

## MEMORY CD4+ T CELLS EXPRESSING HLA-DR CONTRIBUTE TO HIV PERSISTENCE DURING PROLONGED ART

Lee E<sup>1,2</sup>, Hiener B<sup>1,2</sup>, Horsburgh B<sup>1,2</sup>, Bacchetti P<sup>3</sup>, Shao W<sup>4</sup>, Boritz E<sup>5</sup>, Douek D<sup>5</sup>, Fromentin R<sup>6</sup>, Liegler T<sup>7</sup>, Deeks SG<sup>7</sup>, Hecht FM<sup>7</sup>, Milush J<sup>7</sup>, Chomont N<sup>6</sup>, Palmer S<sup>1,2</sup>

<sup>1</sup>The Westmead Institute for Medical Research, Westmead, Australia; <sup>2</sup>University of Sydney, Sydney Medical School, Sydney Australia; <sup>3</sup>Department of Epidemiology and Biostatistics, University of California San Francisco, San Francisco, United States; <sup>4</sup>Leidos Biomedical Research, INC, Frederick National Laboratory for Cancer Research, Frederick, United States; <sup>5</sup>Human Immunology Section, Vaccine Research Center, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, United States; <sup>6</sup>Department of microbiology, infection and immunology, Université de Montréal, Montreal, Canada; <sup>7</sup>Department of Medicine, University of California San Francisco, San Francisco, United States

**Background:** Most measurements of HIV reservoirs are performed using “resting” CD4+ T cells depleted for cells expressing HLA-DR, which is considered an activation marker. However, the possible role of these cells in HIV persistence during ART is undefined. Here, we examine the contribution of HLA-DR+ CD4+ T cells to HIV persistence after prolonged ART (≥15 yrs).

**Methods:** Using LTR-specific qPCR, HIV RNA and DNA were quantified in HLA-DR- (resting) and HLA-DR+ CD4+ T cells from 6 participants (P1-P6) on ART (≥15 yrs). Using single-genome/proviral sequencing, we characterized HIV-RNA/DNA (p6-RT) sequences from HLA-DR-, HLA-DR+, central (CM), transitional (TM) and effector (EM) memory CD4+ T cells. Clonal expansions were defined as ≥2 identical sequences in phylogenetic trees.

**Results:** CD4+ HLA-DR+ and HLA-DR- memory cells contained a median of 3400 and 1000 HIV-RNA copies/million cells; and 36 and 60 HIV-DNA copies/million cells respectively, indicating the HIV transcriptional activity of HLA-DR+ cells is 6-fold higher than HLA-DR-. For one participant treated during acute infection, the amount of defective HIV-DNA and RNA sequences containing stop codons from HLA-DR+ memory T cells was much lower (21% and 0% respectively) than those derived from HLA-DR- memory T cells (50% and 26% respectively). In participants treated during chronic infection, EM more often contained clonally expanded HIV-DNA sequences which were genetically identical to HIV-DNA sequences from HLA-DR+ memory cells (43% P1, 88% P2) than CM and TM (8-18% P1; 8-44% P2; p<0.0001-0.03).

**Conclusions:** CD4+ memory T cells expressing HLA-DR contain measurable HIV-DNA and HIV-RNA. Furthermore, these cells appear to contain more intact virus in the sequenced region than HLA-DR- memory cells. Therefore, measurements of the HIV reservoir during ART should include memory cells expressing HLA-DR. HLA-DR+ and EM cells had clonal expansions of genetically identical HIV-DNA, suggesting these cell subsets replenish the HIV reservoir through proliferation during prolonged ART.