Oral Abstract Session 3: Novel strategies to identify and quantify virus persistence in vivo (biomarkers)

OA3-1

Immunological markers associated with HIV persistence during ART identified by iterated conditional random forests analysis

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Background: The persistence of latently infected cells and residual levels of viral production contribute to HIV persistence and immune activation in HIV infected individuals on suppressive antiretroviral therapy (ART) and represent major barriers to HIV eradication. We hypothesized that HIV persistence on ART was associated with markers of T-cell activation, homing and proliferation.

Methods: Expression of activation/proliferation markers, chemokines receptors, immune checkpoints and their ligands were measured by flow cytometry on PBMCs isolated from 48 HIV-infected subjects on ART for >3 years with HIV viral load < 50 copies/ml and with a CD4 count >350 cells/µL. Two virological markers of HIV persistence were determined by quantitative (q)PCR: the frequencies of CD4 T cells harboring integrated HIV DNA and cell associated unspliced (CA-US) HIV RNA . Chemokines, gamma-c cytokines and sCD14 were quantified in plasma. More than 600 variables were analyzed by fuzzy forests to identify novel biomarkers associated with HIV persistence and that predict low reservoir size. Briefly fuzzy forests first separates the variables into modules that have a similar correlation structure to account for network effects and then performs recursive feature elimination random forests to find the top parameters that are predictive of the outcome.

Results: Using fuzzy forests, we identified the top 100 variables of importance/predictors that were most strongly associated with high frequency of CD4 T cells harboring integrated HIV DNA or CA-US HIV RNA. High CA-US RNA was strongly associated with activating IFN signaling pathway in T cells (pSTAT1-3). High frequency of cells harboring integrated HIV DNA was associated with low CD4 count (p=0.0015) as expected but also with higher frequency of cells expressing markers of proliferation/activation (including expression of 2B4, LAG3, TIGIT on central memory CD4 T cells, p=0.0057, p=0.0016, p=0.0106 respectively and HLA-DR and CD38 on CD8 T cells, p=0.019 and p=0.0252 respectively).

Conclusions: Current assays that measure virus persistence are associated with different immunological pathways. CA-US RNA, a surrogate marker of active viral transcription, was associated with the STAT1-3 downstream of type I interferon signaling pathway while the number of latently infected cells was associated with markers of T-cell activation, proliferation and exhaustion.