Clonal Dynamics within HIV-infected CD4 T Cell Reservoirs after PD-1 Blockade under ART

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Introduction

Background

- Blockade of programmed cell death protein-1 (PD-1) can reverse HIV latency in CD4 T cells and promote antiviral T cell responses\(^1,2\), potentially contributing to a shock-and-kill effect in HIV cure strategies

- PD-1 blockade could also promote proliferation of some CD4 T cells\(^3,4\)

- Clonal dynamics of HIV-infected CD4 T cells after checkpoint inhibition \textit{in vivo} have yet to be characterized in detail

- The effects of PD-1 blockade on the central nervous system HIV reservoirs are under study in the clinical trial NCT03239899

Study objective

- Evaluate the effects of PD-1 blockade on HIV-infected CD4 T cell reservoirs in blood in ART-treated participants from this trial

I. Study timeline

- αPD-1: n = 6
- CD4 counts: >350 cells/µL

II. Sort CD4 T cell subsets

III. Characterize CD4 T cells

- Transcriptome analysis
- TCR sequencing analysis

IV. Characterize virus genomes

- HIV env subgenomic PCR and Sanger sequencing
- Multiple-displacement amplification-single genome sequencing with integration site analysis
- Bulk ISA
- Intact proviral DNA assay

Study Design

- Longitudinal analysis of people with chronic HIV infection on ART receiving a one-time dose of 200 mg pembrolizumab

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<th>Sex</th>
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<th>Nadir CD4</th>
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Transcriptional Profile of CD4 T Cells After PD-1 Blockade

- Gene ontology enrichment analysis

Increased signatures of cell proliferation in EM CD4 T cells at week 3 after treatment
EM CD4 TCR clonotypes are more diverse and evenly distributed week 3 post-infusion.
HIV Reservoir Before/After Pembrolizumab

Infection frequency

Absolute number of HIV-infected CD4 T cells

HIV DNA distribution

Relative shift of HIV-infected CD4 T cell pool to EM subset after PD-1 blockade in some participants
HIV Transcripts After PD-1 Blockade

- HIV transcription profiling assay

Increased HIV-transcriptional initiation at week 3 post-treatment
HIV DNA Diversity in Pembrolizumab-Treated Participants

Decreased diversity of HIV DNA sequences from EM CD4 T cells after anti-PD-1 treatment
Evidence of HIV-infected CD4 T cell proliferation after PD-1 blockade in some cases.
HIV Unique Integration Sites Across Timepoints

Differences in the composition of unique IS were detected between week 3 and week 24 after pembrolizumab.
HIV Provirus Intactness

- Intact proviral DNA assay

No clear change in the number of intact proviruses after PD-1 blockade
Summary

- Increased cell proliferation in EM CD4 T cells at week 3 following PD-1 blockade
- Changes in the distribution of HIV DNA after PD-1 blockade in some participants
- PD-1 blockade induced HIV-transcriptional initiation, possibly due to latency reversal or proliferation of cells that are "initiation-competent"
- Reduced diversity of cell-associated HIV DNA in EM CD4 T cells
- Evidence of clonal expansion and changes in the population of unique integration sites of HIV-infected CD4 T cells after PD-1 blockade in some cases
- No clear change in the number of intact proviruses after anti-PD-1-treatment

What happens to the reservoir?
Community Summary

• **Key Question:**
  • To characterize the clonal dynamics of the HIV reservoir in blood after PD-1 blockade

• **Key Findings:**
  • PD-1 blockade is associated with changes in the sequence composition of cell-associated HIV DNA pool that may reflect proliferation of some infected cell clones
  • Targeted analysis of genetically intact proviruses does not reveal clear expansion after PD-1 blockade

• **Next Steps:**
  • Detailed characterization of intact proviruses in expanded clones after PD-1 blockade will require deep sampling of the infected pool
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*Study participants*

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