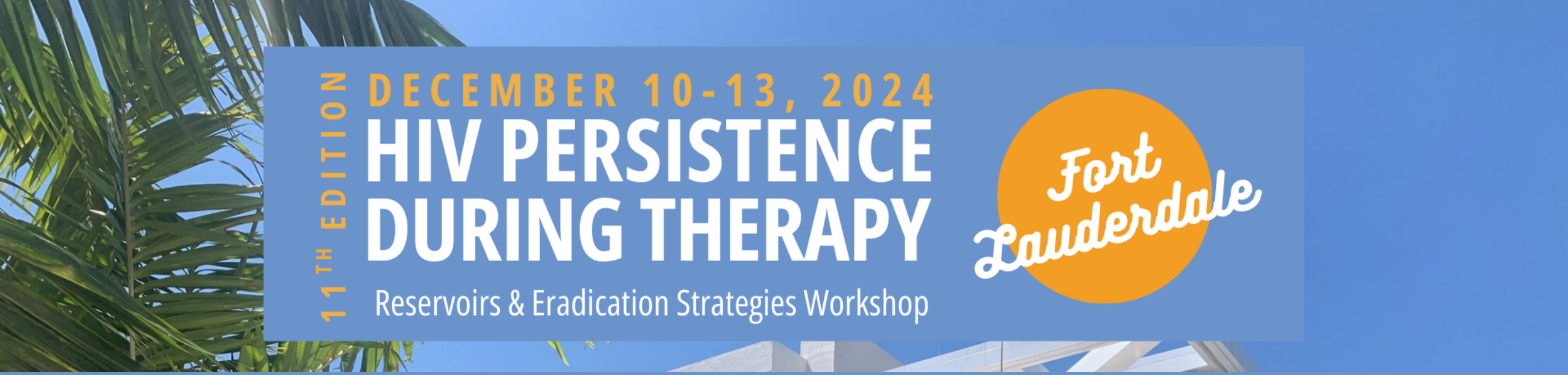

Longitudinal analysis in early treated individuals reveals alteration in the HIV-1 integration site landscape and composition of the inducible reservoir

Tine Struyve[1], Marion Pardons[1], Jozefien De Clercq[1], Liesbet Termote[1], Laurens Lambrechts[1], Ytse Noppe[1], Mathias Lichterfeld[2], Sofie Rutsaert[1], Linos Vandekerckhove[1]

[1]HIV Cure Research Center, Department of Internal Medicine and Pediatrics, Ghent University Hospital, Ghent University, 9000 Ghent, Belgium
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CONFLICTS OF INTEREST

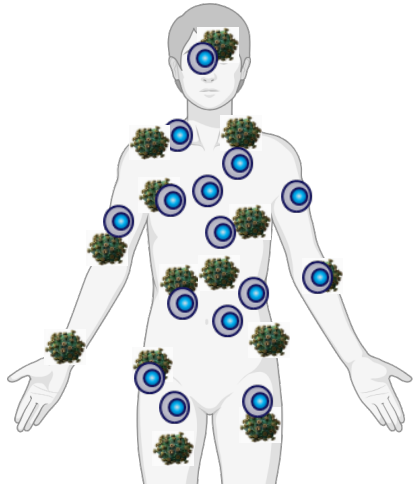
Tat-LNP is provided by Janssen Pharmaceutica

Introduction

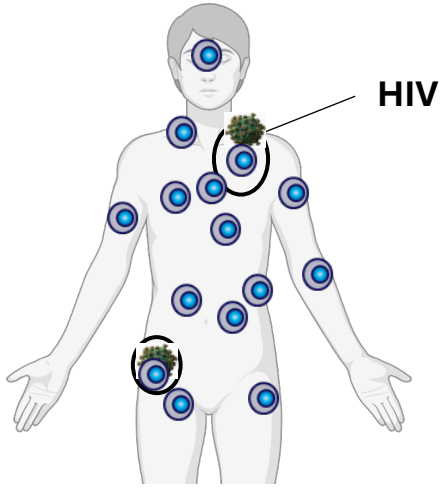
HIV-1

CD4 T-cell

Major barrier to a cure



Person living with HIV
no therapy

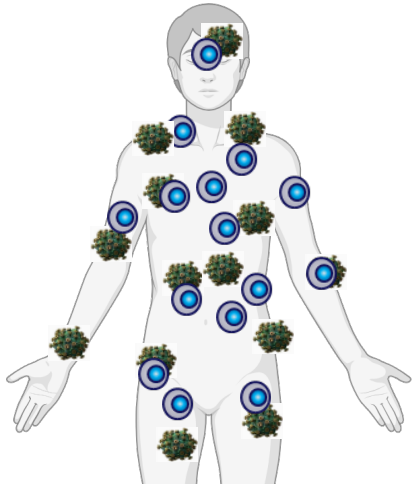


Person living with HIV
on therapy

Early initiation of ART:

- Limits seeding of the viral reservoir
(Archin et al. 2012, Buzon et al. 2014)

- Limits genetic diversity
(Josefsson et al. 2013, Kearney et al. 2014)

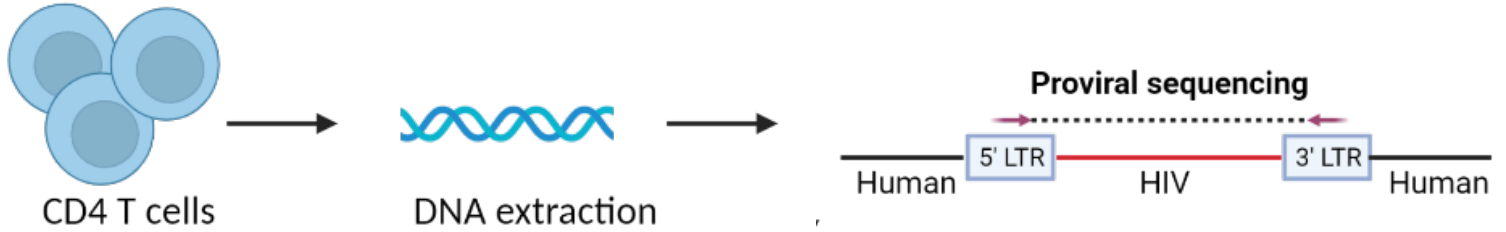


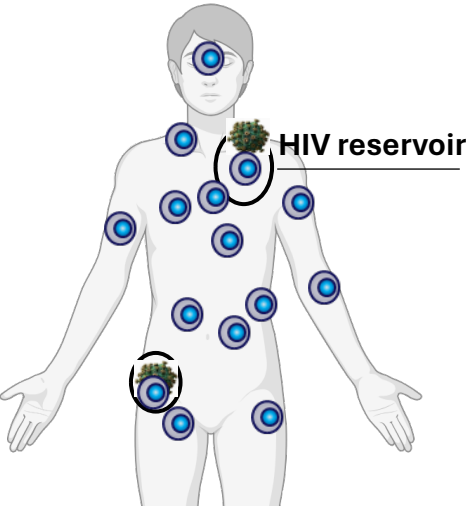
Treatment interruption
→ viral rebound
(Colby et al. 2018, Henrich et al. 2017)

Study the **composition** of the **viral reservoir** on ART and the mechanisms contributing to its **persistence** in **early-treated people living with HIV**.

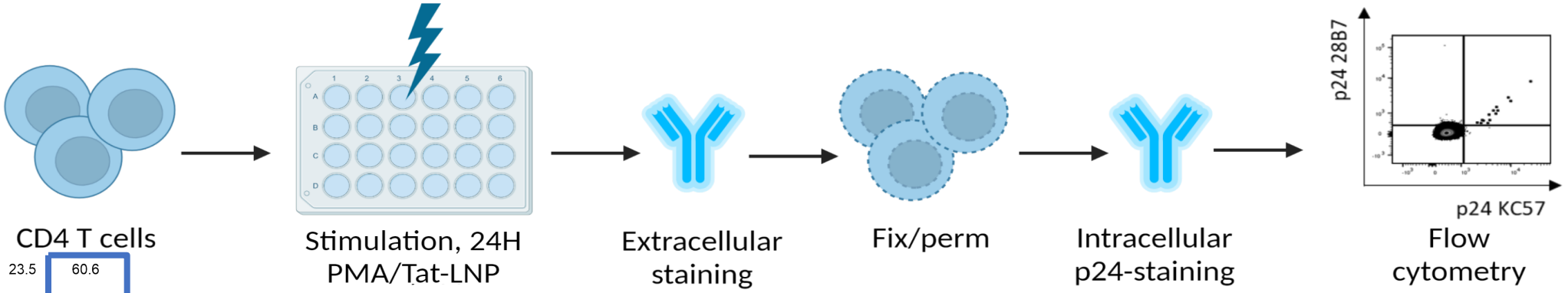
Aims of the study – longitudinal study in early-treated people

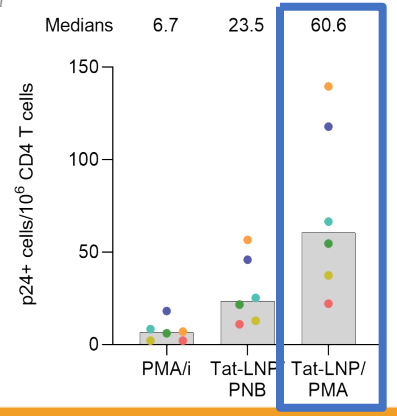
HIV-1 integration site landscape



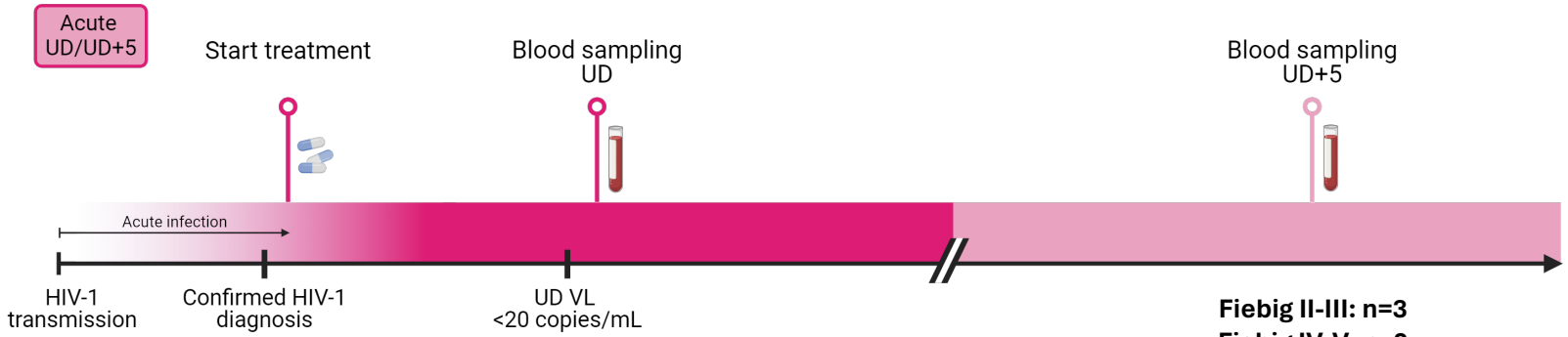


HIV-1 inducible reservoir





Participants sampling timeline



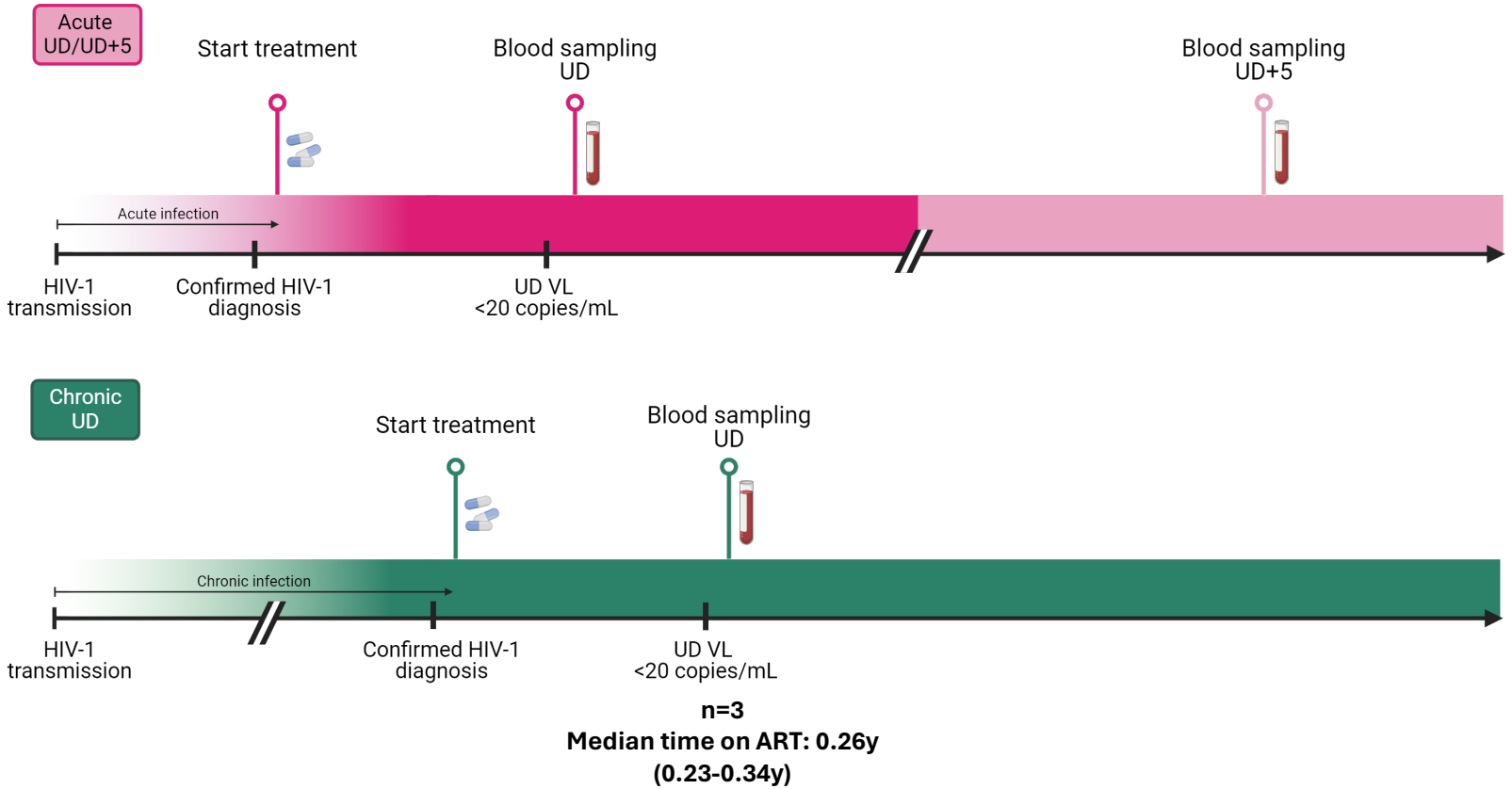
Fiebig II-III: n=4
Fiebig IV-V: n=4

Median time on ART: 0.72y
(0.31-1.93y)

Fiebig II-III: n=3
Fiebig IV-V: n=2

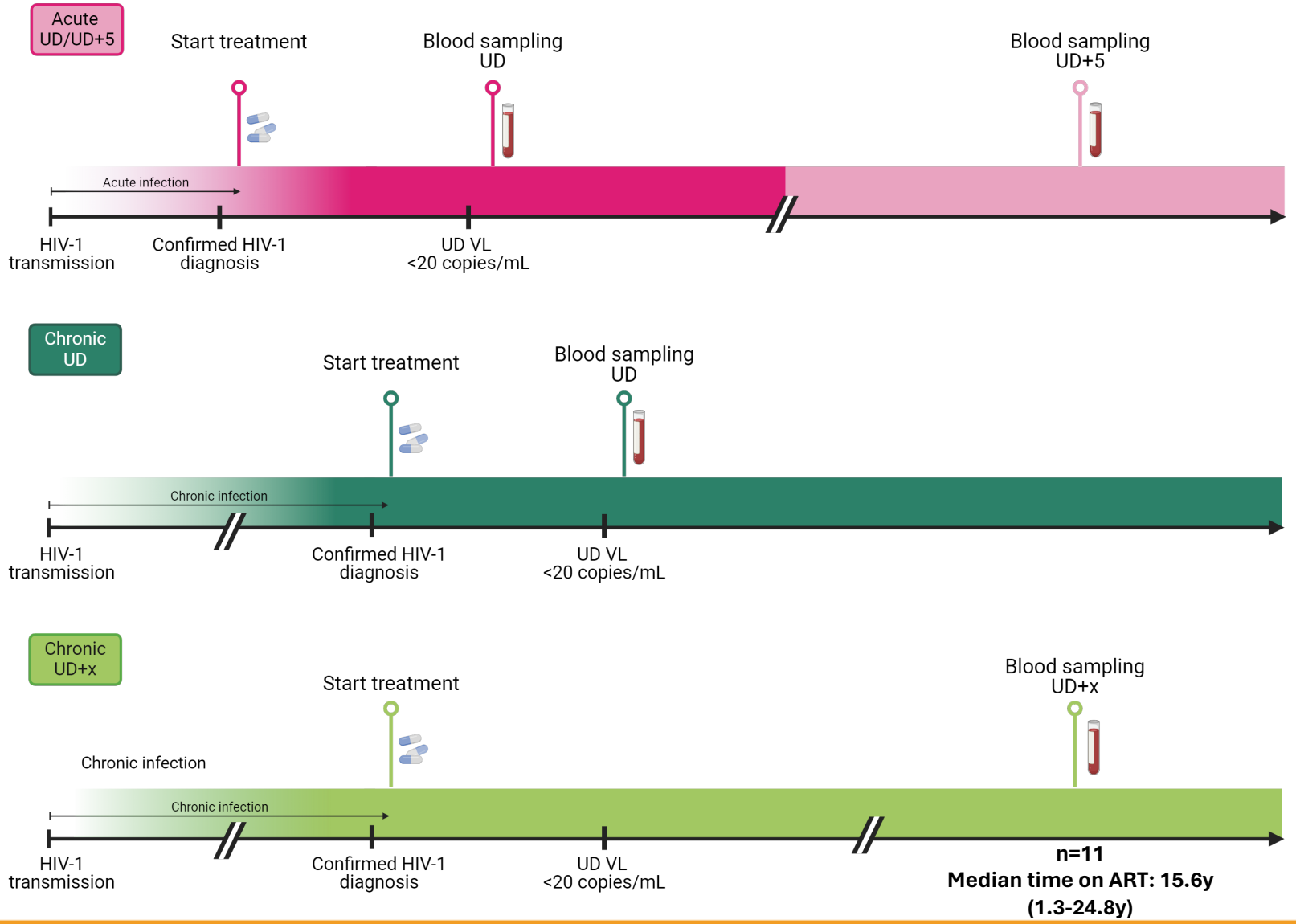
Median time on ART: 5.3y
(3.41-7.42y)

Participants sampling timeline



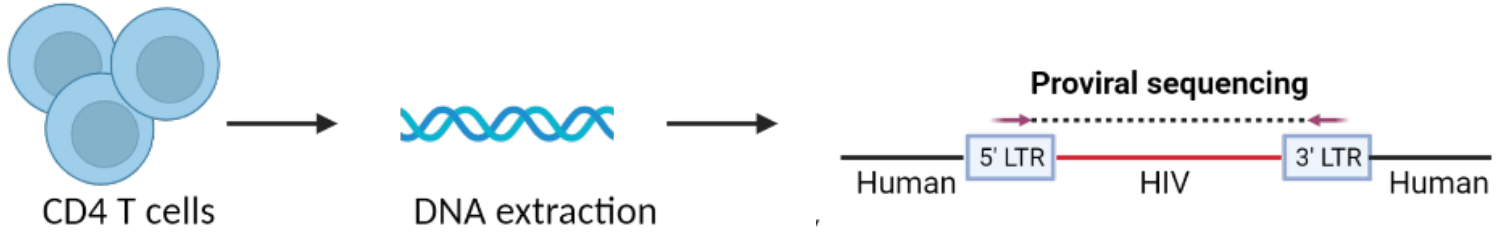
n=3
Median time on ART: 0.26y
(0.23-0.34y)

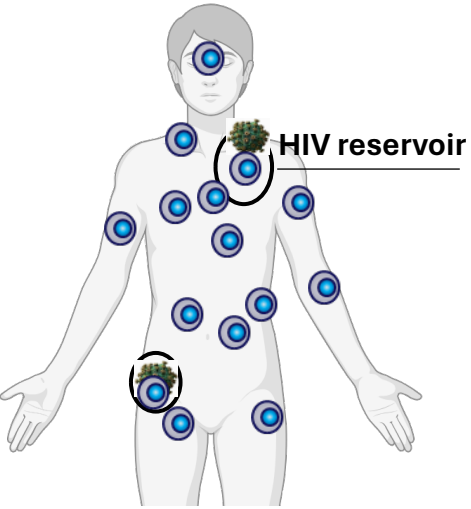
Participants sampling timeline



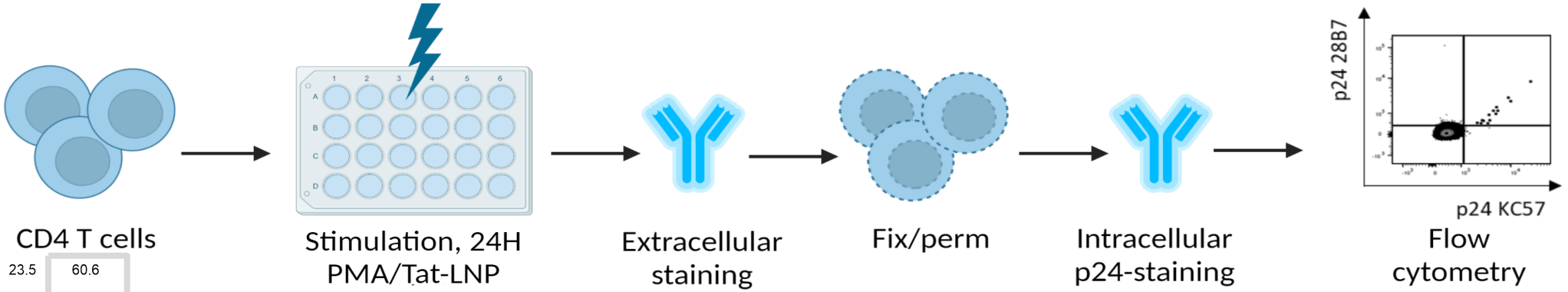
Aims of the study – longitudinal study in early-treated people

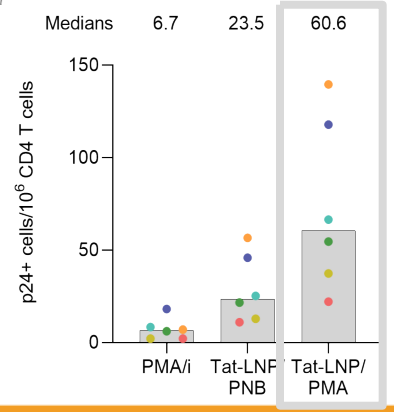
HIV-1 integration site landscape



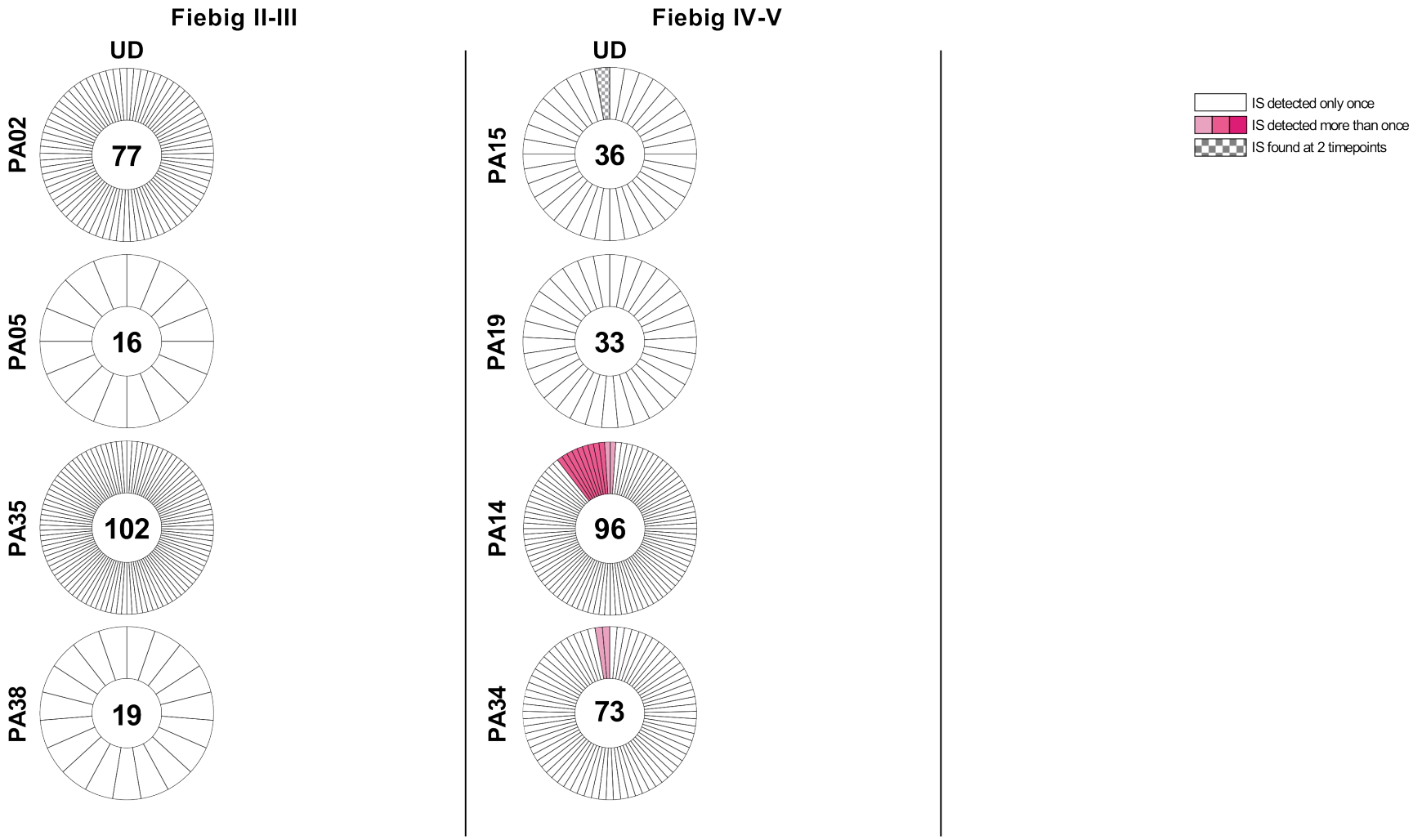


HIV-1 inducible reservoir

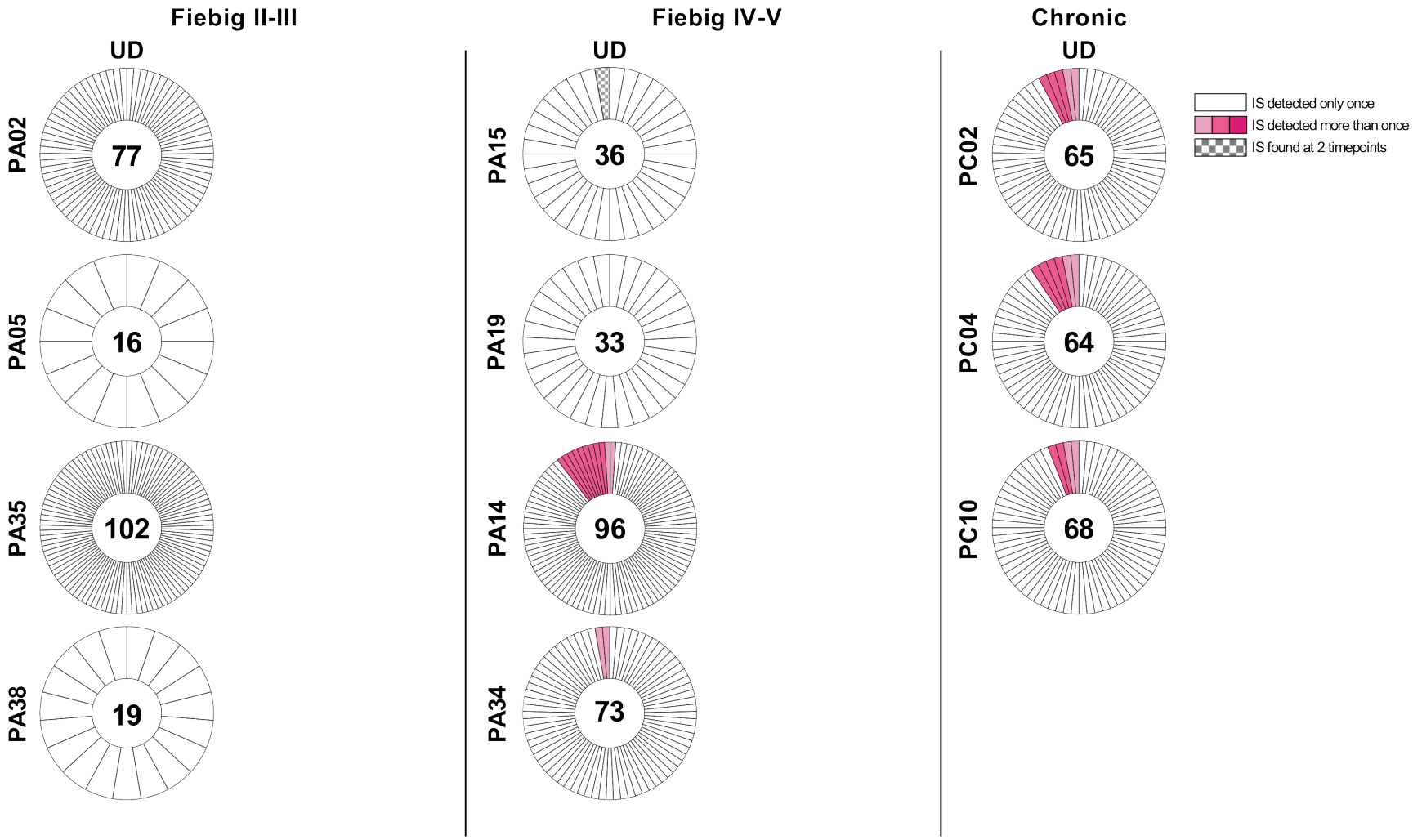




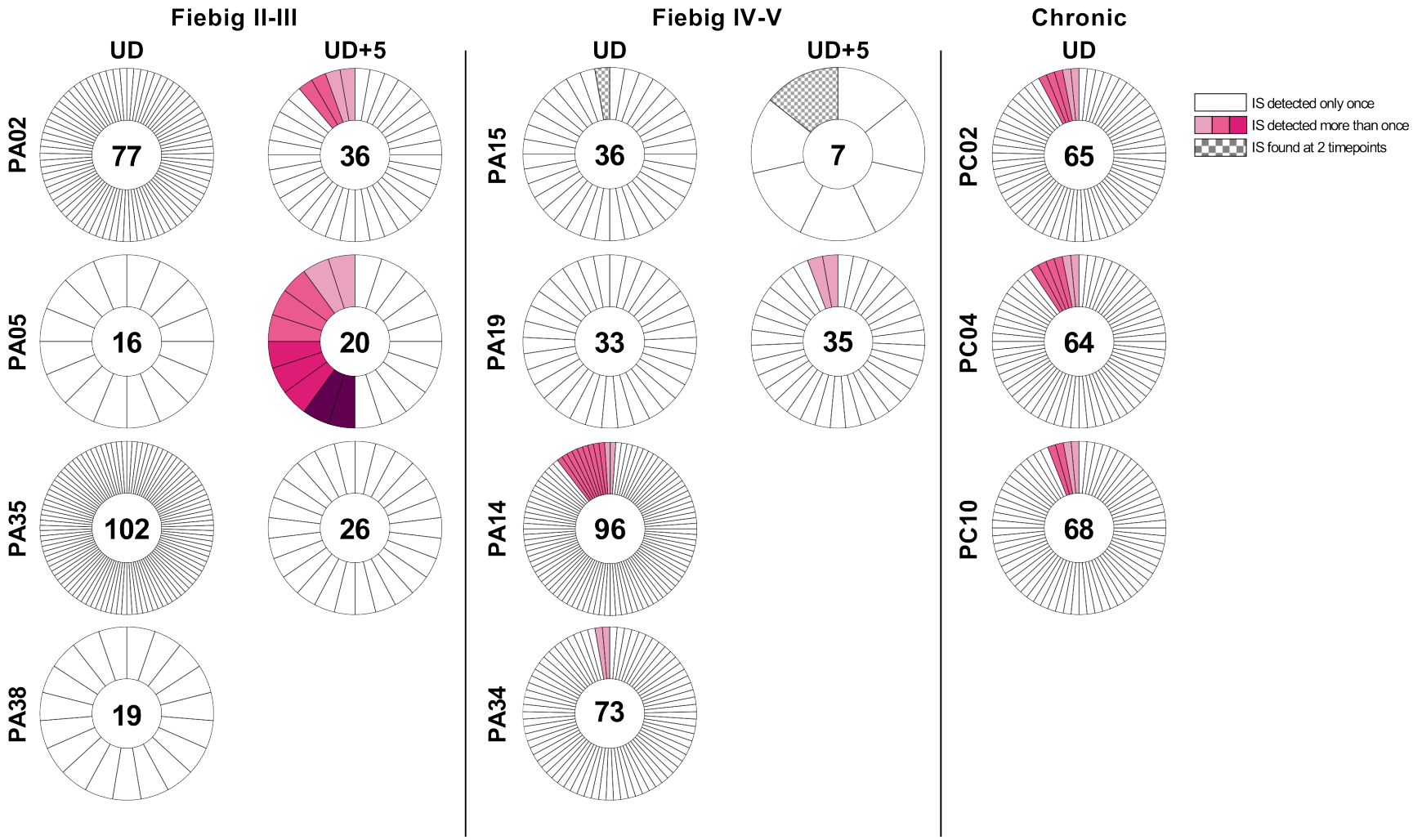
Clones appear after time on ART in early-treated individuals



Clones appear after time on ART in early-treated individuals

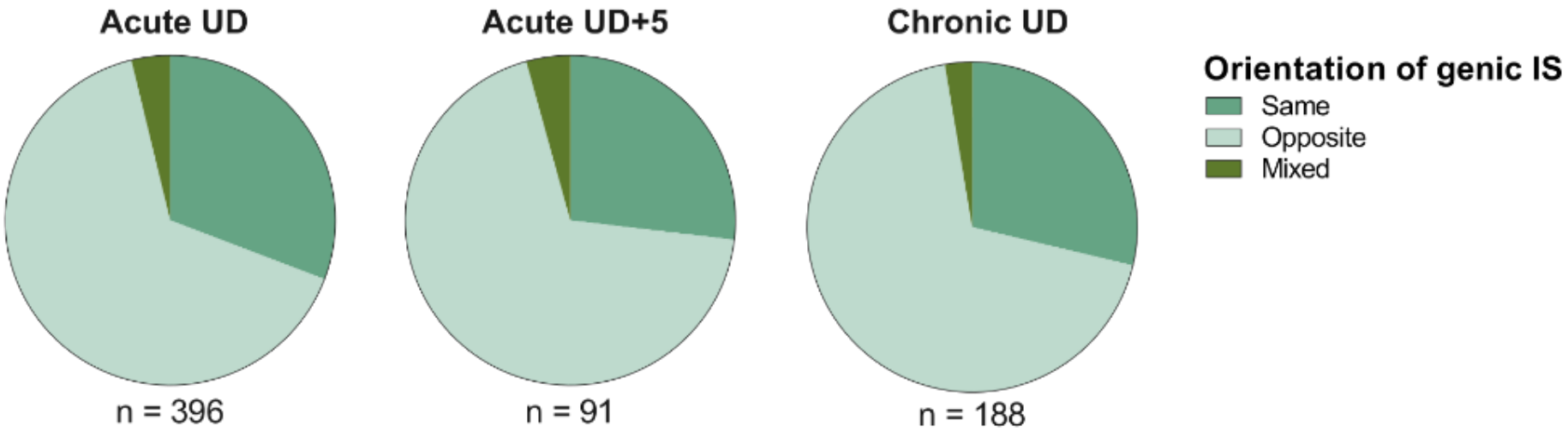


Clones appear after time on ART in early-treated individuals



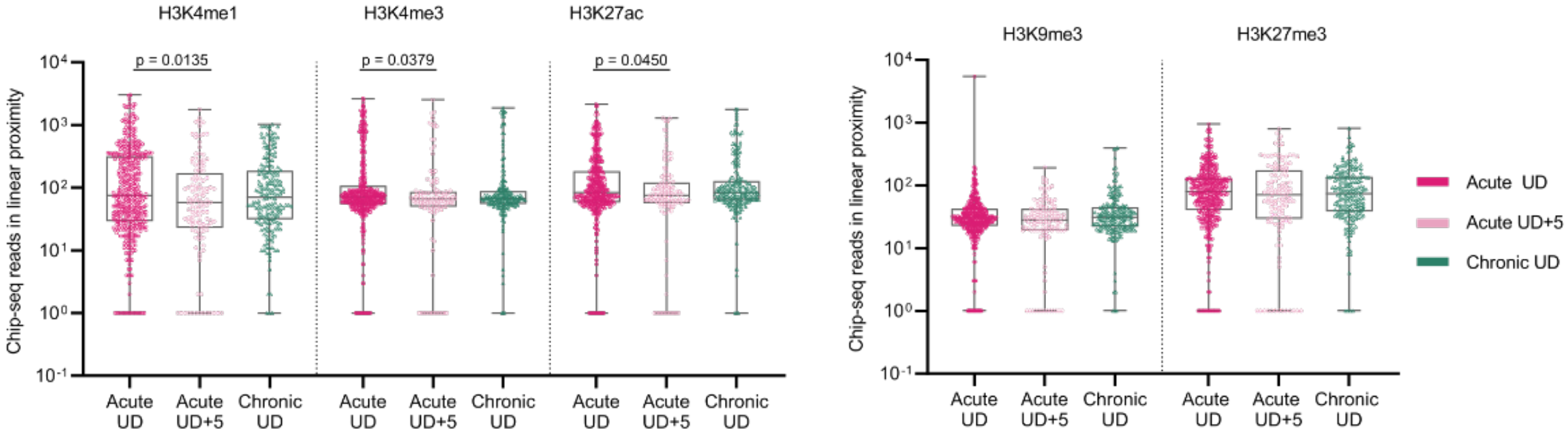
These observations suggest that the detection of clones increases with (i) time since infection and (ii) time on ART.

Progressive selection of proviruses in heterochromatin regions over time on ART in early-treated people



A significant increase in the proportion of proviruses integrated into centromeric/satellite DNA after five years on ART.

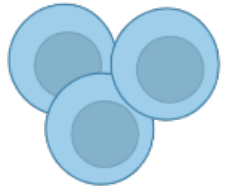
Progressive selection of proviruses in heterochromatin regions over time on ART in early-treated people



- At UD: similar integration site patterns irrespective of treatment initiation timing.
- A significant decrease of proviruses integrated in regions with active transcription after five years on ART.

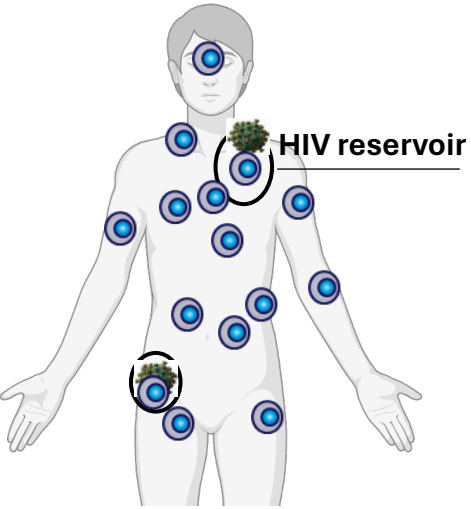
Aims of the study – longitudinal study in early-treated people

HIV-1 integration site landscape

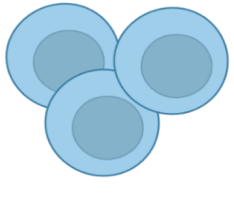


CD4 T cells

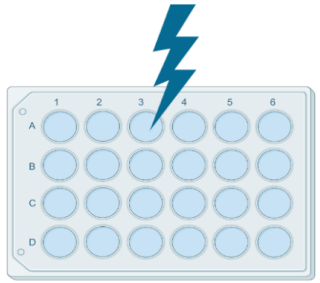
DNA extraction



HIV-1 inducible reservoir



CD4 T cells

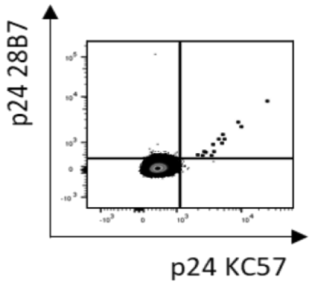


Stimulation, 24H
PMA/Tat-LNP

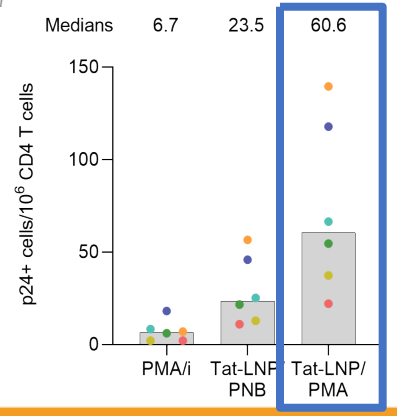
Extracellular staining

Fix/perm

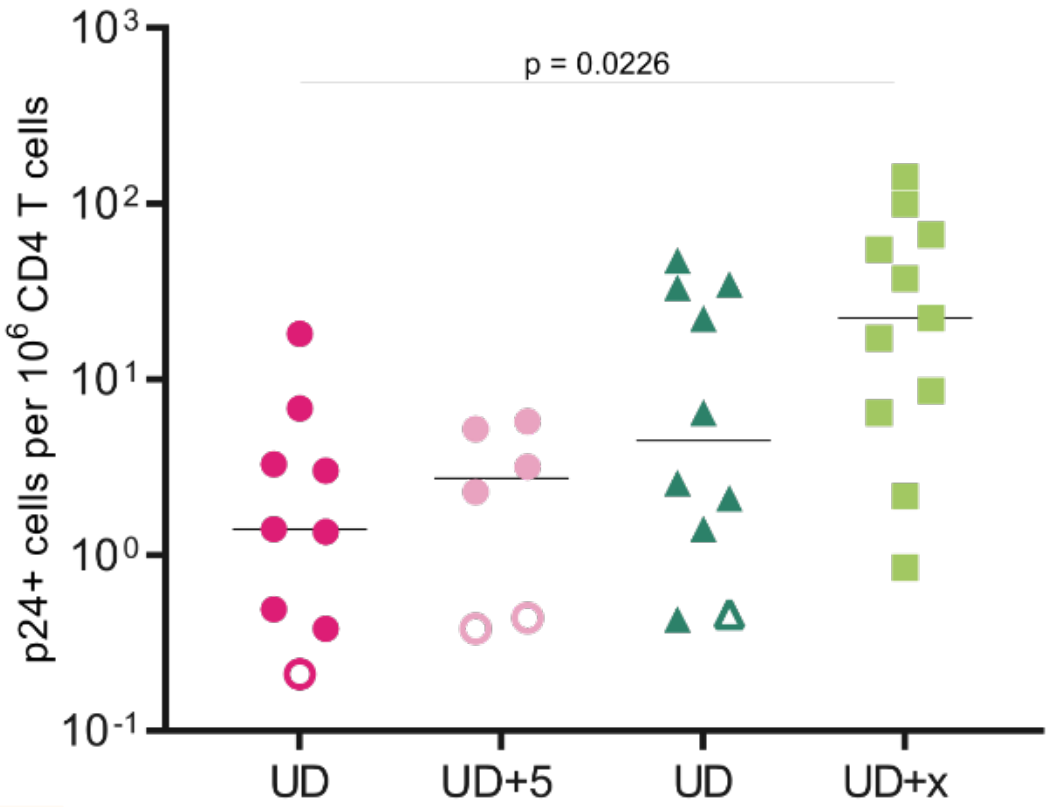
Intracellular p24-staining



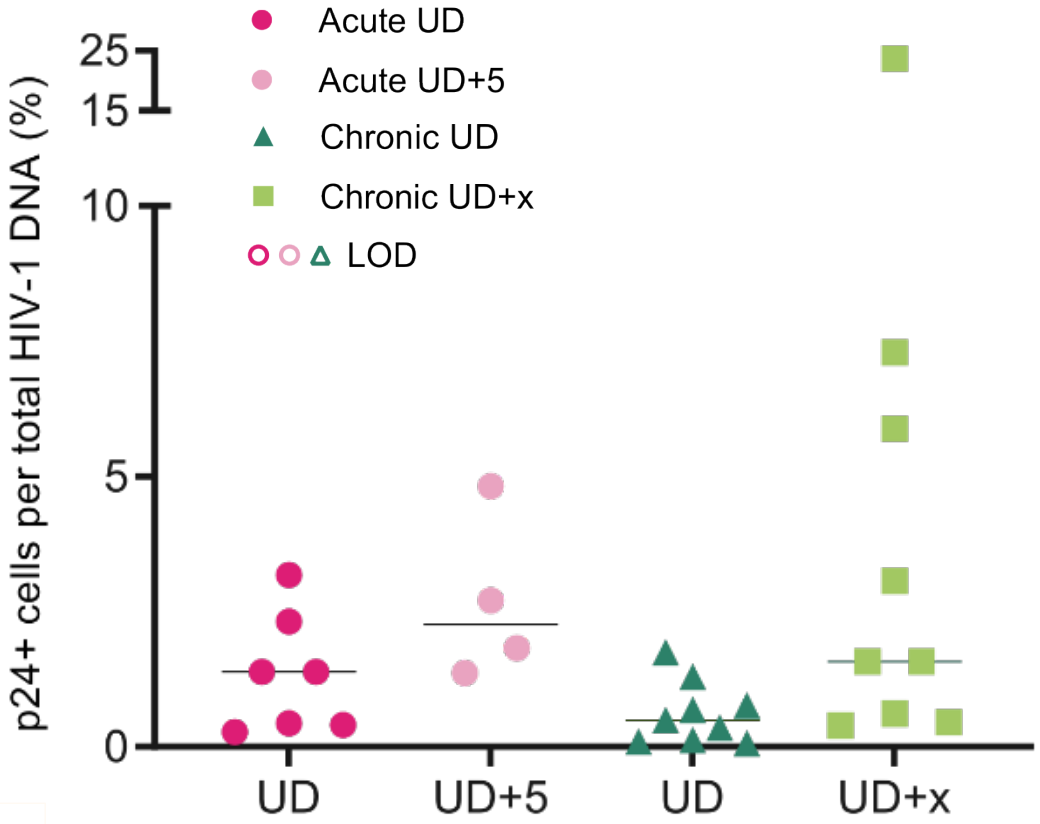
Flow cytometry



Inducibility of the viral reservoir in acute and chronic cohorts

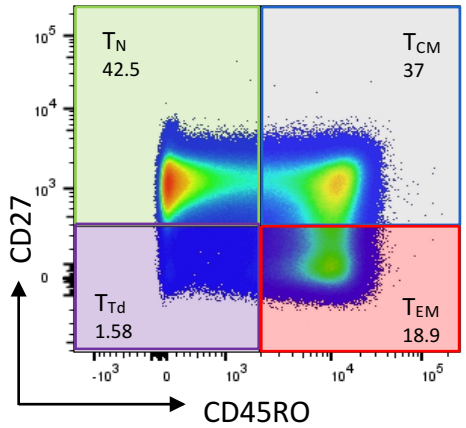


Trend towards lower frequency of p24+ cells in the acute cohorts compared to chronic cohorts.

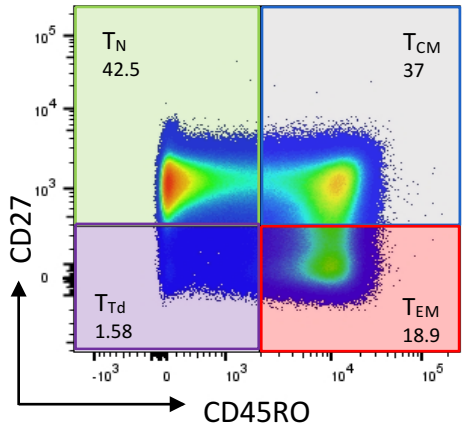


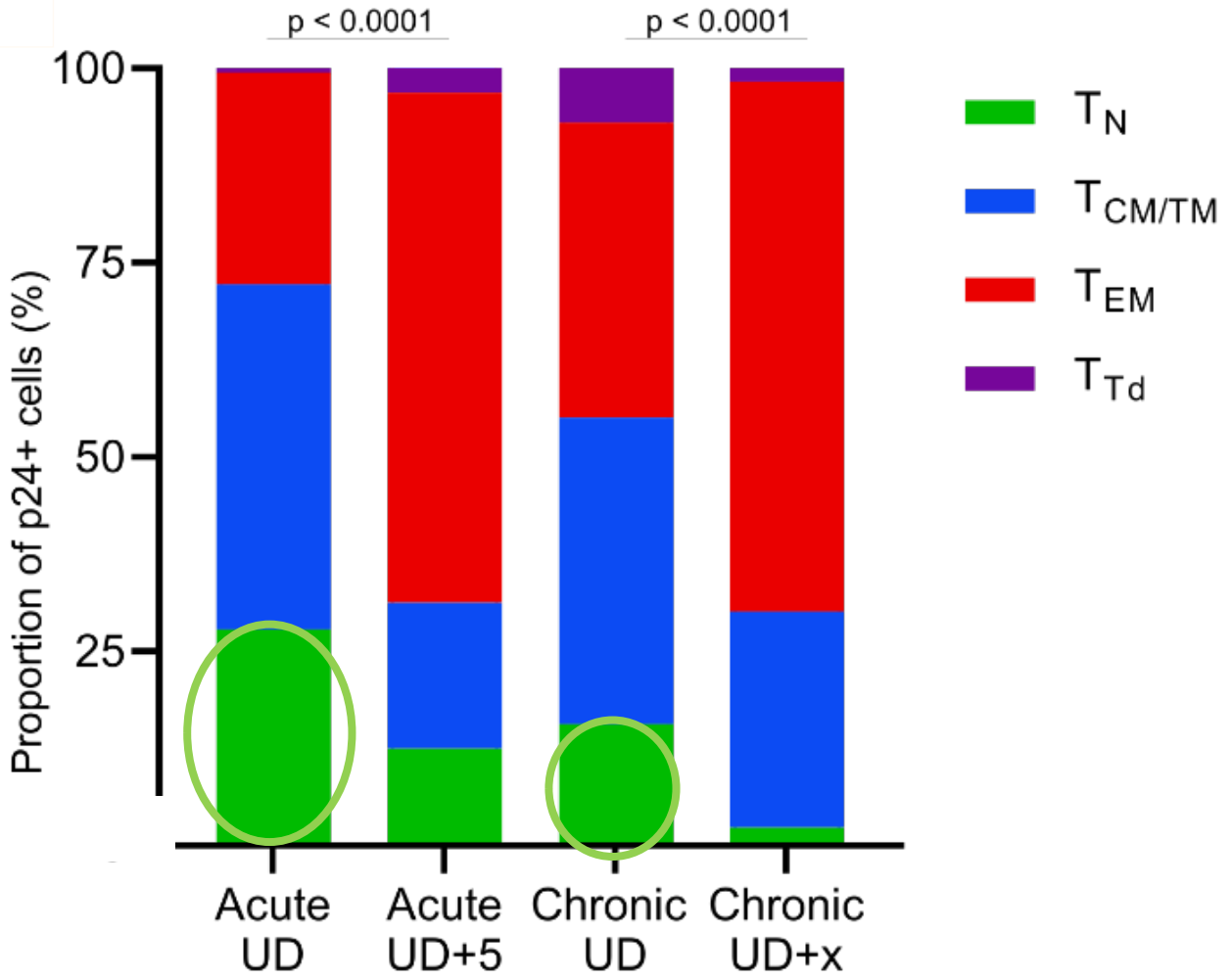
Similar proportions of infected cells with an inducible provirus between all cohorts.

Phenotypic differentiation of p24+ cells is observed after time on ART



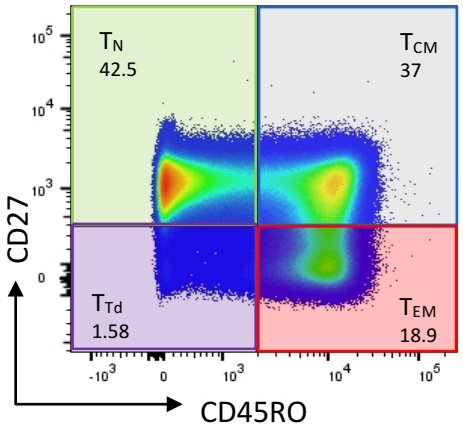
Phenotypic differentiation of p24+ cells is observed after time on ART

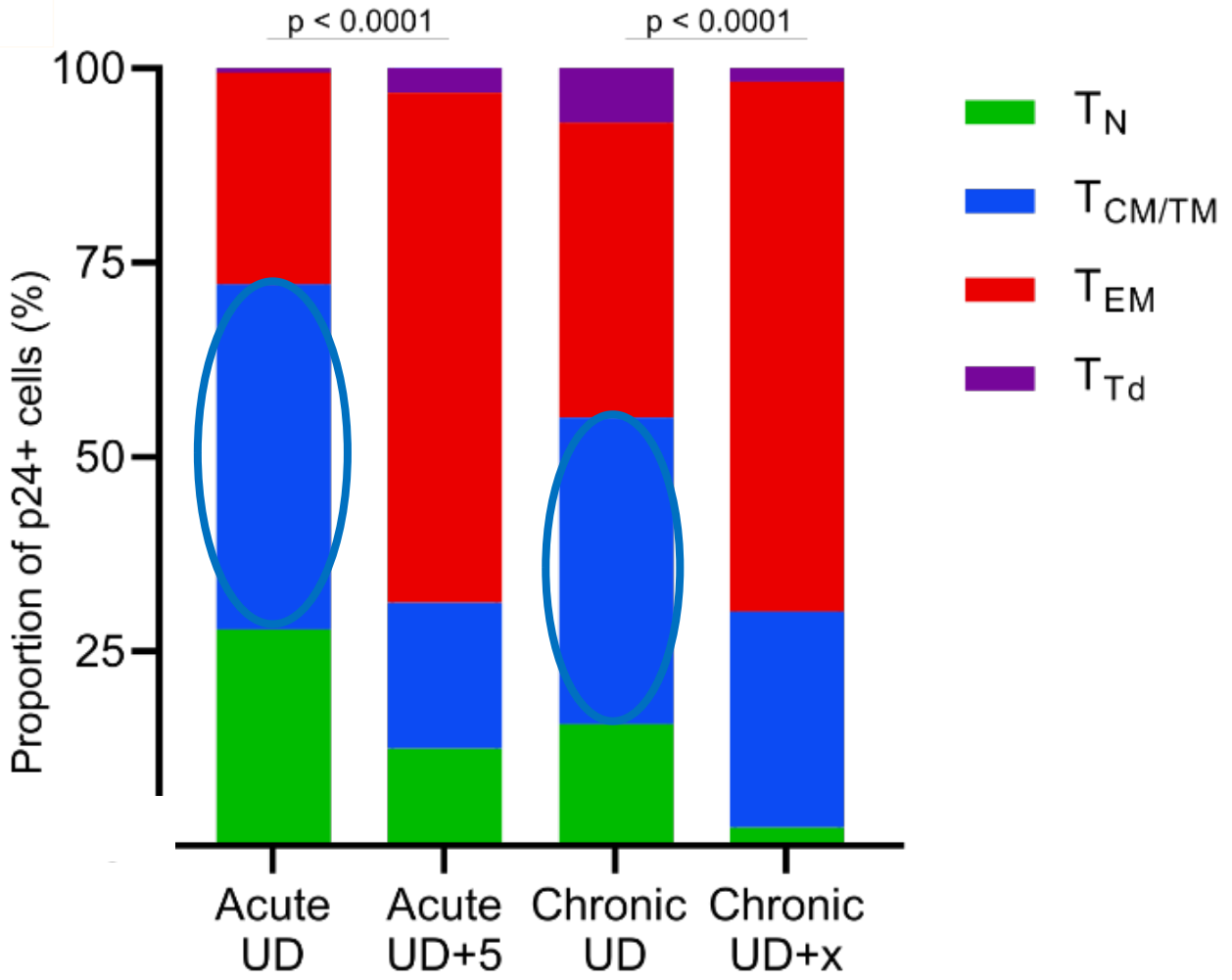




The acute and chronic cohorts at UD displayed a higher fraction of p24+ cells residing in the **naïve (TN)** subset compared to the UD+5/UD+x timepoints.

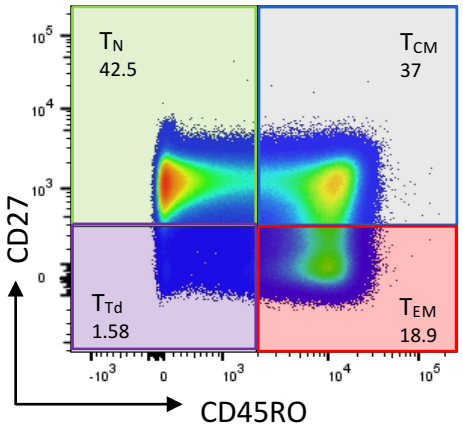
Phenotypic differentiation of p24+ cells is observed after time on ART

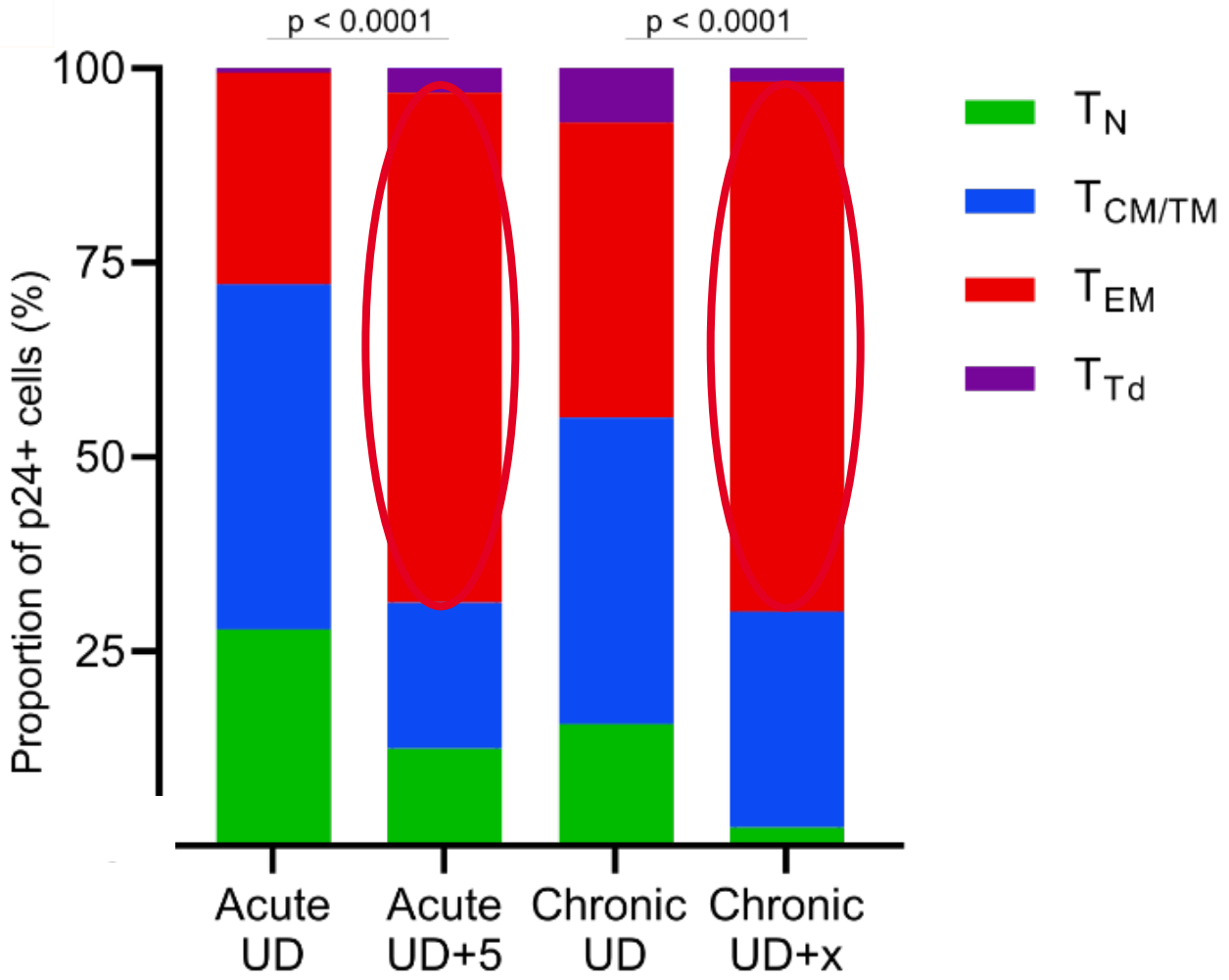




The **same trend** was observed for the proportion of p24+ cells residing in the central memory/transitional memory (**TCM/TTM**) subsets.

Phenotypic differentiation of p24+ cells is observed after time on ART



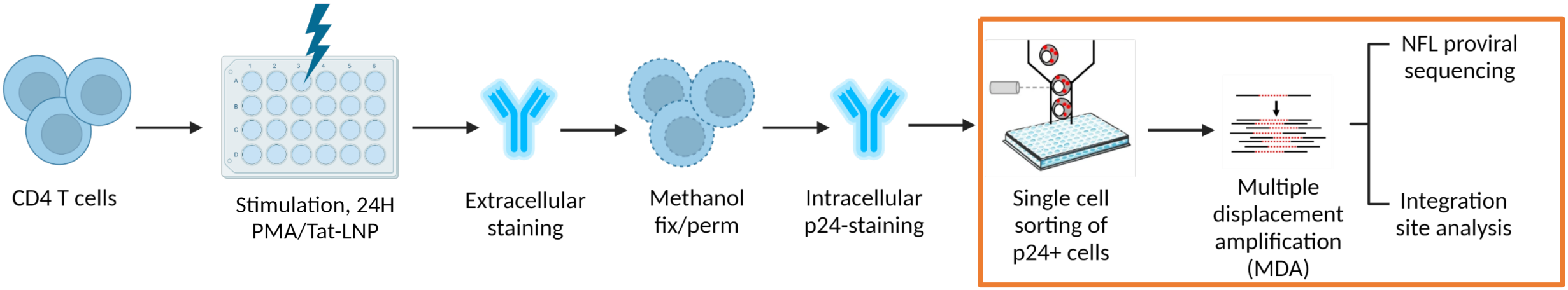


In contrast, the Acute UD+5 and Chronic UD+x groups displayed a higher frequency of p24+ cells residing in the **TEM** fraction compared to the Acute UD and Chronic UD groups.

→ **Shift** in the subset composition of the inducible reservoir towards **more differentiated** cellular phenotypes

Integration sites and proviral sequences from single p24+ cells

Inducible reservoir

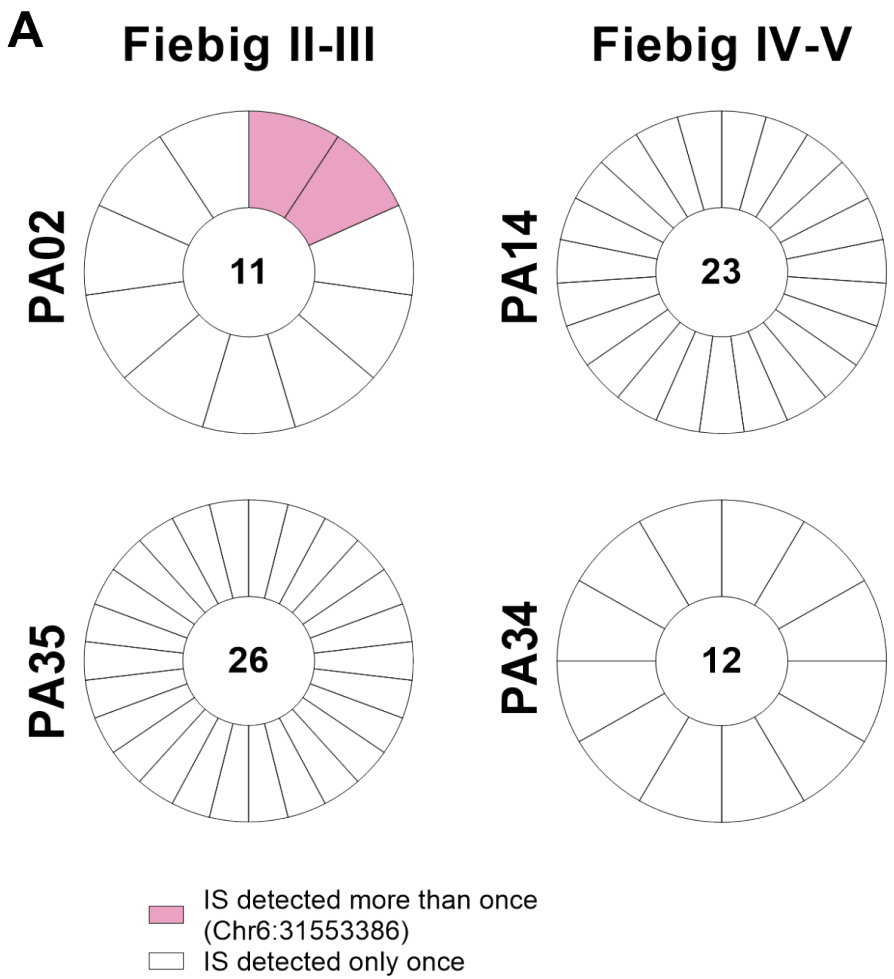


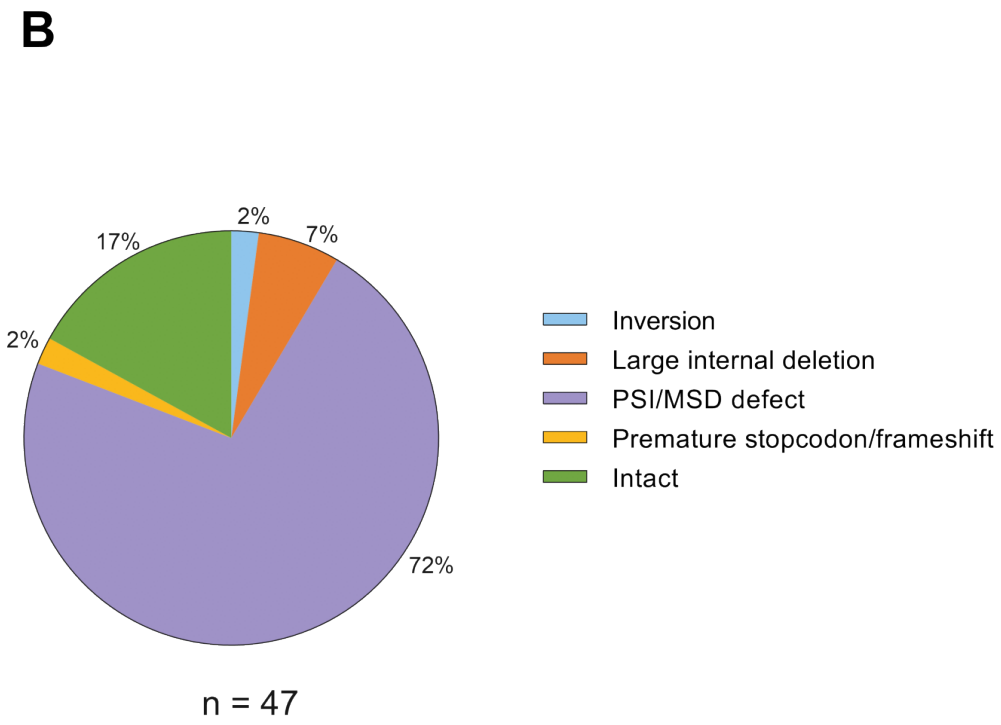
STIP-Seq (Cole et al., 2021)

Acute UD

4 participants with the highest frequencies of p24+ cells

Integration sites and proviral sequences from single p24+ cells

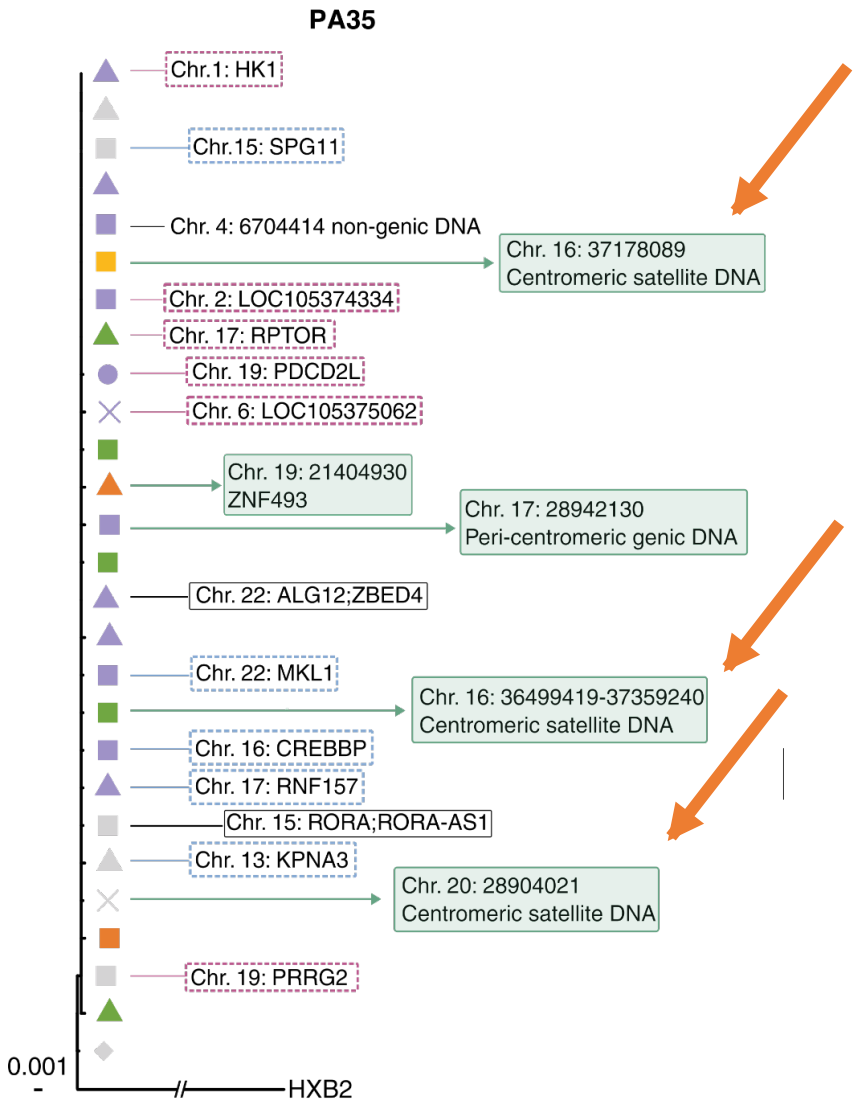




Limited clonal expansion among p24+ cells.
~ IS from bulk CD4 T cells

Majority of proviruses harbor PSI/MSD defects.

Integration sites and proviral sequences from single p24+ cells



Proviruses in centromeric regions can be reactivated from latency following PMA/Tat-LNP stimulation.

Subset
- T_N
- $T_{CM/TM}$
- T_{EM}
- T_{Td}
- Unknown

NFL class
- Incomplete
- Intact
- Large deletion
- Premature stopcodon/frameshift
- PSI/MSD deletion

Integration site
- Same orientation
- Opposite orientation
- Mixed orientation
- (Peri-) centromeric DNA/ZNF gene

Conclusions

- **Early treatment** initiation **limits clonal** expansion.
- **Progressive enrichment** of proviruses integrated in **heterochromatin regions** with time on ART in the acute cohort.
- **PMA/Tat-LNP** enables the detection and characterization of the **inducible reservoir** in participants with **small reservoir sizes.**
- **Lower** inducible reservoir **size**, but **similar inducibility** in acute versus chronic cohorts.
- Shift towards a higher proportion of p24-expressing cells with a **more differentiated phenotype** after time on ART.

Acknowledgement

All the participants from the study

HCRC
Marion Pardons
Liesbet Termote
Jozefien De Clercq
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Ytse Noppe
Sofie Rutsaert
Linos Vandekerckhove

Ragon Institute
Mathias Lichterfeld

Flow cytometry core from Ghent University and
NXTGNT sequencing core

HIV CURE Research Center

GHENT UNIVERSITY

FACULTY OF MEDICINE AND HEALTH SCIENCES

UZ GENT

NIH
National Institutes of Health

fwo
Research Foundation Flanders
Opening new horizons