus 4%; $p < 0.001$) [94]. However, the durability of control varied, with approximately 55% of identified post-treatment controllers maintaining control for two years and approximately 20% for at least five years. These observations are important when interpreting cure trials because post-treatment control does not necessarily indicate eradication of replication-competent virus, and spontaneous or intervention-associated post-treatment control may complicate attribution of ART-free remission to an experimental intervention [95]. Consequently, clinical trials should interpret ATI outcomes alongside baseline characteristics, treatment history, reservoir measurements, and the duration and pattern of post-interruption viral control.

Because undetectable plasma HIV-1 RNA during ART does not establish eradication of replication-competent virus, reservoir measurements alone cannot reliably demonstrate that an intervention has achieved durable remission. Analytical treatment interruption (ATI), conducted within a carefully monitored clinical research protocol, provides a means of assessing whether viral replication resumes after ART withdrawal and whether control can be maintained without ongoing treatment. Previous clinical studies have documented both transient ART-free remission followed by viral rebound and more prolonged remission after allo-HSCT, demonstrating that the duration and trajectory of post-treatment control are essential to interpreting clinical outcomes [90,96]. ATI should therefore be regarded as a clinical efficacy assessment rather than as a substitute for reservoir characterization or a routine component of HIV care.

ATI endpoints should be selected according to the intended therapeutic outcome. Time to confirmed viral rebound is informative for interventions intended to delay reactivation, whereas the post-interruption viral-load trajectory, peak viraemia, and subsequent level of viral control are relevant when evaluating interventions intended to promote immune-mediated control. The duration of ART-free control, preservation of $CD4^+$ T-cell counts, clinical safety, and the proportion of participants maintaining control at prespecified time points provide complementary measures of benefit. Protocol-defined ART restart criteria are essential to participant safety and should be reported transparently alongside virological outcomes. The distinction between time-to-rebound and viral-set-point ATI designs is emphasized in current consensus recommendations for HIV cure trials [97,98]. The major therapeutic strategies targeting persistent HIV-1 reservoirs, including their mechanisms of action, potential benefits, and limitations, are summarized in Table 2.

Table 2. Therapeutic Strategies Targeting Persistent HIV-1 Reservoirs: Mechanisms, Potential Benefits, and Limitations.

Strategy	Mechanism of Action	Potential Benefits	Major Limitation	Why Reservoirs Persist
Therapeutic Vaccination	Enhances HIV-specific $CD8^+$ and $CD4^+$ T-cell responses following latency reversal	Strengthens antiviral immunity; complements LRAs	Limited reservoir reduction despite robust immune responses	Deeply latent cells express insufficient antigen for immune recognition; viral escape and impaired effector function can further limit clearance [99,100]
Immune checkpoint blockade (PD-1, CTLA-4)	Restores exhausted T-cell function and may enhance viral transcription	Improves immune effector activity; may augment shock-and-kill	Inconsistent clinical efficacy and immune-related toxicity	Reactivated cells often remain below the threshold required for efficient immune clearance, particularly within tissue reservoirs [101,102].
Broadly Neutralizing Antibodies (bNAbs)	Neutralize virus and promote Fc-mediated effector functions (e.g., ADCC)	Delay viral rebound; enhance immune-mediated clearance	Activity depends on viral Env expression; viral escape may occur	Latently infected cells lacking Env expression remain invisible to antibody-mediated elimination [103,104]
Engineered T-cell therapies (e.g., CAR T cells)	Redirect T cells to recognize HIV-associated surface antigens and kill antigen-expressing infected cells.	May improve targeted cytotoxicity and potentially overcome limitations of naturally occurring HIV-specific T cells.	Antigen dependence, viral escape, persistence and trafficking of engineered cells, and potential toxicity; clinical efficacy remains to be established.	Latent cells may not express sufficient target antigen, and tissue reservoirs may be difficult for effector cells to reach [105–108].
Natural killer (NK)-cell-based approaches	Enhance innate cytotoxicity against infected cells through antibody-mediated effector mechanisms or engineered NK cells.	May complement $CD8^+$ T-cell responses and antibody-based strategies through partly distinct recognition pathways.	Inconsistent recognition of infected cells, variable NK-cell function, and limited clinical evidence for reservoir reduction.	Low viral antigen expression, inhibitory signals, and restricted access to tissue-associated reservoirs can limit NK-cell activity [109–112].
Cytokine-based immunomodulation (e.g., IL-15 pathway agonism)	Promotes activation, expansion, or cytotoxic activity of selected immune effector populations.	May strengthen NK-cell and $CD8^+$ T-cell responses and improve the activity of combination immunotherapies.	Systemic immune activation and toxicity; increased effector activity does not necessarily translate into reservoir depletion.	Effector activation alone cannot reliably eliminate cells that fail to express recognizable viral antigens or that are poorly accessible [113–115].
Innate immune activation (e.g., TLR agonists)	Activates innate immune pathways and may enhance antigen presentation, antiviral signaling, and downstream adaptive responses.	May promote immune activation and complement latency-reversing or antibody-based interventions.	Responses can be context-dependent; inflammation and immune activation may occur without meaningful reservoir reduction.	Incomplete antigen induction, immune evasion, and inadequate killing of reactivated cells can allow infected cells to persist [116–120].
Gene-editing strategies	Use targeted nucleases or other genome-editing systems to disrupt proviral sequences or modify host genes involved in HIV entry or persistence.	Could potentially inactivate integrated proviruses or confer resistance to infection.	Efficient delivery to all relevant reservoir cells, editing specificity, off-target effects, and incomplete editing remain major challenges.	Reservoirs are distributed across heterogeneous cellular populations and anatomical sites, making comprehensive delivery and editing difficult [121–123]
RNA-targeting strategies	Targets viral RNA or regulates its processing, stability, translation, or expression using approaches such as antisense oligonucleotides or RNA interference.	May suppress viral gene expression or reduce production of viral proteins and particles.	Delivery, durability, sequence variation, and access to tissue reservoirs may limit efficacy.	Transcriptionally silent proviruses produce little or no target RNA, while incomplete delivery may leave other infected cells unaffected [124–127]
Host restriction and reservoir-maintenance pathways	Modulates host factors involved in HIV transcription, viral replication, infected-cell survival, or clonal maintenance.	May expose host-cell vulnerabilities and complement latency-directed or immune-based interventions.	Host pathways may be shared with essential cellular functions, creating toxicity and selectivity concerns.	Host-cell signaling and tissue heterogeneity may shape reservoir persistence and treatment responses; the contributions of monocyte activation and TNFAIP2–CSF-1R signaling remain to be defined [128–130].

Interpretation of analytical treatment interruption (ATI) outcomes must account for the fact that delayed rebound does not establish reservoir eradication: residual infected cells may persist despite prolonged suppression, and plasma viraemia may re-emerge beyond the observation period. Accordingly, cure-directed interventions should be evaluated by integrating clinical outcomes with complementary measures of reservoir integrity, inducibility, and replication competence, while distinguishing pharmacodynamic responses from sustained ART-free control. Follow-up duration and safety outcomes should also be explicitly reported.

7. Emerging Technologies Redefining HIV-1 Reservoir Biology

Emerging single-cell, spatial, and genome-engineering technologies are extending HIV-1 reservoir research beyond bulk measurements toward the resolution of individual infected cells and their tissue environments. These approaches address complementary questions: which cellular and proviral states persist, where infected cells are located, and whether defined viral or host mechanisms can be therapeutically manipulated. Their value lies not simply in generating higher-dimensional datasets, but in linking reservoir heterogeneity to testable mechanisms of persistence and potential therapeutic vulnerabilities. However, molecular characterization, spatial localization, and successful reservoir elimination represent distinct levels of evidence and should not be treated as interchangeable outcomes.

7.1. Single-Cell and Spatial Multi-Omics

Single-cell analyses have demonstrated that HIV-1 reservoirs contain heterogeneous proviral and cellular states that are not adequately represented by population-level measurements. Einkauf et al. integrated HIV-1 RNA expression, proviral sequencing, and integration-site information to characterize infected cells at single-cell resolution, revealing molecular heterogeneity that bulk assays can obscure [43]. These findings support a model in which HIV-1 persistence encompasses multiple regulatory states rather than a uniform condition of transcriptional silence.

Spatial transcriptomics, tissue imaging, and complementary proteomic approaches offer a further dimension by placing infected cells within their local cellular and anatomical environments [131–133]. This is particularly relevant to lymphoid and other tissue compartments, where interactions with neighboring immune cells, local signaling, and tissue architecture may influence viral expression and immune-mediated clearance. Combining spatial information with cell identity and proviral characterization could therefore help distinguish reservoir populations that differ in immune accessibility or potential therapeutic responsiveness. Nevertheless, spatial proximity or a particular transcriptional signature does not, by itself, demonstrate a causal role in reservoir maintenance; such associations require functional validation.

The principal opportunity is to integrate single-cell and spatial measurements with assays of proviral integrity, inducibility, and immune-cell function. This would help determine whether specific cellular or tissue states are associated with persistence, rather than inferring reservoir importance solely from cell abundance or viral RNA detection. Ultimately, these methods may guide the selection of reservoir populations for targeted interventions, but their translational value will depend on validation in relevant human tissues and demonstration that the identified populations can be meaningfully affected by treatment.

7.2. Genome Editing and Precision Epigenetic Engineering

CRISPR-based approaches are being investigated to disrupt or excise integrated HIV-1 proviruses and to target viral regulatory sequences, including the long terminal repeat

(LTR) [134–136]. In parallel, CRISPR-based epigenetic repression using nuclease-inactive dCas9 fused to transcriptional repressor domains, such as KRAB, has been shown to suppress HIV-1 transcription and latency reactivation while promoting repressive chromatin modifications at the LTR in experimental models [137,138]. These strategies represent distinct therapeutic objectives: genome editing seeks to alter or eliminate viral genetic material or a defined host target, whereas epigenetic engineering seeks to modify the regulatory state governing proviral expression.

Neither approach should yet be assumed to achieve complete reservoir elimination or durable, treatment-free remission. Major translational barriers include efficient delivery to diverse infected-cell populations, tissue penetration, cellular specificity, off-target effects, genomic safety, and the durability of the intended modification [139].

Integrating genome engineering with single-cell and spatial reservoir profiling may help align an intervention with the cellular populations and anatomical compartments in which its target is present. However, this remains a prospective precision-medicine framework rather than an established clinical capability. Its utility will depend on demonstrating target engagement in relevant reservoir cells, assessing effects on intact and inducible proviruses, and establishing the durability and safety of the intervention.

Taken together, these technologies provide complementary tools for moving from reservoir description toward mechanism-based intervention. Single-cell and spatial methods can identify candidate persistence-associated states, whereas genome editing and epigenetic engineering can test whether selected molecular vulnerabilities are actionable. The central challenge is to connect these levels of analysis through functional validation and clinically relevant outcomes, including changes in the intact and inducible reservoir and, where appropriate, sustained control after antiretroviral therapy interruption.

8. A Unified Model of HIV-1 Latency and Persistence

HIV-1 persistence is best understood as the outcome of interacting molecular, cellular, immunological, and anatomical determinants rather than a single latency mechanism [10,25]. In this review, reservoir evolution refers to changes in the composition, clonal representation, proviral states, and tissue distribution of infected-cell populations, with or without detectable genetic evolution of viral sequences. Evidence of infected-cell clonal expansion and persistence of intact or replication-competent proviruses during suppressive antiretroviral therapy (ART) supports this distinction [2,3,52].

At the molecular level, epigenetic regulation and restricted transcriptional elongation generate heterogeneous patterns of viral expression and inducibility [10,140]. Their consequences depend on the host cell: survival and clonal expansion sustain infected $CD4^+$ T-cell populations [1–3], while human brain studies provide evidence of persistent HIV infection and, in selected samples, intact or inducible replication-competent proviruses in microglia [6,57]. The relative contribution of these myeloid populations to systemic viral rebound remains unresolved.

Immune pressure further modifies reservoir composition, but its effects depend on both immune access and infected-cell properties. Limited accumulation of HIV-specific cytotoxic T lymphocytes at sites of viral replication [58], impaired cytolytic features of follicular $CD8^+$ T cells and associated immune-regulatory signals [131], and antigen-responsive expansion of infected $CD4^+$ T-cell clones [51] are distinct processes that can influence clearance and reservoir maintenance. Meanwhile, heterogeneous antiretroviral distribution within mesenteric lymph nodes in treated nonhuman primates demonstrates that tissue exposure can constrain pharmacological control [133]. Together, these findings indicate that the fate of an infected cell cannot be inferred from proviral transcription, immune recognition, or anatomical location considered in isolation.

Figure 4 integrates these relationships into four interdependent determinants: proviral visibility, host-cell fitness, immune pressure, and tissue context. Their interaction explains why changes in viral expression or total proviral DNA alone cannot establish reservoir depletion. Reservoir assessment instead requires complementary evidence of proviral integrity, inducibility, and replication competence, interpreted alongside relevant clinical outcomes.

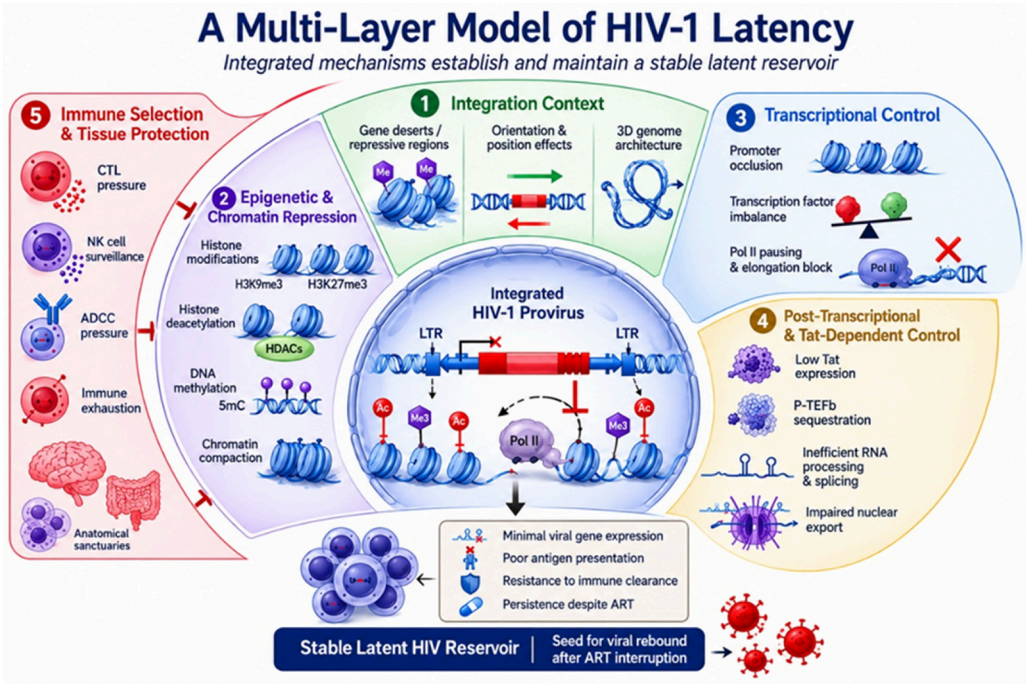


Figure 4. A unified model of HIV-1 latency and persistence. HIV-1 persistence emerges from the interaction of molecular regulation, cellular fitness, immune selection, and tissue compartmentalization. Epigenetic repression, chromatin organization, and transcriptional and post-transcriptional regulation constrain viral gene expression and antigen availability. At the same time, immune surveillance preferentially targets infected cells with greater viral expression, altering the composition of surviving reservoir states. These processes interact with the distinct biological properties of $CD4^+$ T cells and myeloid populations, as well as with tissue-specific microenvironments that regulate immune accessibility, cellular survival, and therapeutic exposure. Together, these interconnected selective forces generate heterogeneous reservoir states that persist despite antiretroviral therapy and can contribute to viral rebound following treatment interruption. Created in BioRender. Abdelnaser, R. (2026) https://BioRender.com/4dej4q1.

The principal unresolved question is which combinations of these determinants sustain clinically consequential reservoirs and contribute to rebound after ART interruption. Addressing it requires longitudinal, cell-resolved analyses that link proviral characteristics to host-cell phenotype, spatial location, immune interactions, and functional viral competence. Therapeutically, this framework favors interventions evaluated against the biological properties of targeted reservoir populations rather than changes in a single molecular marker. Durable remission will require evidence that an intervention either eliminates rebound-competent infection or maintains sufficiently effective control to prevent its re-emergence, with safety and sustained ART-free outcomes assessed independently of intermediate pharmacodynamic responses.

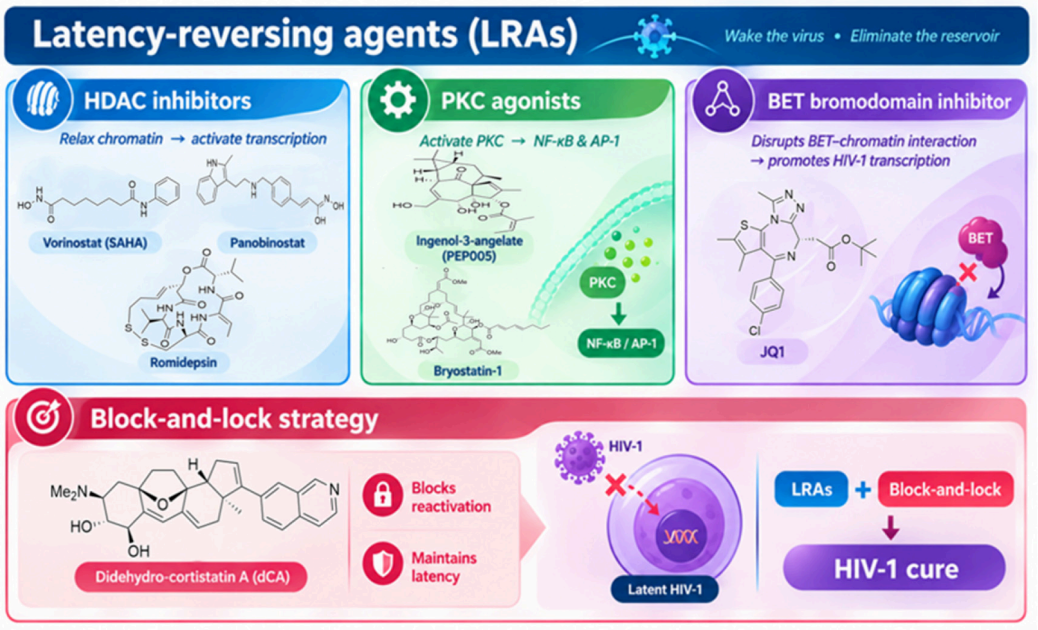
9. Precision Drug Discovery for HIV Cure

HIV-1 persistence despite effective antiretroviral therapy (ART) reflects substantial heterogeneity in proviral regulation, infected-cell phenotype, cellular fitness, immune recognition, and tissue localization. This complexity limits the likelihood that a single

pharmacological intervention will uniformly eliminate all persistent infected cells. Accordingly, HIV-1 cure research must move beyond identifying compounds that suppress viral replication or induce transcription toward identifying and validating molecular vulnerabilities that sustain defined reservoir populations. Small-molecule latency modulators, chemical-biology approaches, targeted protein degradation, structure-guided drug discovery, and artificial intelligence (AI)-assisted computational approaches are emerging as complementary components of this framework. Their translational value should be assessed by whether they identify actionable targets, demonstrate reproducible biological effects in relevant reservoir models, and ultimately improve reservoir control rather than simply produce computational predictions or increase HIV-1 transcription.

9.1. Small-Molecule Latency Modulators

Small molecules represent one of the most extensively investigated pharmacological approaches to HIV-1 cure because they can modulate viral transcription and host-cell regulatory pathways with defined temporal and dose-dependent exposure. Major classes of latency-reversing agents (LRAs) include histone deacetylase (HDAC) inhibitors [83,84,141], protein kinase C (PKC) agonists [142], bromodomain and extraterminal (BET) inhibitors [143], and innate immune agonists targeting Toll-like receptors (TLRs) [76,116–120]. Representative small-molecule modulators of HIV-1 latency and viral transcription are summarized in Scheme 1.



Scheme 1. Chemical structures of representative HIV-1 latency-reversing agents (LRAs) and a block-and-lock compound.

Archin et al. demonstrated that the HDAC inhibitor vorinostat can increase cell-associated HIV-1 RNA in individuals receiving suppressive ART, providing clinical proof of concept that pharmacological latency reversal is achievable in vivo [84]. However, subsequent studies demonstrated that transcriptional induction does not necessarily translate into measurable depletion of the replication-competent reservoir. Rasmussen et al. reported that panobinostat increased HIV-1 transcription during suppressive ART without producing a corresponding reduction in the reservoir [141], while interval dosing of vorinostat enhanced HIV-1 RNA expression without establishing reservoir depletion [83]. These clinical findings illustrate a central limitation of latency-reversal strategies.

PKC agonists provide a mechanistically distinct approach by activating intracellular signaling pathways that promote HIV-1 transcription, including NF-κB-associated signaling. Experimental studies have demonstrated latency-reversing activity for compounds such as prostratin, bryostatin-1, and ingenol derivatives in cellular systems, including primary $CD4^+$ T-cell models [142]. Their translational development remains constrained by the need to separate viral reactivation from nonspecific cellular activation and toxicity, particularly when systemic immune stimulation may compromise tolerability. These limitations support the continued search for compounds with improved selectivity, exposure profiles, and activity against heterogeneous reservoir populations.

BET inhibitors provide another mechanistically distinct approach. Li et al. demonstrated that the BET inhibitor JQ1 can enhance HIV-1 transcription by antagonizing BRD4-mediated restriction of Tat-dependent transcription, thereby facilitating transcriptional elongation [143].

Despite these advances, first-generation LRAs have been limited by incomplete reservoir reactivation, cellular toxicity, and nonspecific immune activation. These limitations have stimulated investigation of alternative epigenetic mechanisms. In a recent study, Li et al. demonstrated that histone decrotonylation represents a distinct regulatory mechanism of HIV-1 latency and identified selective histone decrotonylation inhibitors (HDCRis), including citarinostat, as latency-reversing compounds [144]. These findings extend the range of candidate epigenetic targets, but evidence from cell models and ex vivo samples should not be interpreted as proof of reservoir depletion or clinical efficacy. Further investigation is needed to establish target specificity, reproducibility across reservoir populations, and the relationship between transcriptional induction and infected-cell clearance.

Small molecules can also be used to reinforce rather than reverse HIV-1 latency. Mousseau et al. demonstrated that didehydro-cortistatin A (dCA), a Tat inhibitor, suppresses Tat-dependent transcription and prevents HIV-1 reactivation from latency [85]. Kessing et al. subsequently demonstrated suppression of HIV-1 rebound in an experimental in vivo model, supporting the pharmacological basis of the block-and-lock strategy [86]. Unlike LRAs, latency-promoting agents aim to reduce the probability of viral reactivation; however, sustained transcriptional suppression does not establish irreversible silencing or guarantee that rebound will not occur after treatment withdrawal.

The appropriate endpoints for evaluating latency modulators therefore depend on their intended mechanism. For LRAs, studies should assess viral expression alongside antigen production, immune-mediated killing, and changes in intact or replication-competent reservoirs. For latency-promoting agents, evaluation should establish the durability of repression and the capacity of the virus to reactivate after drug withdrawal. Across both strategies, target selectivity, toxicity, pharmacokinetics, and exposure in relevant anatomical compartments must be considered alongside molecular potency.

9.2. Targeted Protein Degradation and Chemical Biology

Chemical biology has expanded HIV-1 drug discovery beyond conventional occupancy-based inhibition through targeted protein degradation. Proteolysis-targeting chimeras (PROTACs) are bifunctional molecules that recruit an E3 ubiquitin ligase to a target protein, resulting in ubiquitination and proteasomal degradation. Unlike conventional inhibitors, which generally require continued target occupancy, degraders aim to reduce target abundance; whether this produces a more durable biological effect depends on degradation efficiency, target resynthesis, and drug exposure.

Emert-Sedlak et al. demonstrated that PROTAC-mediated degradation of HIV-1 Nef efficiently restores cell-surface CD4 and MHC-I expression and inhibits HIV-1 replication in experimental systems, including donor peripheral blood mononuclear cells [145]. By

counteracting Nef-mediated immune evasion, this approach illustrates how targeted degradation could restore host-cell functions relevant to immune surveillance rather than acting solely through direct inhibition of viral replication.

Targeted degradation has also been extended to host factors required for HIV-1 infection. Newton et al. demonstrated that macrocycle-based PROTACs targeting cyclophilin A (CypA) efficiently reduce CypA abundance and inhibit HIV-1, with enhanced antiviral activity compared with the corresponding non-degrading parental inhibitor in experimental systems including primary human $CD4^+$ T cells and monocyte-derived macrophages [146]. Structural and mechanistic studies have independently established interactions between CypA and the HIV-1 capsid, supporting the biological rationale for targeting this host–virus interface [147,148].

These studies provide experimental proof of concept for targeted degradation of viral or host proteins, but they do not establish that PROTACs eliminate latently infected cells or deplete the persistent HIV-1 reservoir. The current evidence primarily supports effects on immune evasion or viral replication in experimental systems. Major translational challenges include identifying targets that are functionally important during persistent infection, achieving adequate exposure in relevant tissues, minimizing degradation of essential proteins in uninfected cells, and establishing an acceptable safety profile.

In Scheme 1, the compounds are grouped according to their primary mechanisms of action: histone deacetylase (HDAC) inhibitors (vorinostat [SAHA], panobinostat, and romidepsin); protein kinase C (PKC) agonists (ingenol-3-angelate [PEP005] and bryostatin-1); and the bromodomain and extraterminal domain (BET) inhibitor JQ1. These LRAs promote HIV-1 proviral transcription through distinct mechanisms involving chromatin regulation and host-cell signaling. In contrast, didehydro-cortistatin A (dCA), a Tat inhibitor, suppresses HIV-1 transcription and has been investigated as a potential block-and-lock strategy to promote durable viral silencing. The figure distinguishes compounds intended to reverse latency from those investigated to reinforce transcriptional suppression.

9.3. AI-Guided Molecular Discovery and Reservoir Precision Pharmacology

Artificial intelligence (AI) and machine learning (ML) offer opportunities to integrate molecular, genomic, epigenetic, and cellular data to identify therapeutic vulnerabilities associated with HIV-1 persistence. Although these approaches can prioritize candidate compounds and targets, computational predictions alone cannot establish biological activity, target engagement, or efficacy against persistent reservoirs.

Kurczyk et al. demonstrated the potential of computational prioritization in HIV-1 drug discovery by screening approximately 1.5 million commercially available compounds using machine learning and privileged-structure concepts. Experimental testing of 13 selected candidates identified two novel chemotypes with moderate anti-HIV-1 activity [149]. This study illustrates the value of computational screening while highlighting an important distinction: antiviral activity does not necessarily indicate efficacy against latently infected cells or rebound-competent reservoirs.

More directly relevant to reservoir biology, Maldarelli et al. and Wagner et al. linked HIV integration sites to the clonal expansion and persistence of infected cells [2,3], while Simonetti et al. demonstrated that clonally expanded $CD4^+$ T cells can produce infectious HIV-1 in vivo [52]. Antigen-responsive expansion of infected-cell populations provides further evidence of immune-driven clonal selection [51]. Computational models could integrate these findings with cellular and molecular profiles to investigate reservoir maintenance, although these studies do not establish that current AI approaches can predict the long-term behavior or rebound contribution of individual infected-cell clones.

Initial computational studies provide proof of concept for analyzing specific features of HIV biology. Hu et al. developed DeepHINT to predict HIV-1 integration sites using DNA sequence and epigenetic information [150], while a subsequent single-cell multi-omic study applied machine learning to predict HIV reactivation from transcriptomic and chromatin-accessibility features [151]. These approaches may help identify integration- and reactivation-associated states, but they have not yet established validated methods for predicting the long-term survival, transcriptional activity, or clinical rebound contribution of individual proviruses during suppressive antiretroviral therapy (ART).

The emerging opportunity is to extend computational analysis toward vulnerabilities associated with reservoir maintenance, viral expression, and immune evasion. Potential targets include transcriptional regulators, chromatin-modifying enzymes, cellular survival pathways, metabolic dependencies, and host factors influencing immune recognition. However, HIV transcription, proviral DNA detection, and predicted molecular interactions represent distinct levels of evidence and cannot independently establish reservoir integrity, replication competence, or therapeutic susceptibility [152].

Accordingly, candidate targets and compounds require validation in biologically relevant systems, including primary $CD4^+$ T cells, macrophage-lineage models, and tissue-derived or ex vivo reservoir systems, as appropriate to the proposed mechanism. Evaluation should distinguish target engagement and altered viral expression from infected-cell elimination, reductions in intact or replication-competent reservoirs, and changes in rebound potential. Predicted therapeutic combinations or resistance profiles likewise require experimental confirmation [153–155]. Ultimately, reservoir precision pharmacology aims to connect a defined infected-cell population with a validated molecular vulnerability and determine whether targeting that vulnerability reduces rebound-competent infection or contributes to durable ART-free control. Progress will depend on representative datasets, prospective validation, functional experiments, and appropriate clinical outcomes rather than computational performance or changes in individual molecular markers alone.

10. Conclusions and Future Directions

Despite substantial progress in understanding HIV-1 persistence, important uncertainties remain regarding the biological determinants of rebound-competent reservoirs, particularly the contribution of tissue-resident myeloid cells and the relative influence of integration-site architecture, cellular state, immune selection, and anatomical context. Addressing these questions will require longitudinal, cell-resolved studies that link proviral integrity and transcriptional activity with cellular phenotype, spatial distribution, immune interactions, and functional viral competence. Combining complementary approaches, including the intact proviral DNA assay (IPDA), quantitative viral outgrowth assays (QVOA), and near-full-length or full-length proviral sequencing, will be essential because no single assay comprehensively captures reservoir size, inducibility, and replication competence.

For cure research, the central challenge is to translate reservoir heterogeneity into effective and clinically meaningful interventions. Strategies targeting viral expression, immune-mediated clearance, infected-cell survival, and tissue-specific barriers may need to be combined according to the biological properties of the targeted reservoir populations. Single-cell, spatial, and multi-omic technologies, together with computational approaches, may help identify candidate vulnerabilities, but their therapeutic relevance must be established through functional validation and appropriate clinical evaluation. Importantly, molecular target engagement or changes in HIV-1 RNA should not be interpreted as evidence of reservoir elimination. Therapeutic success must instead be assessed through complementary measures of intact and replication-competent reservoirs, time to viral rebound during appropriately monitored analytical treatment interruption (ATI), and sustained ART-free

control. Ultimately, progress toward HIV-1 remission will depend on demonstrating that interventions can eliminate clinically relevant rebound-competent infection or maintain durable ART-free control without unacceptable toxicity.

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Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Full Term
ADCC	Antibody-dependent cellular cytotoxicity
AI	Artificial intelligence
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
BET	Bromodomain and extraterminal domain
bNAbs	Broadly neutralizing antibodies
$CD4^+$	CD4-positive T cells/cluster of differentiation 4-positive
$CD8^+$	CD8-positive T cells/cluster of differentiation 8-positive
CDK9	Cyclin-dependent kinase 9
CHAMP	Control of HIV After Antiretroviral Medication Pause
CNS	Central nervous system
CRISPR	Clustered regularly interspaced short palindromic repeats
CTL	Cytotoxic T lymphocyte
CypA	Cyclophilin A
dCA	Didehydro-cortistatin A
DNA	Deoxyribonucleic acid
Env	HIV-1 envelope glycoprotein
FACS	Fluorescence-activated cell sorting
GANs	Generative adversarial networks
GI	Gastrointestinal
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus type 1
H3K9me3	Histone H3 lysine 9 trimethylation
H3K27me3	Histone H3 lysine 27 trimethylation
IL-10	Interleukin-10
IL-15	Interleukin-15
IPDA	Intact proviral DNA assay
LRA/LRAs	Latency-reversing agent/agents
LEDGF/p75	Lens epithelium-derived growth factor p75
LTR	Long terminal repeat

MD	Molecular dynamics
MHC-I	Major histocompatibility complex class I
MIP-Seq	Matched integration site and proviral sequencing
ML	Machine learning
Nef	Negative factor
NFAT	Nuclear factor of activated T cells
NK	Natural killer
QVOA	Quantitative viral outgrowth assay

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