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Impact of integrase inhibitors on CD8 T cell function and activity

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Background

HIV-specific CD8 T cells play a crucial role in controlling HIV replication. Although they have efficient effector function, CD8 T cells fail to clear HIV infection even in the presence of ART. Here we describe how ART can impact CD8 T cell effector function and therefore interfere -among other factors- with clearance of HIV infected cells.

Material and Methods

PBMCs from HIV+ (n=44) and healthy (n=32) individuals were analyzed via flow cytometry to determine phenotype and functional properties of HIV-specific CD8 T cells in the presence of ART drugs and performed ex vivo proliferation assays of PBMCs from treated HIV+ individuals. We used a viral inhibition assay to determine CD8 T cells clear HIV infected cells and analyzed metabolic profiles using extracellular flux analyzer.

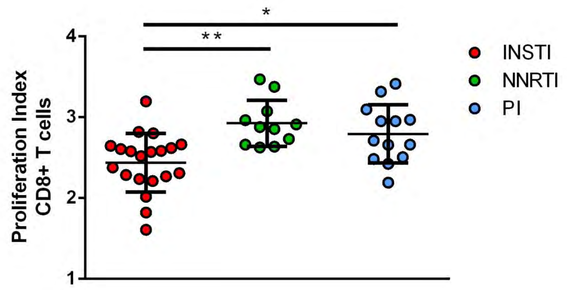
Results

First we assessed the proliferation of ART-treated individuals ex vivo and found a reduction in CD8 T cells of individuals treated with INSTI-based ART regimen (2.43±0.36) compared to both PI (2.79±0.35;p<0.05) and NNRTI (2.92±0.28;p<0.01) based regimens. As we saw a significant impact of INSTI-based regimens on CD8 T cell proliferation, we determined the impact on the multifunctional CD8 T cell responses and could detect a significantly lower expression of IFNγ (p<0.01), IL-2 (p<0.001), TNFα (p<0.01) as well as less migration activity and basal respiration of CD8 T cells after treatment with INSTI. Afterwards, we determined the impact on the killing ability of CD8 T cells and could show the treatment with INSTI-based regimen affects the new synthesis of cytolytic molecules in CD8 T cells.

Conclusion

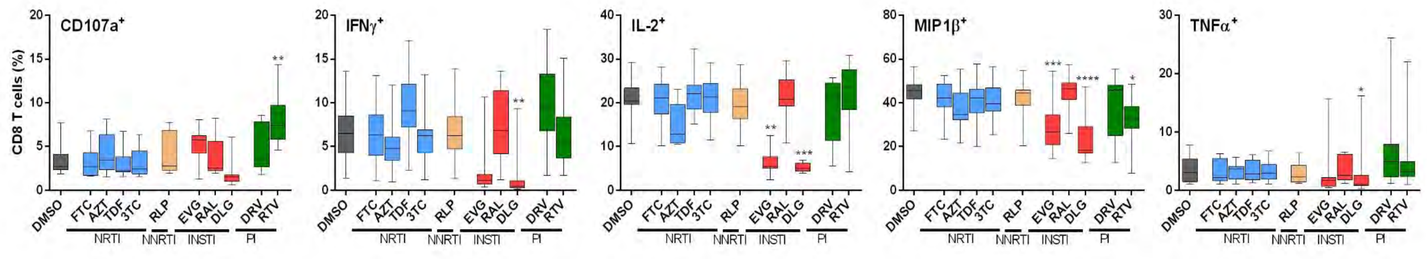
Our data shows that the choice of ART can have a significant impact on CD8 T cell effector function. This may have important implications for HIV eradication strategies.

Figure 1: CD8 T cells treated with INSTI-based regimen showed less proliferation ex vivo



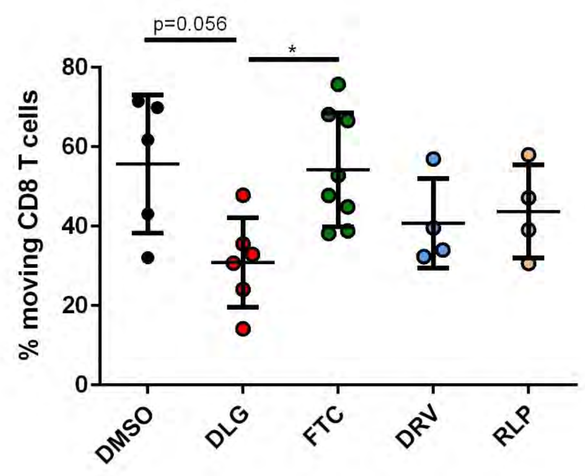
First we assessed the proliferation index of ART-treated individuals ex vivo. PBMC were stained with the cell division tracker carboxyfluorescein succinimidyl ester (CFSE). We found a reduction in CD8 T cells of individuals treated with INSTI-based ART regimen compared to both PI (p<0.05) and NNRTI (p<0.01) based regimens

Figure 2: HAART containing DLG or EVG influences metabolism and functionality of CD8 T cells



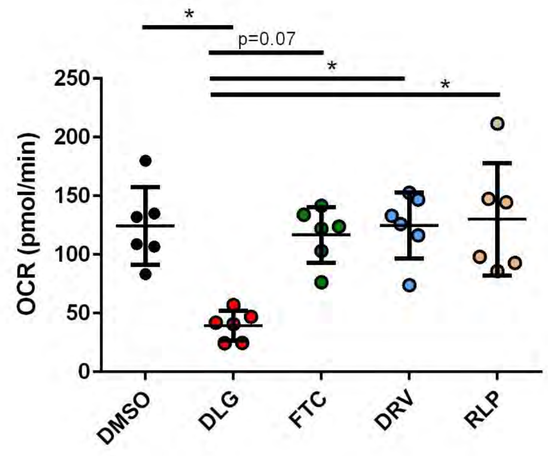
As we saw a significant impact of INSTI-based regimens on CD8 T cell proliferation, we determined the impact on the multifunctional CD8 T cell immune responses of each drug individually. Therefore, we cultured CD8 T cells with individual ART drugs for 3 days and used afterwards a 5 marker functional panel. CD8 T cells had reduced functional properties with significantly lower expression of IFNγ (p<0.01), IL-2 (p<0.001), TNFα (p<0.01) as well as MIP1β (p<0.0001) after treatment with INSTI, but not with other ART drugs.

Figure 3: Dolutegravir-treated CD8 T cells showed less migration capacity



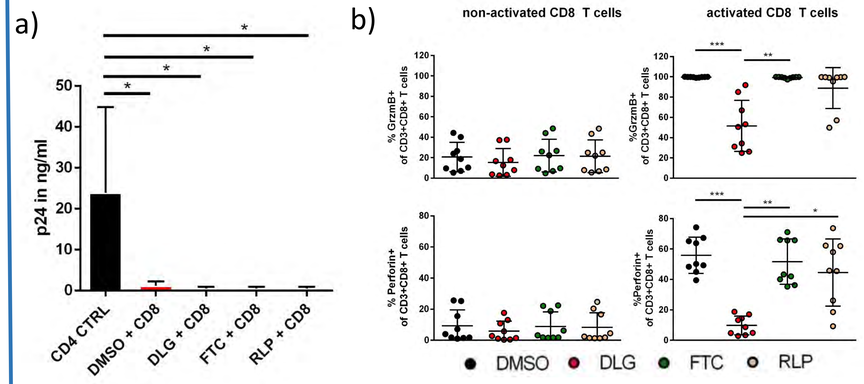
We used a live cell imaging assay to determine the migratory capacity of CD8 T cells treated with different ART regimens. Dolutegravir treated cells showed less migration activity after SDF1α stimulation compared to NRTI regimens (p<0.05).

Figure 4: CD8 T cells treated with INSTI-based regimen had less metabolic activity



We analyzed the basal respiration of CD8 T cells treated with individual ART regimens via the extracellular flux analyser Seahorse XFp. We could observed a close to significant reduction (p=0.07) in INSTI-treated cells compared to NRTI-treated cells and a significant reduction compared to PI- and NNRTI-treated cells (p<0.05).

Figure 5: Treatment with INSTI-based regimen affects the new synthesis of cytolytic molecules in CD8 T cells



We performed a viral inhibition assay to determine the impact on the killing ability of CD8 T cells of each drug. A) Therefore, we cultured CD8 T cells from HIV+ naïve subjects (n=4) with individual ART drugs for 3 days and co-cultured them afterwards with autologous NVP-infected CD4 T cells. Interestingly, we could not detected less killing activity of CD8 T cells treated with Dolutegravir 7 dpi. As we saw no significant difference in the killing ability of CD8 T cells treated with different ART regiments, we wanted to know if Dolutegravir only affect the new synthesis of granzyme B and perforin after activation. B) Therefore we activated PBMCs, treated them with the different drugs for 3 days, re-stimulated them afterwards for 5h and analyzed via FACS. We could show a significant decrease in the GrzmB and Prf expression only in the before activated CD8 T cells.