

11<sup>TH</sup> EDITION

DECEMBER 10-13, 2024

# HIV PERSISTENCE DURING THERAPY

Reservoirs & Eradication Strategies Workshop



Blood and tissue HIV-1 reservoirs display cellular plasticity and lack of compartmentalization in virally suppressed people

Marion Pardons

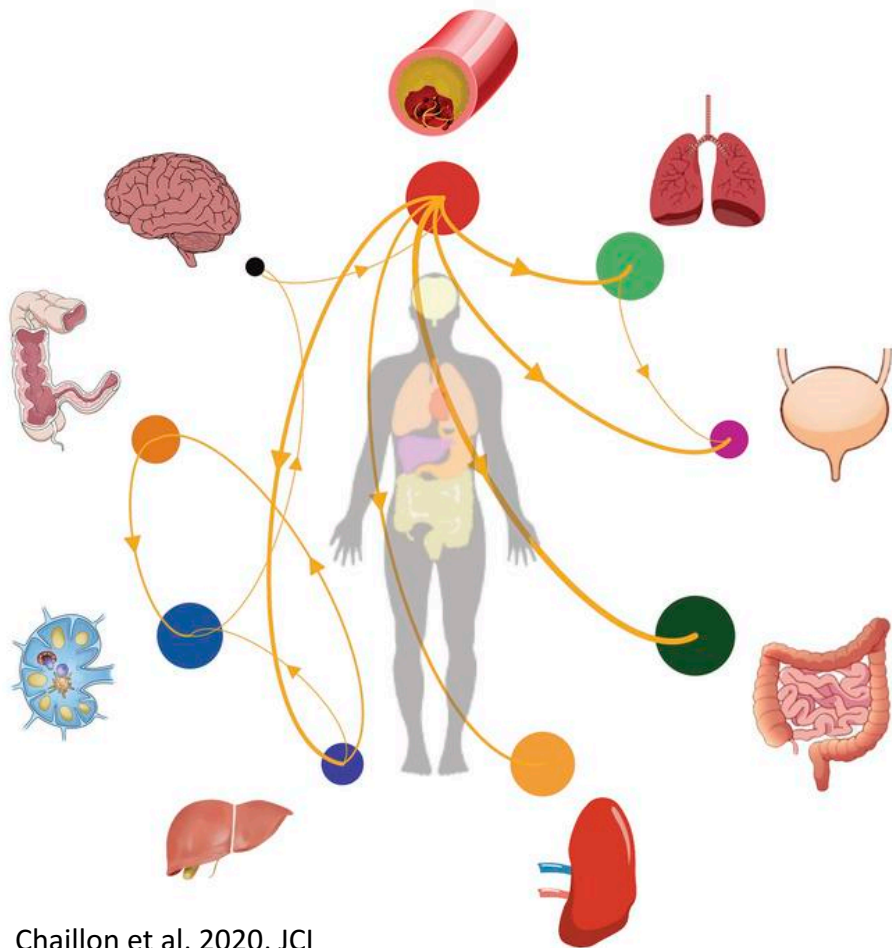
*HIV Cure Research Center, Ghent, Belgium*

[www.hiv-persistence.com](http://www.hiv-persistence.com)

# CONFLICTS OF INTEREST

- The Tat-LNP molecule is provided by Janssen Pharmaceutica

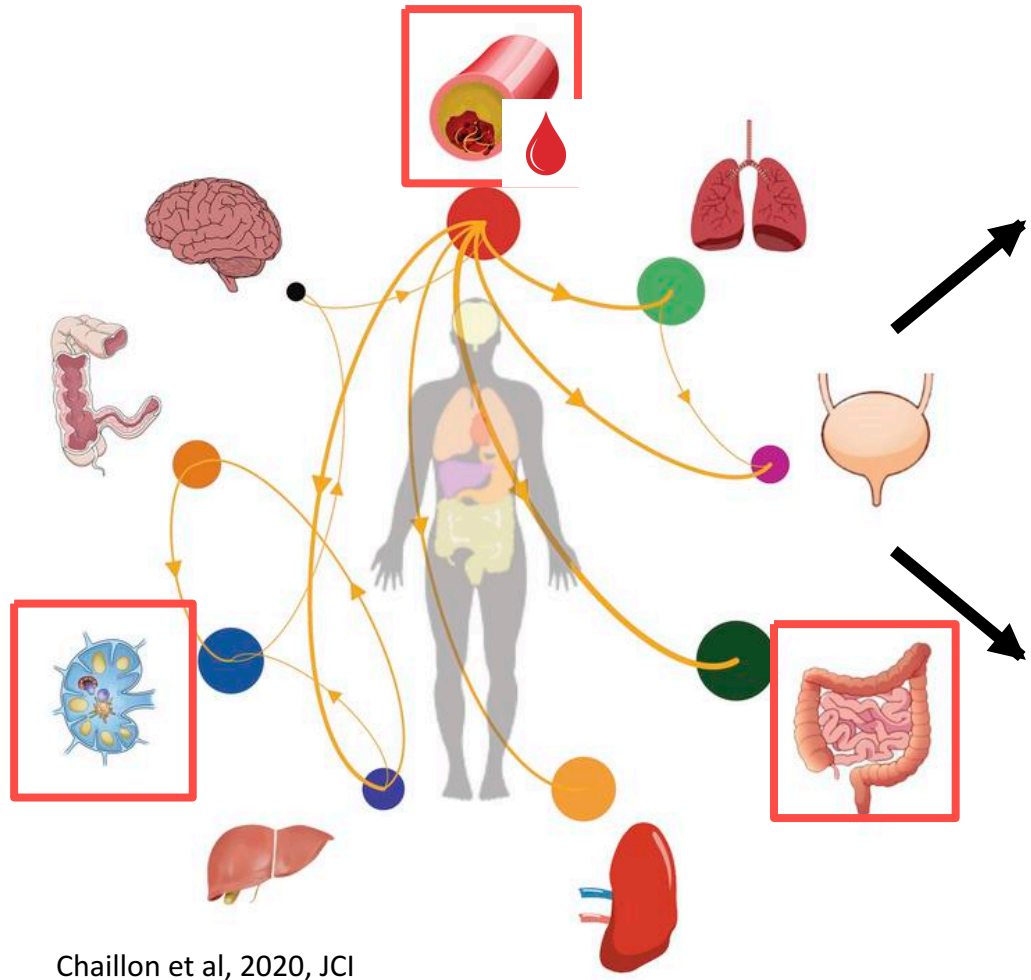
## Tissues serve as ideal sanctuaries for HIV reservoir persistence



Chaillon et al, 2020, JCI

- **Suboptimal penetration** of antiretroviral drugs in anatomical sites, potentially allowing for low levels of ongoing replication  
Fletcher et al, 2014, PNAS; Fletcher et al, AIDS, 2022
- Some anatomical sites have a **naturally reduced susceptibility** to immune responses (e.g. germinal centers in lymph nodes from which the cytotoxic CD8 T cells are excluded)  
Connick et al, 2007, Journal of Immunology
- In human autopsy studies, HIV-infected cells are **detected in almost all tissues**  
Chaillon et al, 2020, JCI; Dufour et al, Cell Reports, 2023
- **Lymph nodes and gastrointestinal tissues** represent hotspots for the persistence of **intact proviruses**  
Sun et al, 2023, eLife

# Tissues serve as ideal sanctuaries for HIV reservoir persistence



Chaillon et al, 2020, JCI

## Phenotypic characterization of the translation-competent reservoir Potent latency reversal by Tat RNA-containing nanoparticle enables multi-omic analysis of the HIV-1 reservoir

[Marion Pardons](#), [Basiel Cole](#), [Laurens Lambrechts](#), [Willem van Snippenberg](#), [Sofie Rutsaert](#), [Ytse Noppe](#), [Nele De Langhe](#), [Annemieke Dhondt](#), [Jerel Vega](#), [Filmon Eyassu](#), [Erik Nijs](#), [Ellen Van Gulck](#), [Daniel Boden](#) & [Linus Vandekerckhove](#) ✉

*Nature Communications* **14**, Article number: 8397 (2023) | [Cite this article](#)

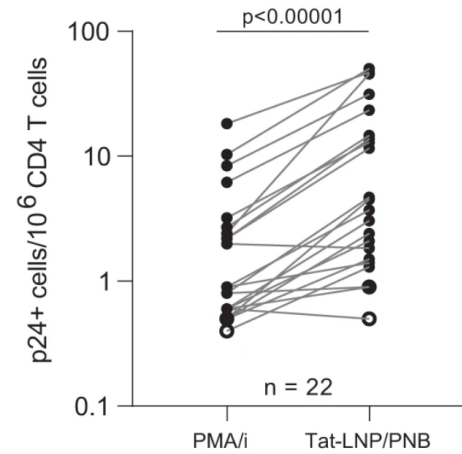
## Virological characterization of the viral reservoir

### HIV-PULSE: a long-read sequencing assay for high-throughput near full-length HIV-1 proviral genome characterization 🧬

[Laurens Lambrechts](#), [Noah Bonine](#), [Rita Verstraeten](#), [Marion Pardons](#), [Ytse Noppe](#), [Sofie Rutsaert](#), [Filip Van Nieuwerburgh](#), [Wim Van Crieking](#), [Basiel Cole](#), [Linus Vandekerckhove](#) ✉ [Author Notes](#)

*Nucleic Acids Research*, Volume 51, Issue 20, 10 November 2023, Page e102,  
<https://doi.org/10.1093/nar/gkad790>

**Published:** 11 October 2023 [Article history](#) ▾



## Key questions

- (i) Do translation-competent reservoir cells in matched blood and lymph nodes exhibit distinct phenotypic features?
- (ii) Are these reservoirs enriched in germinal center T follicular helper (GC Tfh) cells in individuals undergoing treatment for more than 10 years?
- (iii) Does near full-length sequencing reveal compartmentalization between blood and tissues?

# Study design

Patient ID					Age	Gender	Subtype	VL	ART duration				T1 - T0
	B_T0	B_T1	L_T1	G_T1					B_T0	B_T1	L_T1	G_T1	
MRC01					50-60	M	B	<20	18.5	20.5	20.4	20.5	2.0
MRC04					50-60	M	B	<20	18.4	19.8	x	19.8	1.4
MRC08					50-60	M	B	<20	1.3	2.7	2.8	2.7	1.4
MRC15					50-60	M	B	<20	14.7	16.6	16.6	16.7	1.9
MRC16					40-50	F	F01	<20	x	14.6	14.6	x	x
MRC21					60-70	M	B	<20	24.8	26.5	26.5	26.5	1.7
MRC25					40-50	M	B	<20	11.6	12.4	x	12.4	0.8
MRC29					60-70	M	B	<20	x	14.3	x	14.3	x
MRC31					40-50	M	B	<20	x	7.8	x	7.6	x

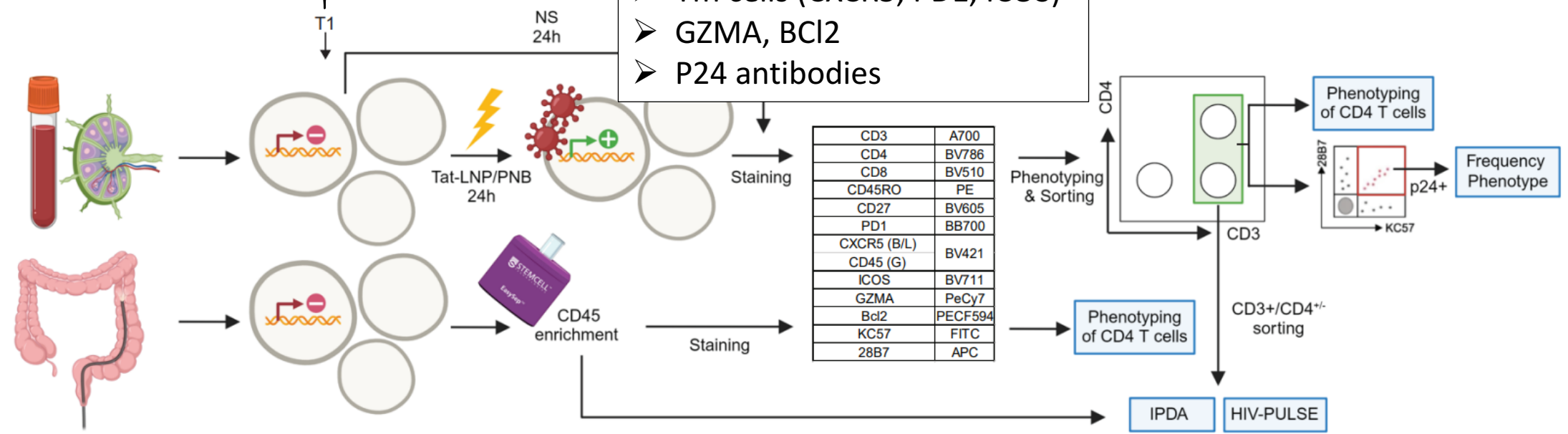
B = blood, L = lymph node, G = gut

# Study design

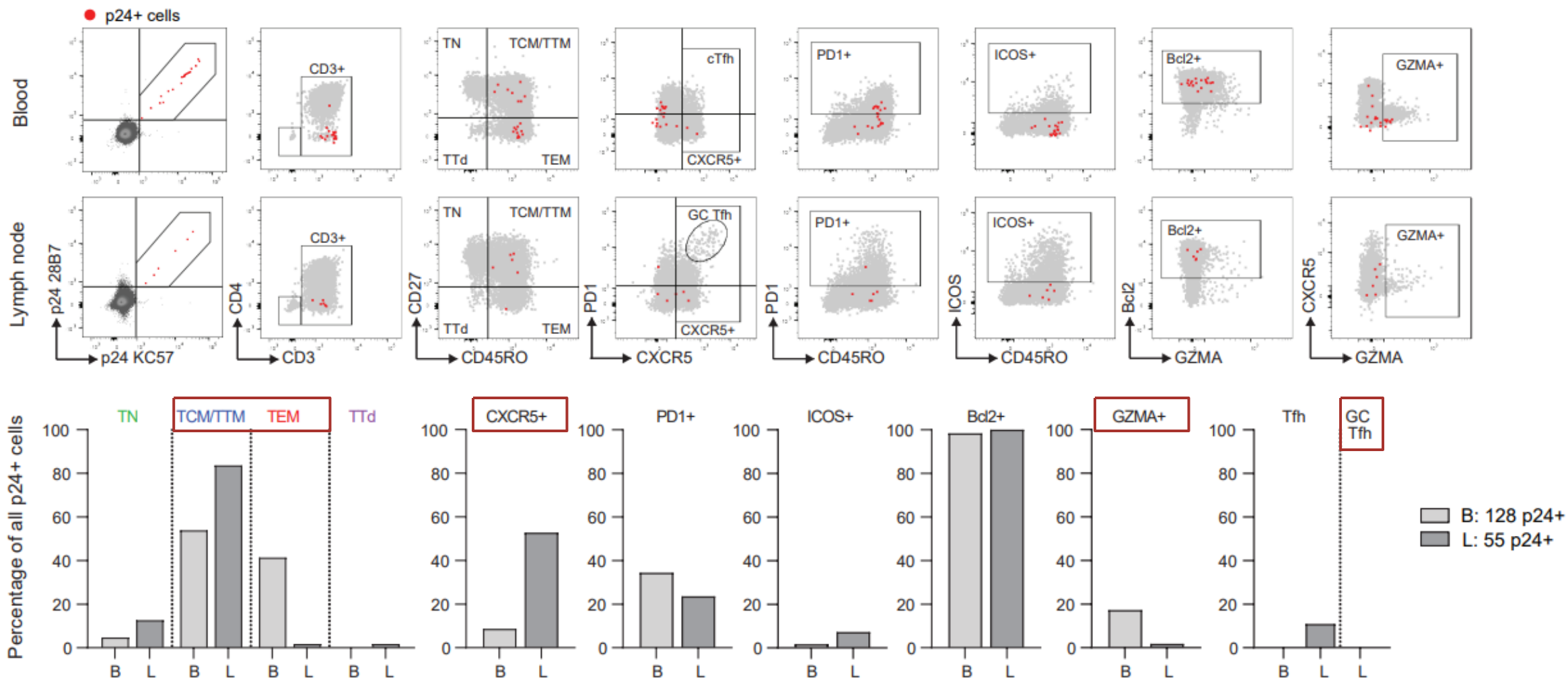
Patient ID	B_T0	B_T1	L_T1	G_T1	Age	Gender	Subtype	VL	ART duration				T1 - T0
									B_T0	B_T1	L_T1	G_T1	
MRC01					50-60	M	B	<20	18.5	20.5	20.4	20.5	2.0
MRC04					50-60	M	B	<20	18.4	19.8	x	19.8	1.4
MRC08					50-60	M	B	<20	1.3	2.7	2.8	2.7	1.4
MRC15					50-60	M	B	<20	14.7	16.6	16.6	16.7	1.9
MRC16					40-50	F	F01	<20	x	14.6	14.6	x	x
MRC21					60-70	M	B	<20	24.8	26.5	26.5	26.5	1.7
MRC25					40-50	M	B	<20	11.6	12.4	x	12.4	0.8
MRC29					60-70	M	B	<20	x	14.3	x	14.3	x
MRC31					40-50					8	x	7.6	x

B = blood, L = lymph node, G = gut

- Memory subsets
- Tfh cells (CXCR5, PD1, ICOS)
- GZMA, Bcl2
- P24 antibodies



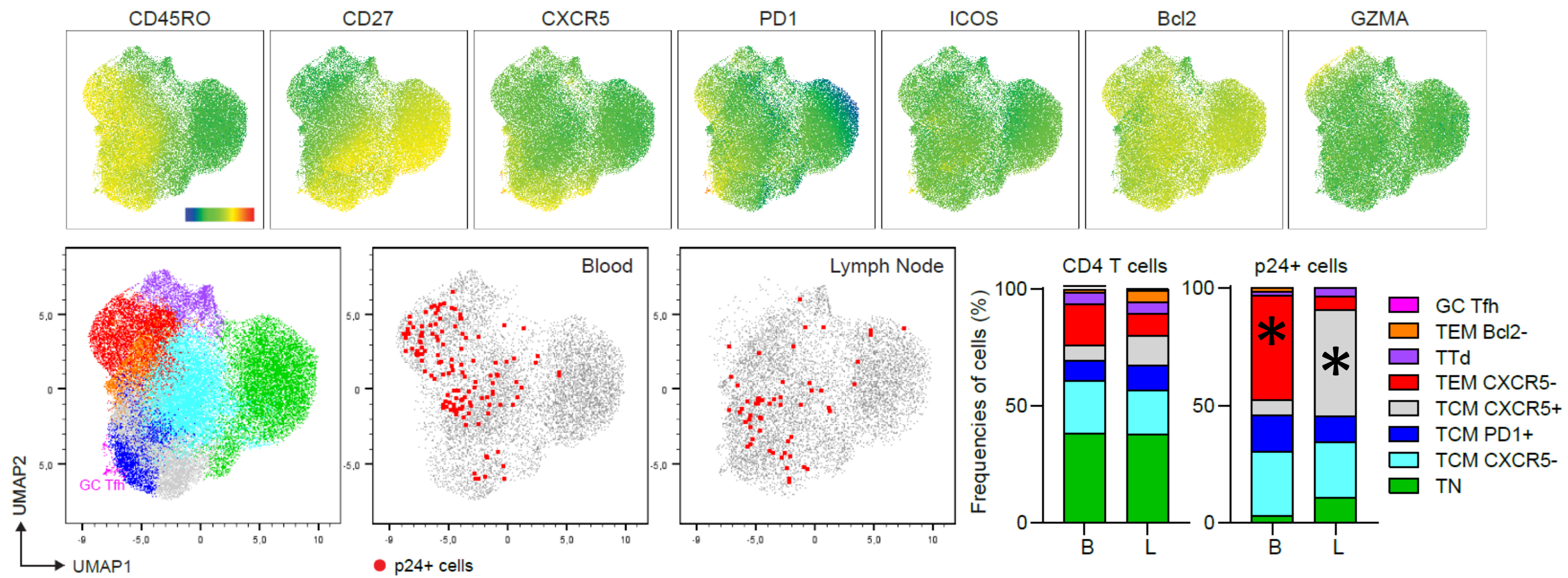
# Phenotypic analysis of p24+ cells in blood and lymph nodes



- P24+ cells from blood and lymph nodes display distinct phenotypes
- GC Tfh cells do not appear to harbor the translation-competent reservoir in long-term suppressed individuals

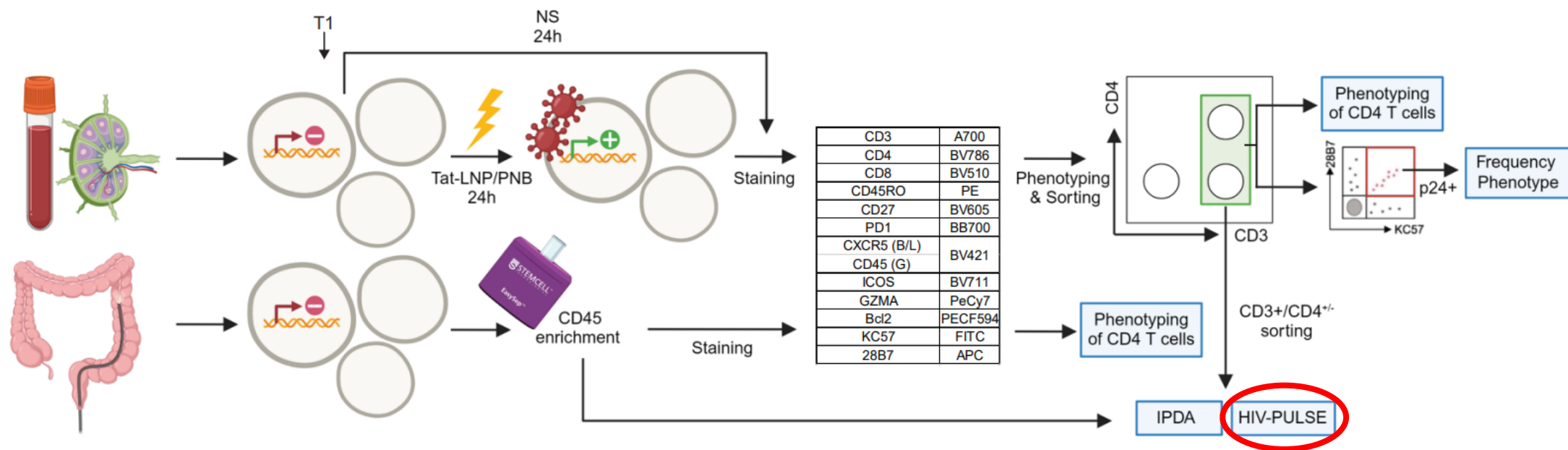


# Unsupervised clustering: FlowSOM analysis



➤ p24+ cells from the blood and the lymph nodes cluster in different subsets of cells

# Near full-length HIV sequencing using HIV-PULSE



## HIV-PULSE: a long-read sequencing assay for high-throughput near full-length HIV-1 proviral genome characterization

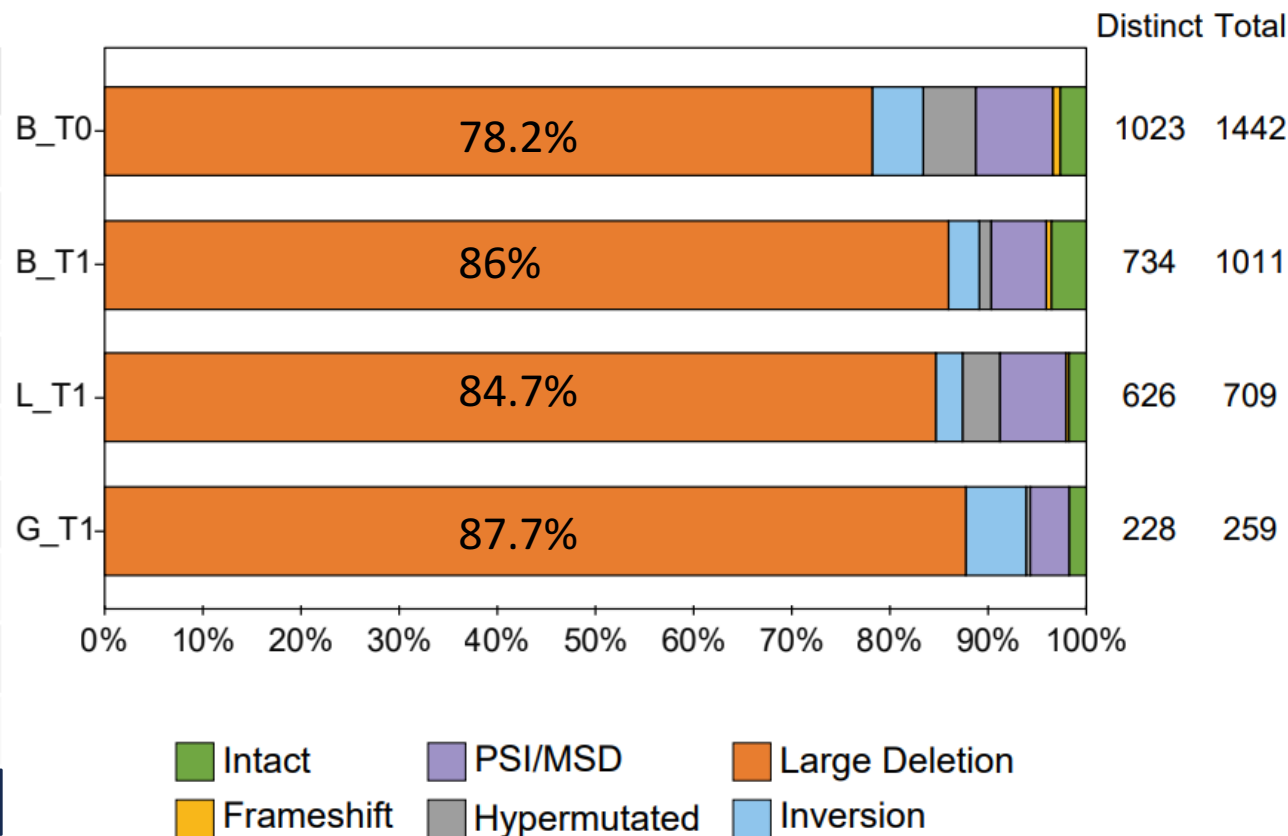
Laurens Lambrechts, Noah Bonine, Rita Verstraeten, Marion Pardons, Ytse Noppe, Sofie Rutsaert, Filip Van Nieuwerburgh, Wim Van Criekeing, Basiel Cole, Linos Vandekerckhove ✉ [Author Notes](#)

*Nucleic Acids Research*, Volume 51, Issue 20, 10 November 2023, Page e102, <https://doi.org/10.1093/nar/gkad790>

**Published:** 11 October 2023 **Article history** ▼

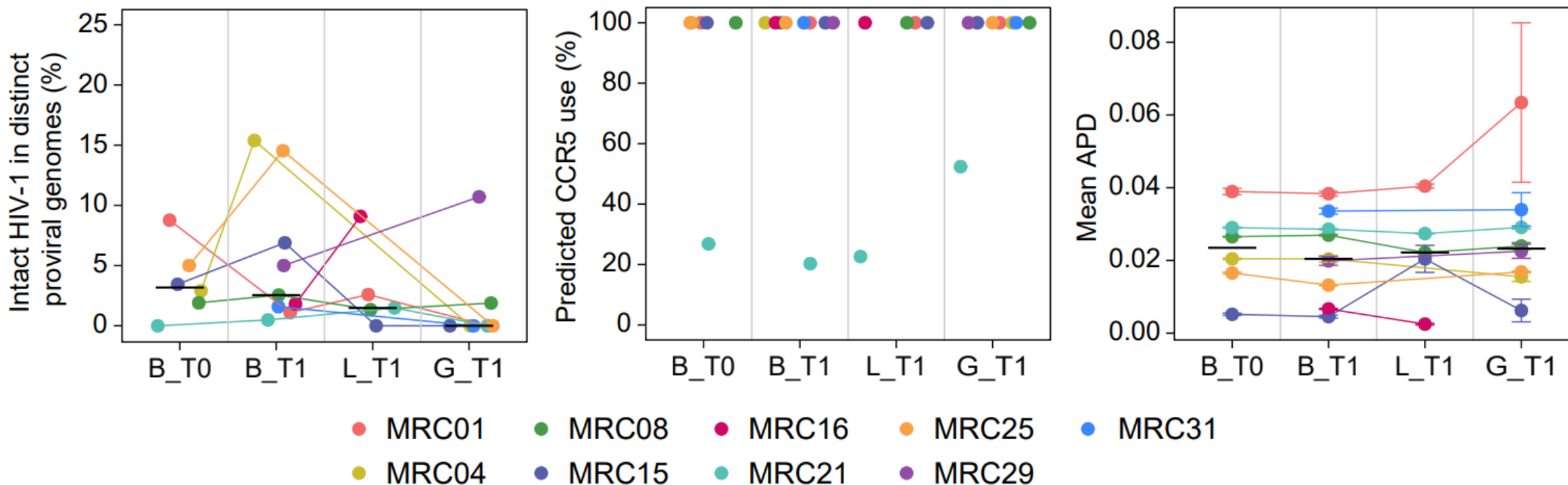
# Near full-length HIV sequencing using HIV-PULSE

Participant	Distinct	Total
MRC01	220	445
MRC04	355	447
MRC08	627	885
MRC15	62	140
MRC16	66	71
MRC21	687	946
MRC25	210	311
MRC29	65	86
MRC31	79	90
<i>Total</i>	<i>2371</i>	<i>3421</i>



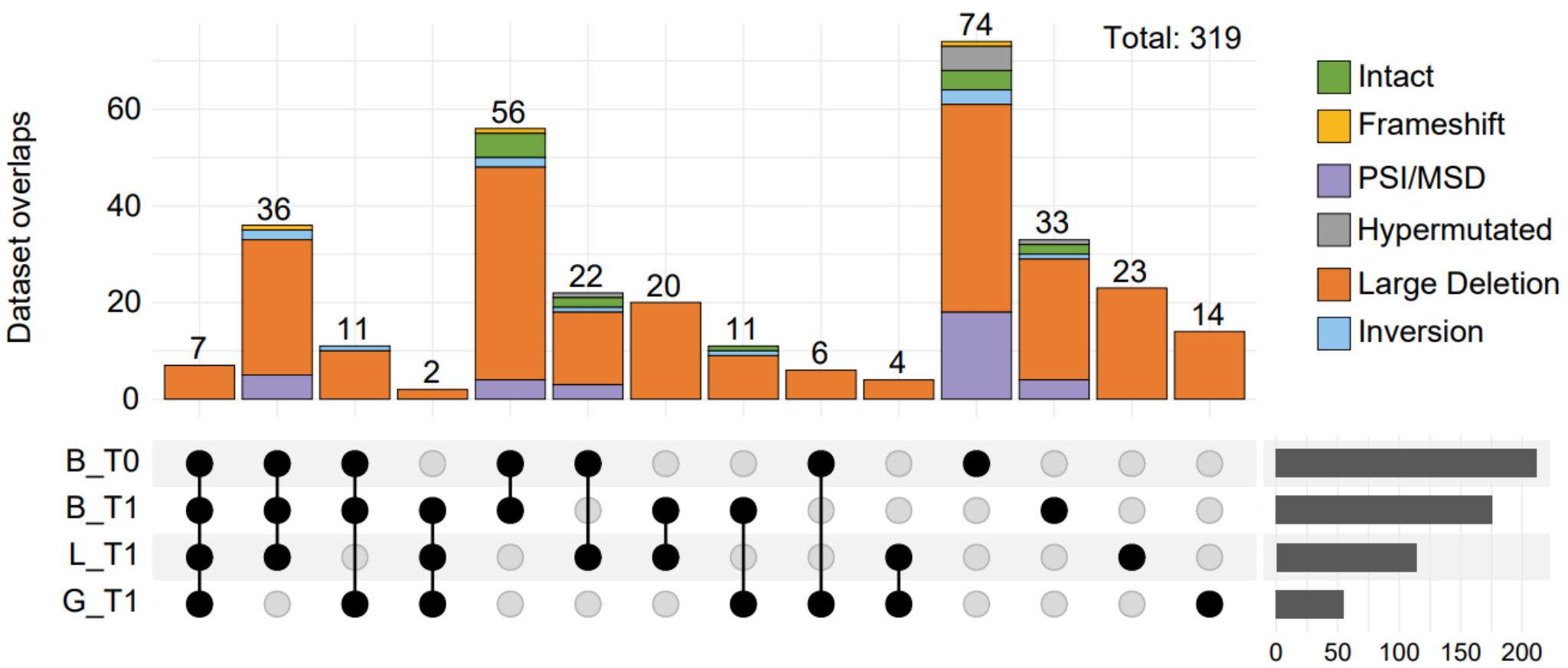
➤ Large deletions are the most prevalent defect in all anatomical compartments

# Proviral landscape in blood and tissues



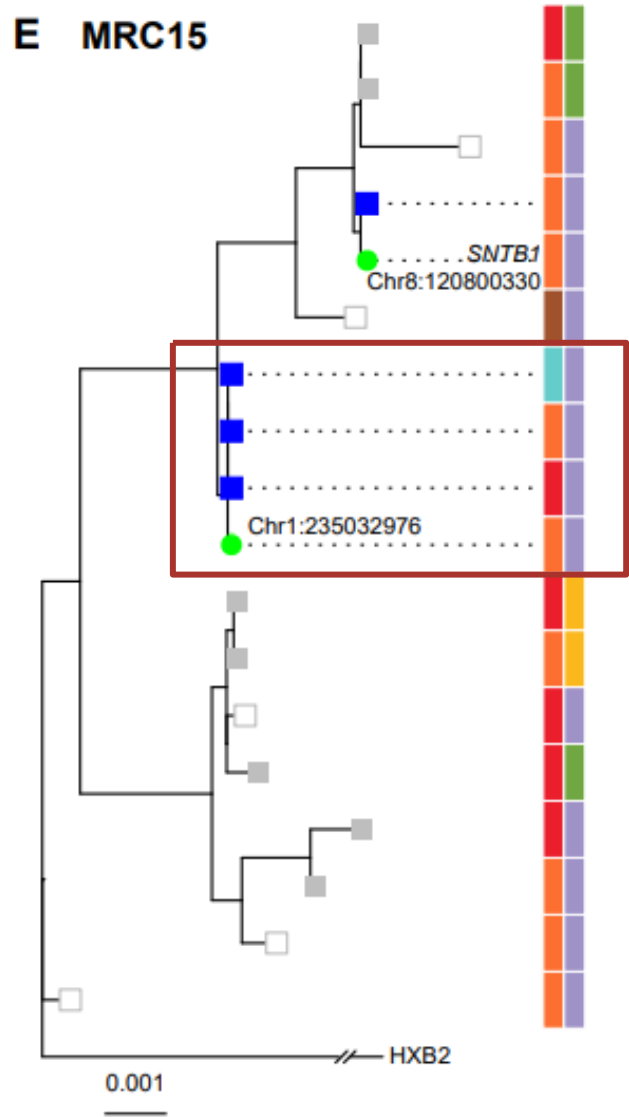
- Similar proportions of intact proviruses are detected between time points and anatomical sites
- For all participants but one (MRC21), all proviruses recovered by HIV-PULSE are predicted to be CCR5-tropic based on their V3 sequence
- The average pairwise distance (APD) is not significantly different between time points and compartments

# Detection of clones between blood and tissues



➤ Clones of infected cells, including those carrying an intact provirus, overlap between time points and anatomical compartments

# Detection of clones between blood and tissues



<b>Dataset</b>	Blood T0	<b>Class</b>	Intact
	Blood T1		Frameshift
	Lymph node T1		PSI/MSD
	Gut T1		
<b>Assay and clonality</b>	Clonal STIP-Seq	<b>Overlaps</b>	STIP-Seq
	Unique STIP-Seq		HIV-PULSE
	Clonal HIV-PULSE		
	Unique HIV-PULSE		

STIP-Seq (Blood T0) = inducible proviruses

- Clones of infected cells, including those carrying an inducible provirus, overlap between time points and anatomical compartments

## Conclusions

- (i) Do translation-competent reservoir cells in matched blood and lymph nodes exhibit distinct phenotypic features? **YES**
  - Blood p24+ cells are found in both TCM/TTM and TEM subsets, lack CXCR5 expression and are enriched in GZMA+ cells.
  - Most lymph node p24+ cells display a TCM/TTM phenotype, with approximately 50% expressing CXCR5 and nearly all lacking GZMA expression.
  - ➔ Plasticity of immune infected cells in response to their microenvironment
  
- (ii) Are these reservoirs enriched in germinal center T follicular helper (GC Tfh) cells in individuals undergoing treatment for more than 10 years? **NO**
  
- (iii) Does near full-length sequencing reveal compartmentalization between blood and tissues? **NO**

# Acknowledgements

## HCRC

**Laurens Lambrechts**

Ytse Noppe

Liesbet Termote

Sofie De Braekeleer

Sarah Gerlo

Linos Vandekerchove

## Janssen

Ellen Van Gulck

Daniel Boden

## Arcturus Therapeutics

Jerel Vega

Jinho Park

## All the participants from the study!



AGENTSCHAP  
INNOVEREN &  
ONDERNEMEN



Flow cytometry and sequencing cores from Ghent and Janssen  
Nurses, doctors and leukapheresis core from UZ Ghent