PE19 LB
Time associated changes in cell-associated HIV RNA in HIV-infected subjects on suppressive antiretroviral therapy - implications for clinical trials of cure interventions

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Background: Cell-associated unspliced (CA-US) HIV RNA is an important marker of the HIV reservoir and a common primary end-point in clinical trials of latency reversing agents in HIV-infected subjects on antiretroviral therapy (ART). We observed large baseline variation in CA-US HIV RNA in a recent clinical trial of disulfiram and hypothesised these changes were due to circadian-related alterations in CD4+ T-cell composition, gene regulation or anticipatory stress.

Methods: Blood was collected on three occasions (B1, B2 and B3) from HIV-infected subjects (n=30) on suppressive ART prior to any intervention. B3 was collected immediately prior to administration of disulfiram. We measured CA-US HIV RNA and DNA by real-time PCR and plasma HIV RNA (using a single copy assay) by droplet digital PCR. Plasma cortisol and thyroid stimulating hormone (TSH) levels were quantified by ELISA. PBMC were stained with live-dead dye and antibodies to CD3, CD4, CD8, CD45RA, CCR7, CD27, CD38, HLA-DR, acetylated lysine and acetylated histone-3 and were analysed by flow cytometry. Data were assessed for normality then analysed with Wilcoxon matched-pairs signed rank tests and paired-t-tests.

Results: CA-US RNA was higher in blood collected at B3 compared to B1 and B2 (median 85.63 vs. 28.14 and 34.87 copies/million CD4+ T-cell equivalents; both, p< 0.001). There were little differences in HIV DNA or plasma HIV RNA at these times. B3 was collected earlier in the day compared to B1 and B2 (mean 8.28am vs. 11.38am and 10.21am; both, p< 0.001). Other parameters that were significantly higher at B3 compared to B1 and B2 were cortisol (p=0.001 and 0.011); TSH (p=0.023 and 0.004); CD8+CD38+HLADR- T-cells (both, p< 0.001) and CD4+CD38+HLADR- T-cells, which were elevated at B3 compared to B2 (p=0.012). There were no significant differences in the percentage of T-cell subsets or histone acetylation in the blood collected at these time-points.

Conclusions: Time-associated variation in CA-US HIV RNA seen in HIV-infected subjects on suppressive ART was not associated with significant alterations in CD4+ T-cell subset composition and was suggestive of circadian changes in HIV RNA transcription. Diurnal changes in CA-US HIV RNA may need to be considered in the design of future cure intervention trials.

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