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Preview

A multidimensional HIV-1 persistence model for clonal expansion and viral rebound *in vitro*

Amare Eshetu[1] and Ya-Chi Ho[1,*]
[1]Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT 06519, USA
*Correspondence: ya-chi.ho@yale.edu
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SUMMARY

Using a replication-competent virus for prolonged *in vitro* culture, Matsuda et al. captured the heterogenous HIV-1 genome and integration site landscape, examined viral cytopathic effects and clonal expansion capacity, and tested drugs that can eliminate HIV-1-infected cells.

HIV-1 latently infected cells do not express viral proteins and therefore cannot be recognized and eliminated by immune effectors or therapeutic strategies. By reactivating HIV-1 from latency, HIV-1-infected cells can express viral proteins and induce immune recognition and killing. This shock-and-kill strategy, which involves inducing HIV-1 reactivation by latency reversing agents (LRAs) (shock) for therapeutic targeting (kill), relies on effective HIV-1 latency reversal that can induce maximum HIV-1 gene expression without causing systemic toxicity (Archin et al., 2012).

Developing an effective HIV-1 eradication strategy requires robust *in vitro* models for drug screens before testing candidate compounds *in vivo*. Several HIV latency cell line models and primary cell models have been designed for extensive compound library screens to identify candidate LRAs that can reactivate HIV-1 gene expression (Jordan et al., 2003; Spina et al., 2013; Yang et al., 2009). These HIV-1 latency models involve infecting cells with a HIV-1 reporter virus and maintaining cells for weeks to capture post-integration latency. However, remarkable differences exist between cell model systems and *ex vivo* drug effects, as compounds that reactivate latent HIV-1 in one system frequently fail to do so consistently across different models, particularly in clinical samples (Spina et al., 2013). This discrepancy suggests that *in vitro* models may not fully recapitulate HIV persistence *in vivo*.

Recent advances have revealed that understanding and targeting HIV-1 persistence is more than measuring HIV-1 reactivation alone (Ho et al., 2013; Jiang et al., 2020; Liu et al., 2020; Maldarelli et al., 2014; Mellors et al., 2021; Pollack et al., 2017; Wagner et al., 2014). The previous standard method to measure the size of the latent reservoir is the quantitative viral outgrowth assay (QVOA) (Finzi et al., 1997) where cells from HIV-1-infected individuals are plated at limiting dilution and stimulated with maximum T cell activation to reverse HIV-1 latency. High levels of HIV-1 p24 protein in the culture supernatant after stimulation indicate reactivation of replication-competent HIV-1 as shown by exponential viral outgrowth (Figure 1A). However, many infected cells that harbor intact HIV-1 proviruses were not induced despite maximum T cell activation and cannot be captured by viral outgrowth assays. These intact noninduced proviruses can instead be captured by near full-length HIV-1 single genome sequencing where the genomic DNA from clinical samples are plated at limiting dilution, amplified by overlapping nested PCR using HIV-1-specific primers, and sequenced (Figure 1B) (Ho et al., 2013). Such HIV-1 proviral landscape mapping identifies intact HIV-1 proviruses without reactivation. Furthermore, HIV-1 proviral landscape mapping not only identified intact noninduced HIV-1 proviruses but also found that >90% of the HIV-1 proviruses were defective. These defective HIV-1 proviruses have inactivating mutations including large internal deletions, hypermutations, packaging signal deletions, and point mutations (Ho et al., 2013). These defects occur during the reverse transcription but do not affect HIV-1 integration into the human genome. Some defective proviruses, particularly those with large internal deletions, increase over time because the proviral genome defects hamper viral proteins production and therefore reduce viral cytopathic effects and immune recognition (Pollack et al., 2017). On the other hand, intact HIV-1 proviruses or some defective proviruses (such as those having packaging signal deletion, hypermutation, and point mutations) can produce viral proteins and induce immune recognition. Cells having such proviruses may die of viral cytopathic effect or immune clearance and decrease over time. Overall, HIV-1 proviral landscape mapping identifies differential viral cytopathic effects and immune selection pressure on intact versus defective HIV-1 proviruses.

HIV-1 integration site mapping has revolutionized our understanding on the clonal expansion dynamics of HIV-1-infected cells and HIV-1 integration site-dependent proliferation. Using sonication shearing of the human genome and linker/ligation-mediated amplification (Maldarelli et al., 2014) and loop amplification (Wagner et al., 2014), Maldarelli et al. and Wagner et al. mapped thousands of HIV-1 integration sites in the human genome (Figure 1C). Integration into the same nucleotide in the 3 billion bases of human genome is more likely because of the proliferation of the infected cell and not due to different integration events. Therefore, different cells having the same HIV-1 integration site indicates

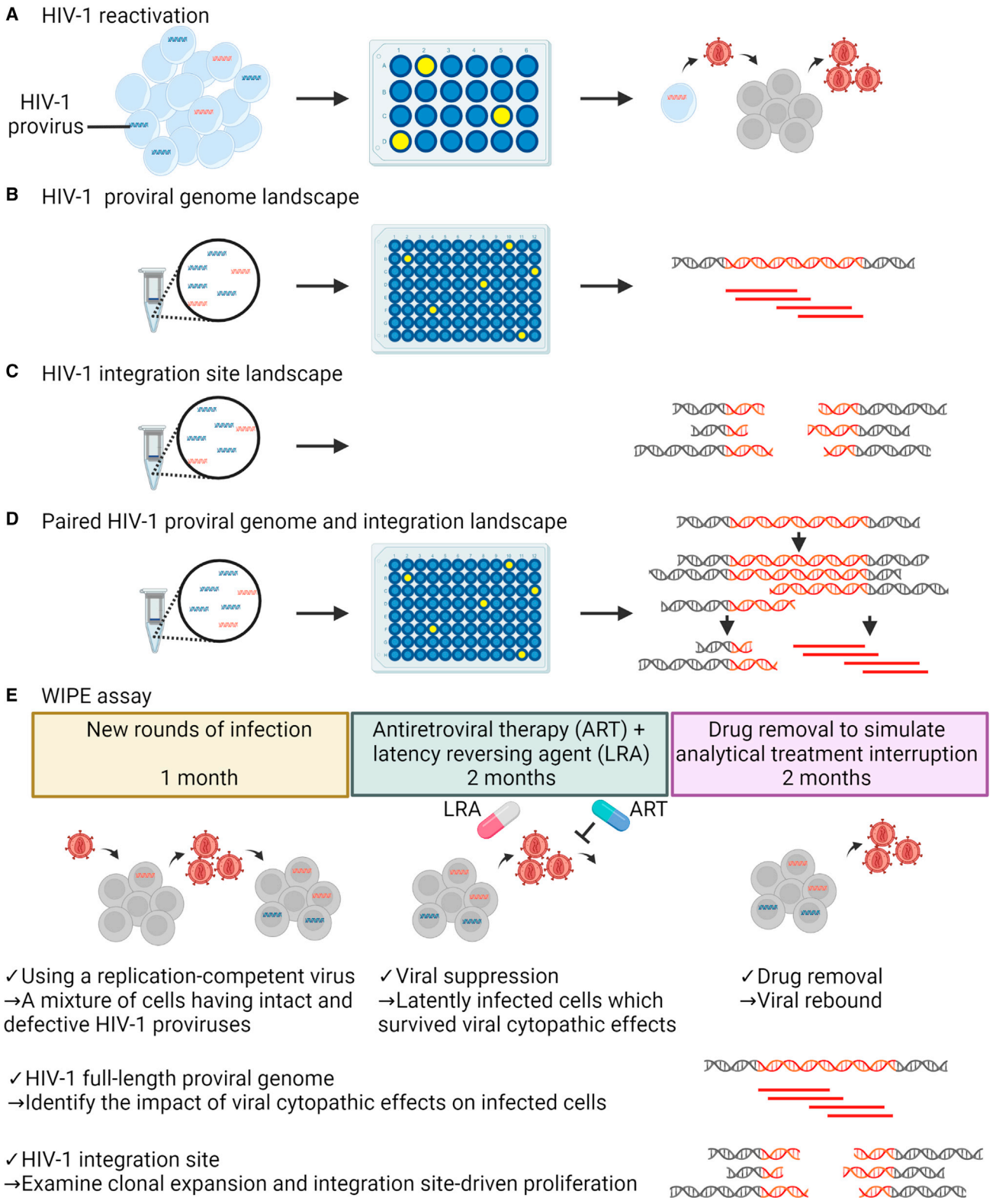

Figure 1. HIV-1 proviral genome landscape and HIV-1 integration site mapping provides mechanistic understanding of HIV-1 persistence on viral cytopathic effects, clonal expansion dynamics, and HIV-1 integration site-dependent proliferation

(A) Limiting dilution quantitative viral outgrowth assay (QVOA) measures HIV-1 reactivation after maximum T cell activation but cannot capture intact noninduced proviruses.

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clonal expansion of the infected cells. When HIV-1 was integrated into certain cancer-related genes in specific locations and in the same orientation as the host transcription unit, there was an enrichment of clonal expansion of the infected cells (Maldarelli et al., 2014; Wagner et al., 2014). This is because, at least in part, HIV-1 drives the proliferation of infected cells through HIV-1-driven aberrant host gene transcription and HIV-1-to-host RNA splicing (Liu et al., 2020). In some rare cases, HIV-1 integration into cancer genes (such as *LCK* and *STAT3*) drives aberrant cancer gene expression and cancer transformation of the infected cells (Mellors et al., 2021).

Given that most HIV-1 proviruses are defective, integration site mapping likely captures integration sites of defective proviruses. No assay could capture the integration sites and HIV-1 genome at the same time to identify the integration sites of intact proviruses until Patro et al. (2019) and Einkauf et al. (2019) used whole genome amplification to identify HIV-1 integration site and proviral genome of the same provirus (Figure 1D). Genomic DNA was plated at limiting dilution so that each well contained DNA from many cells but only one cell having HIV-1 DNA. Separate aliquots of phi29 polymerase-amplified genome were used for integration site sequencing and HIV-1 proviral genome sequencing. Using this method, Jiang et al. (2020) found that intact HIV-1 proviruses evaded immune clearance of the infected cells by residing in the transcriptionally inactive heterochromatins. Overall, simultaneous mapping of HIV-1 integration site and HIV-1 genome reveals how cells harboring intact HIV-1 proviruses survive viral cytopathic effects and immune clearance. These recent advances shift the paradigm that measuring HIV-1 reactivation alone is not sufficient to fully understand HIV-1 persistence. Killing infected cells that survive viral cytopathic effects and targeting HIV-1 integration site-dependent proliferation are two major gaps for a cure. Earlier *in vitro* models were not designed to target these mechanisms of HIV-1 persistence.

In this issue of *Cell Reports Methods*, Matsuda et al. (2021) have now used a replication-competent virus to establish a Jurkat T cell line model and tested the impact of viral suppression by antiretroviral therapy (ART) and HIV-1 reactivation by LRAs on viral cytopathic effect, latency establishment, and the elimination of HIV-1-infected cells (Figure 1E). Because replication-competent HIV-1 infection kills most infected cells and prevents long-term culture, previously reported *in vitro* models used either defective HIV-1 reporter virus for long-term culture (months) (Jordan et al., 2003; Yang et al., 2009) or replication-competent HIV-1 viruses (with ART supplement to prevent new rounds of infection) for short-term culture (weeks) (Martins et al., 2016). Matsuda et al. (2021) infected Jurkat T cells with a replication-competent HIV-1 virus NL4-3 for a total of 5 months of culture, including 1 month of ongoing replication, 2 months of viral suppression, and 2 months of treatment cessation to capture viral outgrowth. The use of replication-competent viruses in this long-term culture recapitulates the diverse mixture of cells harboring intact and defective HIV-1 proviruses *in vivo*.

Using HIV-1 proviral genome mapping, Matsuda et al. (2021) found that during the first month of ongoing replication in this model, the majority of HIV-1 proviruses are intact. After 2 months of ART, viral outgrowth occurs in all culture wells (100%) when ART is discontinued. Importantly, after 2 months of LRA and ART, fewer culture wells have viral outgrowth (36%). In those culture wells having no viral outgrowth, no replication-competent HIV-1 can be found using HIV-1 p24 protein flow cytometry after stimulation, supernatant HIV-1 p24 measurement, and near full-length HIV-1 proviral sequencing. This suggests that the infected cells in these wells are indeed eliminated, most likely through viral cytopathic effects. This model recapitulates *in vivo* HIV-1 infection in which most infected cells die of viral cytopathic effect, some infected cells harbor defective proviruses, and some infected cells harbor latent but inducible proviruses. Some of the latently infected cells may lead to viral rebound after treatment interruptions, as seen *in vivo*.

In addition to HIV-1 gene expression typically measured in existing models, Matsuda et al. (2021) also mapped the HIV-1 proviral genome and integration site landscape over time. They captured an expanded infected cell clone harboring a defective HIV-1 provirus integrated into a cancer-related gene *NSD3* over prolonged culture, suggesting that HIV-1 integration into this location may provide survival benefit for the infected cells to proliferate and persist. Thus, this model recapitulates the persistence of defective HIV-1 proviruses due to the lack of cytotoxicity and HIV-1 integration site-driven clonal expansion.

Overall, the *in vitro* model developed by Matsuda et al. (2021) examines the effect of LRA not only on HIV-1 reactivation but also on HIV-1 elimination. This model provides mechanistic understanding on HIV-1 persistence by recapitulating viral cytopathic effects and clonal expansion dynamics *in vitro* by profiling both HIV-1 integration site and near full-length HIV-1 genome landscape. The caveat of this model is that side-by-side comparison of drugs effects between this model versus *ex vivo* testing in clinical samples was not performed. Validations in *ex vivo* and *in vivo* systems are still required. In addition, viral suppression using one single ART in this model may not be sufficient. Furthermore, this model can be used to test drug effects for a selected list of drug candidates but is not designed for a high-throughput compound library screen. Nevertheless, this multidimensional approach recapitulates multiple mechanisms of HIV-1 persistence

(B) HIV-1 near full-length single-genome proviral sequencing by limiting dilution nested HIV-1 PCR captures HIV-1 proviral genome landscape and immune selection pressure on the infected cells.
(C) Random DNA shearing and linker/ligation-mediated amplification captures HIV-1 integration site and clonal expansion dynamics of the infected cells.
(D) Matched integration site and proviral sequencing by phi29 polymerase-mediated multiple displacement whole-genome amplification captures the integration site and proviral genome of the same provirus.
(E) Widely distributed intact provirus elimination (WIPE) assay by Matsuda et al. (2021) uses a replication-competent virus for long-term culture to capture not only HIV-1 reactivation but also HIV-1 elimination by measuring HIV-1 genome landscape, viral cytopathic effects, HIV-1 integration site, clonal expansion dynamics, HIV-1 integration site-dependent proliferation, and viral rebound. Figure created with Biorender (https://biorender.com/).

and can be applied to other *in vitro* models to improve *in vitro* drug testing for HIV-1 cure strategies before resource-demanding *ex vivo* and *in vivo* studies.

DECLARATIONS OF INTERESTS

The authors declare no competing interests.

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