HIV RNA persists long-term in lymph nodes of individuals initiated on ART in FIEBIG I

Poster 341

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Abstract

One of the major barriers to achieving HIV remission through combination antiretroviral therapy (cART) is virus persistence in reservoirs. The persistence of replicating virus during cART is however not fully defined.

Here, we used flow cytometry, immunohistochemistry (IHC) and RNA *in-situ* hybridization assays to phenotype and localize HIV infected cells in the lymph nodes of early treated individuals. Virus evolution was further assessed by comparing plasma-derived viral sequences to sequences obtained from lymph node cells.

From IHC results, there was a positive correlation between the magnitude of germinal centers (GCs) and HIV plasma viral load (r=0.6, p=0.07) in untreated individuals. Interestingly, the excessive GC T follicular helper (GCTfh) cells' expansion observed in chronic HIV was significantly attenuated by early treatment (p=0.01). HIV Gag p24 antigen was detected almost exclusively in the GCs even after one year of cART mediated viral suppression. This was also confirmed by multiplexing RNAscope *in-situ* hybridization for *Gag-Pol* with BCL-6 staining. Importantly, viral sequence evolution was observed in two early treated individuals for whom we had plasma HIV sequences prior to cART initiation and LN HIV sequences after more than 1 year of suppressive cART.

Taken together, our results demonstrate the persistence of low level viral replication in the lymph nodes of early treated HIV infected individuals. This study highlights the need for future interventions directed at eliminating residual virus replication in tissue sanctuaries during cART.

Patient characteristics

Table 1: Summary patient characteristics

	HIVneg	UnTx	EarlyTx
n	8	8	17
Male	0	1	0
Female	8	7	17
AGE (Median, IQR)	21 (20.5-22)	24 (21-37)	21 (19-22)
CD4 Count (cells/ul)	N/A	357 (355-359)	772 (657.5-833.5)
Viral load (cps/ml)	N/A	12000 (290-22000)	<20
Time post Tx to LN excision (weeks)	N/A	N/A	355 (21-479)

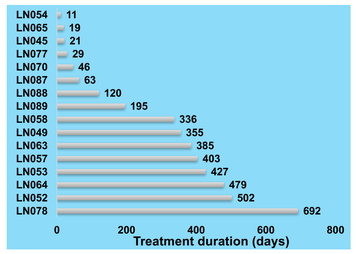
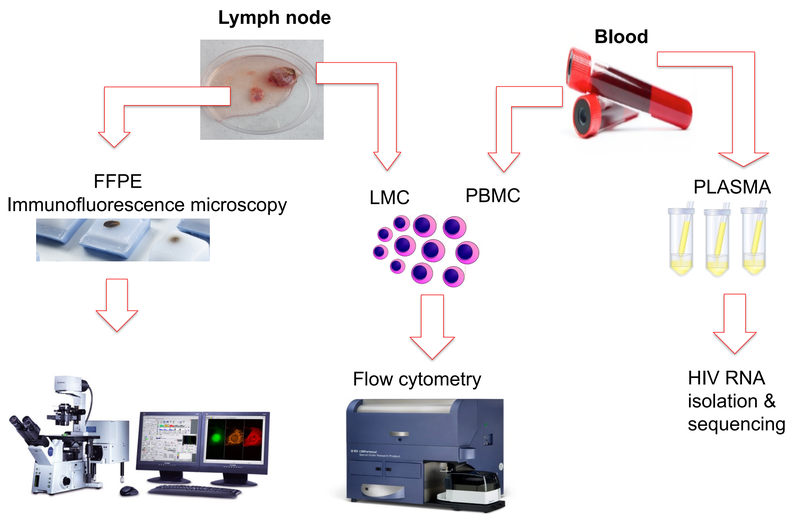


Figure 1: Treatment duration at recruitment

Experimental design



Results

Significant expansion of GCTfh cells in early treated HIV-1 infected individuals

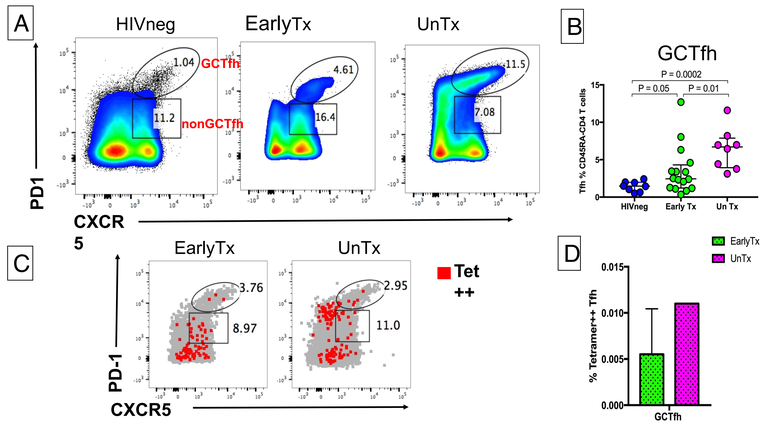


Figure 2: A) FACS plots showing gating strategy and **B)** Frequency distribution of GCTfh subsets across HIVneg, EarlyTx and UnTx groups. **C)** FACS plot and **D)** summary plot showing the distribution of tetramer positive cells (red) within Tfh subsets in EarlyTx and UnTx individuals. P values from Mann U-Whitney tests are reported.

HIV Gag p24 co-localizes with Tfh cells within germinal centers in lymph node tissue sections from early treated patients

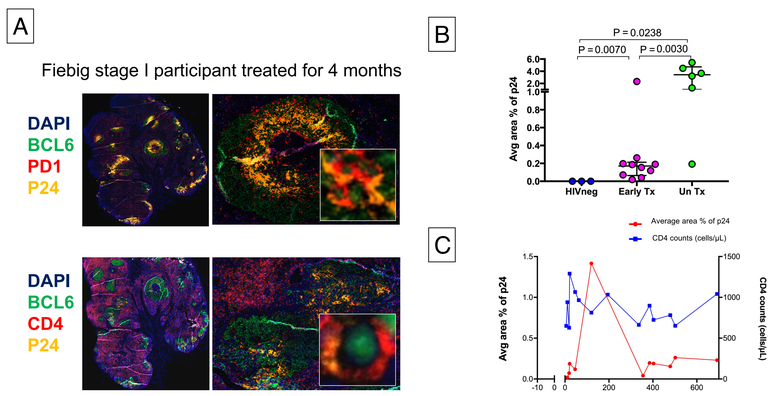


Figure 3: A) Images from multiplex immunofluorescence microscopy staining for BCL-6 (green), Gag p24 (orange) and PD-1 (upper panel, red) or CD4 (lower panel, red) in lymph node tissues sections. **B)** Summary plots comparing the average area percentage staining of Gag p24 on LN sections for HIVneg, EarlyTx and UnTx individuals. **C)** Kinetics of CD4 counts and Gag p24 average area percentage staining in LNs based on days on treatment. P values from Mann U-Whitney tests are reported.

Multiplex RNAscope with immunofluorescence staining of lymph node sections confirms germinal center localization of HIV-RNA

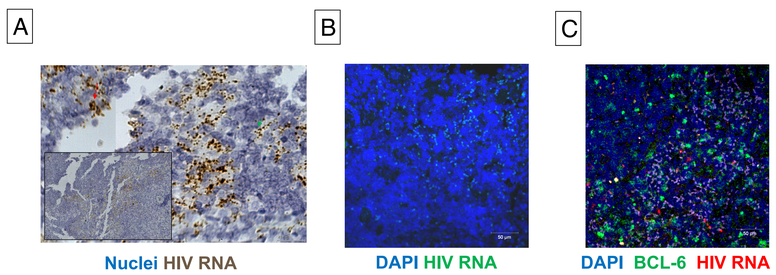


Figure 4: A) A representative LN section from an early treated participant hybridized with HIV Gag-pol RNA (brown). **B)** A representative LN section from an early treated participant hybridized with HIV Gag-pol RNA (green) only or **C)** co- stained with BCL-6 (red). (*Blue: nuclei).*

HIV persistence in lymph nodes drives germinal center formation in early treated HIV-1 infected individuals

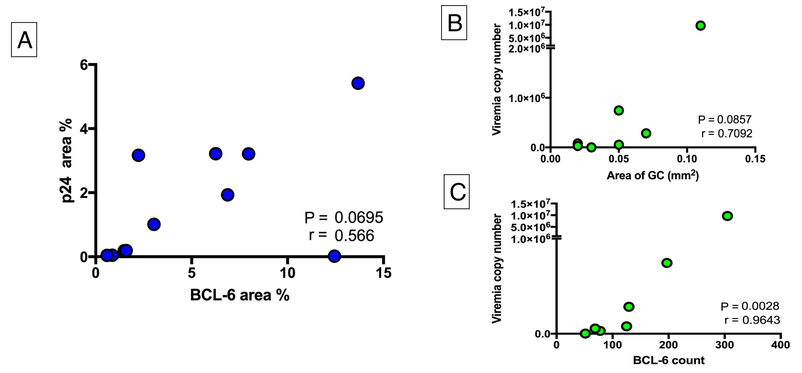


Figure 5: A) The area percentage staining intensity of Gag p24 is correlated to that of BCL-6 for each LN section. **B)** Correlation between viremia copy days and area of GCs or **C)** BCL-6$^+$ cell counts in GCs in FFPE LN sections of EarlyTx individuals. Spearman rho (r) values and p values are reported.

HIV sequences isolated from lymph nodes diverge from transmitted/founder virus sequences isolated at onset of infection

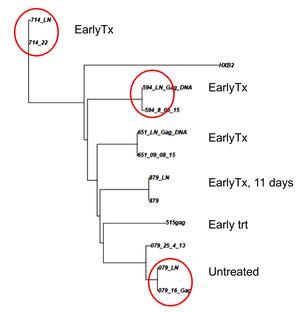


Figure 6: Phylogeny tree was plotted using virus sequences isolated from blood sample and lymph nodes of early treated individuals

Conclusions

- There is a significant expansion of GCTfh cells in early treated individuals, however, this expansion is significantly lower than what was obtained in the chronic HIV-1 infected group.
- HIV Gag p24 antigen and HIV RNA persists in the germinal centers of fully suppressed early treated individuals.
- Despite the early induction of antiretroviral therapy in HIV-1 infected individuals, germinal centers are formed due to antigen persistence in the lymph nodes.
- Evidence of virus sequence diversity suggests ongoing virus replication in lymph nodes of early treated individuals.
- Future cure strategies should focus on eliminating ongoing virus replication.

Acknowledgments

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