# IN-DEPTH TRANSCRIPTOMIC ANALYSIS OF THE TRANSLATION-COMPETENT HIV-1 RESERVOIR

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**Marion Pardons**[1#], Basiel Cole[1#], Laurens Lambrechts[1], Willem van Snippenberg[1], Sofie Rutsaert[1], Ytse Noppe[1], Nele De Langhe[1], Annemieke Dhondt[2], Jerel Vega[3], Erik Nijs[4], Ellen Van Gulck[4], Daniel Boden[5], Linos Vandekerckhove[1]

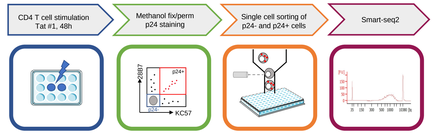
[1]HIV Cure Research Center, Ghent Belgium; [2]Department of Nephrology, Ghent University Hospital, Ghent, Belgium, [3]Arcturus Therapeutics, San Diego, California; [4]Janssen infectious diseases and diagnostics, Beerse, Belgium; [5]Janssen Biopharma, South San Francisco, USA

## BACKGROUND

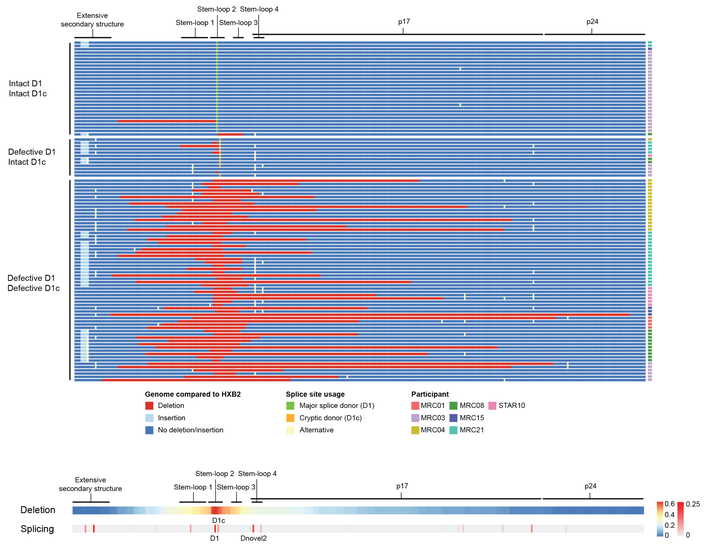
We previously described a Tat mimetic (Tat #1) that induces HIV reactivation in a higher fraction of latently-infected cells compared to PMA/ionomycin, without impacting cell viability nor modifying the transcriptome of the cells. Here we took advantage of these unique properties to study viral and cellular transcripts in p24+ cells following reactivation.

## METHODS

CD4 T cells from 5 ART-treated individuals were treated for 48h with Tat #1. A total of 108 p24+ cells and 109 p24- cells were single-cell sorted and subjected to single-cell RNA sequencing by Smart-seq2. Transcriptomic differences between p24+ and p24- cells were validated at the protein level using flow cytometry.

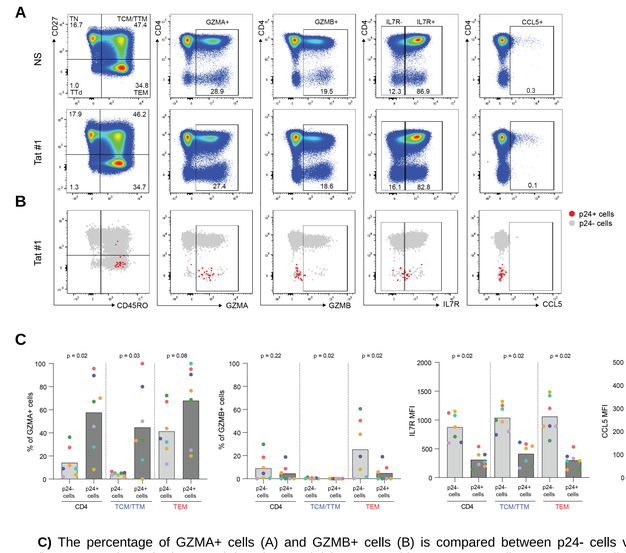



### Proviruses with a defective major splice donor (D1) site and cryptic donor (D1c) site utilized alternative splice sites up- and/or downstream of the MSD




Virogram depicting the 5' UTR region of the viral sequences retrieved with Smart-seq2. Viral sequences are grouped into 3 categories: intact D1 and D1c, defective D1 but intact D1c, defective D1 and D1c.

### Flow cytometry analyses confirmed that p24+ cells expressed higher levels of CCL5 and GZMA proteins while expressing lower levels of IL7R and GZMB proteins compared to p24- cells

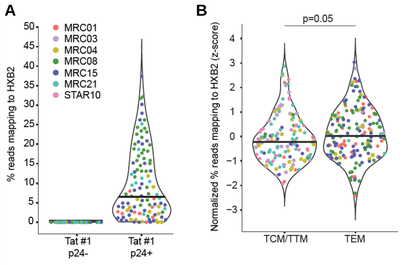



**A)** Representative FACS dot plots showing CD4 T cell subsets (TN, TCM/TTM, TEM, TTd) as well as the expression of GZMA, GZMB, IL7R and CCL5 in absence of stimulation or following a 48h-treatment with Tat #1.

**B)** Representative FACS dot plots showing the phenotype of p24+ cells (in red) overlaid to p24- cells (in grey).

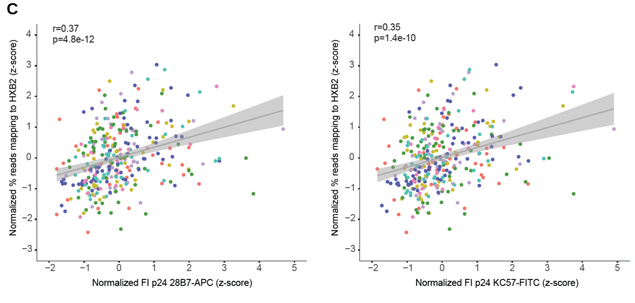
**C)** The percentage of GZMA+ cells (A) and GZMB+ cells (B) is compared between p24- cells versus p24+ cells; The mean fluorescence intensity for IL7R (C) and CCL5 (D) is compared between p24- cells versus p24+ cells. This comparison is made for all CD4 T cells (left), the TCM/TTM subset (center) and the TEM subset (right).

### Among p24+ cells, 6.7% of the detected reads mapped to the HIV reference genome HXB2



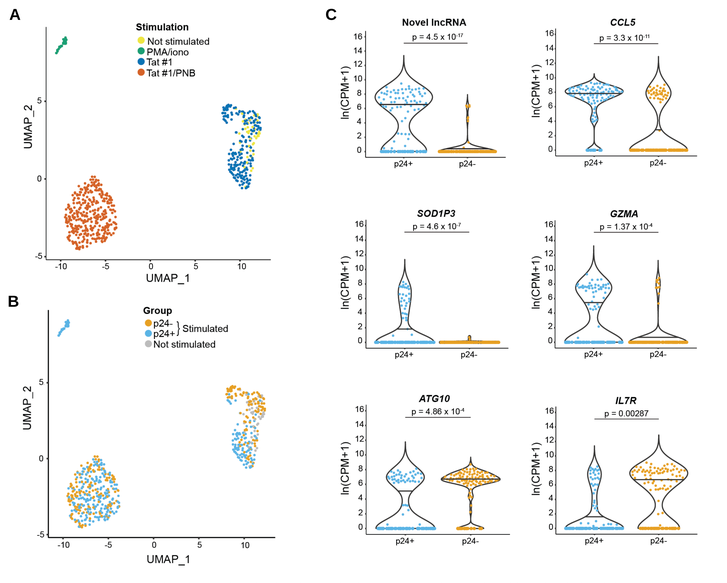

**A)** Violin plots showing the percentage of reads mapping to the reference genome HXB2 in p24- versus p24+ cells following stimulation with Tat #1.
**B)** Violin plots depicting the normalized percentage of reads mapping to HXB2 in the TCM/TTM and TEM subsets.

### Levels of HIV transcripts positively correlated with the intracellular p24 production per cell



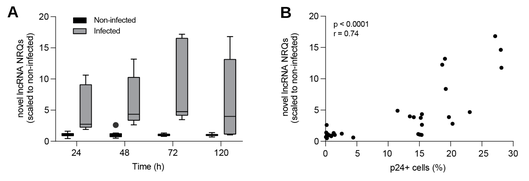

**C)** Correlations between the normalized percentage of reads mapping to HXB2 and the normalized fluorescence intensity (FI) for p24 28B7-APC (left) and for p24 KC57-FITC (right). Each dot represents a sorted p24+ cell analyzed by Smart-seq2.

### Compared to p24- cells, p24+ cells significantly expressed higher levels of a previously undescribed long non-coding (lnc) RNA, *SOD1P3*, *CCL5* and *GZMA*, while expressing lower levels of *ATG10* and *IL7R*



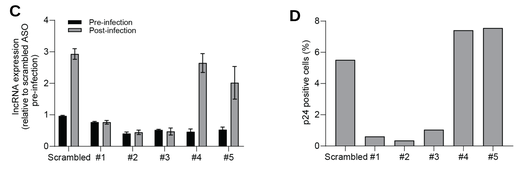

**A-B)** Uniform manifold approximation and projection (UMAP) of Smart-seq2 data, colored by stimulation (A), or p24 expression (B).
**C)** Significantly differentially expressed genes (DEG) between p24+ and p24- cells following a 48h-stimulation with Tat #1.

### In vitro infection of primary CD4 T cells with the virus strain 89.6 induced a strong upregulation of the lncRNA expression




Infection time course in CD4 T-cells of 3 healthy donors using wild-type HIV-1 virus strain 89.6.
**A)** Box plots showing the normalized relative quantities (NRQs; normalized to at least 2 stable reference genes) measured by RT-qPCR.
**B)** Correlation plot between the expression levels of the lncRNA (scaled NRQs) and the percentage of p24 positive cells measured by flow cytometry at 48 and 120h post-infection.

### Pre-treatment of CD4 T cells with three different antisense oligonucleotides targeting the lncRNA prior *in vitro* infection resulted in the efficient silencing of the lncRNA while reducing the percentage of p24+ cells compared to scrambled ASO




CD4 T cells were pre-treated for 2 days with 5µM of anti-sense oligos (ASOs) targeting the lncRNA before infection with HIV-1 89.6 (n=1).
**A)** Box plots showing the expression of the lncRNA pre- and post-infection for each ASO condition.
**B)** Box plots showing the percentage of p24+ cells for each ASO condition.

## CONCLUSIONS

Using single-cell RNA sequencing, we unravelled alternative splicing mechanisms and showed that p24+ cells display a distinct transcriptional signature compared to non-infected cells. We identified a lncRNA as preferentially expressed by p24+ cells and provided evidence this lncRNA may play in role in regulating HIV gene expression.

## ACKNOWLEDGEMENTS

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## ADDITIONAL KEY INFORMATION

Contact: marion.pardons@ugent.be

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HIV Cure Research Center
ARCTURUS Therapeutics
Janssen
UNIVERSITEIT GENT
AGENTSCHAP INNOVEREN & ONDERNEMEN