PE18

Improved assays to measure the inducible latent HIV reservoir

M. Massanella[1], C. Yek[1], S.M. Lada[1], M.C. Strain[1], D.D. Richman[1,2]
[1]UCSD, Pathology, La Jolla, United States, [2]Veterans Affairs San Diego Healthcare System, La Jolla, United States

Background: Precise and practical assays that can reliably measure the impact of a candidate treatment strategy are essential. We improved the standard quantitative viral outgrowth assay (QVOA) and developed a new assay, which promises to be faster, more sensitive, and higher throughput than the standard QVOA.

Methods: Freshly isolated CD4 T cells from 7 ART-suppressed subjects treated during chronic infection were analyzed for total HIV DNA by droplet digital PCR (ddPCR, gag) and our newly developed assays for the inducible HIV reservoir - modified QVOA (mQVOA) and inducible cell-associated RNA expression in dilution (iCARED). For mQVOA, CD4 T-cells in limiting dilution were activated with anti-CD3/CD28 antibodies. After 2 days of culture, MOLT-4/CCR5 cells were added to the culture and cell-free (cf-) RNA was quantified by real-time PCR (Pol) at day 7. Similarly, we used CD3/CD28 co-stimulation for the iCARED assay in the presence of raltegravir. After 3 days of culture, cell-associated (ca-) RNA was quantified by ddPCR (gag and tat-rev). In both cases, we used a magnetic-bead based RNA extraction system (HologicTM) to specifically extract HIV RNA molecules, making it more sensitive than conventional methods and allowing the testing of large volumes of both cells and culture supernatant.

Results: The median for total HIV DNA was 168 [103-332] copies/106 PBMCs and for mQVOA was 5 [1.7-7.3] infectious units/106 CD4 T cells. There was only a 42-fold difference between the two measures; substantially less than what has been reported previously. In the iCARED assay, the median frequency of cells with inducible ca-RNA was 45 [20-61] cells/106 CD4 T cells, which was 10 times more than the median frequency measured by mQVOA and 4 times less than the median frequency given by total HIV DNA. The latently infected cells detected by iCARED assay was highly correlated with quantification by mQVOA (R=0.89, p=0.007) and HIV DNA (R=0.95, p=0.01).

Conclusions: iCARED is a simple method to quantify the transcriptionally competent latent HIV reservoir. Our results suggest that iCARED, which is more rapid (4 days), less expensive, less cell-demanding and hands on time than QVOA, could prove to be a useful tool for clinical investigations.