ART-Treated Subjects with Low Viral Reservoir Show Unusual HIV Latency Distribution

**BACKGROUND**

Small-kilocase viral reservoirs are predominantly found in HIV-1 controllers and individuals treated during acute/early HIV-1 infection. However, other HIV subjects could naturally also harbor low viral reservoirs. We have previously established a cohort of Low Viral Reservoir ‘Treated’ subjects (LoViReT), which represent about 5% of individuals on ART. Here, we aim to study the mechanisms that cause these unusual low reservoirs.

**METHODS**

- Subjects: 42 HIV-1-infected individuals under cART and <100 HIVDNA copies/mL PBMCs constitutes the LoViReT cohort, 12 LoViReT subjects treated in the chronic phase of infection (4 months after infection) with 100 HIVDNA copies/mL PBMCs were compared with 13 chronic controls >100 HIVDNA copies/mL PBMCs. All 14 LoViReT subjects were selected to comprehensively analyze the viral persistence in blood and tissues. Individuals were selected from Hospital Universitari Germans Trias i Pujol and Hospital Clinic.

- Protocol: total HIV-DNA was longitudinally measured in peripheral PBMCs, CD4+ T cells, CD8+ T cells, and CD45RA+ (naive) and CD45RO+ (memory) cells by droplet digital PCR amplifying gag and LTR regions. The RPPR30 gene was quantified in parallel to normalize sample input.

- Replication-competent reservoir: Leukaphereses were obtained in 14 LoViReT to measure the number of infected units per million (UMU) in 38 million CD4+ T cells with a detection limit of 0.2185 UPM.

- Residual viremia: 6 ml of plasma were ultracentrifuged and HIV RNA quantified using Abbott v3 platform.

- Isolation of cell subsets and flow cytometry: At least 100 million PBMCs were stained with monoclonal antibodies against CD4, CD8, CD45RA and CD27, CD45RA*, CD27*. naïve CD4+ T cells, CD45RA*, CD27* central memory CD4+ T cells, CD45RA- CD27*, CD27+ effector memory CD4+ T cells, CD45RA* CD27- effector memory CD4+ T cells, CD45RA- CD27- central memory CD4+ T cells, and CD45RA- CD27- terminally differentiated CD4+ T cells were determined by FACSAria.

**RESULTS**

1. **Longitudinal analysis of total HIV-DNA**

![Figure 2: Longitudinal measure of total HIV-DNA in CD4+ T cells by ddPCR](image)

2. **Viral persistence in LoViReT subjects**

![Figure 3: Viral persistence performed on the indicated cell or tissue obtained from LoViReT subjects. HIV DNA in viral CD4+ T cells and lymph node (LN) CD45RA+ were only assessed in LoViReT with an LN PUM >0.3. Median values are indicated by an horizontal black line. Open symbols represent undetectable values. In those cases, the limit of detection for the samples varied on the basis of cell dilution input, and that value is represented by an horizontal black line.](image)

3. **Distribution of HIV-1 reservoirs in CD4 T-cell subsets**

![Figure 4: HIV latency distribution in T-cell subsets in LoViReT subjects. (A) Total HIV-DNA measured by ddPCR and (B) contribution of each subset to the HIV reservoir.](image)

**CONCLUSIONS**

- LoViReT individuals have abnormally low HIV reservoirs even in the absence of cART.

- 71% of LoViReTs did not show replication-competent virus and harbored limited provirus in tissue sanctuaries under cART.

- A cause of this exceptional low reservoir could be the high contribution of the short-live T_HM and T_EM cells in the total HIV reservoir.

- This individuals are the perfect candidates to try new HIV remission strategies.

**ACKNOWLEDGEMENTS**

This study was supported by Merck Sharp & Dohme Spain S.A. (ISB 54925). CG was supported by the predoctoral fellowship of the Spanish Education, Culture and Sport Ministry (IFPU15/03998). All the patients the study.