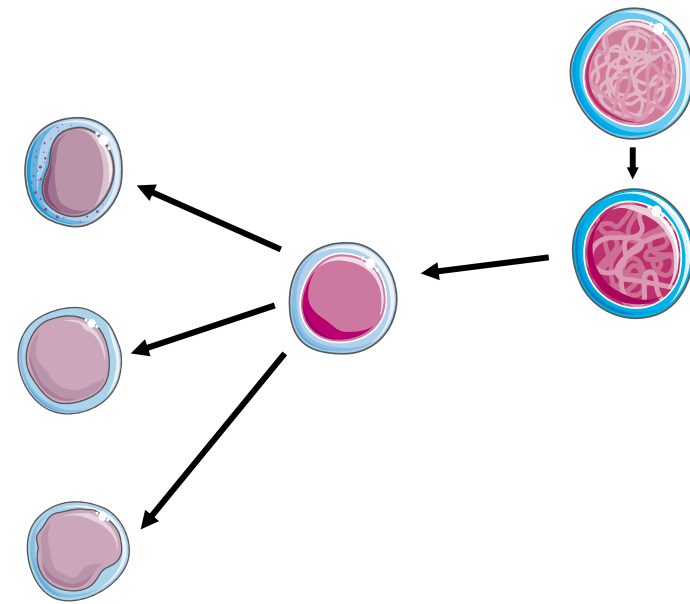


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Persistence during Therapy  
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**Purging the HIV-1 Reservoir Through the Disruption of the PD-1  
Pathway**

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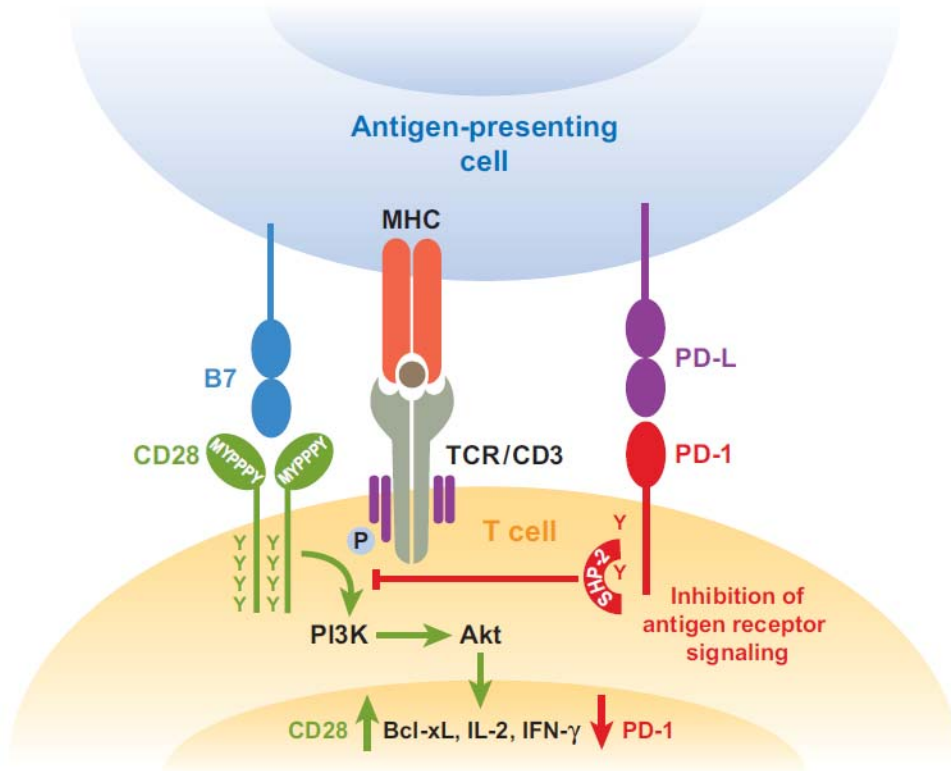
## Introduction

- Small pool of  $T_{CM}$  (*central memory*) and  $T_{TM}$  (*transitional memory*) latently infected CD4+T cells that persist in patients receiving HAART: main obstacle to HIV-1 eradication (N. Chomont et al, Nat. Med, 2009).
- Development of targeted strategies aimed at purging these reservoir cells needed.
- Mechanisms implicated in the establishment and persistence of the HIV reservoir: not fully understood.
- Factors that interfere with CD4+T cells activation and proliferation: major impact on the establishment and/or the maintenance of the HIV-1 reservoir ?
- Negative regulator PD-1 is highly expressed by CD4+T cells from HIV infected patients .

### Hypothesis:

**Role for PD-1 in the establishment and/or maintenance of a cellular reservoir for HIV?**

## PD-1 negative pathway



M Keir, Annu Rev Immunol, 2008

- PD-1 : member of the CD28/CTLA-4 family of T cell regulators

- PD-1 is expressed on activated CD4 and CD8 T cells, B cells and myeloid cells including monocytes and DC

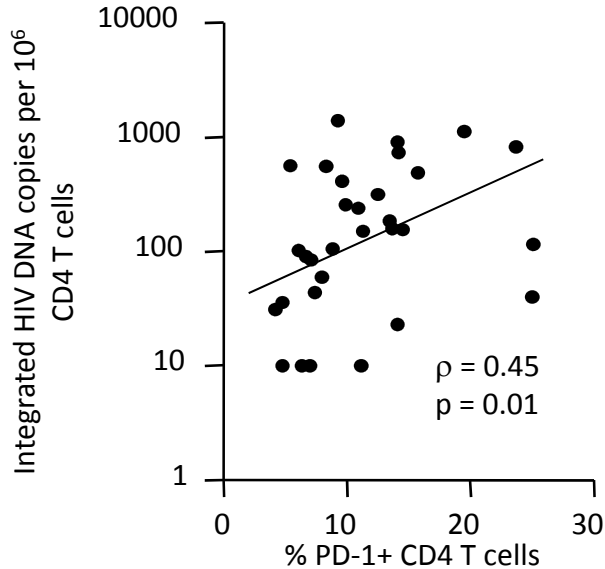
- 2 known ligands for PD-1: PD-L1 and PD-L2, mostly expressed on APCs.

- PD-1 and its ligands negatively regulate immune responses

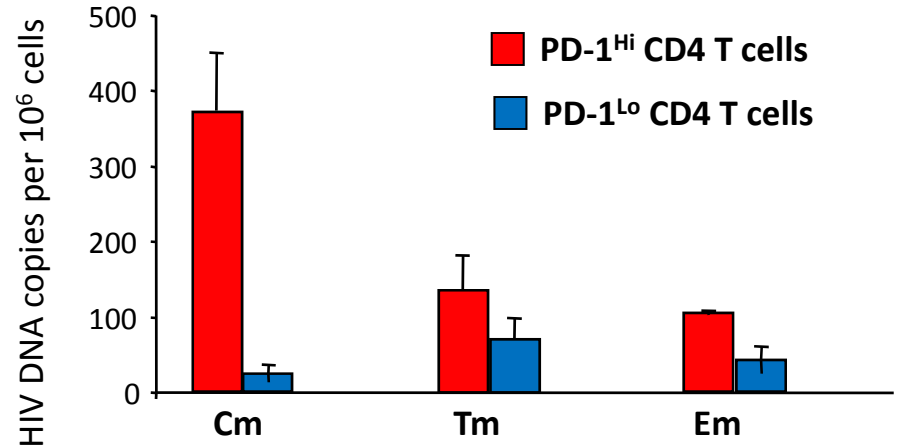
- PD-1/PD-L1 interaction inhibits activation, expansion and acquisition of effector functions of virus specific CD8<sup>+</sup> T cells. (L. Trautmann et al, Nat. Med, 2006 – Day et al. Nature 2006)

- PD-1 inhibits the TCR activation signal through SHP-1 and 2. The PD-1 pathway affects a global inhibition of the T lymphocytes function.

# Sorted PD-1<sup>high</sup> cells preferentially harbor HIV-1 integrated DNA when compared to their PD-1<sup>low</sup> counterparts



PD-1 expression correlates with the reservoir size.  
Impact of PD-1 signaling on the maintenance and/or establishment of the HIV reservoir?



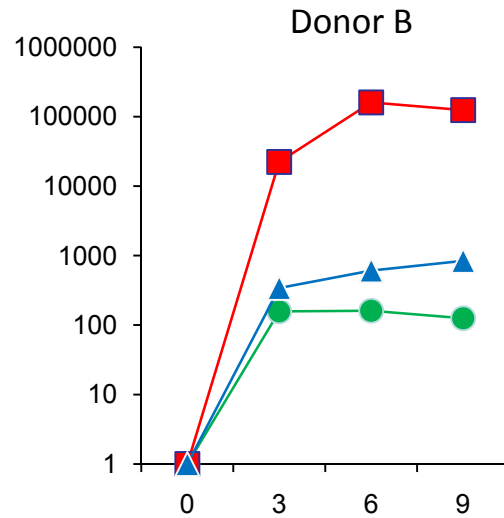
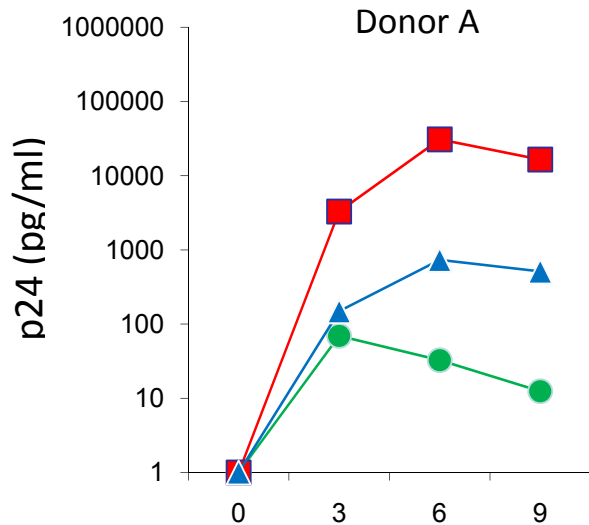
Sorted PD-1 high cells are enriched in total and integrated HIV DNA compared to sorted PD-1 low cells : PD-1 high cells constitute a preferential reservoir for the virus.

**Hypothesis:** PD-1 plays a role in the establishment of the HIV reservoir

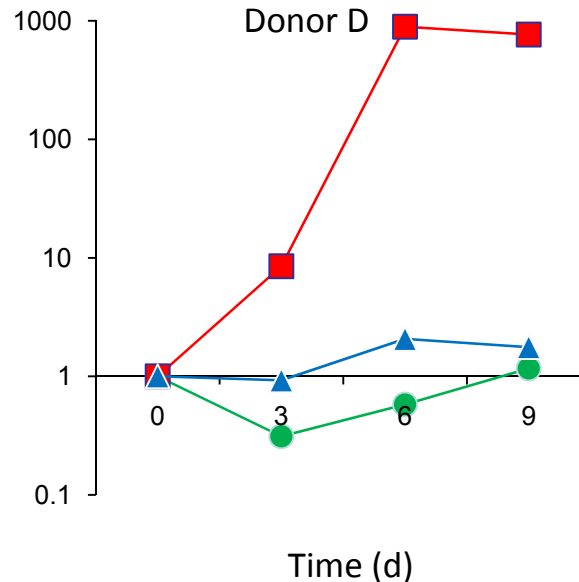
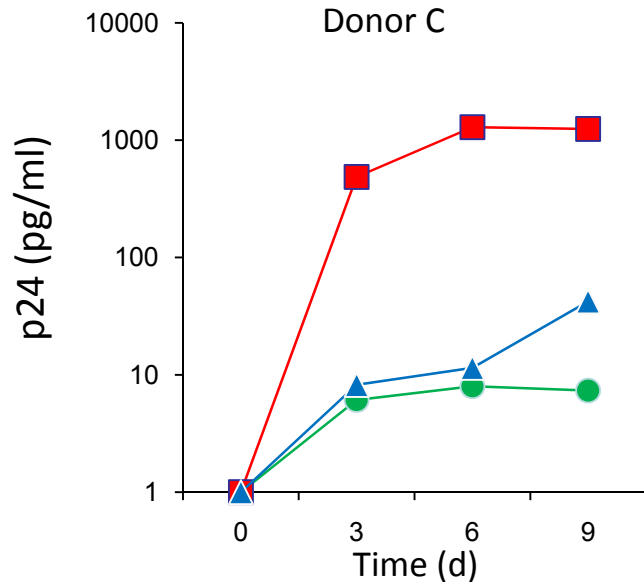
The establishment of a stable reservoir for HIV necessitates the establishment of viral latency = inhibition of viral production.

Does PD-1 triggering inhibit viral production in HIV infected primary CD4+T cells?

# Triggering of the PD-1 pathway inhibits 95% of HIV-1 production in primary CD4+T cells at day 3



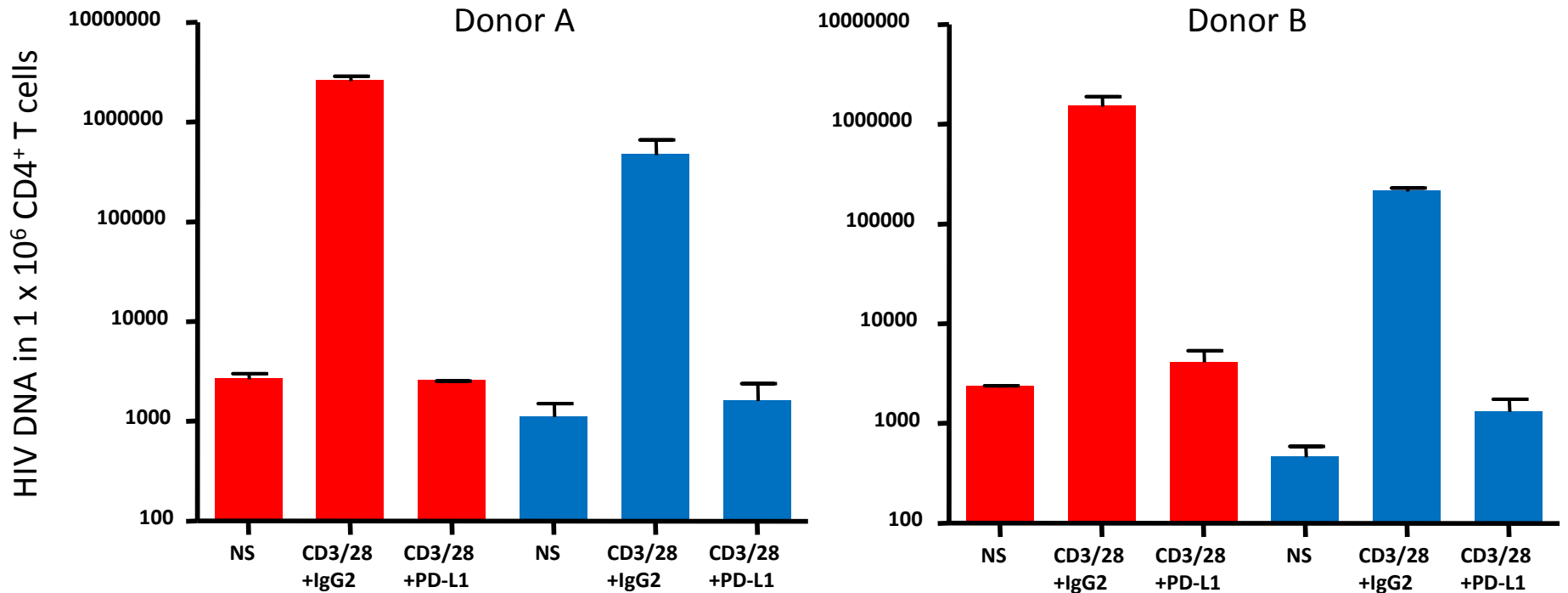
- CD4+T cells from HIV infected viremic subjects purification  
- Stimulated with CD3/CD28 in the presence or absence of PD-L1.  
- Viral production measured by p24 ELISA.



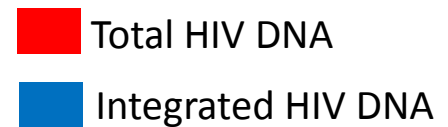
—■— CD3/CD28 + IgG2  
—▲— CD3/CD28 + PD-L1  
—●— NS

The negative signal conferred by the PD-1/PD-L1 interaction inhibits viral production in primary CD4+T cells.

## PD-1 triggering inhibits HIV DNA production and integration

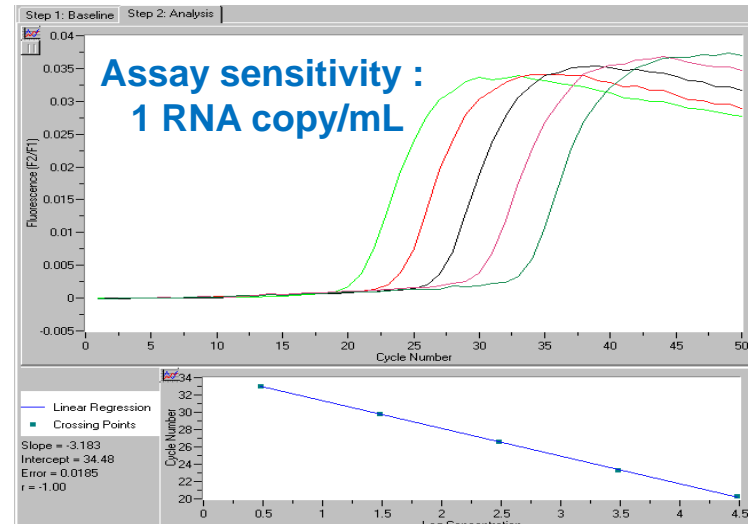
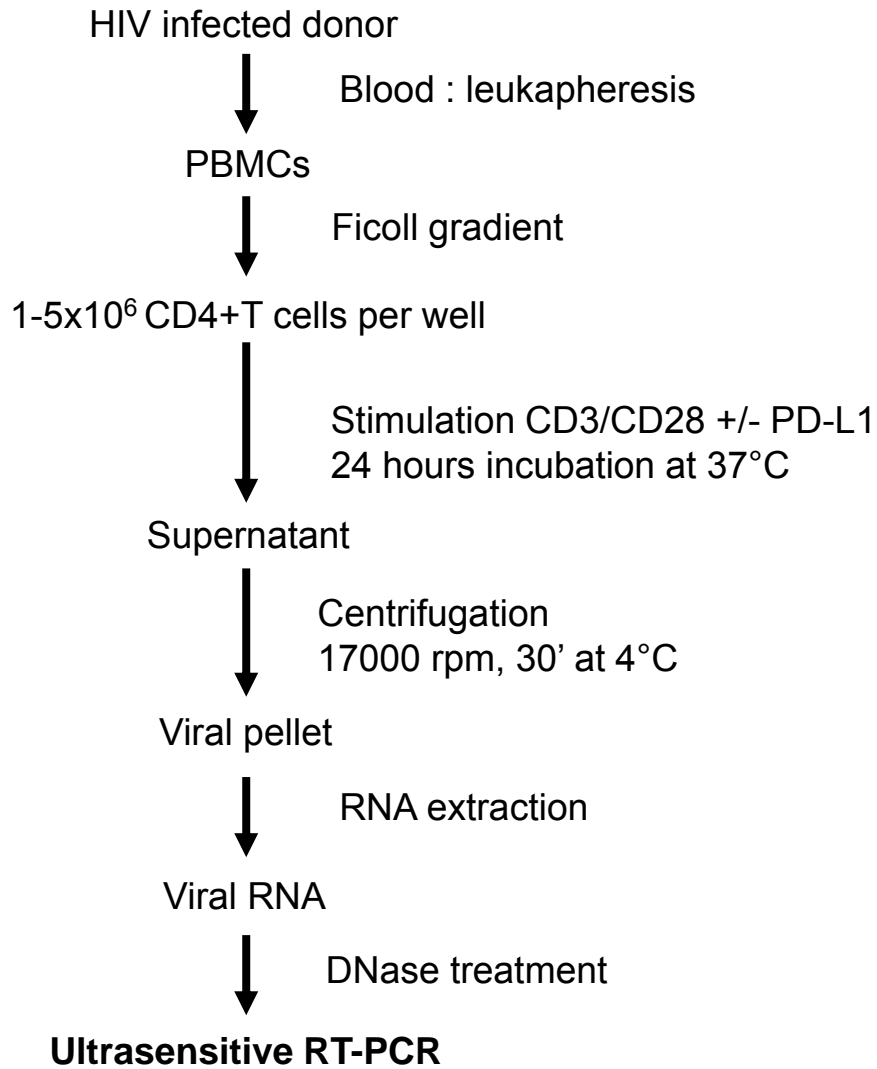


- After 9 days of culture
- Cells lysis with proteinase K
- Real time PCR to quantify total HIV DNA and integrated proviral HIV DNA

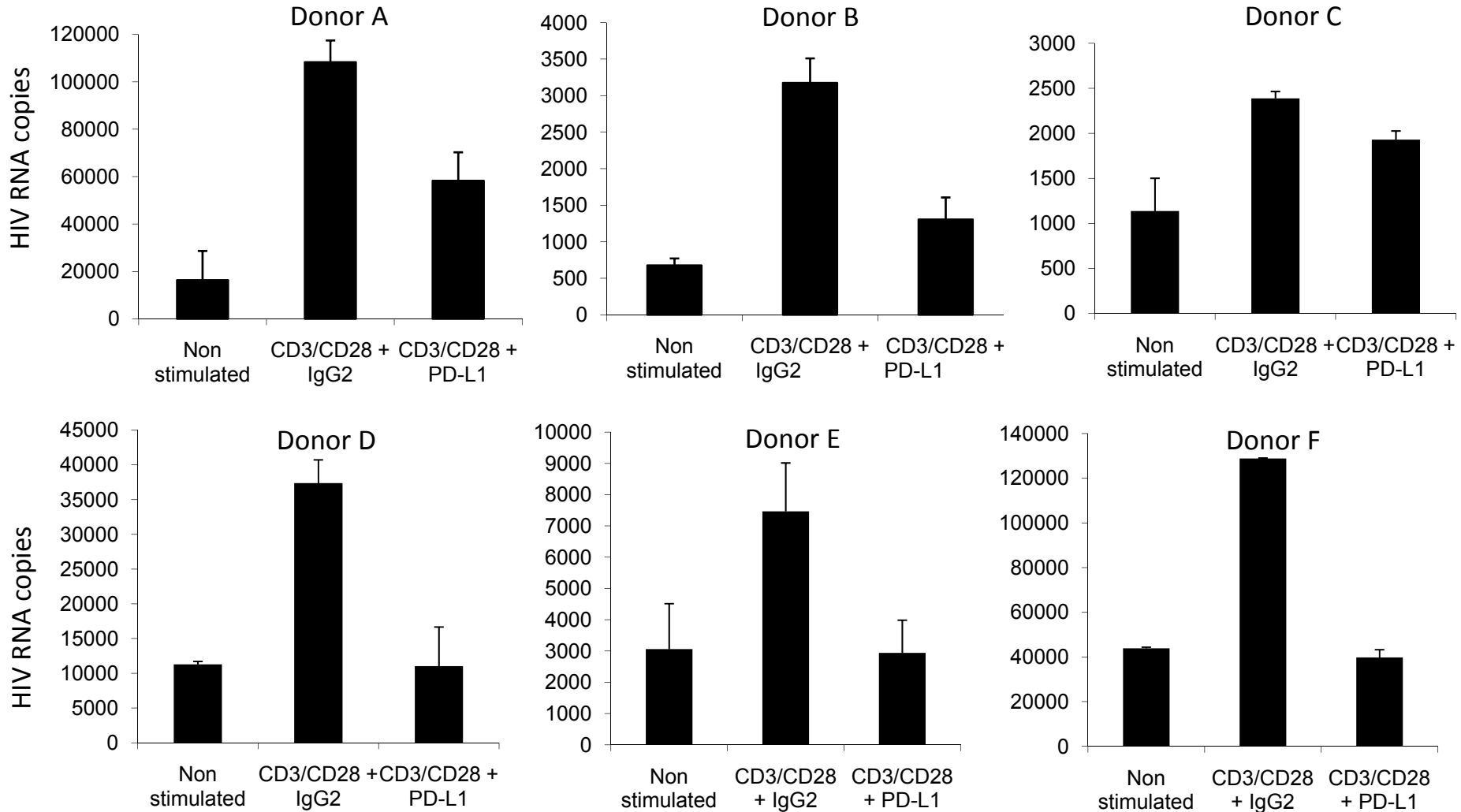


- De novo infection makes difficult to distinguish between two possible mechanisms :
- PD-1 triggering inhibits HIV transcription – direct effect on the viral transcription activity
  - PD-1 triggering inhibits T cell activation of bystander target cells – indirect effect, less target cells for HIV when the PD-1 pathway is triggered

# Ultrasensitive assay to measure viral production



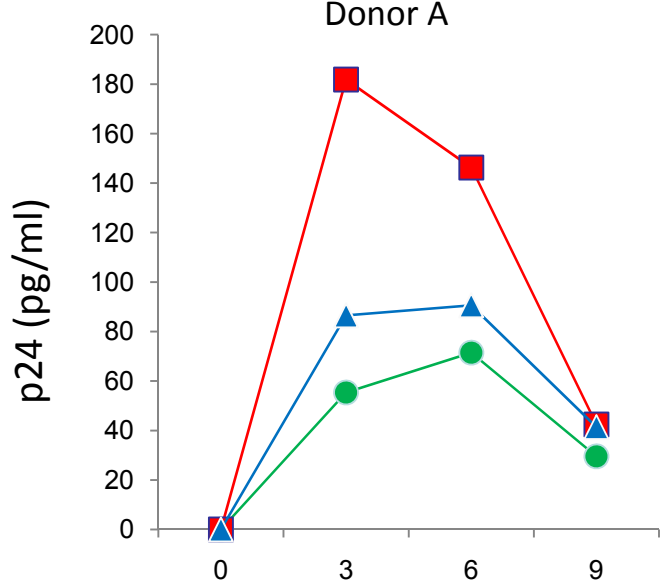
# Triggering of the PD-1 pathway inhibits 60% of HIV-1 production in primary CD4+T cells after 24h of stimulation



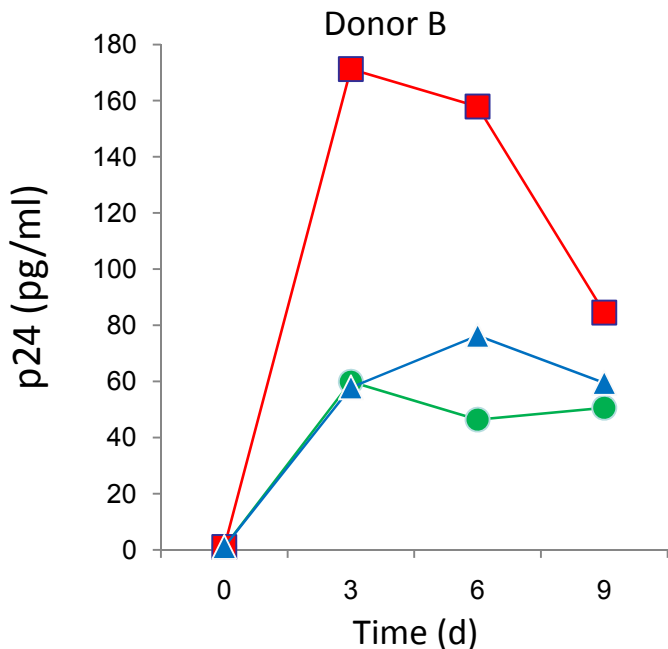
- HAART naïve chronically HIV-infected subjects → Total CD4+T cells negative selection
- TCR triggering (CD3/CD28) with or without co-triggering of the PD-1 pathway with an Ig-PD-L1 chimera.
- Viruses pellet + Viral RNA extraction and quantification by QRT-PCR

**Very short term effect of PD-1 triggering on HIV viral production.  
PD-1 triggering may act directly on the LTR.**

# Triggering of the PD-1 pathway inhibits 45% of HIV-1 production in primary CD4+T cells at day 3 in the presence of ARV



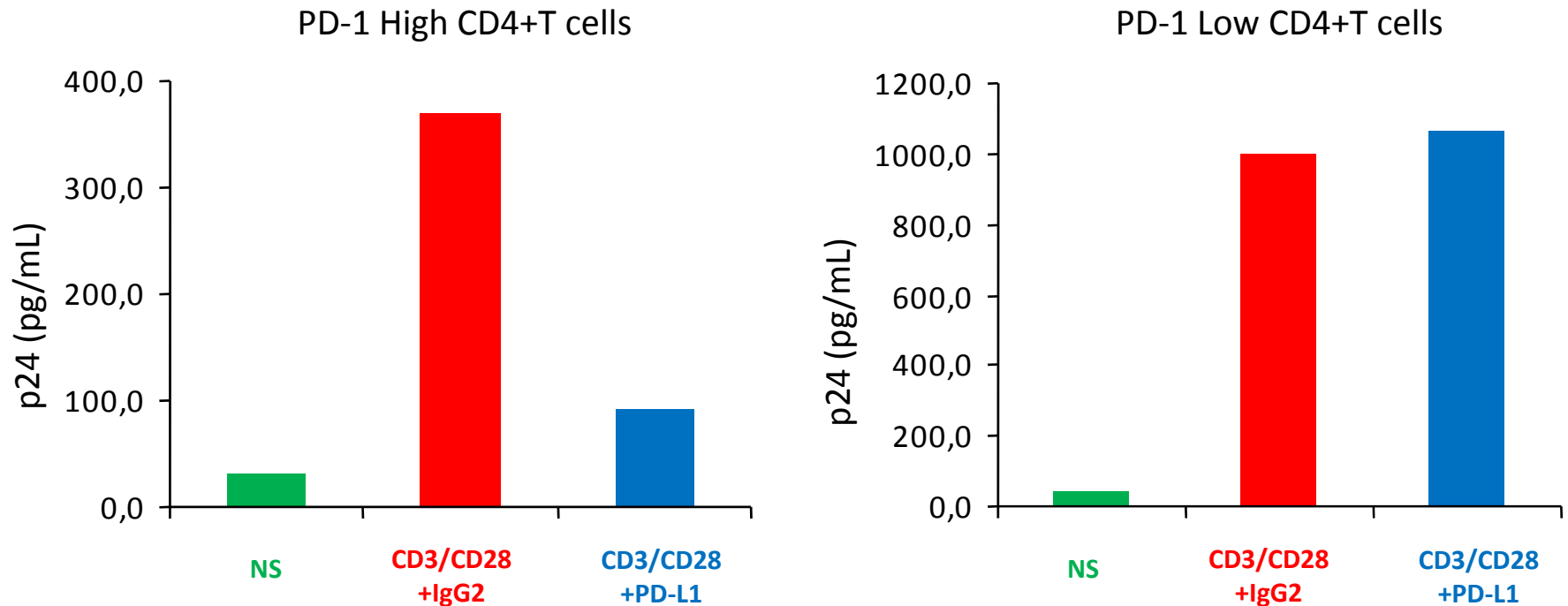
- CD4+T cells from HIV infected viremic subjects purification
- Stimulated with CD3/CD28 in the presence or absence of PD-L1, and ARV
- Viral production measured by p24 ELISA.



- CD3/CD28 + IgG2
- ▲ CD3/CD28 + PD-L1
- NS

- The inhibition mediated by PD-L1 is still observed in the presence of ARV
- PD-1 directly impacts on viral production

## Inhibition restricted to PD-1<sup>high</sup> cells



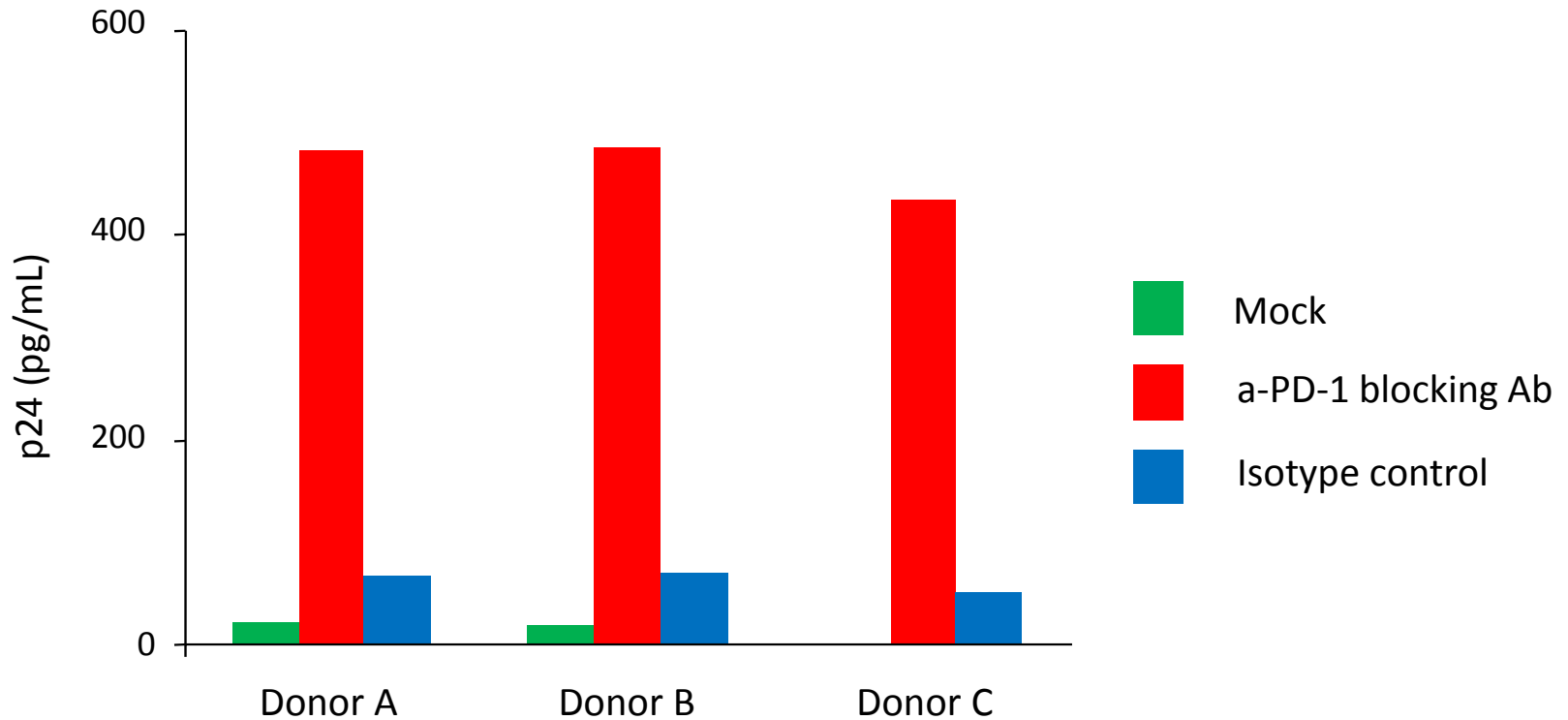
- Total CD4+T cells from viremic HIV infected patients isolation
- PD-1<sup>high</sup> and PD-1<sup>low</sup> subsets highly purified by cell sorting
- Stimulation with different combination of triggering antibodies (CD3/CD28 with PD-L1 or the corresponding IgG isotype).

Inhibition of viral production was exclusively observed in PD-1 high cells indicating the specificity of this pathway

# The disruption of the PD-1/PD-L1 interaction enhances the spontaneous release of HIV-1 virions by CD4+T cells

If PD-1 triggering inhibits viral production, blocking the PD-1/PD-L1 interaction should increase viral production.

- CD4+T cells isolated from HIV infected subjects
- CD4+T cells incubated with an anti-PD-1 antibody that prevents the interaction between PD-1 and its ligands



HIV production is induced after blocking the PD-1 pathway suggesting a role for PD-1 in the maintenance of HIV latency.

## Conclusions

- PD-1+ Central and Transitional memory CD4+T cells are enriched for HIV integrated DNA
- PD-1 receptor may be used as a specific marker to target HIV-1 reservoir cells
- PD-1 receptor triggering inhibits viral production. Role in the establishment of a reservoir?
- Blocking PD-1/PD-L1 interaction induces viral production. Role in the maintenance of a reservoir?

**Can we purge the HIV reservoir by disrupting the PD-1 negative pathway in HAART individuals?**

# Acknowledgments

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