Characterization of the HIV-1 viral reservoir in subtype B early treated individuals

Tine Struyve[1], Marion Pardons[1], Liesbet Termote[1], Jozefien De Clercq[1], Laurens Lambrechts[1], Jerel Vega[2], Daniel Boden[3], Mathias Lichterfeld[4], Sofie Rutsaert[1], Linos Vandekerckhove[1]

*[1]**HIV Cure Research Center**, Ghent University, Ghent, Belgium; [2]Arcturus Therapeutics, Science Center Drive, 92121 San Diego, California; [3]Janssen Biopharma, Johnson and Johnson, South San Francisco, USA; [4]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA*

16/12/2022

CONFLICTS OF INTEREST

The Tat#1 molecule is provided by Janssen.

Introduction

- Early ART initiation limits the size of the viral reservoir, although it does not prevent viral rebound after treatment interruption
  (Archin et al. Proc Natl Acad Sci USA 2012, Buzon et al. J Virol. 2014, Colby et al. Nat Med 2018, Leyre et al. Sci Trans Med 2020)

Introduction

- Early ART initiation limits the size of the viral reservoir, although it does not prevent viral rebound after treatment interruption
  (Archin et al. Proc Natl Acad Sci USA 2012, Buzon et al. J Virol. 2014, Colby et al. Nat Med 2018, Leyre et al. Sci Trans Med 2020)

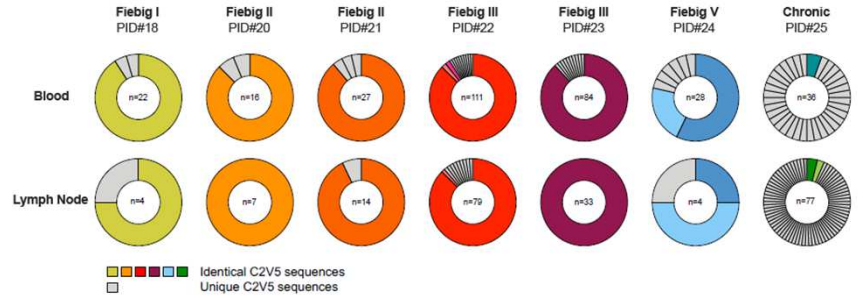
- Integration site analyses:
  - Clonal expansion is rare during acute infection
    (Coffin et al. JCI Insight 2019, Wu et al. JCI Insight 2020, Gantner et al. Preprint 2022)
- Proviral genome analyses:
  - Sequence diversity is low during acute infection
    (Bruner et al. Nat Med 2016, Lee et al. Nat Commun 2019, *Gantner et al. Preprint 2022)*

Table 2. Pre-ART cell-associated HIV DNA, integration sites, and clones in PBMCs from the donors put on ART shortly after infection (groups 1 and 2)

Group	Stage (PID)	HIV DNA[A]	Cells (×10⁶) used for ISA	Unique integration sites	Sites with >1 breakpoint	Clones confirmed
1a	FIII (CH 83-1)	50,000	3	1,409	1	0
	FIII (CH 84-4)	1,700	1.5	176	2	0
	FIV PIT-001	14,000	1.5	149	1	0
	Average per 10⁶ cells	22,000		290	0.67	0
	SD FIII–IV	25,000		210	0.51	0
1b	FIV/V (CH 62-1)	18,000	4.5	709	14	9
	FV (CH 68-5)	5,600	1.5	366	1	0
	FV (CH 91-4)	4,800	4.5	416	3	1
	FV (CH 98-6)	1,900	3	179	2	2
	Average per 10⁶ cells	7,600		120	1.5	0.87
	SD FIV/V–V	7,100		42	1.2	0.9
2	FV (IDFU-192)	17,000	1.5	409	3	0
	FV (JRI)	1,500	3	193	1	1
	FV/VI (AVBIO2-14)	4,300	1.5	144	0	0
	FV/VI (AVBIO2-23)	21,000	1.5	405	3	0
	FVI (AVBIO2-07)	2,100	1.5	48	0	0
	Average per 10⁶ cells	9,200		130	0.89	0.11
	SD	9,100		120	0.94	0.15

[A]Per million cells. F, Fiebig; PID, patient identifier; ISA, integration site analysis.

Coffin et al., JCI Insight 2019



Gantner et al. Preprint 2022

Aims of the study

In early treated individuals:

• The **integration site** and **intactness** of proviral genomes remain **poorly characterized**.

• The **inducible** viral reservoir has been **understudied** due to the **lack** of **latency reversing agents** (LRA) capable of inducing **potent HIV reactivation**.

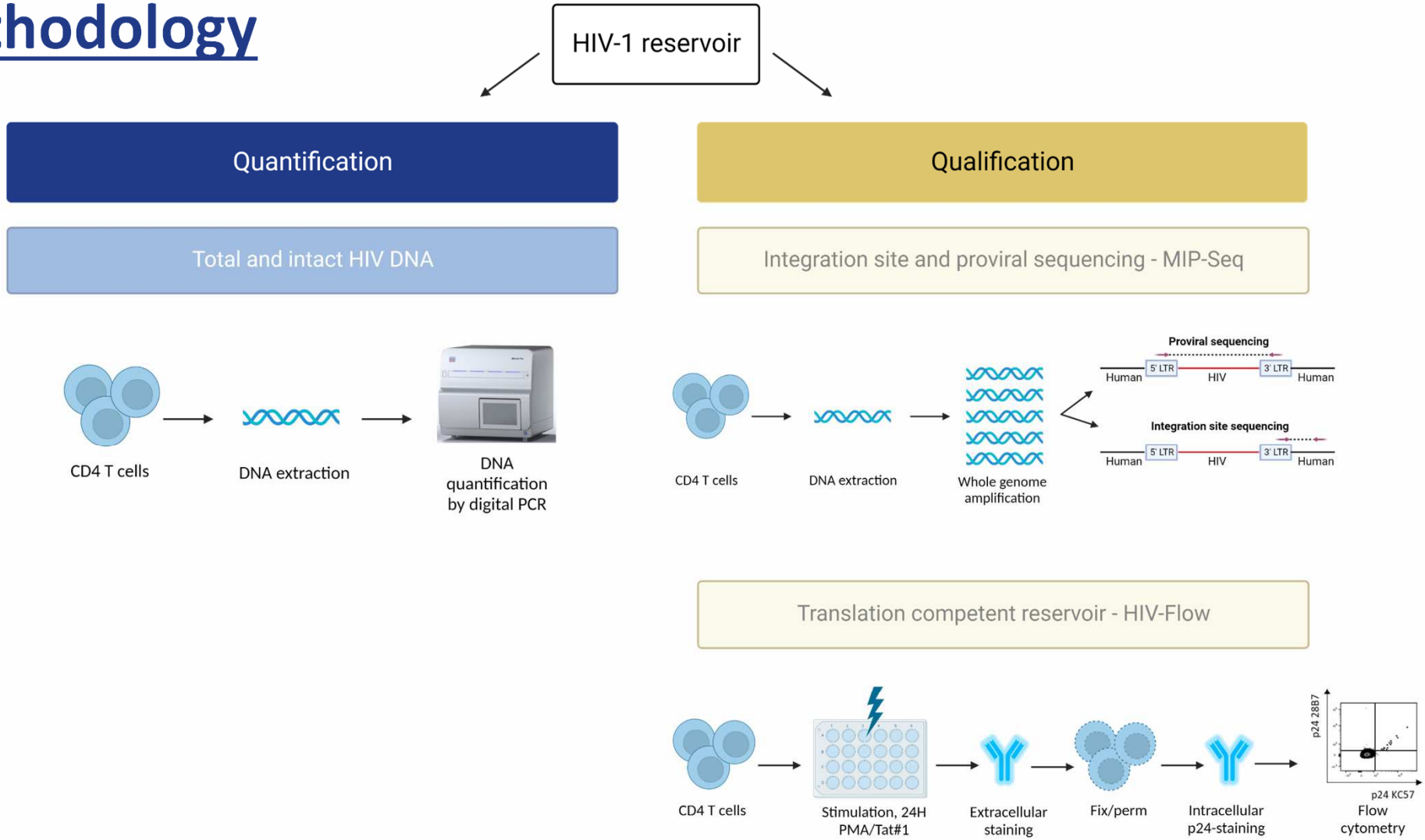
Here, we did an **in-depth assessment** of the **total** and **inducible** viral reservoir in **9 early treated individuals after 1 year of treatment**.

Clinical characteristics of the participants

ID	Age	Gender	Subtype	Fiebig	Time since infection (years)	Time to ART (days)	Time to UD VL (years)	ART duration (years)
ACS002	35	Male	B	Fiebig II-III	1,95	8	1,12	1,93
ACS005	35	Male	B	Fiebig II-III	1,62	7	1,37	1,61
ACS105	33	Male	B	Fiebig II-III	0,79	2	0,32	0,78
ACS107	39	Male	B	Fiebig V	0,97	6	0,75	0,96
ACS108	43	Male	B	Fiebig II-III	0,98	14	0,27	0,94
ACS114	43	Male	B	Fiebig IV	0,52	10	0,16	0,49
ACS404	58	Male	B	Fiebig II-III	0,40	NA	NA	NA
ACS405	46	Male	B	Fiebig II-III	0,48	NA	NA	NA
ACS408	28	Male	B	Fiebig II-III	0,32	NA	NA	NA

- All participants are men and subtype B
- Mostly Fiebig II-III
- ART duration: 0.49 - 1.93 years

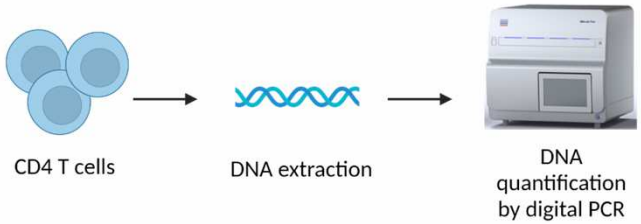
Methodology



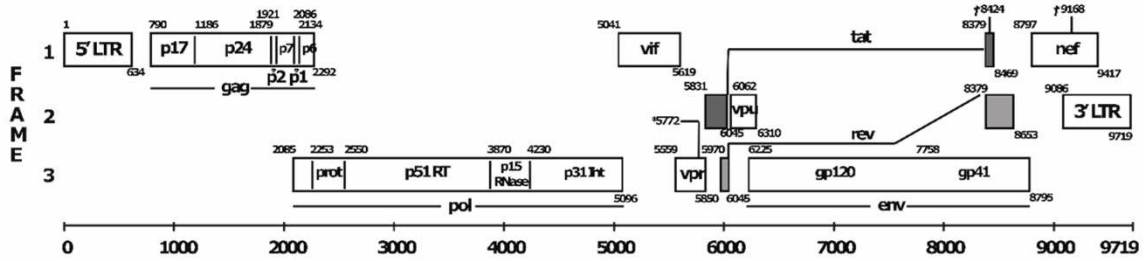
Quantification of the viral reservoir: methodology

Quantification

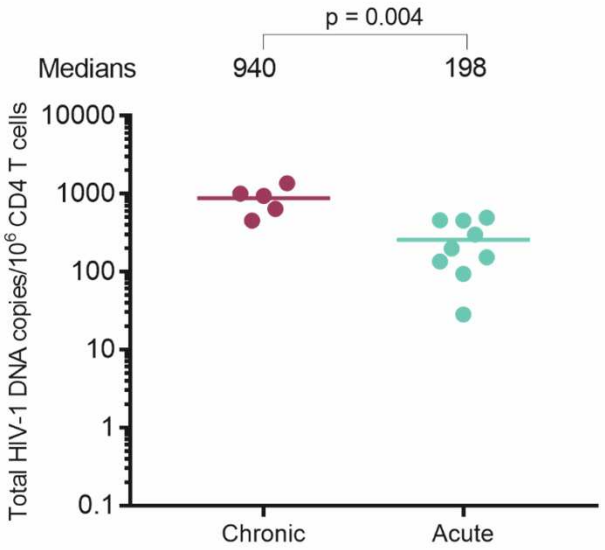
Total and intact HIV DNA



QIAcuity (Qiagen)

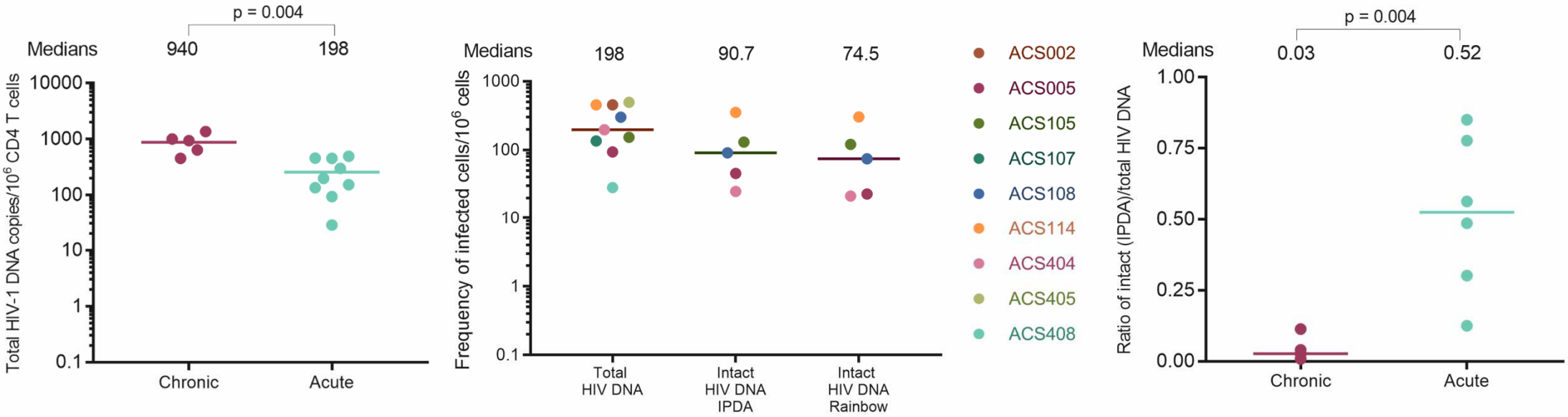


Early ART initiation limits the size of the viral reservoir



- In early treated individuals, **the frequency of total HIV-1 DNA copies per million CD4 T cells** is **lower** than in individuals who initiated ART in chronic infection.

Early ART initiation limits the size of the viral reservoir

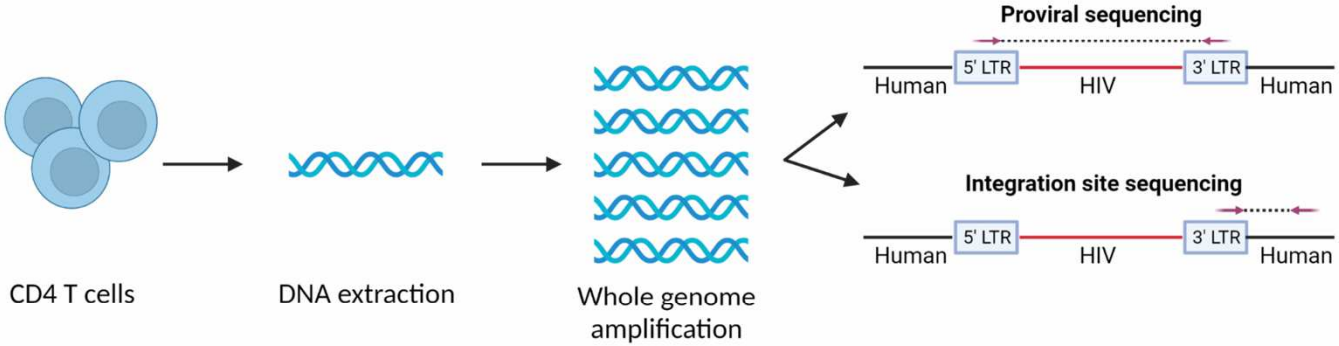


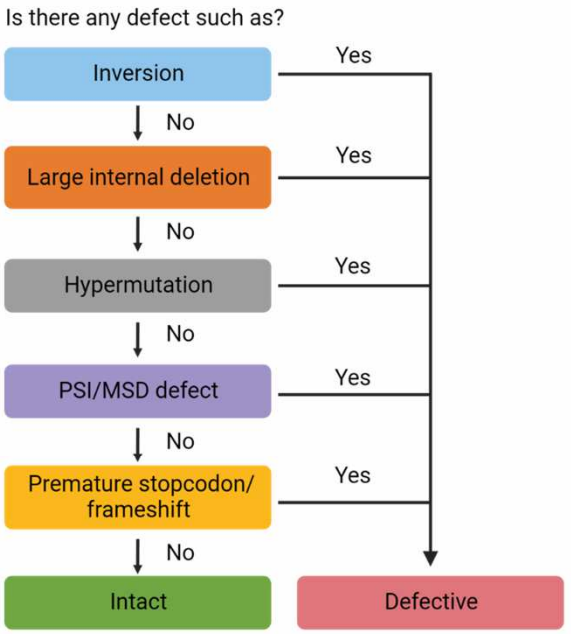
- In early treated individuals, **the frequency of total HIV-1 DNA copies per million CD4 T cells** is **lower** than in individuals who initiated ART in chronic infection.
- Early ART initiation does not prevent the establishment of the intact viral reservoir.

IS and proviral sequencing: methodology

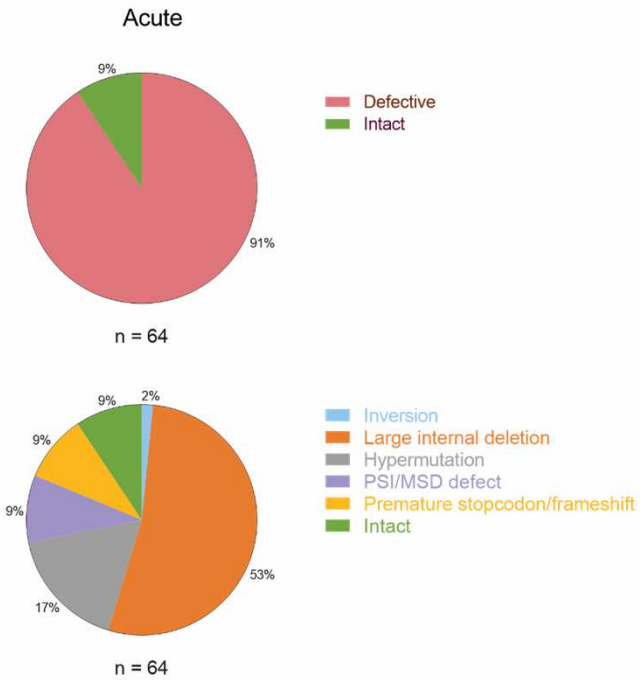
Qualification

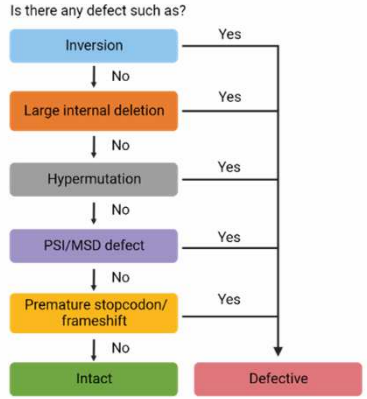
Integration site and proviral sequencing - MIP-Seq





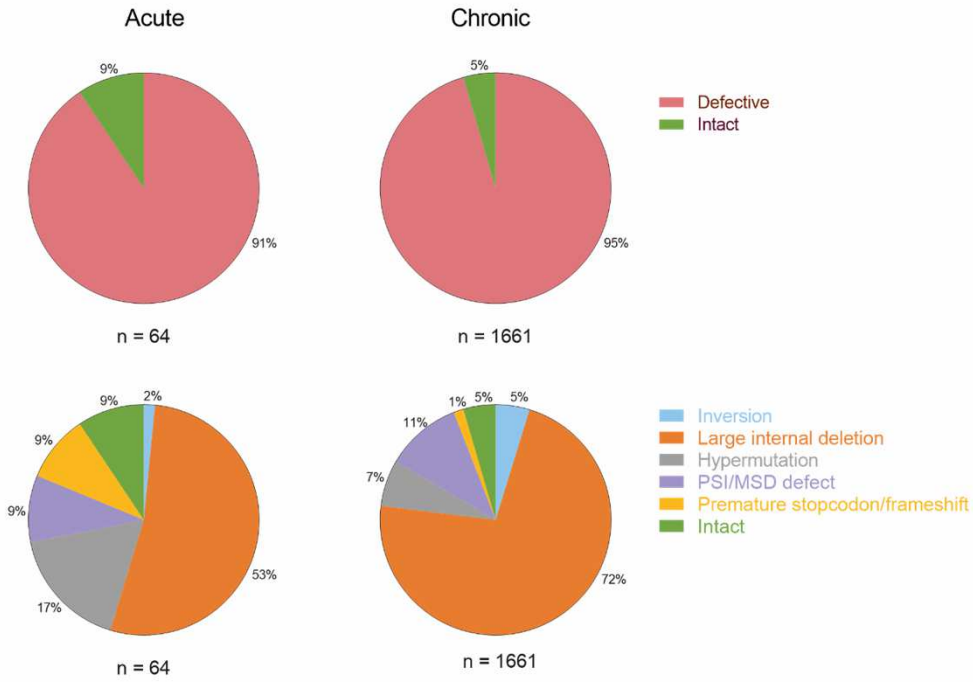
Proviral classification

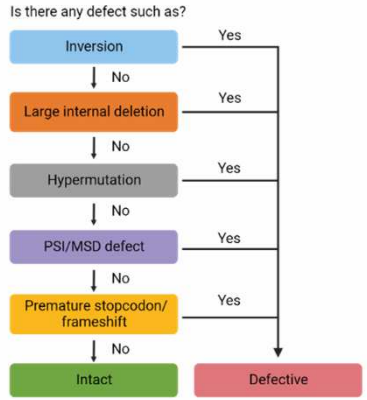




- After a median of 0.96 years [0.49-1.93y] of ART, 9% of proviruses are intact.

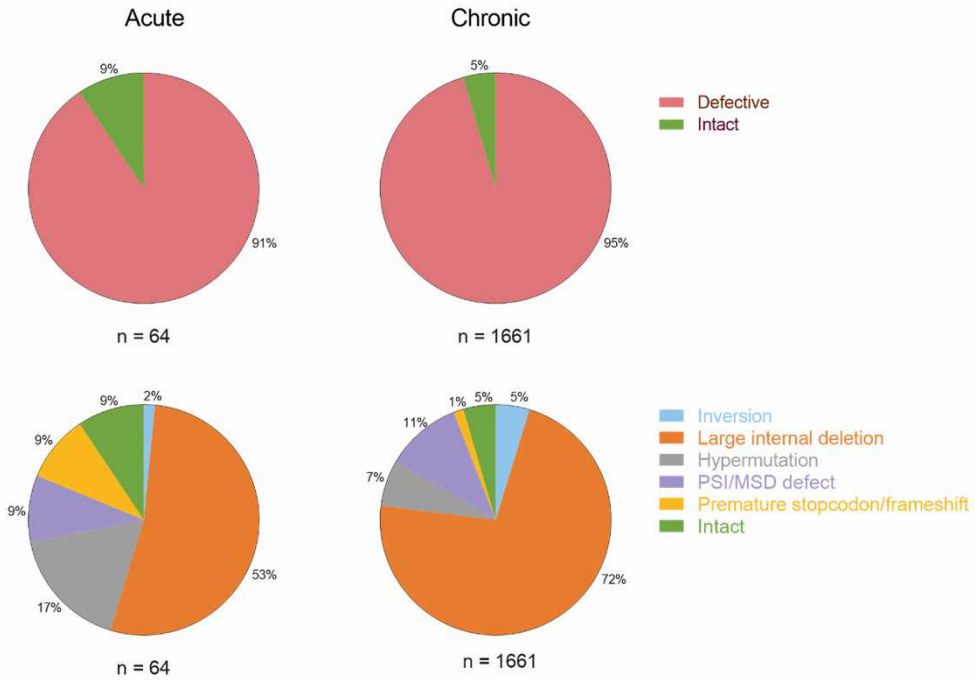
Proviral classification

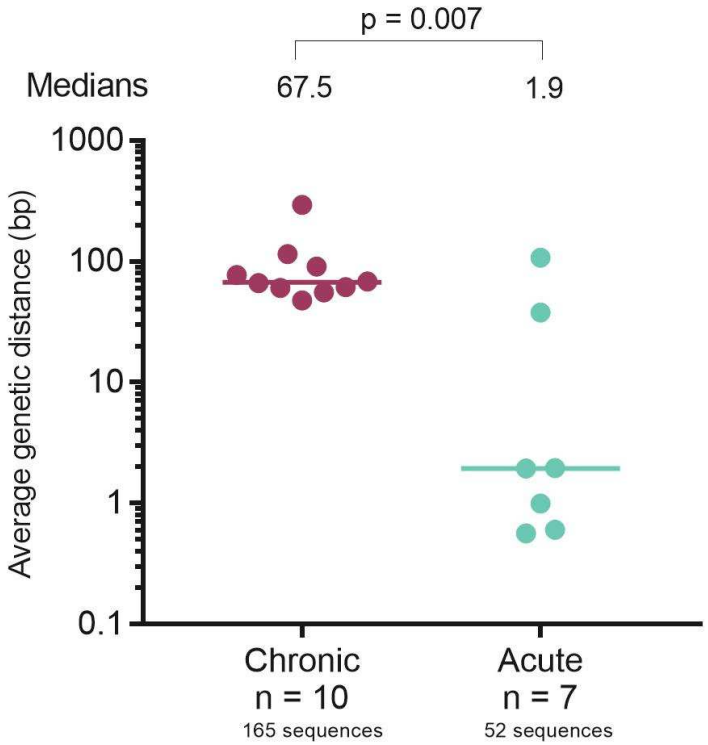




- After a median of 0.96 years [0.49-1.93y] of ART, 9% of proviruses are intact.
- Early treated individuals have a **higher fraction of intact and hypermutated proviruses**, and a **lower fraction of deleted proviruses** compared to chronically treated individuals.

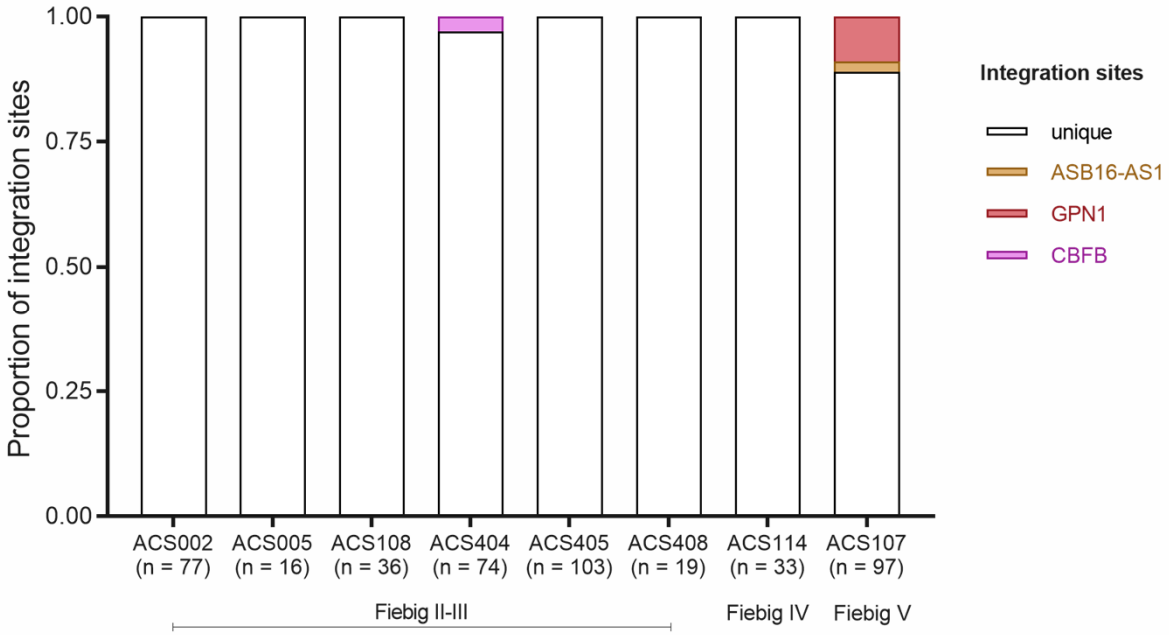
Limited genetic diversity after 1 year of ART





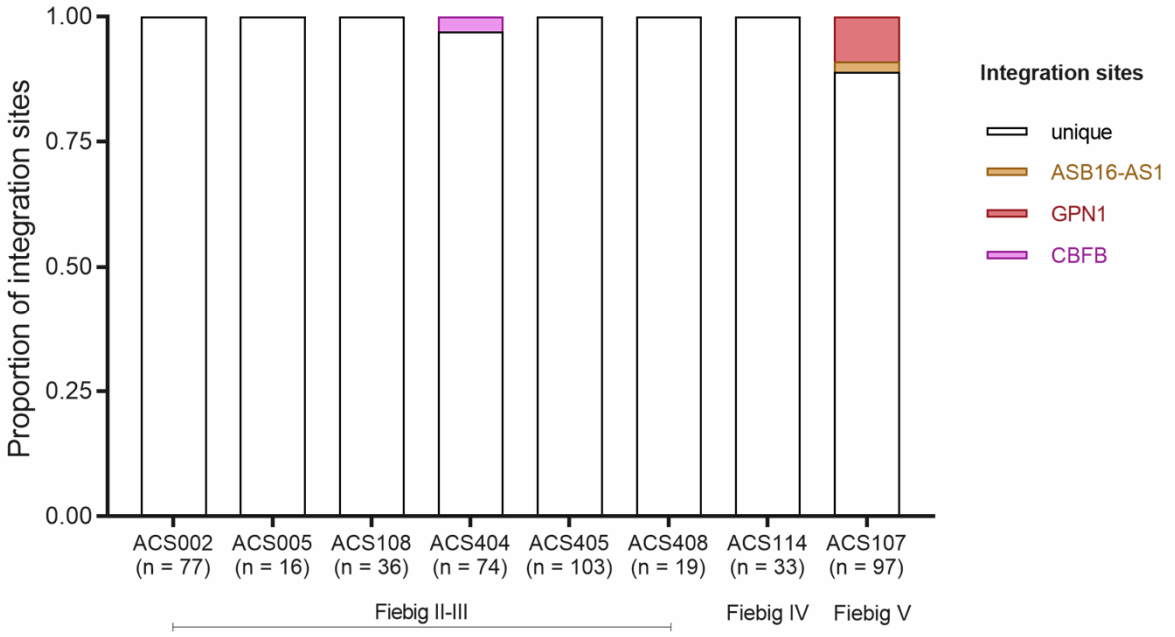
- After a median of 0.96 years [0.49-1.93y] of ART, 9% of proviruses are intact.
- Early treated individuals have a **higher fraction of intact and hypermutated proviruses**, and a **lower fraction of deleted proviruses** compared to chronically treated individuals.
- The analysis of a 4.5 kb region at the 3' end of the provirus revealed that the **intra-individual genetic diversity** is **limited**.

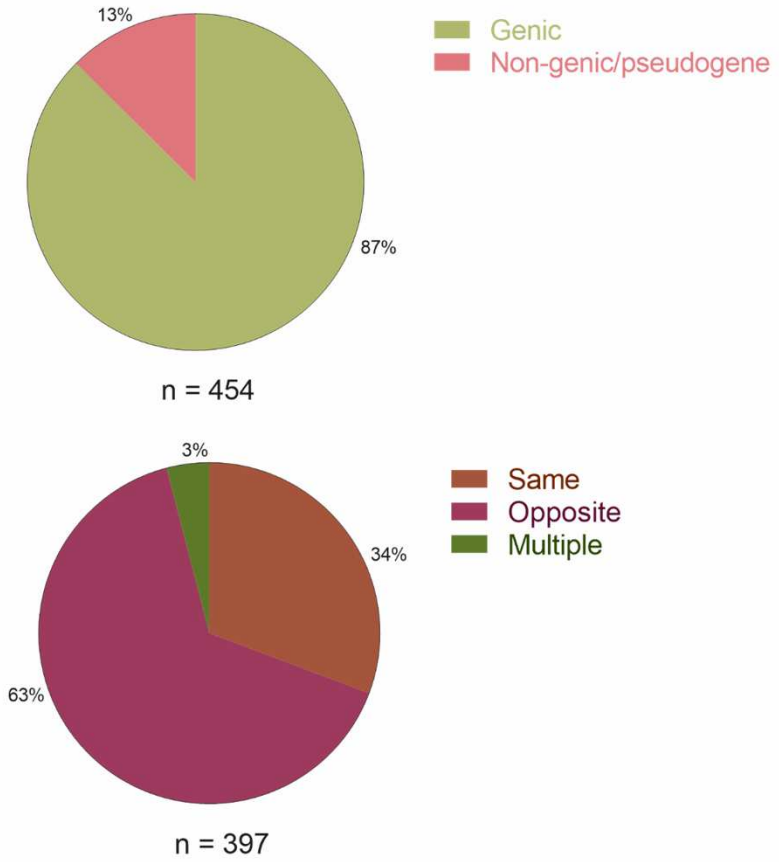
Minimal clonal expansion after 1 year of ART



- The majority of integration sites are unique (97%).
- Clonally expanded cells were retrieved in only 2 out of 8 participants and accounted for 3% of total integration sites.

Minimal clonal expansion after 1 year of ART



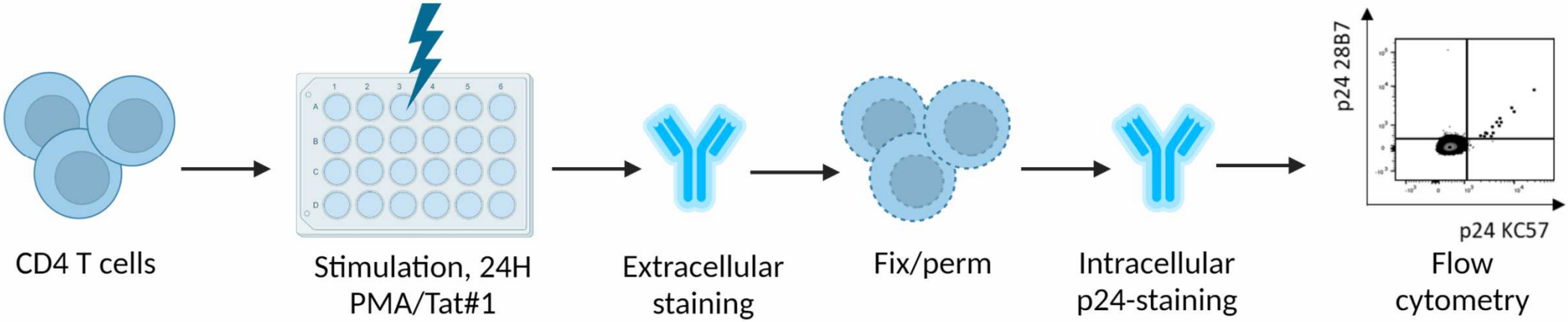


- The majority of integration sites are unique (97%).
- Clonally expanded cells were retrieved in only 2 out of 8 participants and accounted for 3% of total integration sites.
- Among proviruses integrated in genes, opposite orientation relative to the host gene was approximately twice as common as same orientation

HIV-Flow: methodology

Qualification

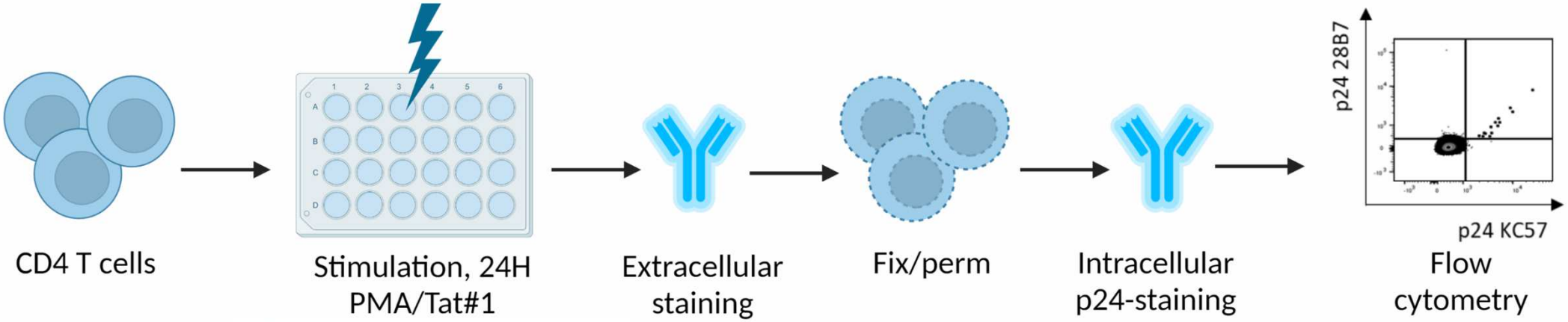
Translation competent reservoir - HIV-Flow

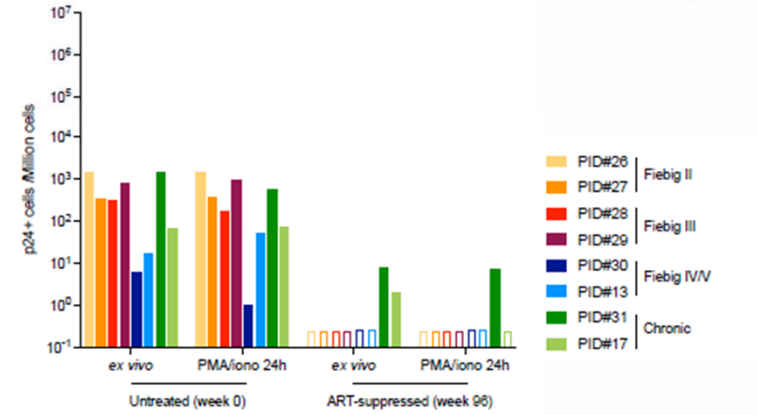


HIV-Flow: methodology

Qualification

Translation competent reservoir - HIV-Flow



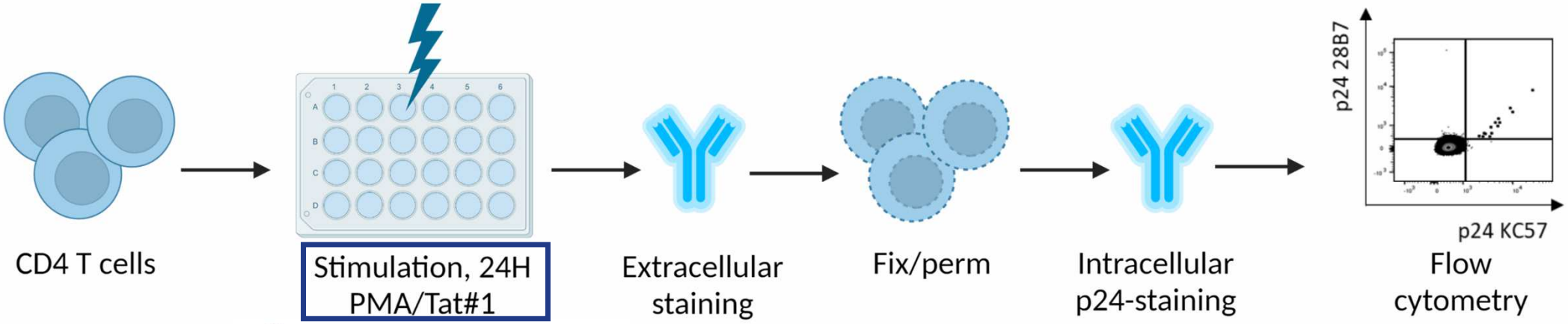


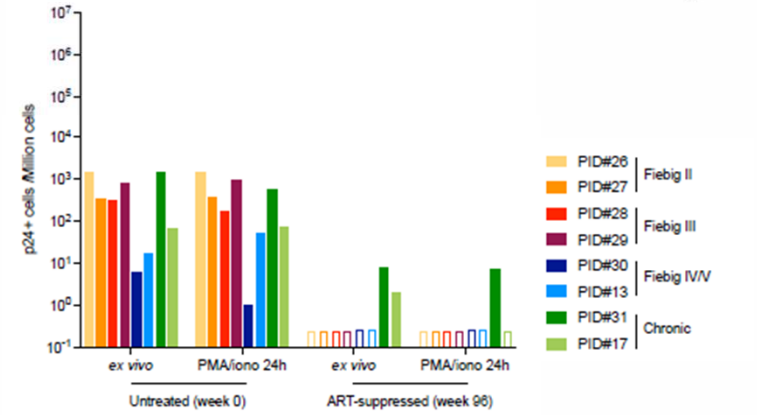
Gantner et al. Preprint 2022

HIV-Flow: methodology

Qualification

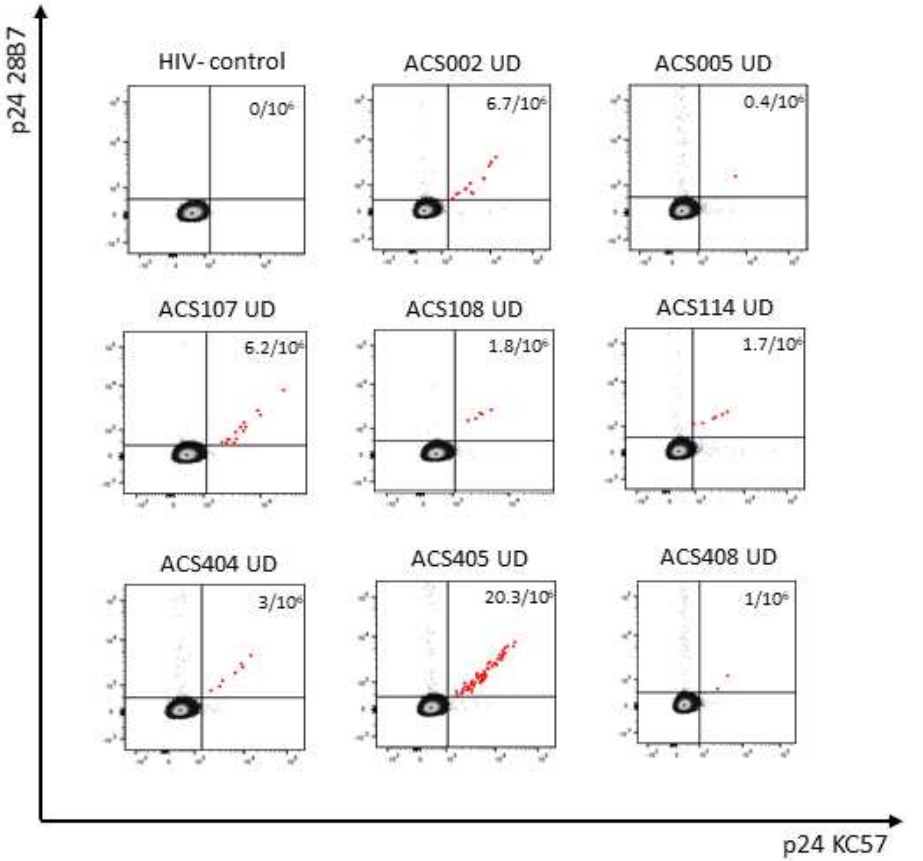
Translation competent reservoir - HIV-Flow





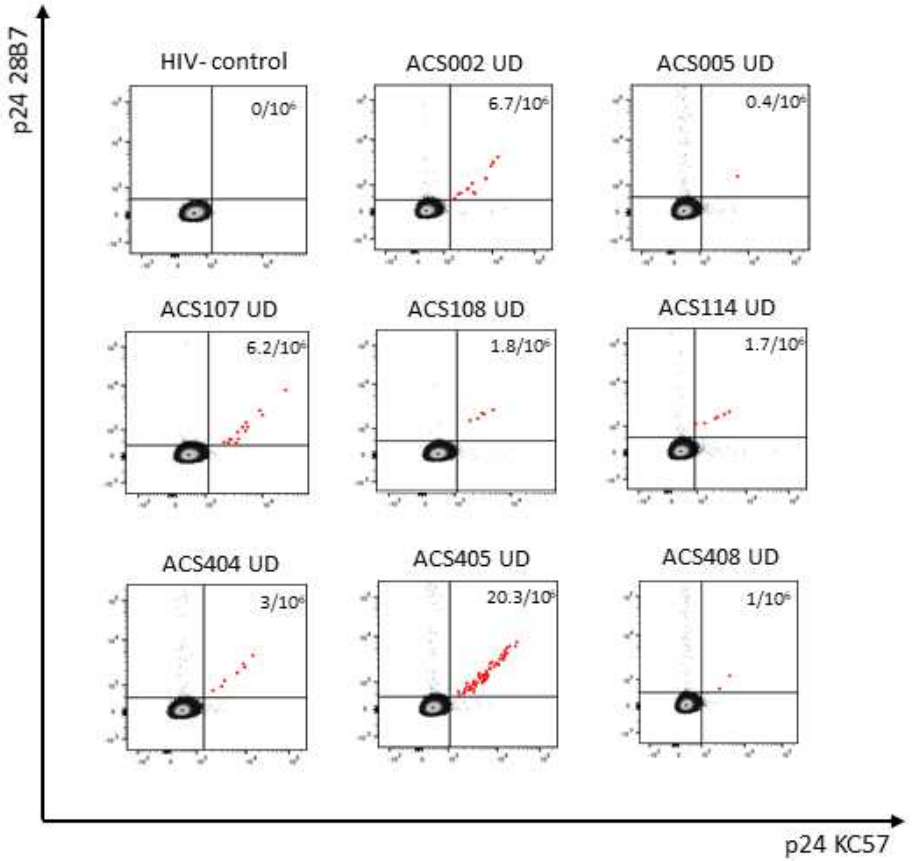
Gantner et al. Preprint 2022

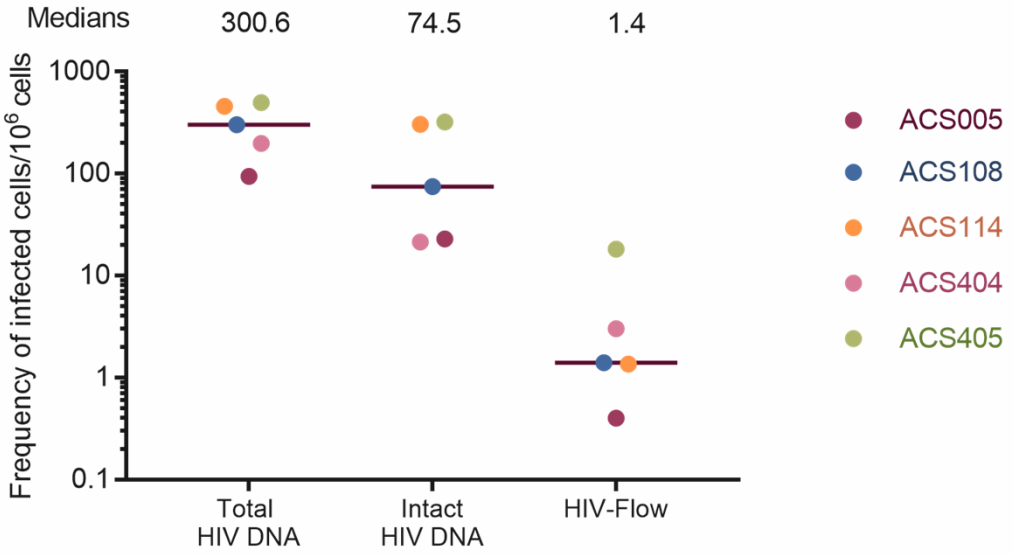
Frequencies of p24+ cells



- Following PMA/Tat#1 stimulation, the frequency of p24+ cells ranges between 0.4-20 p24+ cells.

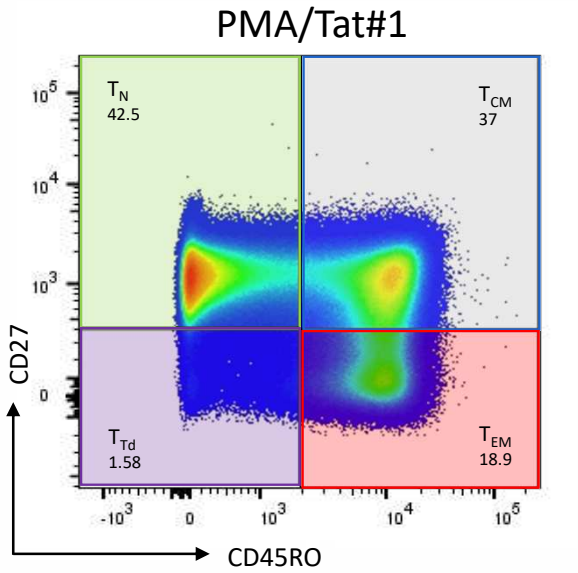
Frequencies of p24+ cells



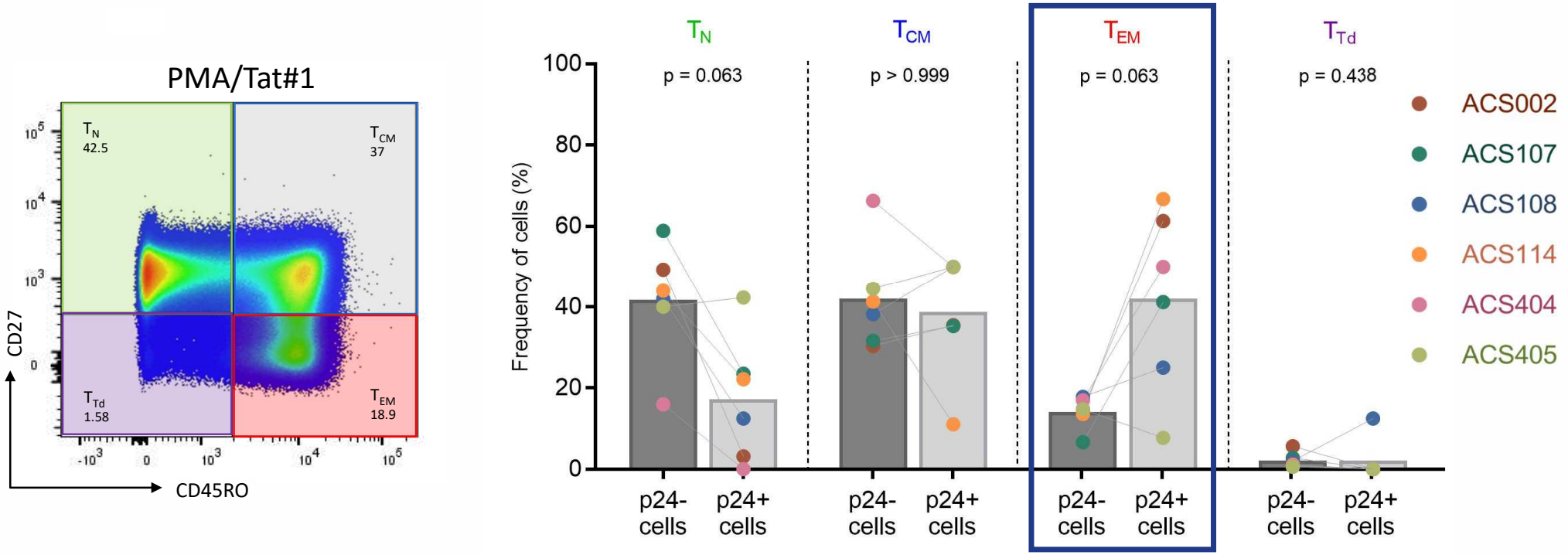


- Following PMA/Tat#1 stimulation, the frequency of p24+ cells ranges between **0.4-20 p24+ cells**.
- The median frequency of p24+ cells is lower than the frequency of cells harboring intact HIV DNA, indicating not all proviruses are inducible.

Phenotype of p24+ cells during ART

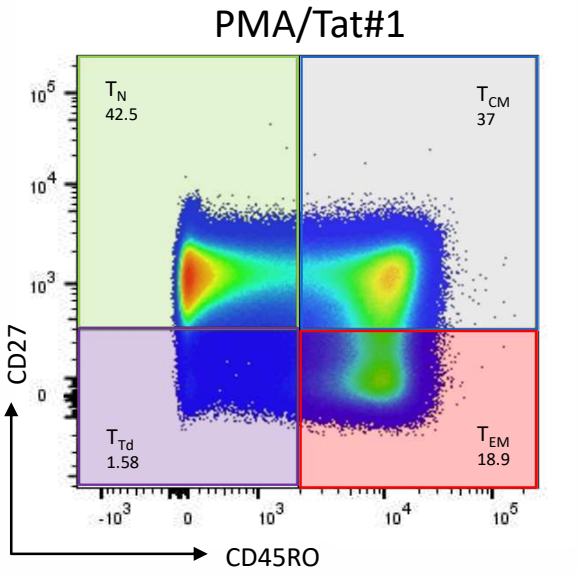


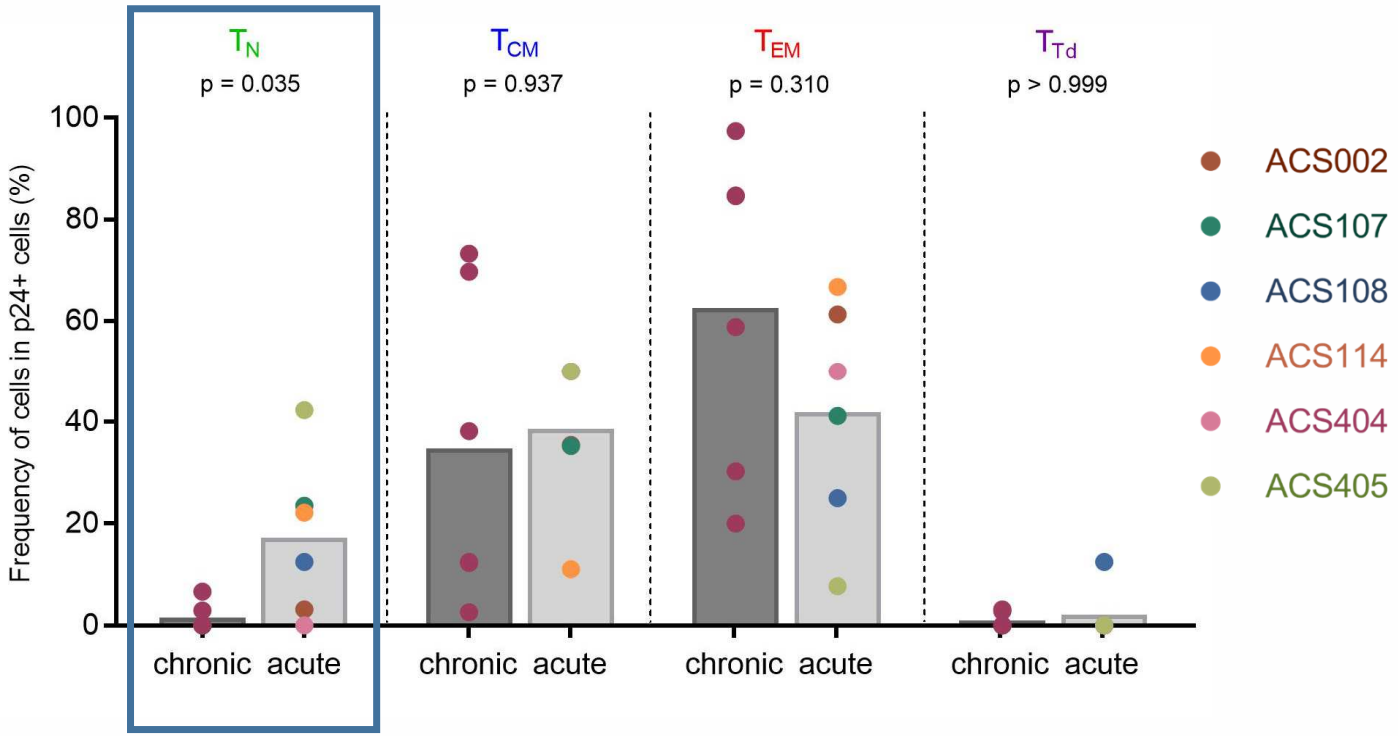
Phenotype of p24+ cells during ART



- p24+ cells reactivated by PMA/Tat#1 tend to be enriched in the **TEM fraction**

Phenotype of p24+ cells during ART





- In early treated individuals, a significantly **higher frequency of p24+ cells** resides in the **naïve T cell fraction** compared to chronically treated individuals.

What is the impact of early ART initiation on the viral reservoir?

- **Early ART** does **not prevent** the **establishment of the viral reservoir** (9% of viral genomes are intact).

- After a median of 0.96 years of ART, the majority of infected cells are clonotypically unique.
  →**Minimal contribution** of **clonal expansion** to persistence of the viral reservoir

- The **genetic diversity** of early treated individuals is **limited** compared to chronically treated individuals.

- **PMA/Tat#1** is a potent combination of LRA, which enables to study the **translation-competent reservoir** in early treated individuals.

What are the next steps?

- Study the translation-competent reservoir more in-depth
- How does the viral reservoir evolve over time in early treated individuals?

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

Janssen
Ellen Van Gulck
Erik Nijs
Daniel Boden

Arcturus Therapeutics
Jerel Vega
Jinho Park

HIV Cure Research Center

GHENT UNIVERSITY

FACULTY OF MEDICINE AND HEALTH SCIENCES

UZ GENT

NIH National Institutes of Health

All the participants from the study

Flow cytometry core from Ghent University and NXTGNT sequencing core

fwo Research Foundation Flanders
Opening new horizons