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Viral markers for HIV cure trials: are we getting any closer?

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The definite proof of concept of an effective treatment aimed at HIV cure would be a very prolonged or permanent HIV remission after interruption of treatment. However, treatment interruption studies have generally resulted in a quick viral rebound in nearly all patients with the exception of a few isolated cases of long-term post-treatment control. Consequently, biomarkers that could predict whether a cure strategy has any profound impact are needed [1].

Most cure strategies aim at minimising the so-called persistent reservoir of latently infected cells. The reservoir is quickly established after HIV infection and persists life-long, regardless of the subsequent suppression of viral replication by antiretroviral treatment. This reservoir comprises cells with replication-competent proviral HIV-DNA that is integrated within the chromosomal DNA [2].

Different methods have been described to measure the amount of replication-competent provirus. The most obvious method is the quantification of the HIV-DNA burden in blood. However, most of the integrated HIV-DNA is not replication competent, as it contains genetic mutations originating from the error-prone viral reverse transcriptase or from innate host defence mechanisms (e.g. APOBEC3G). Hence, a mere quantification of HIV-DNA may not correlate with the relatively small pool of replication-competent provirus [2]. An alternative method consists of measuring viral transcription by quantifying cell-associated HIV-RNA. Yet, transcriptional activity of proviral HIV-DNA may not always lead to virus production. The most direct method to assess replication-competent virus is to quantify the amount of virus that effectively replicates after an *ex vivo* stimulation of blood CD4 T cells in quantitative viral outgrowth assays (qVOA) [3,4]. However, this method is laborious, time-consuming and requires large amounts of blood. Recently a combination of viral outgrowth and cellular RNA quantification, or TILDA, has been described (*tat/rev* induced limiting dilution assay) [5]. This assay is based on the isolation and *ex vivo* stimulation of patient-derived cells, similarly to the viral outgrowth assays, and measures cell-associated HIV-RNA production rather than productive infection. TILDA is more sensitive and faster compared to qVOA; however, the reactivated provirus may be transcriptionally active yet not replication competent.

With the increasing number of assays available to measure the HIV reservoir, there is a growing debate as to which marker is most predictive of viral cure, or may be applicable in large-scale clinical studies. The problem is two-fold. First, there have not been many clinical trials that have studied HIV cure as a clinical endpoint so far (i.e. the absence of viral rebound after treatment interruption), and the studies that have done so have mostly resulted in a quick viral rebound of nearly all study participants. Secondly, the few studies that have performed a structured treatment interruption have only quantified a limited set of reservoir markers, mostly HIV-DNA and HIV-RNA by PCR. Despite these limited data, there are some interesting lessons to be learned from previous treatment interruption trials.

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Because the majority of patients experience viral rebound following treatment interruption, the time to viral rebound has been suggested as the next best measure to assess biomarkers. Two recent treatment interruption trials (SPARTAC and ANRS 116 SALTO) have shown a link between levels of total HIV-DNA load and time to viral rebound by correlating biomarkers with the time to viral rebound [6,7]. This suggests that HIV-DNA quantification may represent a predictive marker, despite the concern that most of the quantified DNA is derived from non-replication-competent proviral HIV-DNA. Azzoni's treatment intensification study provides additional support to this concept, as a decline in integrated HIV-DNA levels was shown in study participants who had sustained viral control after treatment interruption following prior treatment intensification with pegylated interferon alpha-2a [8]. In contrast to these findings, a recent pooled analysis by Li *et al*. of six treatment interruption trials, including 124 study participants, has indicated that cell-associated HIV-RNA levels, measured in patients on ART before treatment interruption were predictive for the time to viral rebound. The levels of total HIV-DNA were not predictive in this study. [9]. These findings suggest that the transcriptional state of the viral reservoir may also be a promising marker to predict viral rebound.

It should be noted that currently available data should be treated with caution, as most correlations in terms of biomarkers are the results of *post hoc* analyses from clinical studies. These studies may provide preliminary proof-of-concept data, but results should be validated in clinical trials designed for this purpose. Consequently, future trials involving treatment interruption studies will benefit from a more comprehensive analysis of HIV reservoir markers, i.e. viral DNA, RNA, qVOA and/or TILDA measures. Moreover, there should also be the aim to organise storage of sufficient amounts of blood in good conditions to enable long-term preservation of viable cells, which will facilitate the analysis of further biomarkers in the future.

In addition to this, it should be noted that the time to viral rebound might not necessarily represent the best measurement to assess progress towards a functional cure. The timing of viral rebound may only be a function of the remaining population of latently infected cells and their propensity to reactivate within a given time frame. Post-treatment viral control may be independent of this phenomenon, and only depend on a successful immune response that suppresses viral replication from reactivated proviruses.

In conclusion, the end of the long and winding road towards a cure for HIV-1 is not yet in sight. The lack of predictive markers to establish firm clinical endpoints is certainly an important factor for this. To foster progress in the field, we will have to analyse a broad range of viral reservoir markers in well-structured clinical trials without pre-emptively focusing on a single parameter that may not prove predictive of outcome or may lack power.

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