Commentary

Relevance of multiply spliced HIV-1 RNA measurement in assessing the efficacy of viral latency-reversing strategies

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Latently infected CD4+ T cells provide a reservoir for the human immunodeficiency virus type 1 (HIV-1) that persists for life even in patients on effective combination antiretroviral therapy (ART). When ART is interrupted, HIV-1 soon rebounds from its latent reservoir (LR) and disease progresses, hence people living with HIV-1 (PLWH) must take ART indefinitely, facing the risk of cumulative drug toxicity and occurrence of chronic immune activation, cancer, and other morbidities. In the attempt to find a sterilizing HIV-1 cure, various strategies for eliminating the LR have been developed and are being clinically tested. The primary intervention strategy is often referred to as ‘shock and kill’, in which latency reversing agents (LRAs) induce reactivation of quiescent provirus (the shock) and cells that exit viral latency are eliminated either by host HIV-specific CD8+ T cells and natural killer cells or virus-induced apoptosis (the kill) [1]. Some histone deacetylase inhibitors (HDACis) with LRA ability, namely Vorinostat, Panobinostat, and Romidepsin, have already been administered to ART patients resulting in an increase of cell-associated (CA) HIV-1 RNA in CD4+ T cells and, in some cases, of plasma HIV-1 RNA, yet failing to reduce the number of latently infected cells in blood [2]. These and other concluded LRA-based trials have clearly shown the need for more effective latency-reversing interventions as well as for reinforcing immune-mediated killing of reactivated HIV-1+ cells; toward this goal, combinations of two functionally distinct LRAs and additional immunological approaches, such as therapeutic HIV-1 vaccination, antibody-based and immunomodulatory strategies, are under development [3]. Another aspect that is crucial for the success of HIV-1 eradication trials is availability of methods that accurately measure the LR size before and after interventions. Several assays based on quantitative PCR (qPCR), immunoassays, and cell culture systems are currently available to measure the total amount and integrity of proviral DNA, the content of provirus competent for transcription, translation, and expression of infectious virions [4].

Every method has its own advantages, such as sensitivity or rapidity, but also drawbacks including under- or over-estimation of the LR size, time and/or cost intensiveness. Most importantly, results produced by different assays are often divergent, therefore reaching to a general consensus on which methods should be used for measuring LR in HIV-1 cure trials has become a priority.

In a study reported in EBioMedicine, Sharon R Lewin and colleagues compared expression of two CA HIV-1 RNA species, unspliced (US) and multiply-spliced (MS) RNAs, in CD4+ T cells from PLWH on ART for their association with the supernatant (SN) or plasma HIV-1 RNA levels upon LRA-induced latency reversal ex vivo and in vivo, respectively [5]. In ART patients, US RNA (genomic and Gag-coding) levels are high, reflecting the number of cells harboring transcription-competent LR, whereas MS RNA (Tat/Rev/Nef-coding) mark productively infected rare cells with potentially high level of viral protein and virus production. Results showed that ex vivo stimulation with HDACi (Romidepsin, Panobinostat, but not Vorinostat) or the JQ1 bromodomain inhibitor increased US and MS RNA levels as well as SN RNA levels (SN RNA levels were not altered by JQ1), yet MS but not US RNA correlated with SN RNA (both as total amounts and fold change increase) [5]. The authors also showed that, in 11 participants who received Panobinostat in a clinical trial (NCT01680094), MS RNA levels were significantly higher in samples with detectable plasma HIV RNA as compared to undetectable, concluding that MS RNA can serve as a biomarker for latency reversal in both pre-clinical and clinical studies. This assumption was corroborated by the finding that defective proviral sequences published in database could be rarely detected with the MS-specific primers as opposed to US-specific primers employed in this work (8% vs 40% defective proviruses), an instructive analysis that could be generally applied to qPCR-based assays in latency-reversal studies.

Of note, the authors found that total as compared to resting CD4+ T cells from PLWH on ART had higher HIV-1 DNA and US RNA levels, indicating that transcriptionally-active LR is not restricted to cells with a resting phenotype as commonly assumed, in agreement with a recent study demonstrating the wide distribution of the LR across CD4+ T-cell subsets in PLWH, including those expressing activation markers [6].

Overall, results presented in this article support the biological and clinical relevance of MS RNA measurement in determining the size of the LR, along with previously reported evidence that, upon ART interruption, MS RNA may serve as predictor of viral rebound [7] or CD4+ T cell loss [8]. Nonetheless, this method has limitations because,
analogously to US RNA assay, it does not measure the translational and replication competence of the provirus; indeed, most proviruses in CD4⁺ T cells of PLWH on ART are defective [9] and, in addition, translation-incompetent viral RNAs may result from host promoter-driven readthrough transcription [10], thus measuring MS RNA overestimates the size of the LR that can actually produce antigenic viral proteins and/or infectious virions. Powerful methods for measuring intact HIV-1 genomes, viral protein expression, and replication-competent virus are continuously being developed [4]. Likely, only the combination of various viral measurements will provide a full picture of the LR dynamics during HIV-1 eradication trials and a reliable tool for evaluating the efficacy of such interventions.

Declaration of Competing Interest

The author declares no conflict of interest.

Contributors

MD conceived and wrote the commentary.

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