HIV-1 elimination from reservoirs - viral dynamics during suppressive ART

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Abstract message

- In pre-therapy situations CXCR4-tropic HIV-1 variants increase over time and with stage of disease, whereas under therapy CXR4-tropic variants become markedly reduced or vanish*
- The study aim was to identify, which lymphoid compartment(s) are responsible for the observed selective elimination of the CXCR4 infected cells looking at the homing potential of cells from the blood periphery
- Preliminary data from cell sortings reveal that Lymph node homing as well as gut homing properties of cells coincide with higher proviral loads
- Of note: most gut homing memory T-cells possess also lymph node homing properties
- tSNE visualization is a suitable tool for identifying essential marker co-expression on infected cells

Background

In the absence of any therapy X4 (CXCR4)-tropic HIV-1 is found to increase over time of infection, associated with an accelerated disease progression. More recent analyses during ART show that in most successfully treated patients the opposite is true: X4 viruses are diminished while R5 (CCR5) viruses are stable*. Antiretroviral therapy itself and the recovering immune system seem to readily detect and clear cells infected with X4 viruses . Our intention is to look at long lived memroy T-cell compartments in the periphery of patients during untreated and treated time points to determine which cells are involved in the viral dynamics and which lymphoid tissue is responsible fo reservoir formation, stability and viral clearance with special focus on X4- and R5- tropic virus compartmentalization.

Material & Methods

Our pilot study used MACS technology for the analysis of peripheral blood of arbitrarily chosen HIV-positive patient samples from the Swiss HIV cohort study. Non-relevant CD8+ (CTLs) and CD19+ (B-cells) were depleted and CD8-CD19- cells were selected for CD4 and Integrin B7 (gut homing) or CCR7 (lymph node homing). Proviral loads (pVLs) were determined by validated qPCR. For multi-dimensional data visualization the tSNE plugin of FlowJo was used.

Results

MACS preliminary results:
In order to evaluate which lymphoid homing marker might be of highest relevance, proviral loads of MACS sorted fractions revealed that CD4+CCR7+/-CD8-CD19- and CD4+B7+CD8-CD19- cells were clearly enriched for proviral DNA copies.

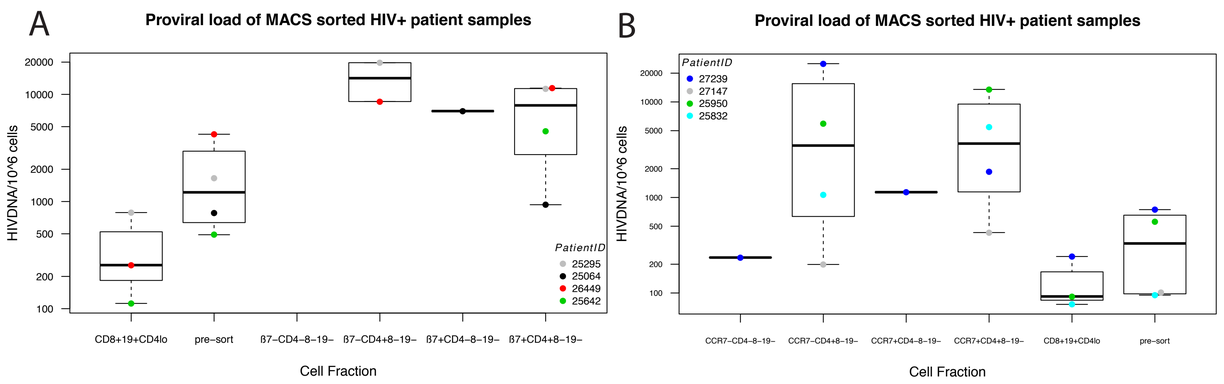

Figure 1. Proviral loads of MACS sorted patient cell fractions. A) shows Lymph node homing selected cells and B) selected gut homing cells: Boxplot depicts proviral distributions of sorted patient cell samples which are highlighted as individual data points in each graph. Median is indicated by black line inside each boxplot.

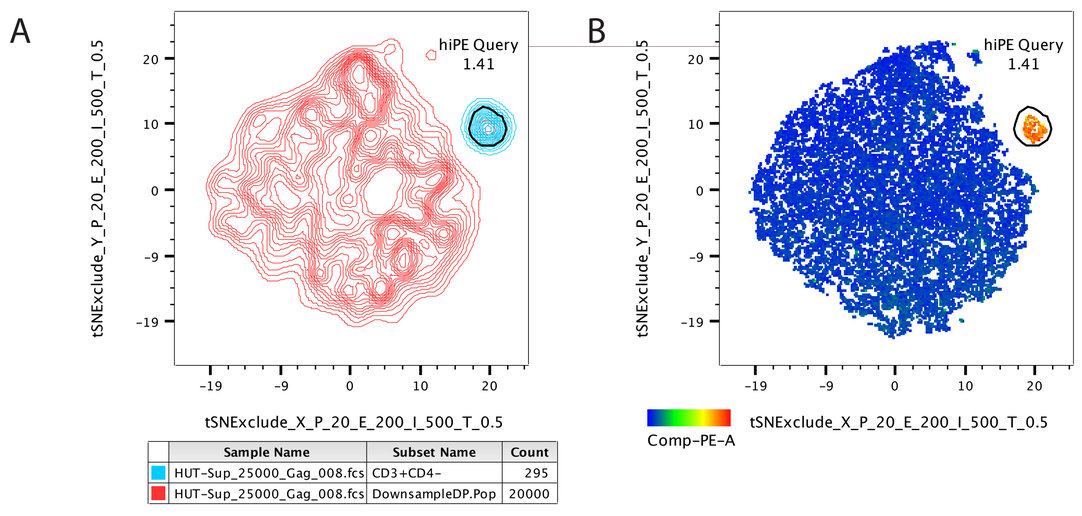

Figure 2. tSNE multi-parameter visualization for HIV-1 patient simulation. A) Depicts the clustered cell mixutre of 1% Gag expressing cells (HUT4-3: CD3+CD4-) titrated into uninfected Lymphocyte cell line (SupT1: CD3-CD4+) . Overlay shows CD3+CD4- highlighted in blue which is only expressed by infectious cells. The Heatmap of section B) shows PE-expression levels (HIV-1 Gag) in the clusterd cells. All PE high cells are clustered as a distinct, isolated population.

For the better interpretation of the complex multi-parameter data the tSNE algorithm was tested on patient simulated condition (1% HIV-1 infected cells) using HIV Gag::PE intracellular staining. As seen in Figure 2B all high PE expressing cells cluster in one population. Further analysis showed that those cells are indeed the infected cells. Further simulation of lower dilutions remain to be tested.

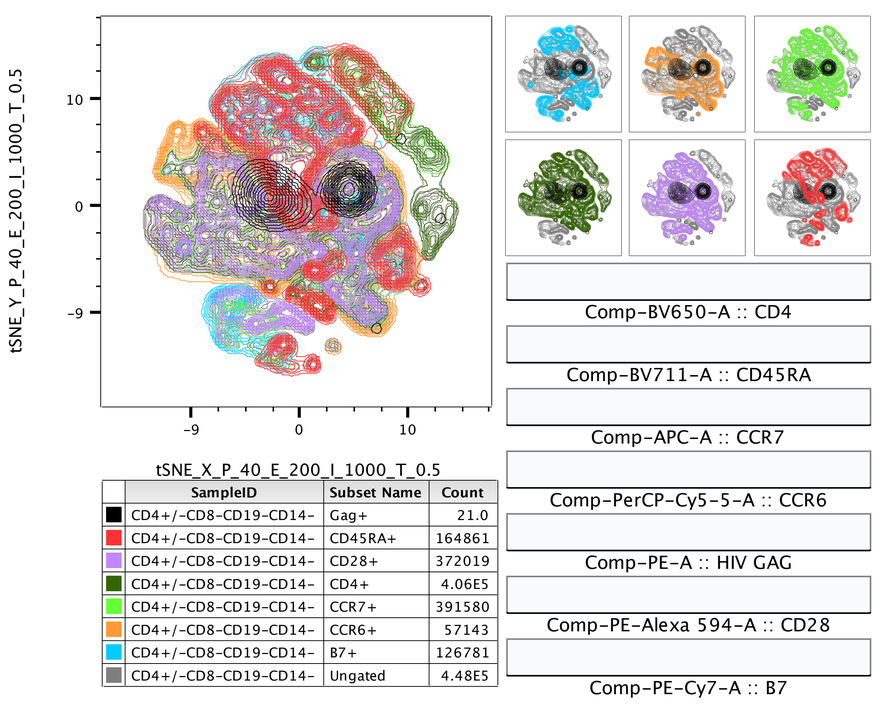

Figure 3.
tSNE multi-parameter visualization of patient sample. Left graph shows contour plot of clustered cells (~500'000 cells). Cells were pre-gated on CD3+CD4+/-CD8-CD19-CD14- events. Each colour shown in table underneath shows respective positive fraction of each differentiation maker. Right top: tSNE overlays of each single marker superimposed on HIV-1 Gag and all other clustered events. Histoplot show marker expression of each gated poplation. Black events illustrate intracellular HIV-1 Gag expressiom as a proxy for infected cells that have late gene translation as well as viral budding from cell surfaces.

Patient condition (~200 HIV DNA copies/10^6 cells) multi-parameter stain on ~500'000 cells of interest (CD3+CD4+/-CD8-CD19-). HIV Gag+ cells are highlighted in plot as black. Data implies for this condition that HIV Gag+ cells co-express CD4, CCR7, CD28 and some Integrin B7.

Outlook

- *HIV Envelope broadly neutralizing antibodies will be used for tSNE analysis to identify potential intact proviruses of patients under suppressive cART and high proviral loads with much higher precision*
- *tSNE results will then highlight reservoir markers of interest for subsequent live cell sorts and the selective reactivation of latent proviruses*
- *scRNA sequencing of sorted cell fractions will give further insights into the role of immuno-modulating transcription profiles in HIV infected cells*
- *Finally, integrating all data from these cells, circulating in the periphery, will then be followed by a detailed analysis of the respective tissue(s) of interest (GALT/LN biopsies) using CyTOF imaging*

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* Bader, J. et al. Therapeutic immune recovery prevents emergence of CXCR4-tropic HIV-1. Clin. Infect. Dis. ciw737 (2016).