$CD4^+$ T cell subsets respond differently to latency reversal agents

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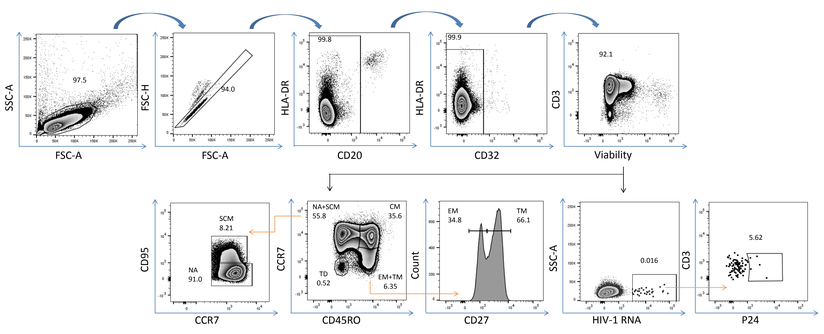
Background: Several pharmaceutical compounds, such as the Latency reversal agents (LRAs), can reactivate HIV expression from infected cells and might help to reduce the pool of latently infected $CD4^+$T cells. However, the capacity of the LRAs to reactivate all infected CD4+T subpopulations is currently unknown.

Methods: Viral reactivation assays were performed using freshly-isolated $CD4^+$ T cells obtained from whole blood donations of n=9 ART-suppressed patients. A total of 13 conditions, including controls, Ingenol (ING) and Bryostatin-1 (BRY-1) (PKC agonists), Romidepsin (RMD) and Panobinostat (PNB) (HDAC inhibitors) and JQ1 (Bromodomain inhibitor), and the combinations of LRAs from different families, were set up per patient. Intracellular HIV-1 RNA and p24 production were evaluated by the flow-RNA/FISH technology within different $CD4^+$T cell subsets (Naïve, T_{NA}; Stem Cell Memory, T_{SCM}; Central Memory, T_{CM}, Effector memory, T_{EM}; Transitional Memory, T_{TM}; and Terminally Differentiated, T_{TD}) after 22h in culture. Synergisms, antagonisms and additive effects between compounds were calculated using the Bliss independence model.

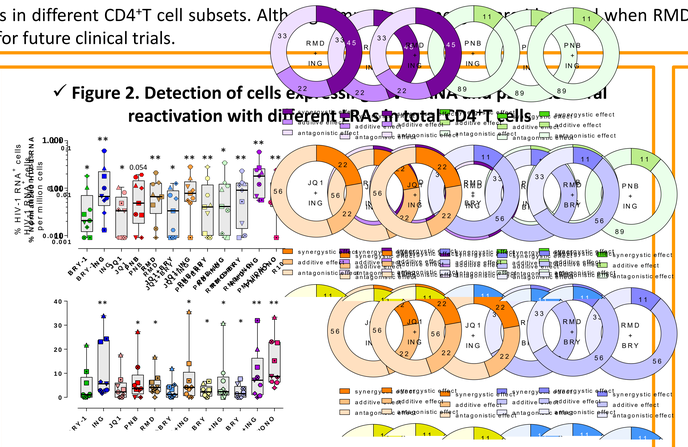
Results: In general, a median of 16.28% of the whole HIV-reservoir in $CD4^+$T cells induced HIV-1 RNA after viral reactivation but only 10.1% of these reactivated cells produced p24. The highest levels of HIV-RNA and the viral protein p24 were induced by ING, RMD and PNB followed by BRY and JQ1. Moreover, the combination of RMD+ING induced a reactivation by 3.50-fold compared to the negative control. Concerning $CD4^+$T cell subpopulations, most memory subsets were reactivated by Romidepsin and the highest susceptibility was observed in the T_{EM} cells (FC=4.24). PNB was more efficient at reactivating T_{CM} (FC=2.11) and T_{EM} cells (FC=2.32) while ING was performing better in T_{CM} (FC=4.05) and T_{TM} (FC=5.27) but not in T_{EM} cells. Regarding the combinations of LRAs, RMD+ING became the most effective condition in all tested $CD4^+$T cell subsets, primarily in T_{TM} and T_{EM} (FC=6.72 and 6.96), but with no effect in T_{SCM}. Contrarily, an antagonistic effect in terms of HIV transcription was observed when PNB+ING were added.

Conclusion: Our results endorse the efficiency of the flow-RNA/FISH protocol to assess the potency of LRAs in different $CD4^+$T cell subsets. Alth[illegible] when RMD and ING are combined, existing LRAs present limited capacity to induce HIV transcription in the most important reservoir cells. This study emphasizes the need to generate new drugs with wide reactivation ability in all $CD4^+$T cells for future clinical trials.

✓ Figure 1. Gating strategy used for the identification of $CD4^+$ T cell subpopulations expressing HIV-1 RNA transcripts and p24 in samples from HIV-infected individuals

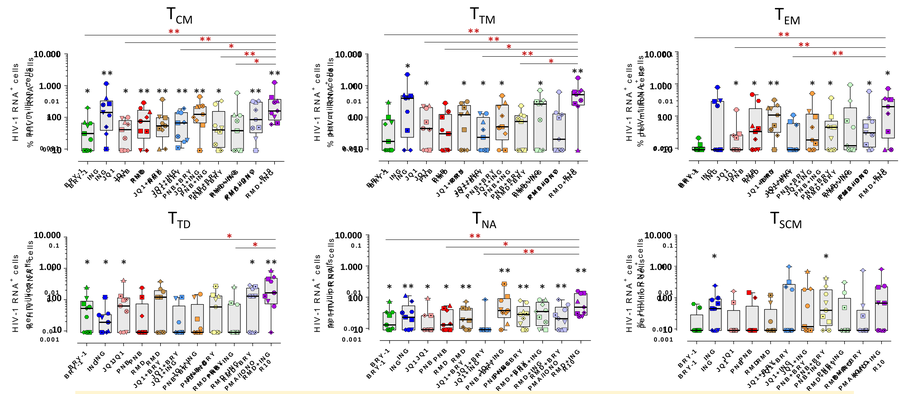


✓ Figure 2. Detection of cells expressing HIV-1 RNA and p24 after viral reactivation with different LRAs in total $CD4^+$T cells



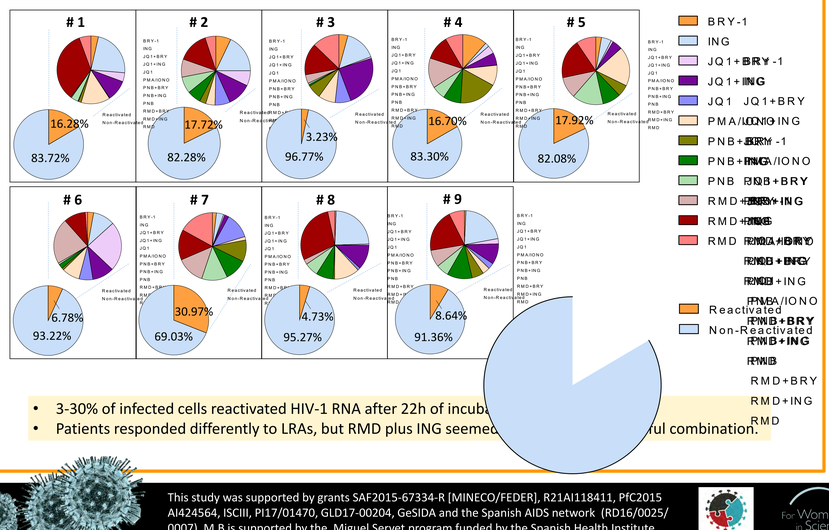
- RMD plus ING induced the highest levels of HIV-1 RNA in all patients 22h after reactivation.
- HIV-1 RNA expression did not always translate into p24 production.

✓ Figure 3. Proportion of cells expressing HIV-1 RNA after viral reactivation in different $CD4^+$ T cell subpopulations



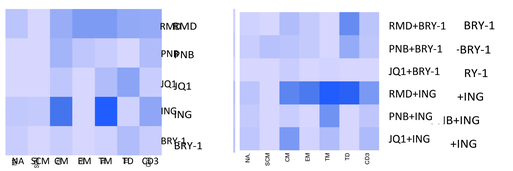
- $CD4^+$ T cell subsets presented different susceptibilities to LRAs.
- Most subsets were susceptible to reactivate HIV in presence of the combination of Romidepsin and Ingenol.
- T_{CM} and T_{NA} presented broader susceptibility to different families of LRAs.

✓ Figure 4. Quantification of the inducible reservoir

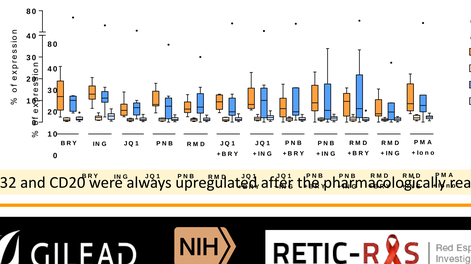


- 3-30% of infected cells reactivated HIV-1 RNA after 22h of incubu[illegible]
- Patients responded differently to LRAs, but RMD plus ING seemed[illegible]rul combination.

✓ Figure 5. Summary heatmaps of HIV-1 reactivation

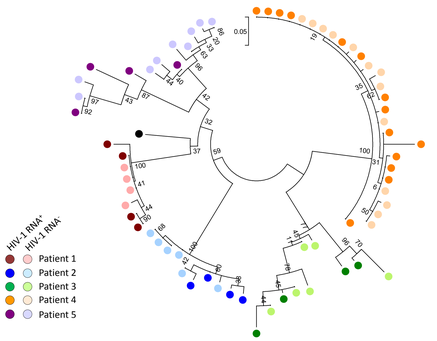


✓ Figure 6. Percentage of cells expressing $CD20^{dim}$ or $CD32^{dim}$ in HIV-1 RNA^+ and HIV-1 RNA^- $CD4^+$ T cells



CD32 and CD20 were always upregulated after the pharmacologically reactivation of HIV.

✓ Figure 7. Envelope sequencing of HIV-1 RNA^+ and HIV-1 RNA^- $CD4^+$ T cells after viral reactivation with LRAs



Viral quasispecies found in both sorted subpopulations of $CD4^+$ T cells were intermingled in the phylogenetic trees.

- ❖A total of 3-30% of the cell reservoir can be reactivated to transcribe HIV-1 RNA with LRAs, however only 5-10% of these cells produce p24.
- ❖Specific $CD4^+$ T subsets respond differently to LRAs and their combinations.
- ❖The synergistic combination of RMD and ING seems to impact most $CD4^+$ T cell subsets (in terms of HIV-1 RNA).
- ❖The combination of some drugs has an antagonist impact on HIV reactivation (PNB+ING).
- ❖Reactivated viral variants are not selected by specific LRAs.

This study emphasizes the need to generate new drugs with wide reactivation ability in all $CD4^+$T cells for future clinical trials

This study was supported by grants SAF2015-67334-R [MINECO/FEDER], R21AI118411, PfC2015 AI424564, ISCIII, PI17/01470, GLD17-00204, GeSIDA and the Spanish AIDS network (RD16/0025/0007). M.B is supported by the Miguel Servet program funded by the Spanish Health Institute Carlos III (CP17/00179).

PRESENTED AT CROI 2019
Seattle, USA