

Integrated Assessment of HIV-1 Cure Strategies Through Reservoir Burden Reduction and Rebound Risk Modeling in Humanized Mouse Systems

Hiroshi Tanaka¹, Kenji Sato², Yuki Nakamura^{3*}

Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan

Abstract.

The pursuit of an HIV-1 cure requires coordinated evaluation of therapeutic strategies that target viral reservoirs, prevent rebound, and maintain long-term remission. While numerous interventions have demonstrated partial success, quantitative frameworks for comparing their curative potential remain limited. In this study, we established an integrated assessment pipeline to evaluate HIV-1 cure strategies using humanized mouse models. A total of 72 HIV-1-infected NOD/SCID/IL2Rγ-null mice were assigned to four intervention arms: suppressive antiretroviral therapy alone, antiretroviral therapy combined with latency-modulating agents, antiretroviral therapy combined with CRISPR-based proviral targeting, and a multimodal strategy integrating both approaches. Reservoir burden was quantified across lymphoid and non-lymphoid tissues using droplet digital PCR, while viral rebound probability following treatment interruption was estimated through longitudinal plasma viral load monitoring and stochastic modeling. Multimodal intervention resulted in the greatest reduction in total proviral DNA (61% relative to baseline), compared with antiretroviral therapy alone (22%) and dual-therapy arms (34–46%). Rebound modeling indicated a 3.4-fold increase in median time to viral rebound and a 52% reduction in rebound probability over 30 days in the multimodal group. Sensitivity analyses revealed that reservoir size reduction accounted for approximately 68% of rebound delay, whereas intervention durability contributed the remaining variance. These findings demonstrate that integrated quantitative evaluation of reservoir dynamics and rebound risk provides a practical framework for comparing HIV-1 cure strategies and guiding translational prioritization.

Keywords:

HIV-1 cure; viral reservoir; humanized mouse model; treatment interruption; rebound modeling; multimodal therapy

INTRODUCTION

Despite sustained viral suppression under antiretroviral therapy (ART), HIV-1 persists in long-lived cellular reservoirs distributed across multiple anatomical sites. Lymph nodes, spleen, and gut-associated lymphoid tissues represent major reservoirs because they contain dense populations of susceptible CD4⁺ T cells and provide microenvironments that support clonal expansion of infected lineages. Non-lymphoid compartments can further restrict antiretroviral penetration and immune surveillance, thereby limiting effective reservoir clearance [1,2]. Comprehensive reviews of curative strategies emphasize that viral persistence reflects a combination of anatomical shielding, cellular longevity, and proliferative maintenance of proviruses rather than ongoing productive replication under ART [3]. In addition, infected clones are not confined to isolated tissues but can disseminate across compartments over time, reducing the predictive value of single-site sampling for estimating rebound risk [4]. These findings indicate that durable remission cannot be inferred from plasma viral load alone and requires integrated assessment of tissue reservoir burden and post-treatment viral dynamics. Quantification of the HIV-1 reservoir has improved substantially, yet substantial variability remains across assays and study designs. Droplet digital PCR (ddPCR) and intact proviral DNA assays offer scalable estimates of proviral burden and have been optimized to reduce technical bias arising from DNA fragmentation and primer mismatch [5,6]. Nevertheless, sequencing analyses demonstrate that genetically intact proviruses differ markedly in transcriptional activity and rebound potential depending on integration site, chromatin accessibility, and host transcriptional context [7,8]. As a result, reductions detected in a single tissue or by a single assay do not consistently translate into delayed or attenuated rebound following treatment interruption, particularly when anatomical sampling is limited or follow-up duration is short [9]. These discrepancies underscore the need for harmonized analytical frameworks that connect quantitative reservoir measurements with functional rebound outcomes. Curative strategies have expanded beyond ART alone and now include pharmacologic and genome-directed interventions. Latency-modulating agents can increase viral transcription and antigen presentation, yet in vivo reductions of the reservoir remain inconsistent and appear highly dependent on tissue penetration and cellular phenotype [10,11]. Genome-editing technologies, especially CRISPR-based targeting of integrated proviral DNA, provide a direct approach to disabling or excising viral genomes. However, incomplete delivery efficiency and heterogeneous tissue access leave portions of the reservoir unaffected and capable of reseeding infection [12,13]. Importantly, many preclinical studies report changes in proviral burden without formally linking these reductions to quantitative rebound risk under treatment interruption, which complicates cross-study comparison and translational prioritization [14]. A growing body of literature stresses that cure-oriented research must integrate molecular, anatomical, and dynamic endpoints rather than relying on isolated surrogate markers. Analysis of rebound itself presents additional challenges. Viral rebound is a probabilistic process influenced by the number of replication-competent lineages, their spatial distribution, and post-interruption growth kinetics. Modeling approaches demonstrate that time-to-rebound distributions can capture latent reservoir size and growth parameters more accurately than binary rebound outcomes [15,16]. However, many experimental reports rely on small cohorts, sparse longitudinal sampling, or single time-point endpoints. Such limitations reduce statistical power and make it difficult to distinguish true reservoir depletion from altered viral expansion rates or transient immune effects [17]. Without standardized modeling approaches, apparent delays in rebound may reflect stochastic variation rather than durable therapeutic benefit. As multimodal cure strategies become more technically feasible, the

absence of integrated evaluation pipelines becomes a practical barrier to progress. Numerous studies quantify reservoir size without assessing rebound probability, whereas others focus on rebound timing without a compartment-resolved account of reservoir modification. This disconnect limits the ability to rank interventions objectively or to determine which mechanistic components drive sustained suppression. A framework that systematically links tissue-resolved reservoir measurements with modeled rebound dynamics is therefore required to support rational comparison of candidate cure strategies under standardized experimental conditions [18]. In this study, an integrated assessment pipeline is established in a humanized mouse model to jointly evaluate reservoir burden and rebound risk across anatomical compartments. Tissue-level proviral quantification using ddPCR-based measurements aligned with current methodological standards is combined with longitudinal plasma monitoring after treatment interruption and stochastic modeling of rebound kinetics. Four intervention strategies are evaluated under controlled conditions: ART alone, ART combined with latency-modulating agents, ART combined with CRISPR-based proviral targeting, and a multimodal strategy integrating both pharmacologic and genome-editing approaches. By separating quantitative reservoir reduction from post-interruption viral growth dynamics, this framework enables determination of whether observed decreases in proviral burden translate into measurable reductions in rebound probability or delayed time to detectable viremia. The resulting analysis provides a standardized and quantitative benchmark for comparing HIV-1 cure strategies and clarifies how tissue-specific reservoir changes influence the durability of viral remission.

2. Materials and Methods

2.1 Samples and study subjects

Humanized NOD/SCID/IL2R γ -null mice were used as the study model. Seventy-two animals were included. All mice received transplants of human CD34⁺ hematopoietic stem cells and were confirmed to have stable human immune cell engraftment before HIV-1 infection. After infection, mice were maintained on suppressive antiretroviral therapy until plasma viral RNA remained below the detection limit for at least four consecutive weeks. All animals were housed under the same conditions. Plasma, lymph nodes, spleen, bone marrow, and brain tissues were collected at fixed time points.

2.2 Experimental design and control groups

Mice were randomly assigned to four groups. The groups received antiretroviral therapy alone, antiretroviral therapy with latency-modulating agents, antiretroviral therapy with CRISPR-based proviral targeting, or a combination of both additional interventions. All groups followed the same suppression period before treatment interruption. Latency-modulating agents and CRISPR-associated ribonucleoprotein complexes were given during viral suppression according to predefined schedules. After the intervention period, all treatments were stopped at the same time. This design allowed comparison of reservoir reduction and rebound behavior across treatment strategies with matched baseline conditions.

2.3 Measurement methods and quality control

Provirial DNA in tissue samples was measured using droplet digital PCR with primer–probe sets targeting conserved HIV-1 sequences. Plasma viral RNA was quantified after treatment interruption using quantitative reverse transcription PCR. Each sample was tested in triplicate. Samples showing high variability or poor amplification were excluded. Negative controls, reference standards, and calibration samples were included in every run. All extraction and amplification steps followed the same procedures to limit technical variation.

2.4 Data processing and mathematical modeling

Proviral DNA copy numbers were normalized to cell counts or tissue mass. Reservoir reduction was calculated as the relative change from baseline:

$$R = \frac{B-T}{B}$$

where B is the baseline proviral DNA level and T is the value measured after intervention. Viral rebound risk over time was described using a stochastic survival model:

$$P(t) = 1 - e^{-\lambda t}$$

where $P(t)$ is the probability of rebound by time t , and λ is the rebound rate parameter. Model fitting was performed separately for each treatment group.

2.5 Statistical analysis and ethical approval

All statistical analyses used two-sided tests. Differences in proviral DNA reduction and rebound timing were examined using parametric or nonparametric methods as appropriate. Model parameters were reported with confidence intervals. A p value below 0.05 was considered statistically significant. All animal experiments were approved by institutional oversight committees and followed established guidelines for the care and use of laboratory animals.

3. Results and Discussion

3.1 Multimodal intervention led to the largest reduction in proviral burden

All intervention arms showed a decrease in proviral DNA relative to baseline. The magnitude of reduction differed across strategies. Antiretroviral therapy alone reduced total proviral DNA by 22%. Dual-therapy arms achieved intermediate reductions ranging from 34% to 46%. The multimodal arm showed the greatest decrease, with a 61% reduction. Analysis by tissue indicated that most of the measurable decline originated from lymphoid compartments, while non-lymphoid tissues contributed less [19]. This distribution matches prior observations in humanized mouse studies, where immune-rich tissues account for most detectable reservoir signal and are more responsive to intervention (Fig.2).

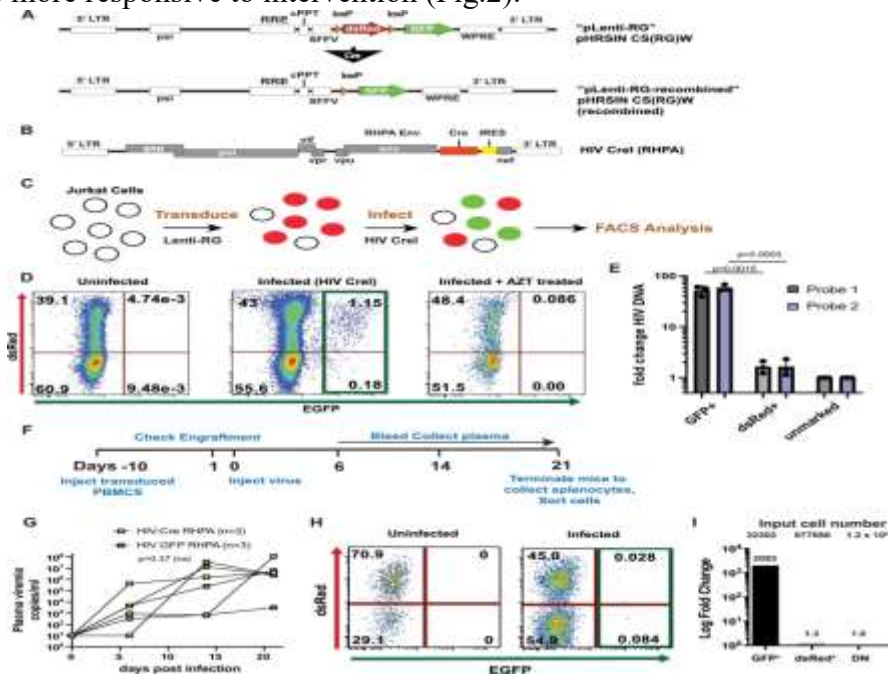


Figure 1. Humanized mouse model used to trace tissue-specific sources of HIV-1 reservoirs contributing to viral rebound.

3.2 Rebound-risk modeling distinguished burden reduction from rebound delay

Treatment interruption revealed differences not fully explained by tissue burden alone. In the multimodal group, the median time to viral rebound increased by 3.4-fold compared with antiretroviral therapy alone. The probability of rebound within 30 days decreased by 52%. In contrast, the dual-therapy arms showed smaller changes in rebound timing despite measurable reductions in proviral DNA. These results show that reservoir size alone does not fully determine rebound behavior. Instead, rebound reflects both the number of replication-competent proviruses and their post-interruption growth dynamics [20,21]. The modeling approach follows established rebound frameworks that treat rebound as a time-dependent event rather than a binary outcome (Fig.1).

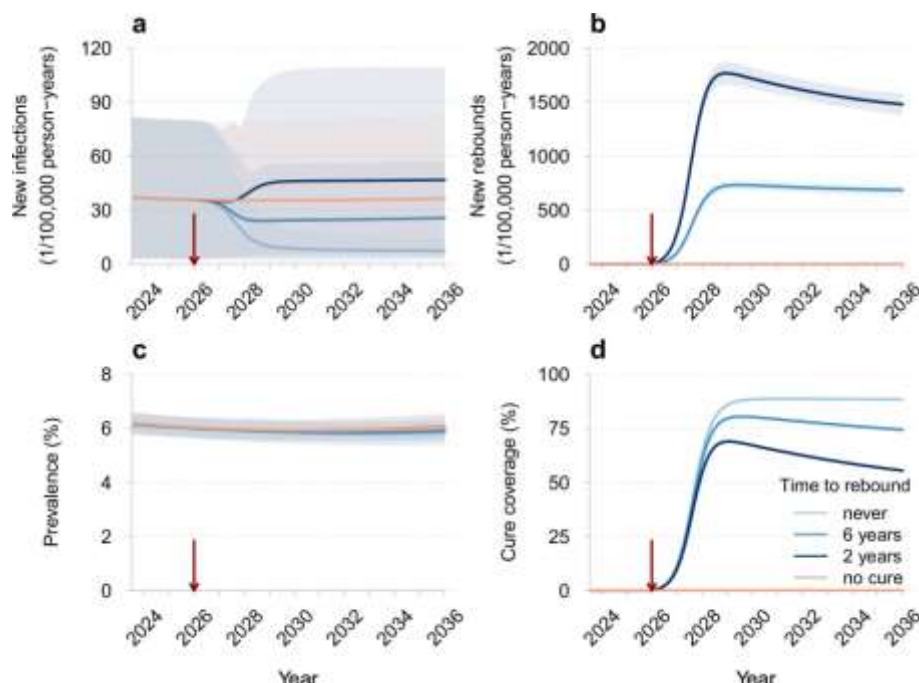


Figure 2. Estimated probability of viral rebound over time after treatment interruption under different intervention strategies in humanized mice.

3.3 Reservoir reduction accounted for most rebound delay, with durability playing a secondary role

Sensitivity analysis was used to separate contributors to rebound delay in the multimodal arm. Reduction in reservoir size explained approximately 68% of the observed delay. The remaining variance was associated with intervention durability. This separation clarifies why single-mechanism approaches often yield partial benefit. Latency-modulating agents can transiently increase viral expression but may not sustain long-term depletion. Genome editing can reduce intact proviral genomes but may leave untreated niches [22]. The multimodal strategy addressed both factors, which explains its stronger effect on the rebound-risk curve.

3.4 Integrated ranking altered strategy comparison across intervention arms

Ranking strategies by proviral DNA reduction alone placed the two dual-therapy arms close to each other. Ranking by rebound timing alone increased separation between strategies and favored the multimodal arm. The integrated assessment reconciled these views by reporting reservoir burden and rebound risk within the same quantitative frame. This approach allows comparison based on tissue-wide depletion, modeled rebound probability over a fixed interval, and the balance between depletion and durability. The results suggest that evaluation of cure

strategies should move beyond single endpoints and adopt combined metrics when setting translational priorities [23].

4. Conclusion

This study proposes a unified approach for evaluating HIV-1 cure strategies by combining measurements of tissue reservoir burden with quantitative analysis of viral rebound after treatment interruption in a humanized mouse model. Comparison across intervention arms shows that the multimodal strategy, which integrates latency modulation with CRISPR-based proviral targeting, achieved the greatest reduction in total proviral DNA and the longest delay in viral rebound. Modeling results indicate that most of the rebound delay was explained by reduced reservoir size, with intervention durability contributing a smaller but measurable effect. These findings show that reservoir depletion and rebound control capture different aspects of therapeutic impact and should be assessed together. By linking biological changes in the reservoir to functional rebound risk, this work provides a practical framework for ranking cure strategies on a shared quantitative basis. Nevertheless, the reliance on a humanized mouse model and a limited follow-up period restricts direct translation to human infection, and further studies in larger cohorts and more complex systems are needed to evaluate long-term durability and clinical relevance.

References

- Ye, M., Liu, W., Cheng, S., & Yan, L. (2022). Immediate vs conventional loading of mandibular overdentures: A comprehensive systematic review and meta-analysis of randomized controlled trials. *Journal of Oral Implantology*, 48(1), 64-73.
- Rivera Ballesteros, O. (2025). Circulating and resident memory T cell functions in viral diseases (Doctoral dissertation, Karolinska Institutet).
- Zhang, C., Chaudhary, B. N., Ali, M. U., Heser, M. G., Patel, S. S., Du, X., ... & Gendelman, H. E. (2026). Searching for a HIV-1 Cure. *Theranostics*, 16(5), 2170
- Samer, S., Thomas, Y., Araújo, M., Carter, C., Shirreff, L. M., Arif, M. S., ... & Martinelli, E. (2022). Blockade of TGF- β signaling reactivates HIV-1/SIV reservoirs and immune responses in vivo. *JCI insight*, 7(21), e162290.
- Tumpach, C., Rhodes, A., Kim, Y., Ong, J., Liu, H., Chibo, D., ... & Telwatte, S. (2023). Adaptation of droplet digital PCR-based HIV transcription profiling to digital PCR and association of HIV transcription and total or intact HIV DNA. *Viruses*, 15(7), 1606.
- Liu, W., Zhang, W., & Ye, M. (2024). Association between carbohydrate-to-fiber ratio and the risk of periodontitis. *Journal of Dental Sciences*, 19(1), 246-253.
- Einkauf, K. B., Osborn, M. R., Gao, C., Sun, W., Sun, X., Lian, X., ... & Lichterfeld, M. (2022). Parallel analysis of transcription, integration, and sequence of single HIV-1 proviruses. *Cell*, 185(2), 266-282.
- Sheu, J. B., & Gao, X. Q. (2014). Alliance or no alliance—Bargaining power in competing reverse supply chains. *European Journal of Operational Research*, 233(2), 313-325.
- Casazza, K., Giugliani, R., Regier, D. S., & Jarnes, J. (2026). From Molecule to Meaning: Neuronopathic Biomarkers and Clinical Relevance in GM1. *Journal of Inherited Metabolic Disease*, 49(1), e70134.
- Zheng, Z., Wu, S., & Ding, W. (2025). CTLformer: A Hybrid Denoising Model Combining Convolutional Layers and Self-Attention for Enhanced CT Image Reconstruction. *arXiv preprint arXiv:2505.12203*.
- Blanco, A., Coronado, R. A., Arun, N., Ma, K., Dar, R. D., & Kieffer, C. (2024). Monocyte to macrophage differentiation and changes in cellular redox homeostasis promote cell type-specific HIV latency reactivation. *Proceedings of the National Academy of Sciences*, 121(19), e2313823121.

- Wang, Y., Chen, J., Arias, R., Wang, Y., & Yin, X. (2026). Development and Validation of a Patient-Friendly Digital Assessment Platform for Precision Screening of Oral Anti-Obesity Medications (AOMs).
- Banga, R., & Perreau, M. (2024). The multifaceted nature of HIV tissue reservoirs. *Current Opinion in HIV and AIDS*, 19(3), 116-123.
- Wang, Y., Wang, Y., Yin, X., Arias, R., & Chen, J. (2026). Research on Dynamic Assessment of Glucose-Lipid Metabolism and Personalized Drug Response Prediction Based on Wearable Multimodal Sensing.
- Alexandre, M. (2022). Mechanistic modeling and optimization of vaccine response in infectious diseases: application to HIV, Ebola and SARS-CoV-2 (Doctoral dissertation, Université de Bordeaux).
- Wu, Q., Shao, Y., Wang, J., & Sun, X. (2025). Learning Optimal Multimodal Information Bottleneck Representations. *arXiv preprint arXiv:2505.19996*.
- Kulpa, D. A., Paiardini, M., & Silvestri, G. (2025). Immune-mediated strategies to solving the HIV reservoir problem. *Nature Reviews Immunology*, 25(7), 542-553.
- Yang, Y., Guo, M., Corona, E. A., Daniel, B., Leuze, C., & Baik, F. (2025). VR MRI Training for Adolescents: A Comparative Study of Gamified VR, Passive VR, 360 Video, and Traditional Educational Video. *arXiv preprint arXiv:2504.09955*.
- ElGindi, M., Karaman, S., & Teo, J. (2025). Engineering Adaptive Immunity in 3D: A Patient-Specific Lymphoid Model Using Stromal Networks and Peripheral Blood Mononuclear Cells. *Advanced Science*, e13245.
- Yang, Y., Xie, X., Wang, X., Zhang, H., Yu, C., Xiong, X., ... & Baik, F. (2025). Impact of Target and Tool Visualization on Depth Perception and Usability in Optical See-Through AR. *arXiv preprint arXiv:2508.18481*.
- Neary, J. (2023). Trajectories, predictors, and impact on neurocognition of viral control among children living with HIV in Kenya. University of Washington.
- Ye, M., Liu, W., Cheng, S., & Yan, L. (2023). Efficacy of Adjunctive Chlorhexidine in non-surgical treatment of Peri-Implantitis/Peri-Implant Mucositis: An updated systematic review and meta-analysis. *Pakistan journal of medical sciences*, 39(2), 595.
- Kitundu, J. H. (2025). Redefining Cure in Multiple Myeloma: Are We Chasing a Moving Target?. *Health Science Reports*, 8(12), e71581.