DIFFERENTIAL RESPONSES OF MEMORY CD4+ T CELL SUBSETS TO HIV LATENCY REVERSING AGENTS

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WHERE DOES HIV PERSIST?
CD4+ T CELLS BEST CHARACTERIZED RESERVOIR

Eradication strategies to target HIV in these subsets will need to address the heterogeneity of this complex population that harbors the latent reservoir.
**LARA: Latency and Reversal Assay Recapitulate CD4+ T Cell Reservoir Heterogeneity in Vitro**

Signaling pathways characteristic of each memory CD4+ T cell subset are maintained in LARA culture.
LARA CONDITIONS GENERATE A SIMILAR HIERARCHY OF INFECTED SUBSETS AS OBSERVED IN VIRALLY SUPPRESSED HIV-INFECTED INDIVIDUALS

LARA in vitro conditions recapitulate the dynamics of the HIV latent CD4+ T cell reservoir in vivo
LARA allows monitoring of latency reversal in total memory, CM, TM and EM CD4+ T cell subsets in a single assay.

LARA generates latently infected cells capable of efficient latency reversal in all memory CD4+ T cell subsets.
Memory CD4+ T cell subsets show range of responses to latency reversing agents

Bryostatin induces highest efficiency of latency reversal in EM and TM subsets
Why do subsets respond differently to LRAs?

Employ a bioinformatics approach to identify pathways for latency reversal in memory CD4+ T cell subsets

Cohort of virally suppressed HIV-infected individuals

Sort memory CD4+ T cell subsets ex vivo
n=4

Activate with LRA

TILDA + RNAseq

TILDA- a quantitative PCR assay that measures the frequency of inducible cell associated multi-spliced HIV RNA

TILDA

HIV mRNA frequency per million CD4+ T cells

CM | TM | EM

n=4

unstimulated, PMA, ionomycin, bryostatin, IL-15

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TRANSCRIPTOMIC ANALYSIS REVEALS LRAS INDUCE DIFFERENT PATHWAYS IN CD4+ T CELL SUBSETS

**LRAs activate pathways that lead to proliferation and effector T cell differentiation:**

- LRAs activate pathways that lead to proliferation and effector T cell differentiation:
  - Transition to EM phenotype as a pathway to latency reversal
  - However, although there is some overlap, LRAs do not up-regulate the same pathways in all subsets
LARA recapitulates the heterogeneity of the CD4+ cell HIV reservoir through the generation of latently infected cells in CM, TM and EM subsets.

LRAs show range of efficiencies in memory CD4+ T cell subsets – potentially explained by the differential cellular pathway induction in response to LRAs in the CM, TM and EM subsets.

Transcriptomic analyses support that the transition to an EM phenotype as a potential pathway to latency reversal.

LRAs can be used to characterize mechanisms that govern HIV latency maintenance and reversal in different CD4+ T cell subsets providing a greater understanding of HIV persistence.

Together, these data provide evidence that latency reversal as a therapeutic strategy will require a better understanding of the mechanisms of latency in these physiologically diverse subsets.
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Study Participants
LRA combinations with different MOA can induce subset specific latency reversal.

LRA with different MOA can be used to delineate CD4+ T cell subset-specific mechanisms of HIV latency maintenance and reversal.