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) [1,2]. 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 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 Board of the Blood Supply Foundation in Amsterdam, The Netherlands, as well as by the Ethics committee 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 below. 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).

EDC 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 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) using the restriction enzymes Tsp509I and/or Msel. Double barcoded LAM-PCR products were deep sequenced with 454 and the MiSeq technology and large scale genome analyses was performed using dedicated bioinformatics applications [3].

In a longitudinal study of virus infection 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 HIV-1 in long-term cART patients (Figure 6).

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).

Discussion

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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