381 Large-Scale Analysis of HIV-1 Integration Sites in Untreated and Treated Patients

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Background

The human immunodeficiency virus 1 (HIV-1) integrates its genome into the chromatin of host cells, allowing the virus to persist in infected cells and hide from the host immune surveillance. Integration is a semi-random process and proviruses in specific genes are proposed to be linked to clonal expansion and persistence of infected cells in patients under long-term antiretroviral treatment (ART) [1-3]. However, very little is known about the integration site (IS) repertoire in the high-viremia phase preceding the administration of combined ART (cART) and in the early phases after therapy start.

We have studied the IS repertoire in 55 HIV-infected patients to allow a high-throughput analysis of HIV-1 integration characteristics in both viremic and short-term cART treated patients.

Materials and Methods

Peripheral blood mononuclear cell (PBMC) samples from HIV-infected individuals (n=55) were used for this study. Written informed consents were obtained from all donors and patients in accordance with the ethical principles set out in the declaration of Helsinki. This study was approved by the Medical Ethics Committee of the Academic Medical Center and the Ethics Advisory Body of the Sanquin Blood Supply Foundation in Amsterdam, The Netherlands, as well as by the Ethics commitee of Freiburg, Germany. Eligible patients for this study were HIV-1 positive, aged 18 years or older, in stable clinical condition and without known adherence problems. The individuals were either untreated or cART was started with emtricitabine or lamivudine plus tenofovir disoproxil fumarate, in combination with either Raltegravir , or another recommended third compound, i.e. a boosted PI or the NNRTI EFV as described before [5]. The study including some of the treated patients was approved by the Frankfurt University Hospital Ethics Committee (Reference: Vote-No. 159-09, as by Nov 12th, 2009; NCT 01168167).

CD4 enrichment and DNA isolation PBMC were isolated from blood using Ficoll-Paque (GE Healthcare Life Sciences) density gradient centrifugation. Subsequently, CD4+ T cells were purified from the PBMC using CD4+ T Cell MicroBead Cocktail (MACS technology, MiltenyiBiotec). Blood sampling in both therapy-naïve and treated patients was performed with intervals ranging from two days to approximately eight months. Total DNA from patients' PBMCs or purified CD4+ cells was extracted.

Integration site analysis WT HIV-1 IS were analyzed from PBMCs or purified CD4+ T cells with linear amplification-mediated PCR (LAM-PCR; Figure 7) [4] using the restriction enzymes *Tsp509*I and/or *Mse*I. Double barcoded LAM-PCR products were deep sequenced with 454 and the MiSeq technology and large scale integrome analyses was performed using dedicated bioinformatics applications [6,7].

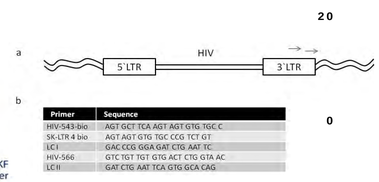


Figure 7: Outline of the LAM-PCR

Results

Integration site analysis yielded a comparable number of IS for both the treated and the untreated patient groups (Table 1). The majority of post-treatment IS data is from the very early time points after therapy start (maximally 1 month after therapy start) (Figure 1) . The overall integration pattern followed that typical of lentiviruses in all the studied groups (Figure 2). HIV-1 long terminal repeat (LTR) ends were found to harbour deletions (>2bp) in 2.7% of the analyzed IS (Figure 3).

	Patient no.	Total no. of IS	IS/ng DNA	Exactly mappable IS	IS in gene	IS in gene +/-10 kb
untreated	43	1106	0.0128	977	571 (58.4%)	607 (62.1%)
ART	2	97	0.0162	91	55 (60.4%)	64 (70.3%)
cART+ PI/NNRTI	17	683	0.0128	611	413 (67.6%)	471 (77.1%)
cART+ IN-inhibitor	13	358	0.0055	319	204 (63.9%)	234 (73.4%)
total	55	2244	0.0107	1998	1243 (62.2%)	1376 (68.9%)

Table 1: Summary of patient groups and integration site information.

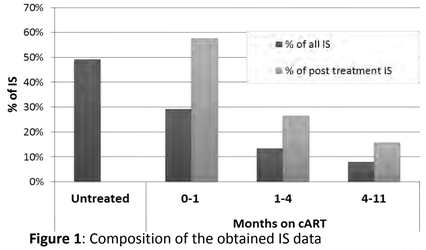


Figure 1: Composition of the obtained IS data

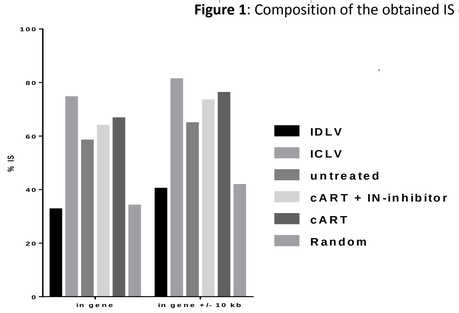


Figure 2: The analysis of HIV integrations compared to integrations of lentiviral vectors

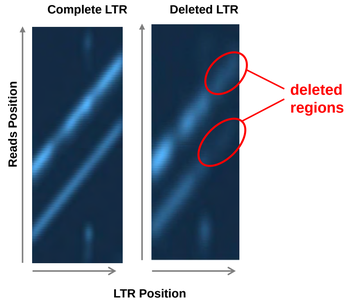


Figure 3: Detection of deletions occurring in the HIV-1 proviral 3'LTRs

In a longitudinal study of virus integration sites we identified persisting cell clones marked by integrations in 17 genes in 9 patients (Figure 4). The genes that have been linked to clonal expansion of infected cells, when harbouring an integrated provirus, were less targeted for integration in our data (Figure 5). Yet IS were found in many same genes in our data than in previously published for HIV-1 in long-term cART patients (Figure 6).

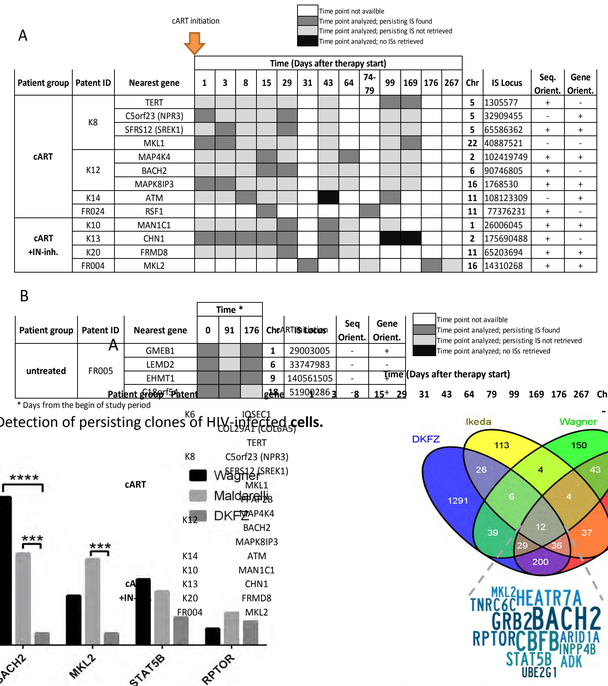


Figure 4: Detection of persisting clones of HIV-infected cells.

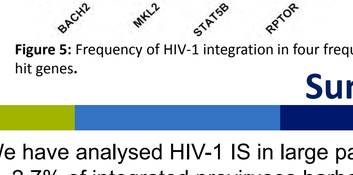


Figure 5: Frequency of HIV-1 integration in four frequently hit genes.

Figure 6: Comparison of unique IS of this study and recently published HIV-1 data

Summary

We have analysed HIV-1 IS in large patient cohorts and found the following:

- 2.7% of integrated proviruses harbour LTR deletions
- HIV-1 integration follows the typical pattern for lentiviruses both in the high-viremia stage and after therapy start
- Genes proposed to link to clonal proliferation of infected cells if harbouring a provirus are less targeted for integration before or early after therapy start than in long-term cART studies
- Long-lived cell clones that are probably expanded can be identified already before cART
- Persistent cell clones can be identified with IS in many cellular genes.

References and acknowledgements

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