Distinct HIV Genetic Populations in Effector Memory T Cells after Prolonged Therapy
Questions about long-lived HIV reservoirs

Effective ART

Effect of very prolonged ART (>15 years) on the genetic composition of HIV-DNA?

Memory CD4+ T cell subsets

Full stimulation

HIV transcripts

Active viral transcription

Relationship between the genetic composition of HIV-DNA and the level of HIV-RNA transcripts?
Method Overview

UCSF:
- 6 Participants on very prolonged ART >15 years:
  - 4 initiated ART during chronic infection (PT1-4)
  - 2 initiated ART during early infection (PT5-6)

Leukapheresis

Rectal biopsies

VGTI:
- CD4+ Naïve T cells ($T_N$)
- CD4+ Stem Cell Memory T cells ($T_{SCM}$)
- CD4+ Central memory T cells ($T_{CM}$)
- CD4+ Transitional memory T cells ($T_{TM}$)
- CD4+ Effector memory T cells ($T_{EM}$)

CD4+ Homing subsets:
- CXCR5+CCR6- (X5+R6-)
- CXCR5-CCR6+ (X5+R6+)
- CXCR5+CCR6- (X5-R6-)

TILDA (tat/rev induced limiting dilution assay)

WMI:
- DNA sequencing by Single-Proviral Sequencing
  - Gag-pol ($p6$-$RT$) sequences

CD4+ T cells
CD8+ T cells
CD3- T cells
CD4-CD8- T cells
**TEM** contains clonal HIV-DNA

Overall % clonal HIV-DNA

TEM was highly enriched with clonally expanded identical HIV-DNA when compared to other cellular subsets

Overall of 40-66% clonally expanded HIV-DNA in participants treated during chronic infection

*Only one sequence; Likelihood p values: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001*
2 Mechanisms of Cellular Proliferation

Memory CD4+ T cell

Antigen specific response

Large phylogenetic group containing clonally expanded HIV-DNA

Stable pool of CD4+ memory T cells

IL-7 IL-2

Small phylogenetic groups containing clonally expanded HIV-DNA
Cellular proliferation by homeostatic response
Effector memory contain 82% clonal hypermutated HIV-DNA

TEM contained 82% clonal hypermutants

100% Hypermutants in $T_{EM}$

$T_{EM}$ contained 82% clonal hypermutants
Effector memory contain 92% clonal wild-type HIV-DNA.
Effector memory contain 51% clonal drug resistant HIV-DNA

Leukapheresis

- Leuka T_N
- Leuka T_SCM
- Leuka T_CCM
- Leuka T_TM
- Leuka T_EM

Homing

- Leuka DN (X5-R6-)
- Leuka R6+
- Leuka X5+
- Leuka DP (X5+R6+)

Gut

- Ileum CD4+
- Rectum CD4+

51% HIV-DNA in T_EM clonal HIV-DNA carrying DRMs

Likelihood p values: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

† Only one sequence
Enrichment of hypermutants in T_{EM} (treatment during early infection)

Overall

PT5

PT6

% Diversity = 2.5%

% Diversity = 0.07%

Diversity = 0.07%
Inducible HIV transcripts in CD4+ memory subsets

TCM, TTM and TEM subsets derived from all participants produced multiply spliced HIV transcripts upon full stimulation.
Conclusions

- The distribution of HIV-1 genetic material among memory subsets varied dramatically across the cohort after prolonged ART.

- Clonal expansions of HIV-DNA can be driven by random antigen-driven cellular proliferation and/or homeostatic response.

- $T_{EM}$ are marked by clonal expansions with distinctive HIV-DNA genetic populations which reflects cellular proliferation induced by antigen-specific response.

- Genetic analysis reveals that proliferative bursts can be attenuated by cellular restriction factors and/or by death of cells expressing replication competent virus.

- The amount of inducible viral transcripts is lower in $T_{EM}$ from an individual with expanded hypermutant populations.
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