Clonal Integration Site Frequency and Replication Competent Virus in Patients on ART

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BACKGROUND

The latent HIV-1 reservoir remains one of the major obstacles to cure HIV. To monitor HIV cure strategies, a validated biomarker is needed that can evaluate the reservoir over time *in vivo*. Consequently, numerous assays are now being investigated to estimate the size of the replication competent provirus.

AIM OF THE STUDY

To map HIV-1 integration sites and assess the percentage of clonally expanded cells in patients under stable ART, who have different amount of inducible replication competent virus and HIV-1 RNA transcription.

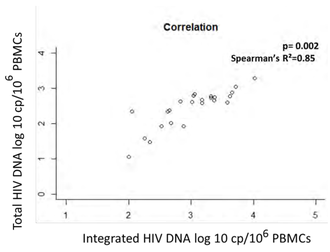
METHODS

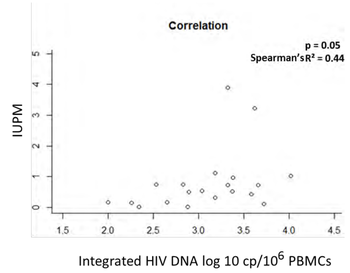
A comprehensive study was designed to evaluate and compare potential HIV-1 reservoir biomarkers. A cohort of 25 ART treated patients was sampled in which plasma viral load (<50 cp/ml) was suppressed for median of 7 years (5-11). Total, integrated HIV-1 DNA and unspliced (us) HIV-1 RNA were quantified in peripheral blood. A quantitative viral outgrowth assay (qVOA) was performed. Subsequently, a nested substudy of 10 patients was set-up to analyse HIV-1 integration sites and tat/rev induced limiting dilutions assay (TILDA). The selected patients had low level of total HIV-1 DNA (<250 cp/10^6PBMCs) or high level of qVOA (> 2 IUPM). The percentage of integration site clonality was estimated based on results from the integration site analysis using integration site loop amplification (ISLA), and the frequency of cells with inducible HIV RNA transcription were estimated using maximum likelihood method.

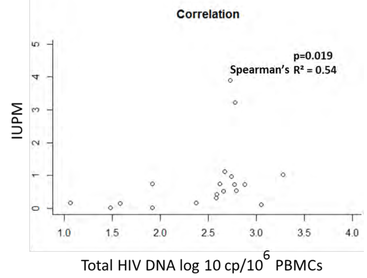
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RESULTS

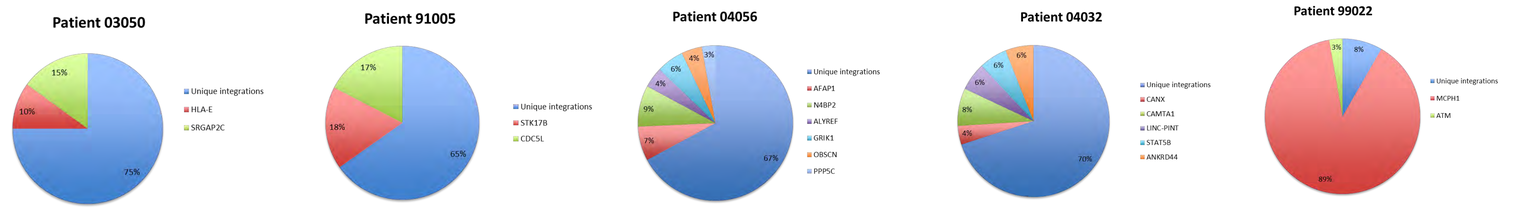
Integrated and total HIV-1 DNA were detected in all patients, and both measures correlated well (p=0.002, R^2=0.85). Replication-competent virus was detected in 80% of patients by the qVOA and this correlated with integrated and total HIV-1 DNA (p=0.05, R^2=0.44; *p*=0.019, R^2=0.54; respectively).



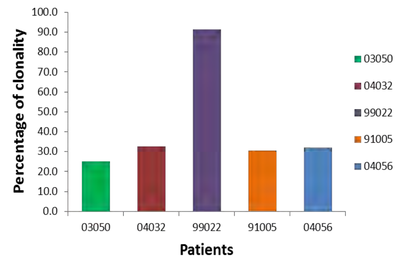


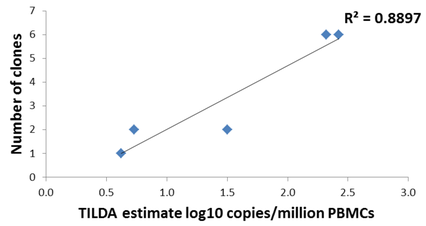


In total 317 integration sites were analysed. 118 (37%) of integrations were detected once, while 17 were detected more than once (62.7%). Among the 5 patients integration sites were found in 134 different genes.



A wide range of percentage of clonality (25.0-91.4%) was observed between patients. The majority of patients had an average of 30% clonality, which on average comprised of 3.4 predominant clonal integration sites per patient. Patients with higher clonal diversity (≥ 6 clones) had higher estimate for TILDA (frequency of cells with inducible msRNA > 200 cells /10^6 PBMCs).





CONCLUSION

We observed a good correlation between VOA and integrated and total HIV-1 DNA. Integration site sequencing revealed that most of the ART treated patients in this study had an HIV DNA reservoir consisting of approximately 30% clonally expanded cells. These levels of clonal provirus did not affect the amount of inducible virus, except for the one patient with >90% clonality.

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