

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

Liliana Pérez

Virus Persistence and Dynamics Section  
Vaccine Research Center, NIAID, NIH

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# Introduction

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## Background

- Blockade of programmed cell death protein-1 (PD-1) can reverse HIV latency in CD4 T cells and promote antiviral T cell responses<sup>1,2</sup>, potentially contributing to a shock-and-kill effect in HIV cure strategies
- PD-1 blockade could also promote proliferation of some CD4 T cells<sup>3,4</sup>
- Clonal dynamics of HIV-infected CD4 T cells after checkpoint inhibition *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

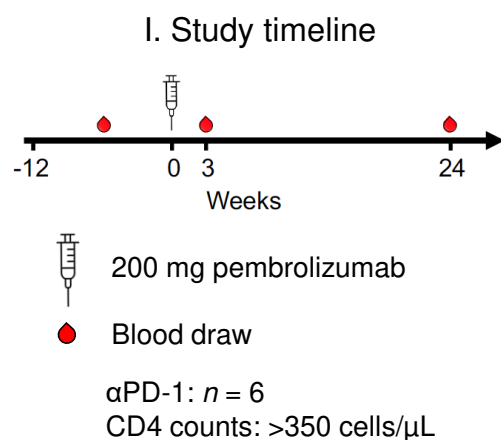
## Study objective

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

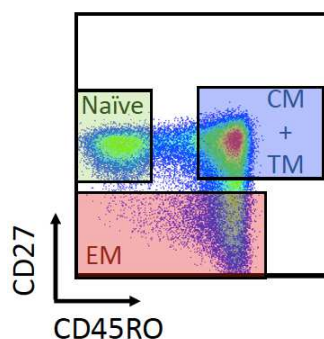
<sup>1</sup>Trautmann et al. Nat Med, 2006; <sup>2</sup>Petrovas et al. J Exp Med, 2006; <sup>3</sup>Day et al. Nature, 2006; <sup>4</sup>Porichis et al. Blood, 2011

# Study Design

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



**II. Sort CD4 T cell subsets**



**III. Characterize CD4 T cells**

- Transcriptome analysis
- TCR sequencing analysis

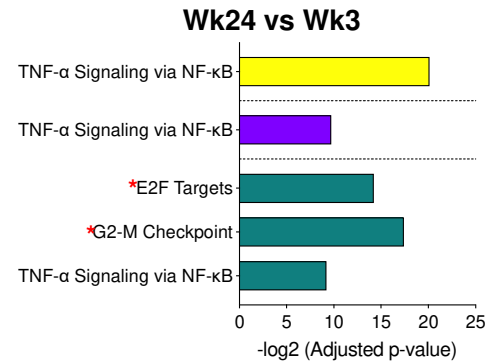
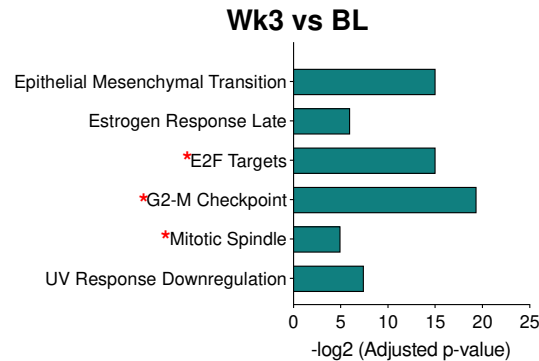
**IV. Characterize virus genomes**

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

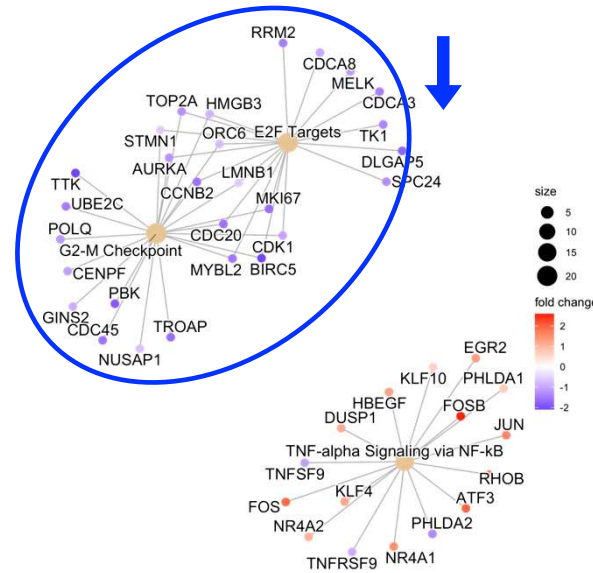
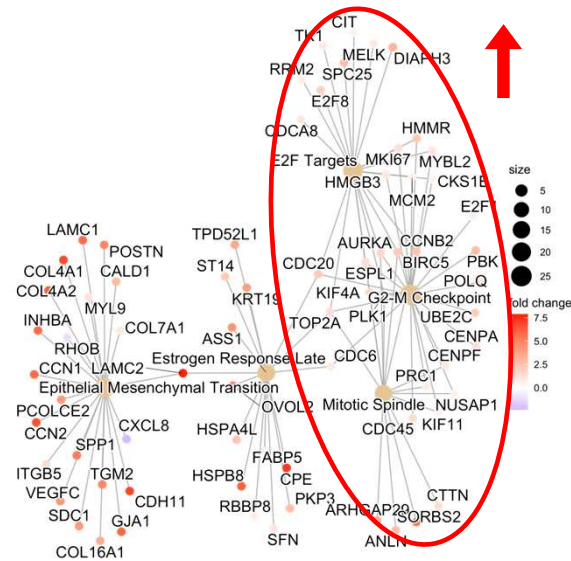
PID	Age	Sex	Ethnicity	Nadir CD4	Year of infection	ART	Current ART
P003	51	F	Black	Unknown	Early 2000	2007	elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide
P004	61	F	Black	Unknown	Unknown	2008	efavirenz/emtricitabine/tenofovir
P005	33	M	Black	<200	2013	2013	abacavir/dolutegravir/lamivudine/tenofovir
P006	55	M	White	190	1984	1990	abacavir/dolutegravir/lamivudine
P008	62	M	White	500	2002	2003	bictegravir/tenofovir alafenamide/emtricitabine
P009	58	M	Black	>200	1986	1991	bictegravir/emtricitabine/tenofovir alafenamide

# Transcriptional Profile of CD4 T Cells After PD-1 Blockade

## ➤ Gene ontology enrichment analysis



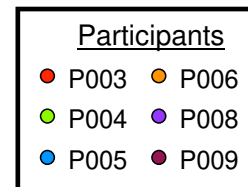
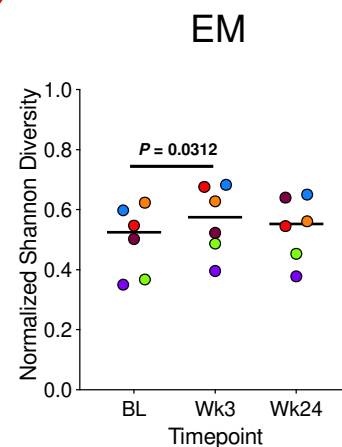
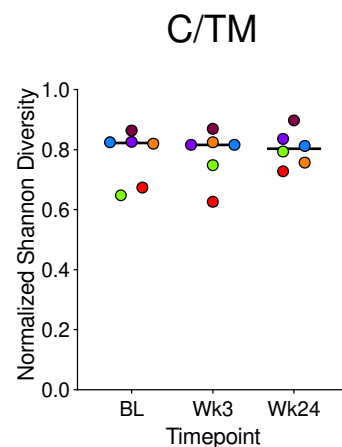
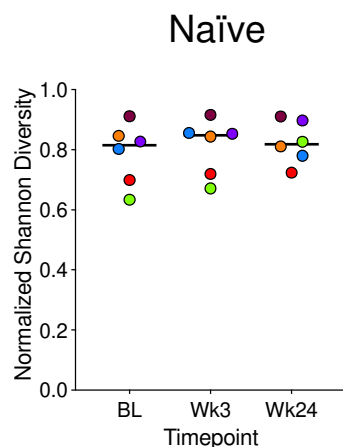
■ N CD4  
■ C/TM CD4  
■ EM CD4



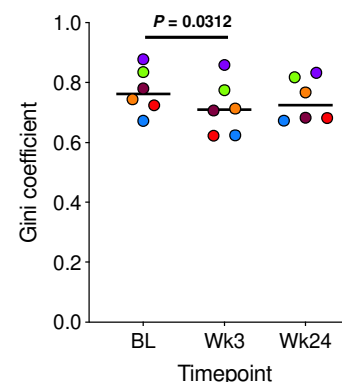
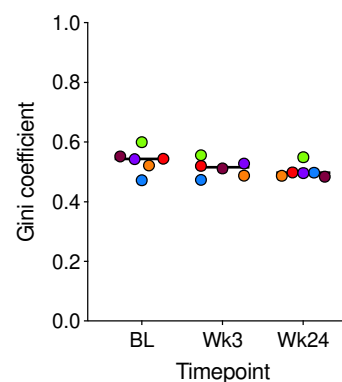
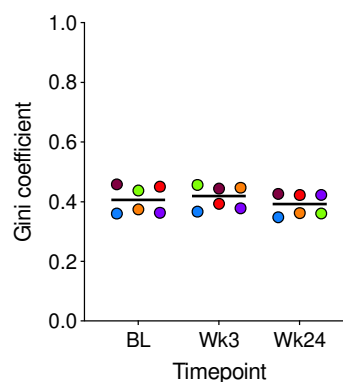
Increased signatures of cell proliferation in EM CD4 T cells at week 3 after treatment

# TCR Repertoire Analysis

Diversity



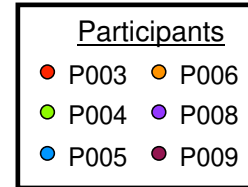
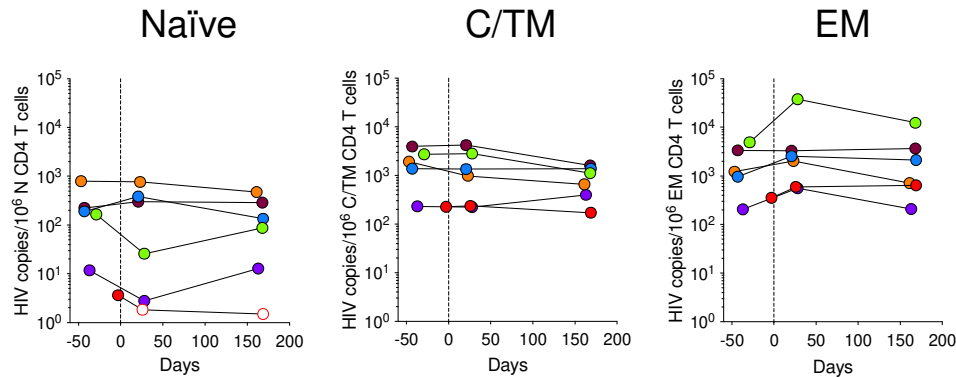
Clonal distribution



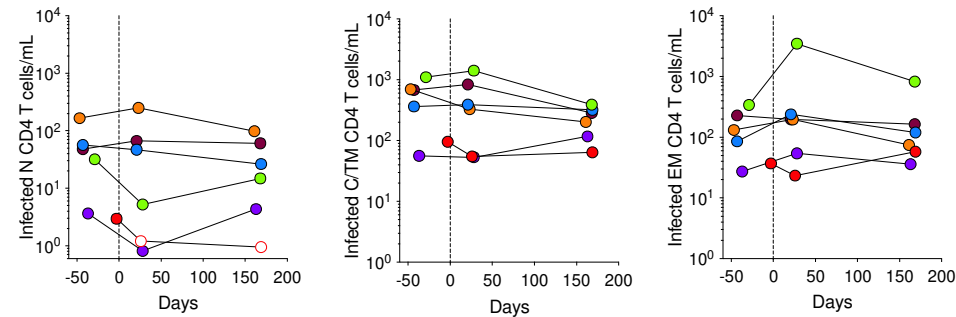
EM CD4 TCR clonotypes are more diverse and evenly distributed week 3 post-infusion

# HIV Reservoir Before/After Pembrolizumab

Infection frequency

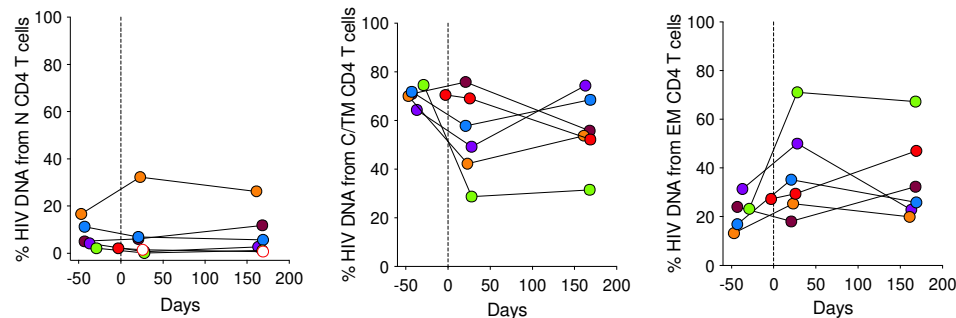


Absolute number of HIV-infected CD4 T cells



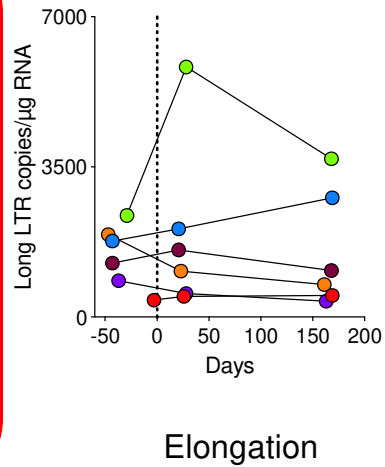
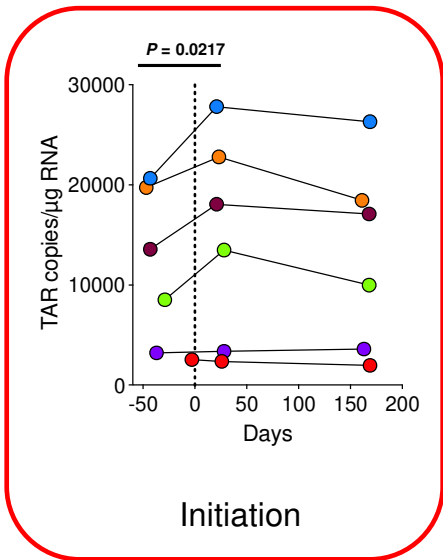
Relative shift of HIV-infected CD4 T cell pool to EM subset after PD-1 blockade in some participants

HIV DNA distribution

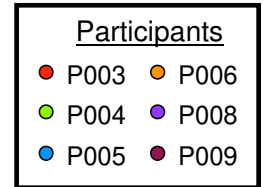
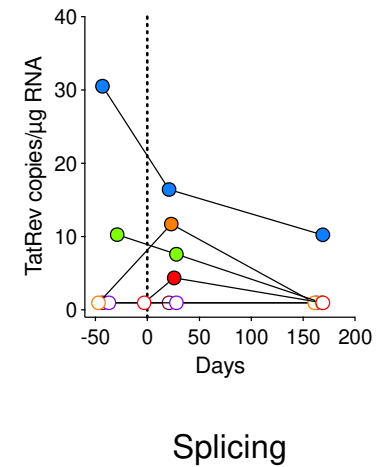
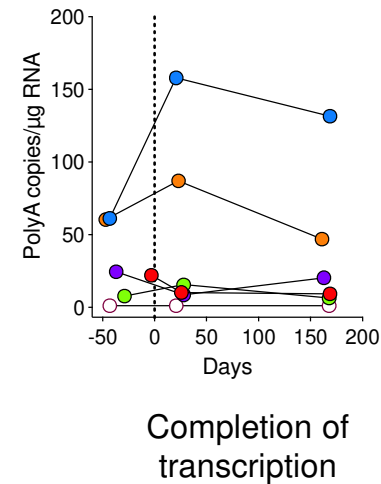
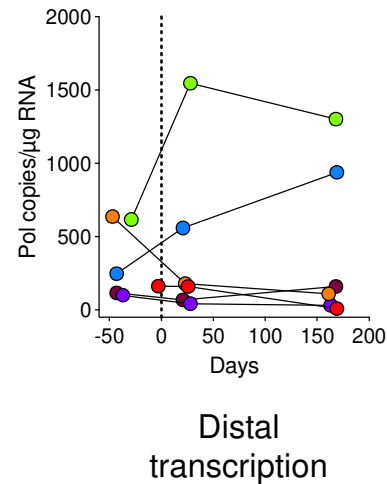


# HIV Transcripts After PD-1 Blockade

➤ HIV transcription profiling assay

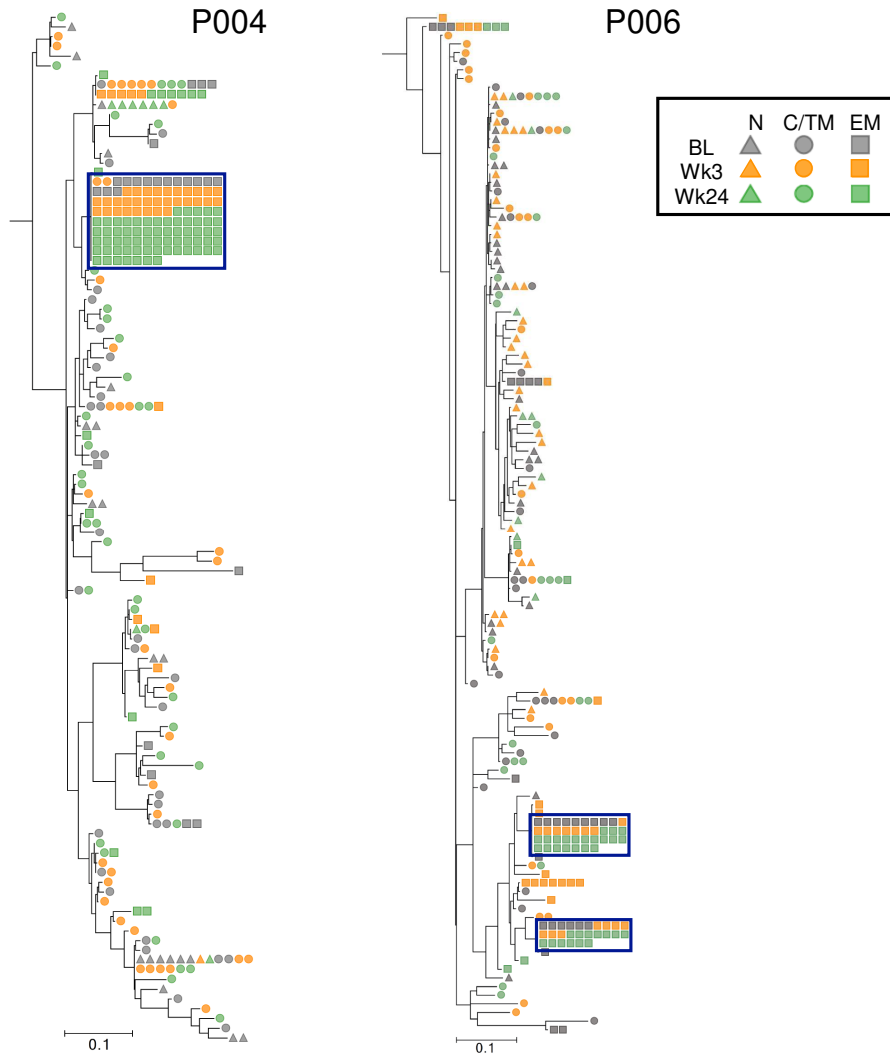


Total CD4 T cells



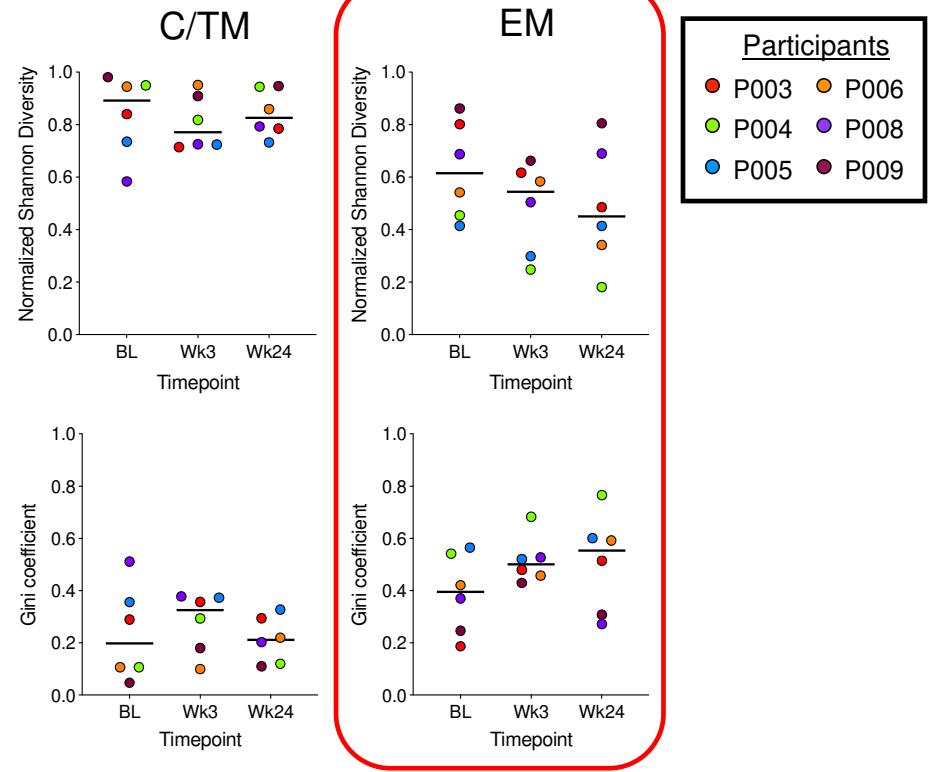
Increased HIV-transcriptional initiation at week 3 post-treatment

# HIV DNA Diversity in Pembrolizumab-Treated Participants



Diversity

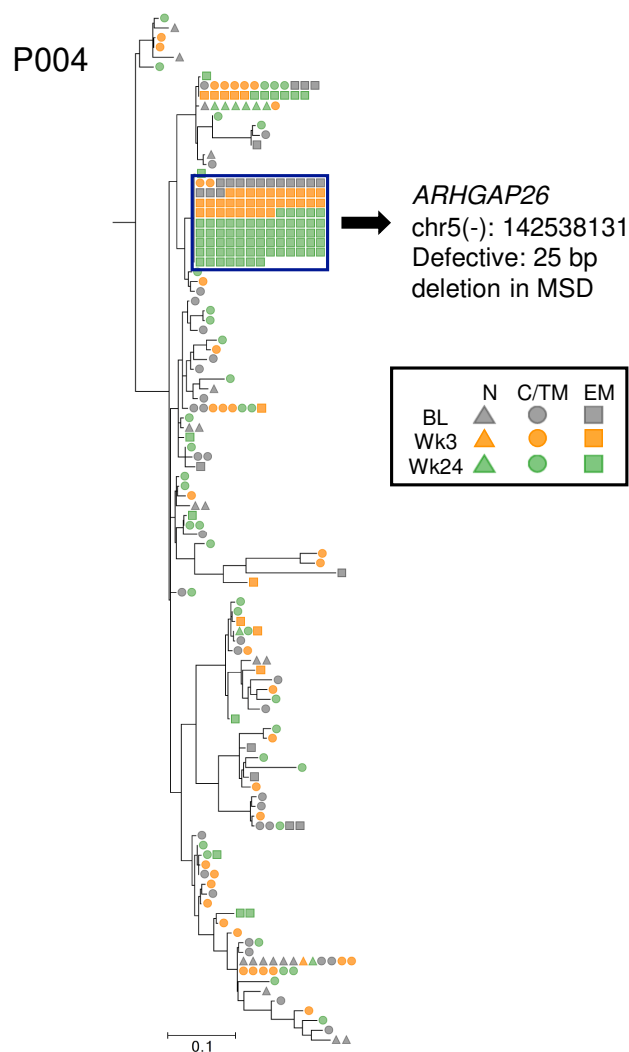
Clonal distribution



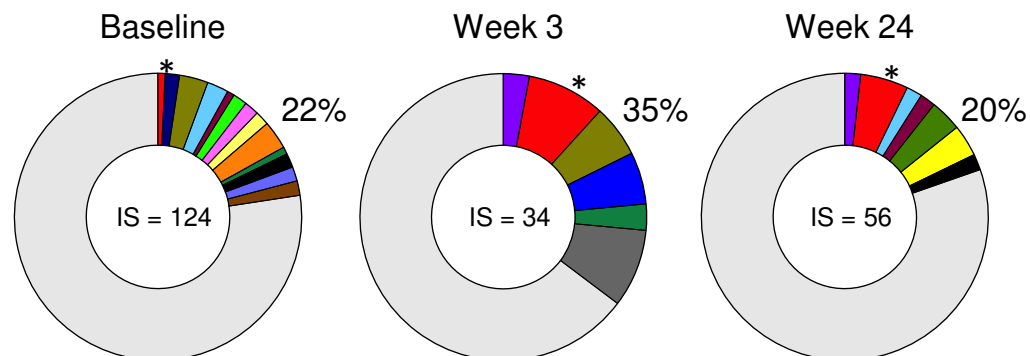
Decreased diversity of HIV DNA sequences from EM CD4 T cells after anti-PD-1 treatment



# HIV Provirus Clonality

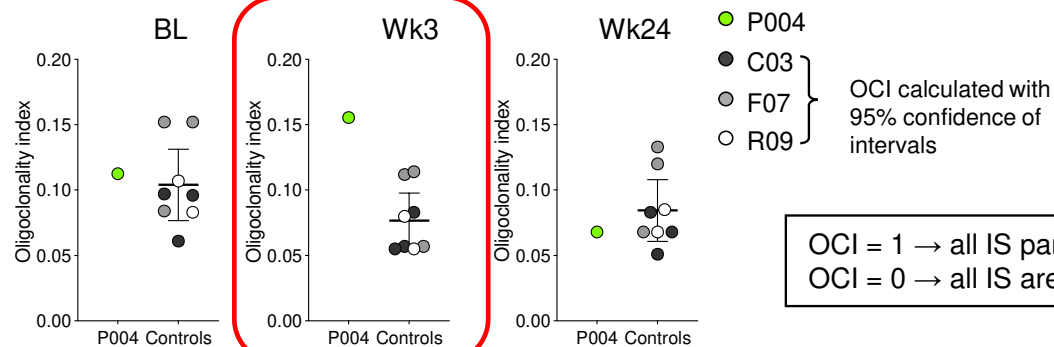


## Bulk ISA of CD4 T cells



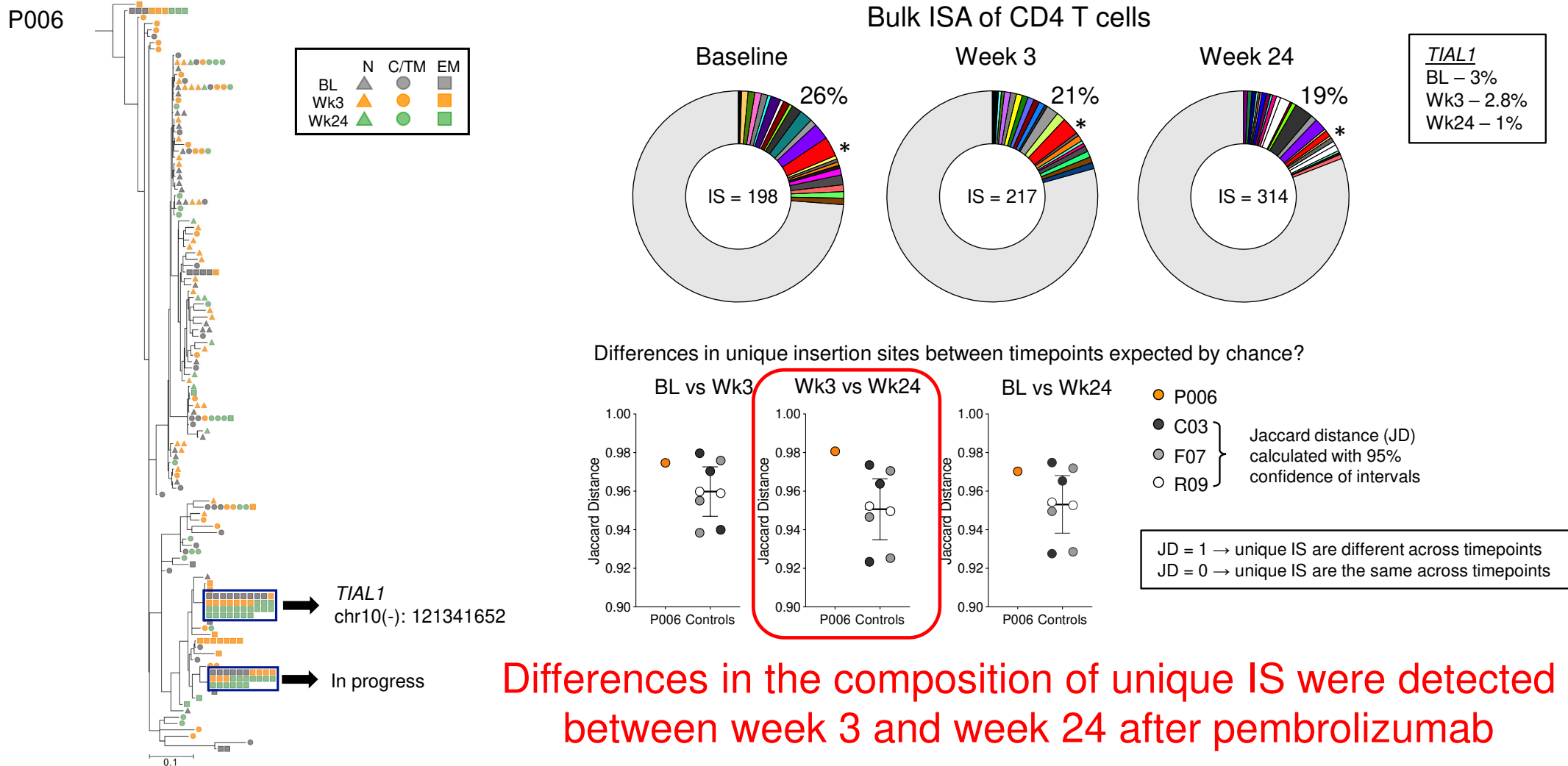
**ARHGAP26**  
BL – 0.8%  
Wk3 – 8.8%  
Wk24 – 5.4%

## Oligoclonality index (OCI) expected by chance?



**Evidence of HIV-infected CD4 T cell proliferation after PD-1 blockade in some cases**

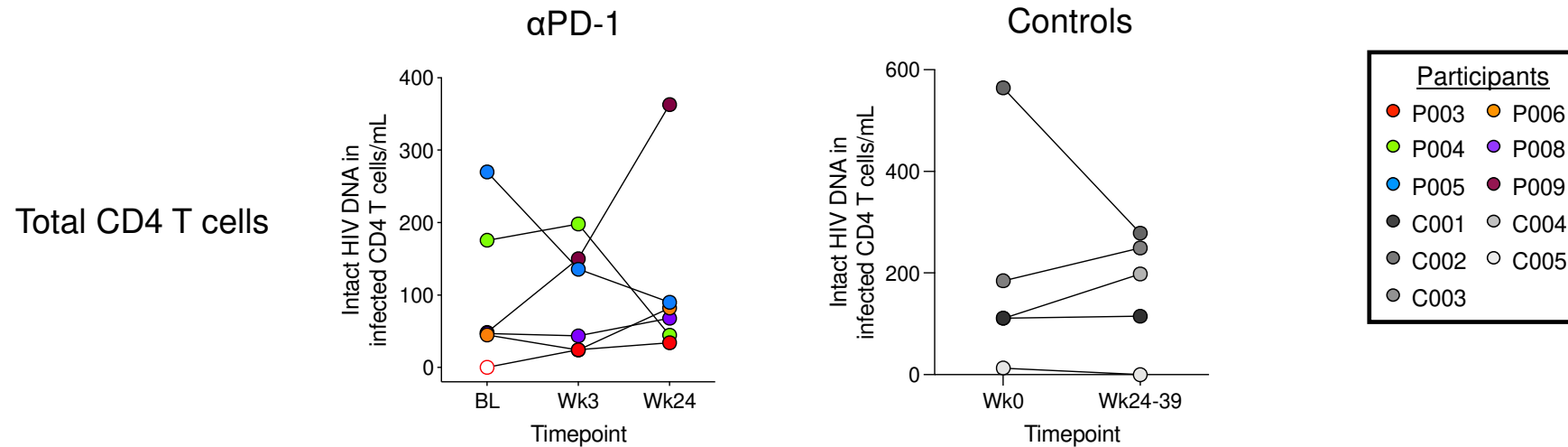
# HIV Unique Integration Sites Across Timepoints



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

# HIV Provirus Intactness

➤ Intact proviral DNA assay

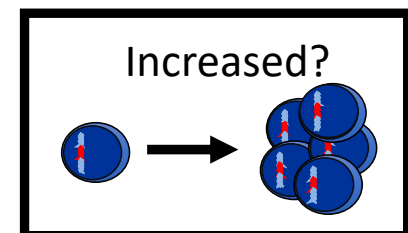
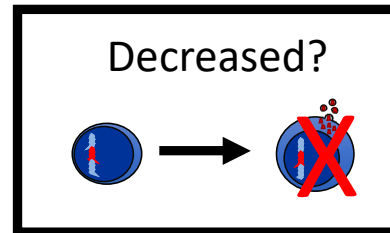


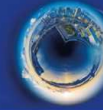
No clear change in the number of intact proviruses after PD-1 blockade

# 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 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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