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Commentary

# How Much HIV is Alive? The Challenge of Measuring Replication Competent HIV for HIV Cure Research

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A critical knowledge gap in the field of HIV cure research is how best to measure the persistence of replication-competent HIV in the form of intact but latent proviruses. One approach to curing HIV is to eradicate all replication-competent HIV from an individual. It is unlikely that any method, now or in the future, will be able to declare with certainty that all intact proviruses have been eliminated from an individual's body. The difficulty in declaring that all intact proviruses have been eradicated is best illustrated by the two Boston patients who underwent allogeneic, hematopoietic stem cell transplantation for uncontrolled malignancy. After successful transplantation and several years of suppressive antiretroviral therapy (ART), HIV DNA or RNA could not be detected in large volumes of blood cells assayed many times from either patient, yet viremia rebound occurred 7–32 weeks after cessation of ART (Henrich et al., 2014). Similarly, it has been difficult to conclude that all HIV has been eliminated even from the Berlin patient who was transplanted with HIV-resistant cells and in whom HIV has not been detected in blood for the past decade off of ART (Yukl et al., 2013). As such, laboratory assays to exclude the persistence of intact proviruses in a person are no more likely to be found than is a therapy that eradicates all HIV from a person.

The more realistic goal of HIV cure research is to reduce HIV reservoirs and enhance immune control of HIV to undetectable levels in the blood after cessation of ART, also termed, HIV remission. Researchers studying interventions aimed at HIV remission struggle to understand how much their interventions are affecting intact proviruses because of the shortcomings of existing methods to measure them. What matters for the goal of HIV cure is how much HIV can replicate. Polymerase chain reaction (PCR)-based methods are used to quantify the amount of HIV DNA and HIV RNA in mononuclear cells from blood and lymphoid tissues. Having detectable HIV DNA, however, does not tell us how much HIV could replicate as most of the DNA is defective (Eriksson et al., 2013). Cell-associated HIV RNA has been used as a proxy for "active" reservoirs since RNA is short-lived and its presence implies recent transcription (Pasternak et al., 2013). Measuring low level HIV RNA in blood or other body fluids is another marker for ongoing viral production (Cillo et al., 2014). However, all these methods are not direct measures of the ability to infect other cells, replicate and produce infectious progeny. The current "gold standard" of such test is the viral outgrowth assay (VOA), a co-culture assay that stimulates resting CD4+ T cells from HIV-infected patients to produce virus capable of infecting HIV-uninfected cells from another donor. However, this method underestimates the amount of replication competent virus by 60-fold compared to detection of intact proviruses by sequence analysis. This underestimation can be reduced by sequential stimulation and co-culture of patient and donors cells to activate a greater fraction of proviruses (Ho et al., 2013), but this approach is impractical because the VOA, even with a single round of stimulation, requires a large blood volume, weeks to complete, and costs more than $1000 per assay for reagents and labor.

In this issue of *EBioMedicine*, Procopio and Chomont et al., proposed the Tal/rev Induced Limiting Dilution Assay or TILDA, a cell-associated HIV RNA PCR that is based on the VOA concept in that it relies on stimulating patient's CD4+ T cells, but instead of the cumbersome co-culture methods, an early product of viral transcription, the tat/rev multi-spliced RNA, is measured (Procopio et al., 2015). When HIV RNA enters the cytoplasm, it reverse transcribes into DNA that penetrates the nuclease to integrate into the host genome. When cells with integrated DNA are activated, they transcribe RNA that is multiply spliced for translation of tat and rev proteins that accelerate transcription and permit genomic viral RNA to exit the nucleus, respectively, which is translated into the structural proteins required for assembly and release of virions from the cell (Pasternak et al., 2013). Because TILDA uses small blood volume and is relatively easy to perform without the expense and labor intensiveness of VOA, it is an attractive option for assessing HIV persistence.

So how good is TILDA in measuring HIV that is capable of replicating? The frequency of CD4+ T cells with inducible RNA by TILDA is within the realm of expectation. First, early treated individuals harbor fewer such cells than chronically treated individuals. Second, the size of inducible virus by TILDA is smaller than HIV DNA by PCR but larger than replication competent virus by VOA, suggesting that TILDA is measuring the induction of more intact proviruses than the standard VOA assay. However, TILDA does not directly measure virus production, either infectious or non-infectious, but rather, it measures inducible multi-spliced transcripts, which is quite proximal to virion release. As the authors note, it is possible that cells with inducible multiply-

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spliced transcripts may not produce infectious virus particles. Indeed, the results of the TILDA assay and the VOA performed on the same samples show a weak, non-significant correlation. Whether this is due to limitations of the TILDA or VOA is unclear and will require further study. Lymphoid tissues are also major HIV reservoir sites (Rothenberger et al., 2015), and it is possible that TILDA, because of its small sample requirement, could be used to measure inducible proviruses in tissue samples. This possibility would be a significant advance given that a sensitive VOA cannot be performed with the limited number of cells available from tissue samples.

TILDA could be a research tool for in vitro screening of drugs such as latency reversing agents for further animal and human trials, and measuring the effects of these drugs in vivo. As testing for HIV remission will require ART interruption (Li et al., 2015), TILDA could be used to identify trial participants with the highest potential for achieving remission (i.e. have no inducible RNA by TILDA). These potential uses require further validation. Future studies should include TILDA as one of the outcome measures of differing interventions (latency reversing agents, immunotherapeutics, gene- and cell-based therapies) and in different patient populations (virally suppressed acutely and chronically infected, children and adults) to assess the broad utility of TILDA. If TILDA or other novel assays can predict the duration of HIV remission off ART, it will accelerate progress towards a cure of HIV.

ART saves lives, and life-long, uninterrupted ART remain the standard of care. HIV remission and eradication are aspiring goals that one day could allow individuals living with HIV to be free of long-term ART, and ultimately, free of HIV. Many progresses have been made in understanding HIV persistence and designing interventions that could mitigate HIV. Developing and validating tools to accurately measure replication competent HIV is among the highest priorities in the HIV cure research field.

## Conflicts of interest

Authors declared no potential conflict of interest relevant to this manuscript.

## Disclaimer

The views expressed are those of the authors and should not be construed to represent the positions of the U.S. Army or the Department of Defense.

## References

Cillo, A.R., Vagratian, D., Bedison, M.A., et al., 2014. Improved single-copy assays for quantification of persistent HIV-1 viremia in patients on suppressive antiretroviral therapy. J. Clin. Microbiol. 52, 3944–3951.

Eriksson, S., Graf, E.H., Dahl, V., et al., 2013. Comparative analysis of measures of viral reservoirs in HIV-1 eradication studies. PLoS Pathog. 9, e1003174.

Henrich, T.J., Hanhauser, E., Marty, F.M., et al., 2014. Antiretroviral-free HIV-1 remission and viral rebound after allogeneic stem cell transplantation: report of 2 cases. Ann. Intern. Med. 161, 319–327.

Ho, Y.C., Shan, L., Hosmane, N.N., et al., 2013. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. Cell 155, 540–551.

Li, J.Z., Smith, D.M., Mellors, J.W., 2015. The critical roles of treatment interruption studies and biomarker identification in the search for an HIV cure. AIDS 29, 1429–1432.

Pasternak, A.O., Lukashov, V.V., Berkhout, B., 2013. Cell-associated HIV RNA: a dynamic biomarker of viral persistence. Retrovirology 10, 41.

Procopio, F.A., Fromentin, R., Kulpa, D.A., et al., 2015. A novel assay to measure the magnitude of the inducible viral reservoir in HIV-infected individuals. EBiomedicine http://dx.doi.org/10.1016/j.ebiom.2015.06.019 (http://www.sciencedirect.com/science/article/pii/S2352396415300487).

Rothenberger, M.K., Keele, B.F., Wietgrefe, S.W., et al., 2015. Large number of rebounding/founder HIV variants emerge from multifocal infection in lymphatic tissues after treatment interruption. Proc. Natl. Acad. Sci. U. S. A. 112, E1126–E1134.

Yukl, S.A., Boritz, E., Busch, M., et al., 2013. Challenges in detecting HIV persistence during potentially curative interventions: a study of the Berlin patient. PLoS Pathog. 9, e1003347.