Combined assays shed new light on HIV-1 proviral sequence and linked integration site

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BACKGROUND

- HIV-1 infection remains incurable due to the establishment of a **persistent viral reservoir**, capable of rebounding upon treatment interruption.
- Evidence has shown that only a small proportion of this reservoir contains intact proviruses, and these are maintained, at least in part, by **clonal expansion**.
- We have generated integration site (IS) and proviral sequence data down to single cell level on subjects on cART using different approaches, **comparing strengths/weaknesses and complementarity of the methods.**

METHODS

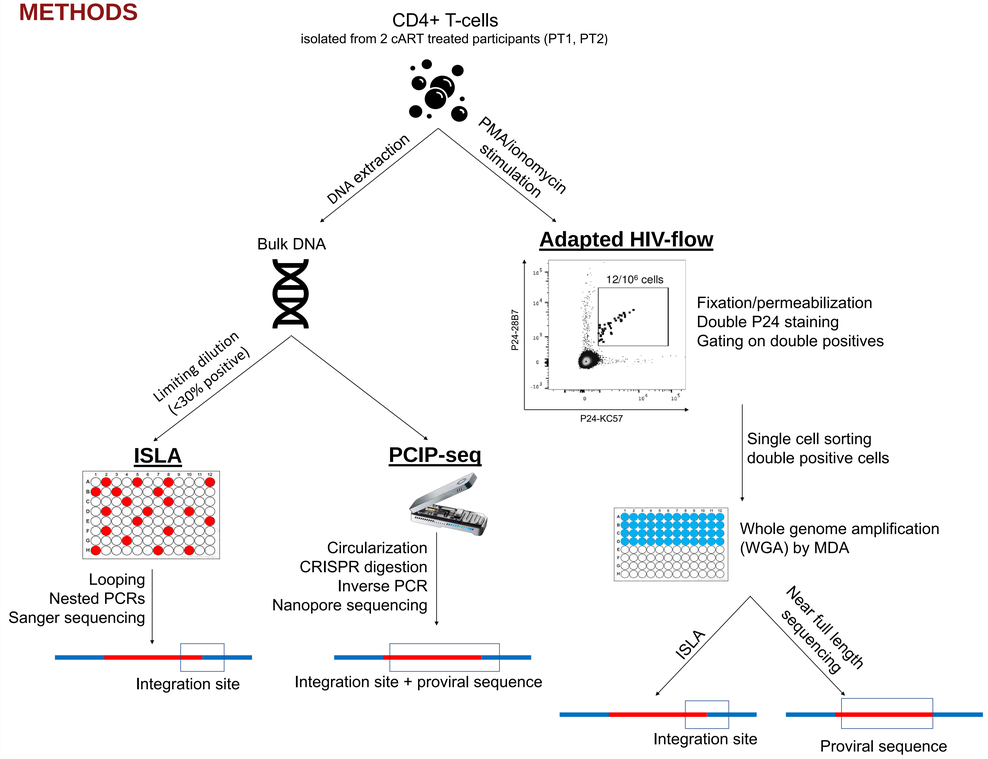


Figure 1: Graphical overview of methods. ISLA = integration site loop amplification, MDA = multiple displacement amplification, PCIP-seq = pooled CRISPR inverse PCR sequencing (detailed methods, see poster #0375).

Table 1: Comparison of techniques.

	ISLA	PCIP-seq	Adapted HIV-flow
Input	Diluted DNA	Bulk DNA	CD4+ T-cells
Throughput	Low: labour intensive, Sanger sequencing	High: bulk input, Nanopore sequencing	Low: a lot of cells needed
Price	High: repeated PCRs	Low	High: MDA, ISLA and NFL
Output	Integration site	Integration site + proviral sequence	Integration site + proviral sequence of inducible proviruses

RESULTS

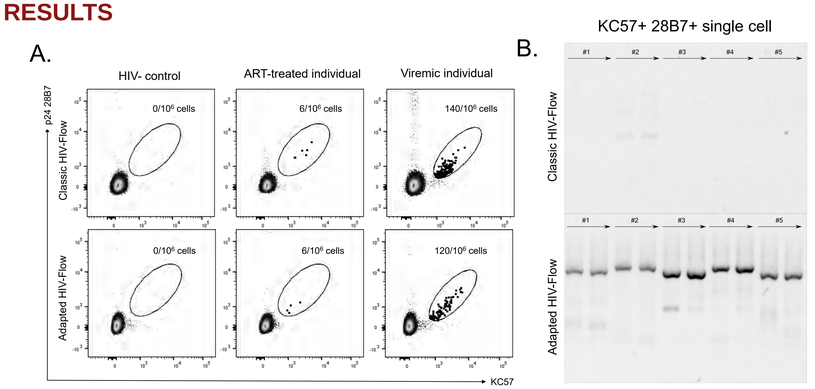


Figure 2: Comparison between classic HIV-Flow (M. Pardons et al., PLOS Path., 2019) and an adapted version. A: representative dot plots showing the co-staining of KC57 and P24-28B7 in purified CD4+ T-cells from one HIV- control, one cART treated individual and one viremic individual. B: five amplicon approach (~2kb overlapping fragments spanning the entire genome) to sequence the near full length proviral genome of a unique single P24+ sorted cell.

- A total of 17 P24 double positive single cells were sorted from PT2, and WGA was performed using MDA.
- Integration sites were recovered with ISLA and the proviral genome was amplified using a 5-amplicon approach.

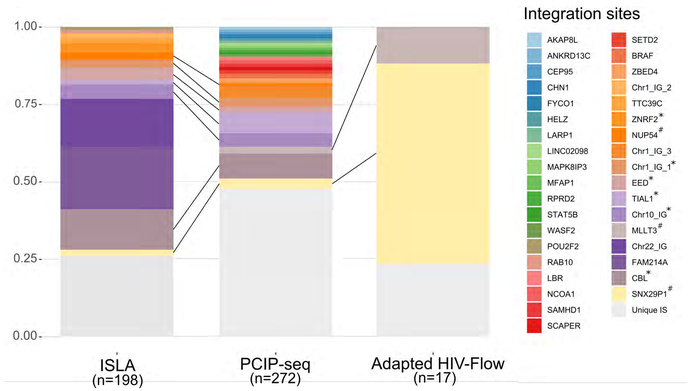


Figure 3: Integration site profiles obtained in PT2 with ISLA, PCIP-seq and adapted HIV-Flow. Lines connect integration sites (IS) found in multiple techniques. *IS found in ISLA and PCIP-seq. #IS found in ISLA, PCIP-seq and adapted HIV-Flow.

- Using ISLA, a total of 134 IS were recovered from PT1 and 86 from PT2. Using PCIP-seq, 80 IS for PT1 and 161 IS for PT2 were recovered.
- Near full-length (NFL) proviruses from 4 clones were detected with both PCIP-seq and ISLA in PT2. Two of these were also found with the adapted HIV-flow. However, both are rendered defective by short deletions.
- The two remaining NFL genomes are intact and located into centromeric regions. Importantly, these were not found with the adapted HIV-Flow assay.
- An NFL provirus is integrated into the 2nd intron of the MLTT3 gene, which protein product is part of the Tat-P-TEFb complex.

CONCLUSIONS

- Comparing PCIP-seq to ISLA, we show that PCIP-seq is a potent method to retrieve both integration site and proviral genome.
- Integration site landscapes are different between methods, however, overlapping integration sites are retrieved with the three methods.
- The combination of these techniques will allow for extensive characterization of reservoirs in patients on cART.

ADDITIONAL KEY INFORMATION

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