Inducible replication-competent HIV proviruses persist in memory CD4+ T cells expressing high levels of α4β1

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CONFLICTS OF INTEREST

No conflict of interest to declare

How to target (and eliminate) the HIV reservoir that causes viral rebound?

- **Why is this important?** A few **intact** (i.e. without any defect) and latent HIV genomes **persist during long-term antiretroviral therapies** (ART) and can reactivate to cause **viral rebound** when treatment is interrupted.
- **What is the objective?** To **identify the cells** in which **intact replication-competent** HIV **hides**.
- **What did we find?** **Intact, reactivable HIV** are hidden in **memory CD4+ T** cells that express **high levels of α4β1** (a cell-adhesion molecule also known as **VLA-4**) on their surface.
- **What are the implications for a cure?** α4β1 **might be targeted** to reduce the size of the HIV reservoir.
- **What are the next steps?** **Testing anti-VLA-4 molecules** in a humanized mice model.

Background

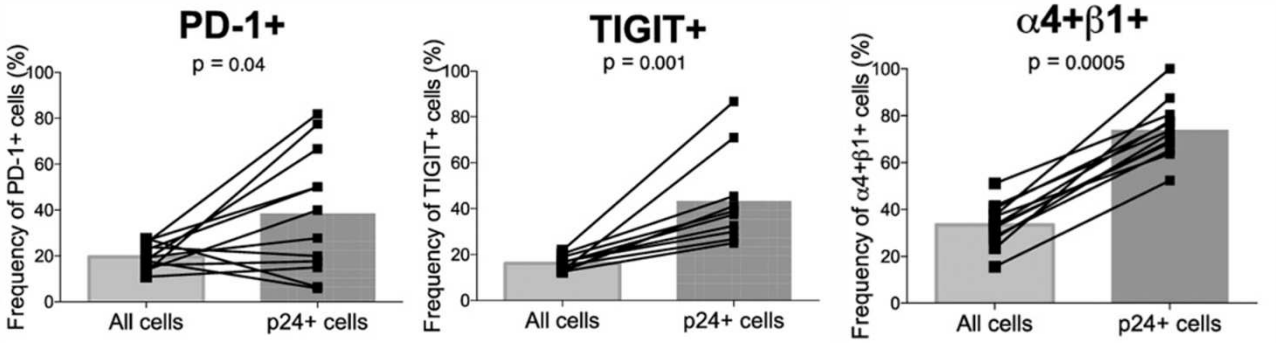
- **HIV persists** during long-term ART, and ART interruption is generally followed by a rapid **viral rebound** (Davey RT et al. PNAS 1999).
- **Inducible, intact, replication-competent** proviruses are the **source** of viral rebound (Finzi D et al. Science 1997; Wong JK et al. Science 1997; Ho YC et al. Cell 2013).

Phenotype	Cellular marker	Cell type	HIV reservoir enrichment	Reported in
Memory	$CD4^+CD45RA^-CD27^{+/-}CCR7^{+/-}$	**T_{CM}; T_{EM}; T_{TM}**	T_{CM} : infected cells T_{TM} : viral RNA T_{TM} & T_{EM} : viral protein T_{EM} : intact HIV genomes	Chomont et al. *Nat Med* 2009 Gálvez et al. *mBio* 2021 Pardons et al. *Plos Path* 2019 Hiener et al. *Cell Reports* 2017
Effector function	$CD4^+CXCR3^{+/-}CCR4^{+/-}CCR6^{+/-}$	**Th1; Th2; Th17; Th1/Th17**	Th17 & Th1/TH17 (CCR6+) : integrated HIV DNA Th1 (CCR4-CCR6-) : intact HIV genomes Th2 (CCR4+CCR6-) : intact HIV genomes	Gosselin et al. *AIDS* 2017 Lee et al. *JCI* 2017 Kuo et al. *J Infect Dis* 2020
Immune checkpoint molecule	**$PD\text{-}1^+$ $LAG\text{-}3^+$ $TIGIT^+$ $CTLA\text{-}4^+$**	T_M; T_{FH}	T_M PD-1+ : integrated HIV DNA T_M CTLA4-+PD-1+ : *gag* SIV DNA T_M PD-1+LAG-3+TIGIT+ : integrated HIV DNA T_M PD-1+ or TIGIT+ : viral protein T_{FH} (T_M PD-1+CXCR5+) : replicative DNA	Chomont et al. *Nat Med* 2009 McGary et al. *Immunity* 2017 Fromentin et al. *Plos Path* 2016 Pardons et al. *Plos Path* 2019 Banga et al. *Nat Med* 2016
Activation	$CD4^+$**$HLA\text{-}DR^+$**	Activated T $CD4^+$	Viral protein Intact HIV genomes	Pardons et al. *Plos Path* 2019 Horsburgh et al. *AIDS* 2020
Adhesion	$CD4^+$**$\alpha4^+\beta7^+/\beta1^+$**	T $CD4^+$	α4β7: susceptibility to infection α4β1 viral prottein p24	Cicala et al. *PNAS* 2009 Sivro et al. *Sci Trans Med* 2018 Pardons et al. *Plos Path* 2019

- There are **no known cellular markers to target specifically** the intact replication-competent **reservoir**.

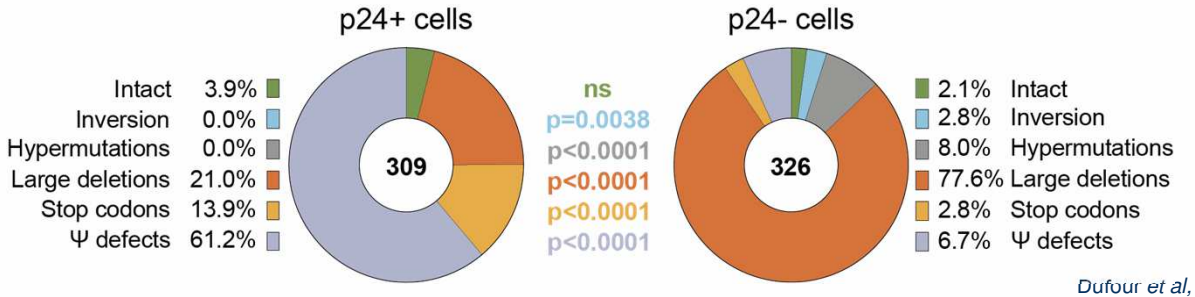
Background

- The **HIV-Flow** assay measures the **inducible translation-competent reservoir (p24+)**, which is enriched in memory CD4+ T cells expressing **PD-1, TIGIT and α4β1** (Pardons M et al. PLoS Path 2019).



Pardons et al, PLoS Path 2019

- Although they carry inducible and translation-competent genomes, **p24+ cells** measured by HIV-Flow **rarely harbor intact** proviruses (Cole B et al. Nat Comm 2021; Dufour C *et al.* CROI 2022).



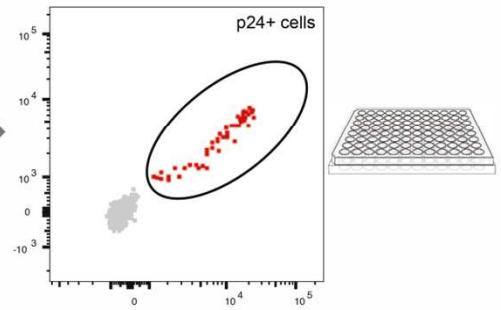
Dufour *et al*, under review

Experimental approach

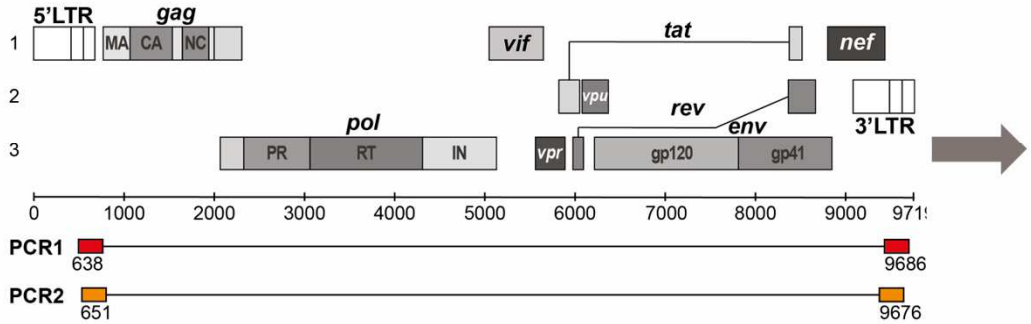
HIV-Flow panel

Marker	Information
p24 KC57	Viral capsid
p24 28B7	
CD45RA	Memory CD4 T cells subsets
CCR7	
HLA-DR	Activation/proliferation
ICOS	
α4	Integrins VLA-4
β1	
PD-1	Immune checkpoints
TIGIT	

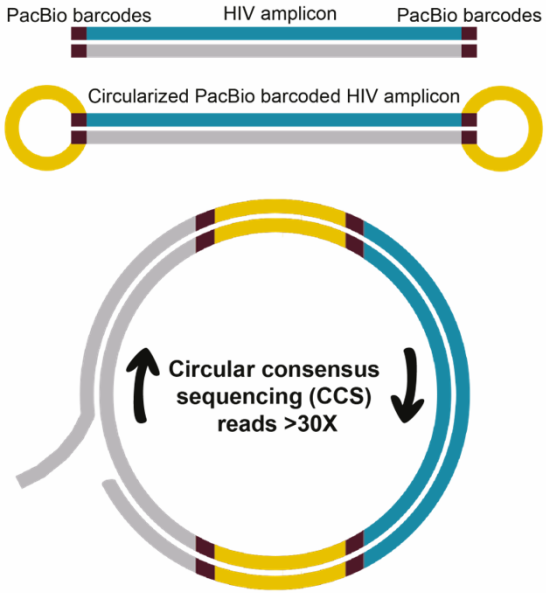
Index single-cell sorting



Near-full length amplification of single HIV genome

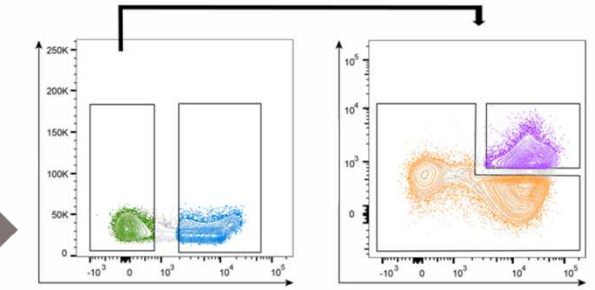


PacBio sequencing

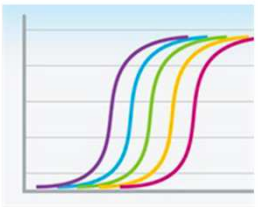


Type of analysis:	Integrity analysis	Expression levels
Methodology:	Is there any defect such as?	FI single cell
	Inversion	MFI population
	or	
	Hypermutation(s)	PD-1 <1 or >1
	or	
	Large internal deletion(s)	TIGIT <1 or >1
	or	
	Stop codon(s)/Frameshift(s)	HLA-DR <1 or >1
	or	
	Packaging signal or major donor site defect(s)	ICOS <1 or >1
	or	α4 <1 or >1
	Small internal deletion(s)	β1 <1 or >1
		CD45RA <1 or >1
	if not	
	Intact	CCR7 <1 or >1
Softwares:	MAFFT Geneious (ref. HXB2) HIV Database "QCTool" HIV Database "GeneCutter"	FlowJo

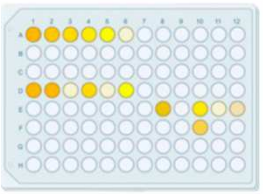
Bulk-sorting of identified population



Integrated HIV DNA qPCR



qVOA



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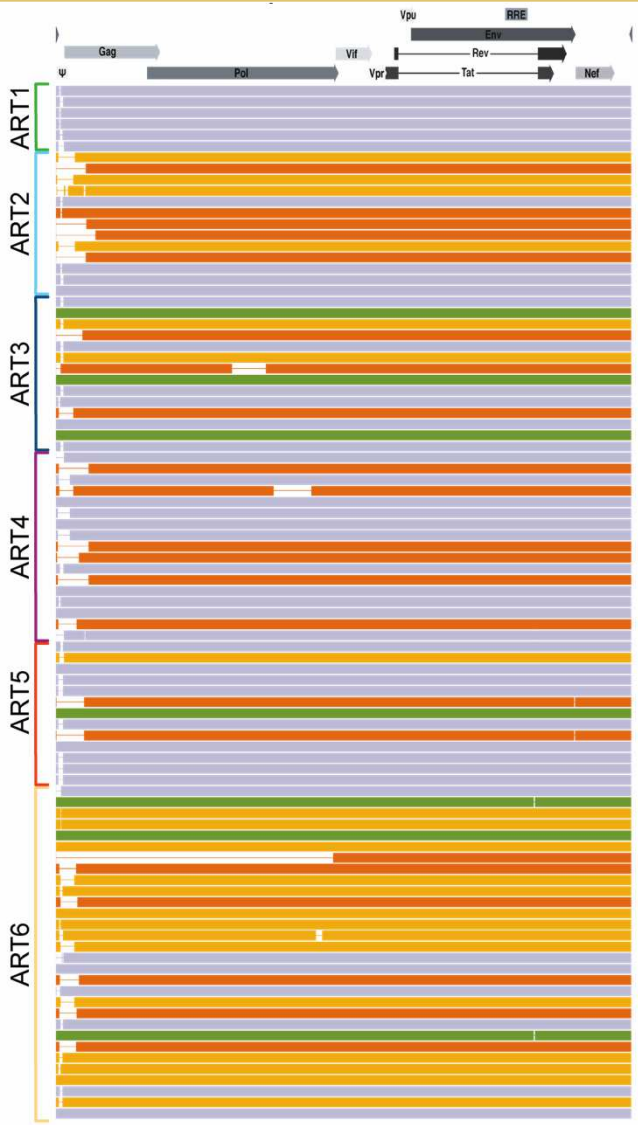
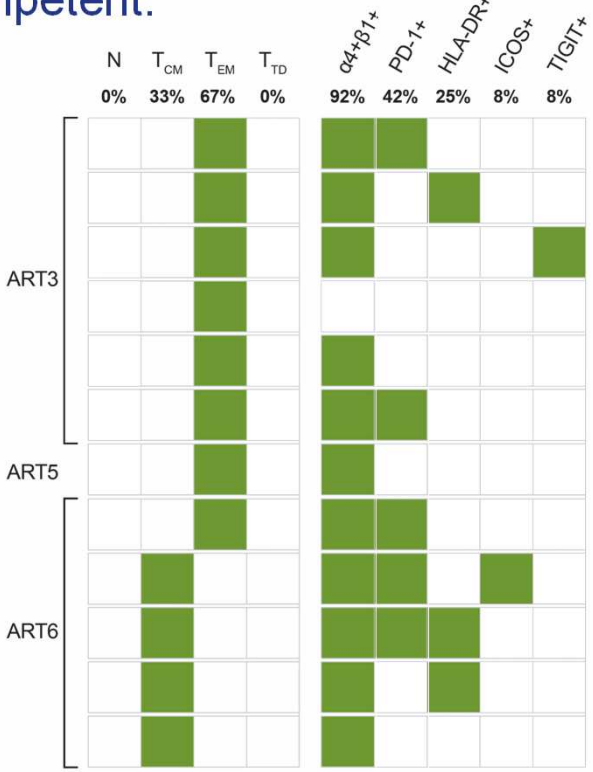
Inducible intact proviruses are in memory CD4+ T cells expressing α4β1



- We sequenced a total of **309 HIV genomes from single-sorted p24-expressing cells** from 6 ART-treated participants.
- **12 proviruses** from these p24+ cells were **intact**, and considered potentially replication-competent.

These p24+ CD4+ T cells harboring intact proviruses:

- **always** display a **memory phenotype** (67% T_{EM}; 33% T_{CM});
- **often** express the combination of integrin **α4β1** (**92%**);
- **sometimes** express **PD-1** (**42%**);
- **rarely** express **HLA-DR** (25%), **ICOS** (8%) or **TIGIT** (8%).



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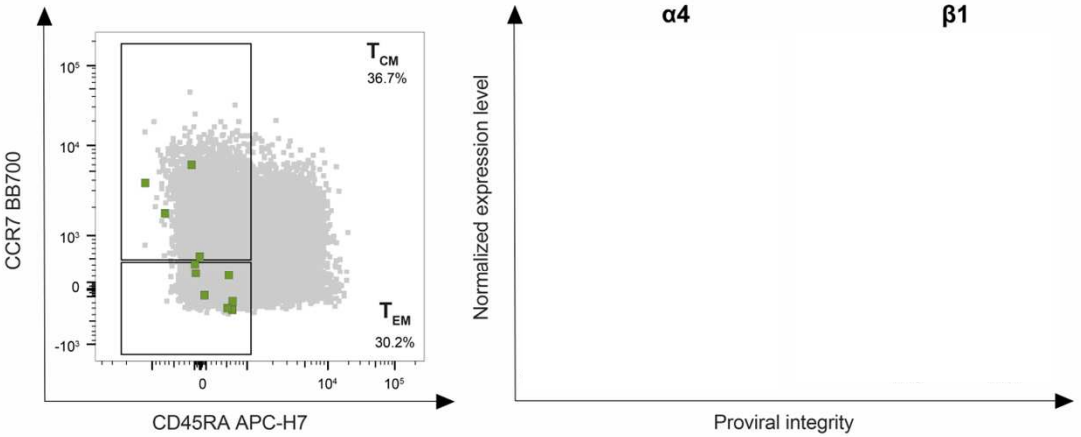
Intact proviruses persist in $\alpha4^{high}\beta1^{high}$ memory CD4+ T cells

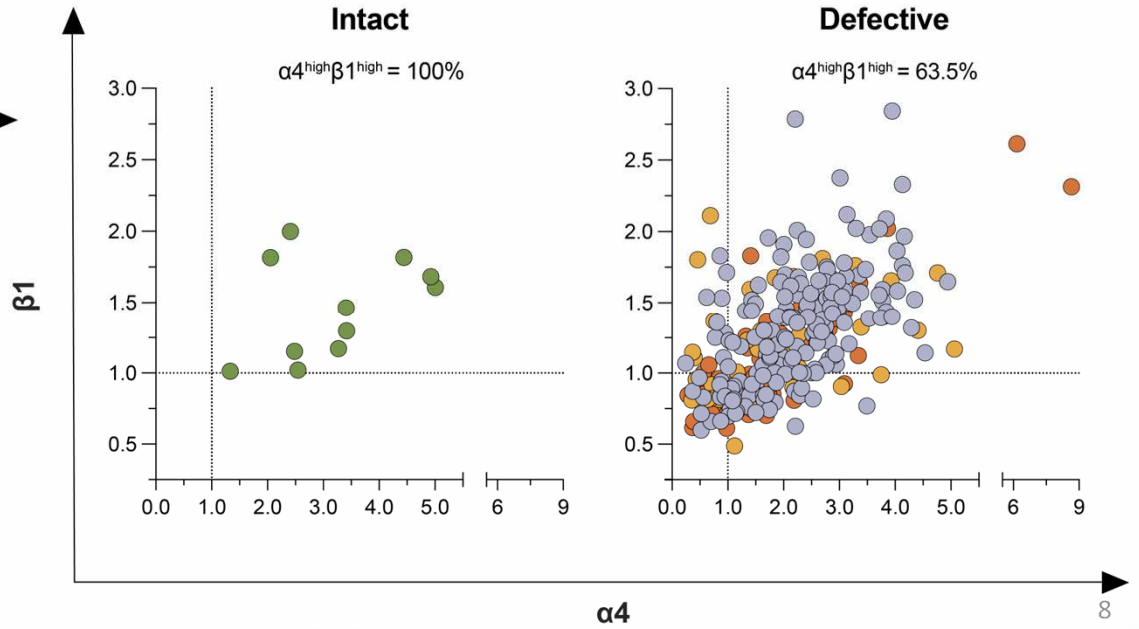
We compared the level of expression of α4 and β1 from each single-sorted p24+ cells with the expression on total CD4+ T cells.



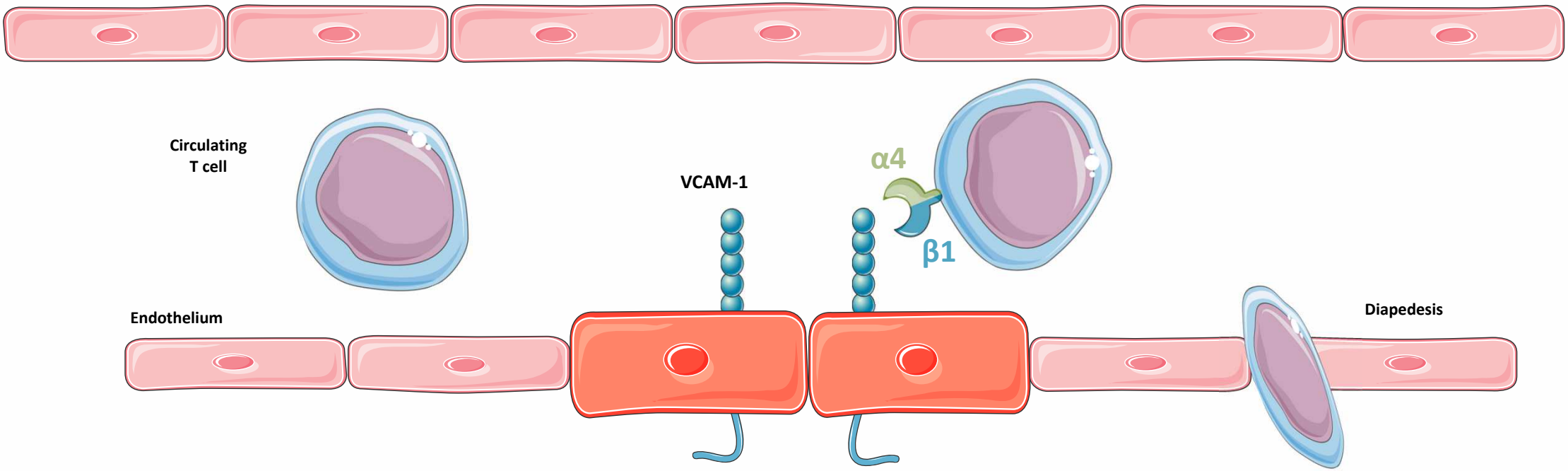
Dufour *et al*, under review

- All intact proviruses were found in cells expressing higher levels of α4β1 than the total CD4+ T cell population, whereas only 63.5% of defective proviruses were in cells expressing $\alpha4^{high}\beta1^{high}$.

- Compared to p24+ cells harboring defective HIV genomes, cells with intact proviruses express 1.62-fold more α4 and 1.20-fold more β1.



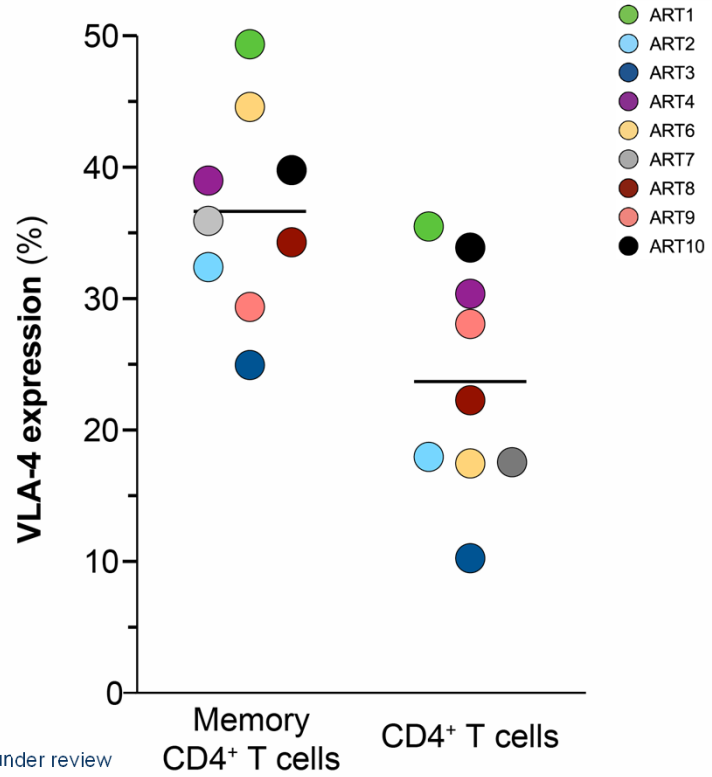
What are α4 and β1?



- **α4** and **β1** together form the integrin known as **VLA-4**.
- **VLA-4** is a **cell-adhesion molecule** involved in the adhesion of T cells to activated endothelial cells (Elices et al. *Cell* 1990), initiating diapedesis.
- Its ligand, **VCAM-1** (Osborn et al. *Cell* 1989), is **expressed at the surface of inflamed tissues**, mostly in the CNS (Engelhardt et al. *Intern Immunol* 1995) and in bone marrow (DeNucci & Shimizu *J Immunol* 2011).

Cells expressing α4β1 in ART-treated PWH are memory CD4+ T cells

We analyzed the phenotype of VLA-4 (α4β1) cells compared to the total CD4+ T cell population



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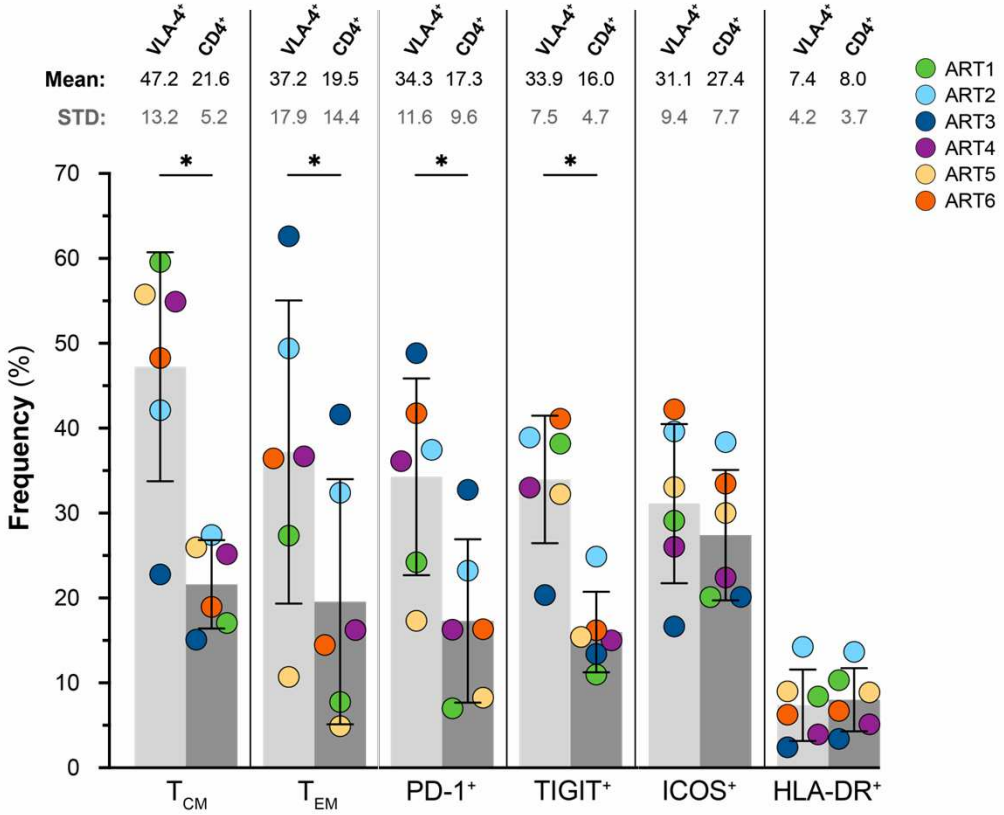
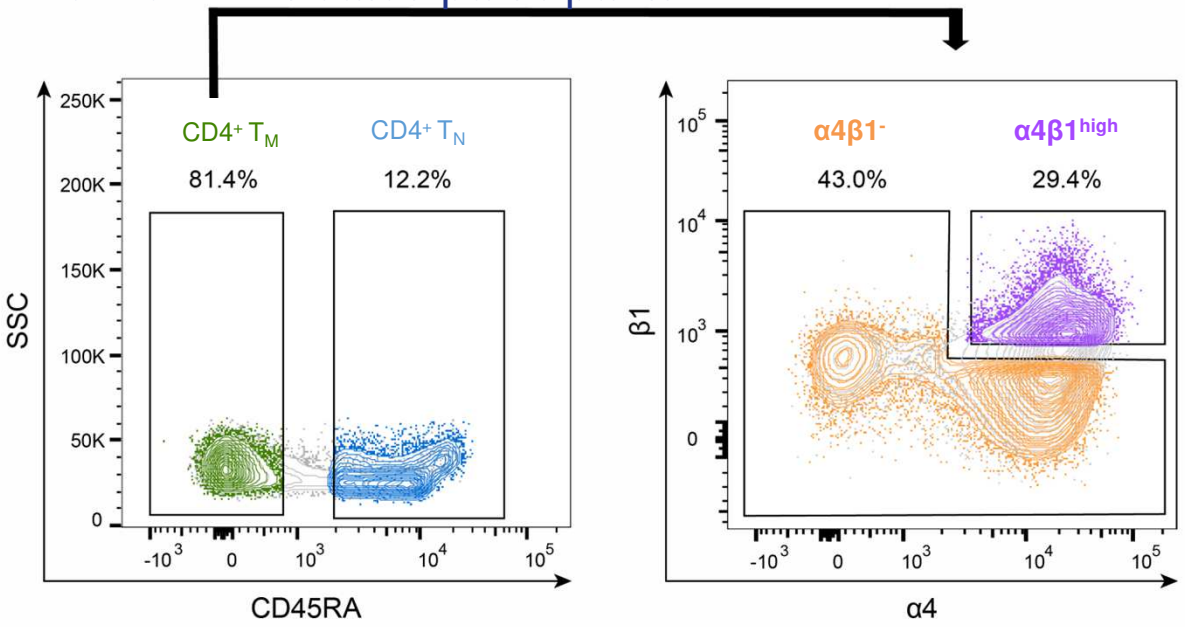


In ART-treated people with HIV:

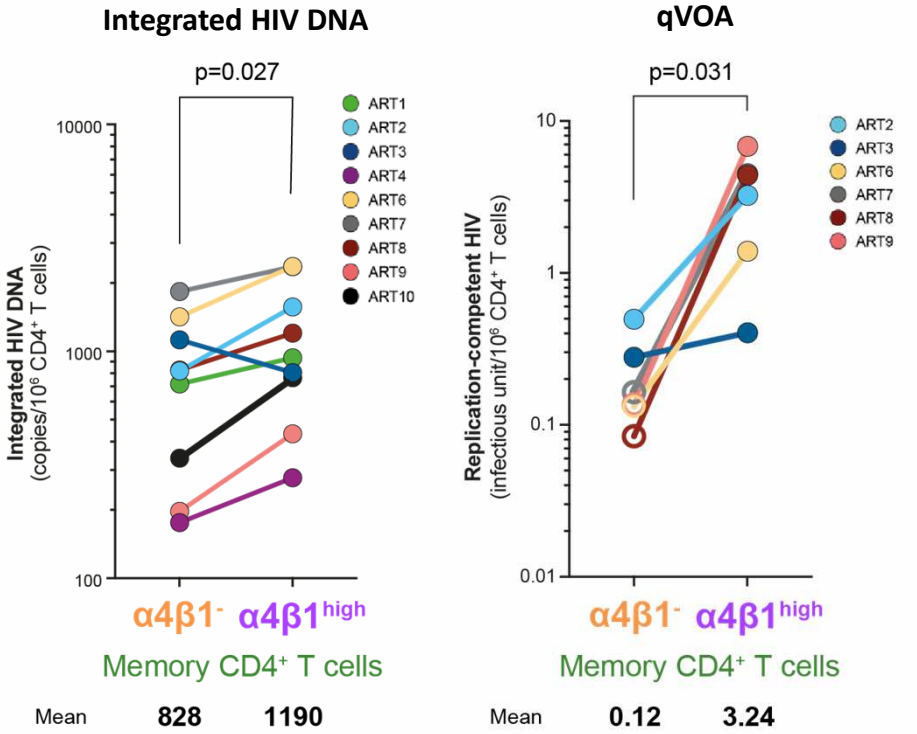
- VLA-4 is more frequently expressed in **memory CD4+ T cells**, and preferentially in T_{CM};
- VLA-4+ CD4+ T cells are enriched for the expression of **PD-1** and **TIGIT**.

α4β1^{high} T_M are enriched in replication-competent proviruses

We bulked-sorted >6X10^6 unstimulated memory CD4+ T cells expressing high or low levels of α4β1 from 6 ART-treated participants



Dufour *et al*, under review



Compared to α4β1$^-$ cells, bulk-sorted **memory CD4+ T α4β1^{high} cells** were:

- **slightly** enriched (**1.14X**) in **integrated HIV DNA**;
- **highly** enriched (**27X**) in **replication-competent virus**
  - 4 out of 6 α4β1$^-$ cell samples displayed undetectable qVOA value.

Conclusions

In PWH under suppressive ART for years, **memory CD4+ T cells** expressing **high levels of VLA-4** are enriched in **intact, inducible and replication-competent HIV genomes**.

Perspectives

- Does this **integrin play a direct role in HIV persistence**?
- Could it be **pharmacologically targeted** to **reduce the size of the reservoir**?

In **collaboration** with **other CanCURE research teams** in Canada, we are currently **exploring whether this finding** can be recapitulated in the **humanized mice model**, which will allow us to test possible interventions.

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