Persistence of HIV DNA in tissues early after transplantation with CCR5Δ32 stem cells

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Background

Cure of HIV infection was observed following stem cell transplantation (SCT) with homozygous CCR5Δ32 donor cells in the Berlin patient (Hutter et al. 2009). In contrast, in the Boston patients, transplanted with a regular donor, HIV rebound was observed after treatment interruption despite loss of detectable HIV DNA in PBMCs (Henrich et al. 2014). It is unclear which reservoir fueled HIV rebound.

Methods

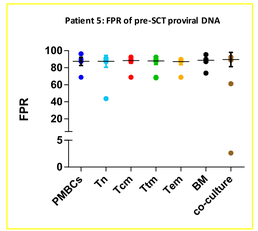
The IciStem collaboration aims to guide clinicians involved in allogeneic SCT procedures in HIV-infected individuals and systematically studies samples from these individuals to understand the biological bases of HIV cure by SCT. Currently 28 patients have been included in the project. Within this framwork, the impact of SCT on the HIV reservoir of IciStem patients 5 and 11 was investigated. SCT was performed using HLA-matched CCR5Δ32 donors. Before and after SCT we performed:

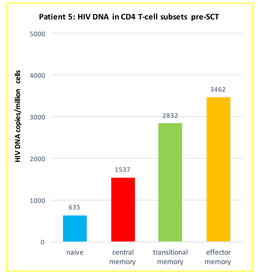
1) Phenotypic and genotypic co-receptor tropism analysis
2) Quantification of the HIV reservoir in CD4 T-cell subsets (Tn, Tcm, Ttm, Tem, and Tscm) and bone marrow using ddPCR
3) Characterization of the HIV reservoir using deep-sequencing
4) Single copy assay (SCA) on plasma.

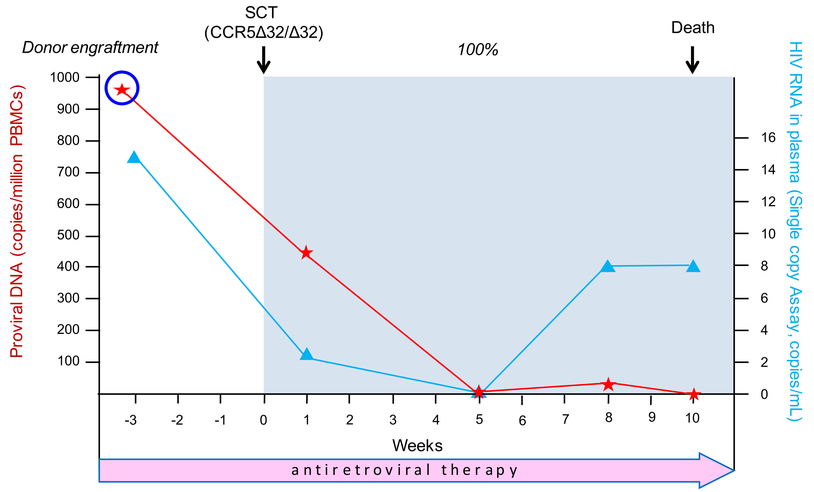
After graft failure, patient 11 was re-transplanted with CCR5Δ32 heterozygous donor cells. Post-SCT viral dynamics and the post-mortem viral reservoir were analyzed using ddPCR.

Results: patient 5 pre-SCT

Patient 5 harboured CCR5-tropic virus (FPR 89%), was treated with ART since 1999, and was effectively suppressed for 5 years prior to SCT. Before SCT, no viral RNA was detected in routine diagnostics while SCA in plasma detected 15 c/ml. HIV DNA was detected in PBMCs and T-cell subsets, showing increased HIV DNA abundance in the more differentiated T-cell subsets. Deep-sequencing and co-receptor tropism genotyping predicted mostly R5 variants except for one minor X4 sequence from culture.

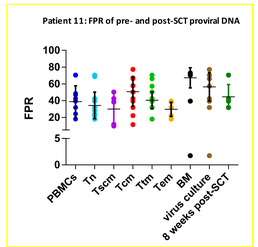


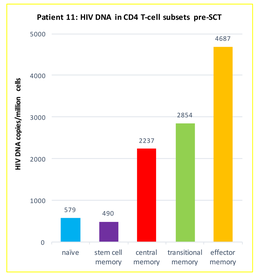


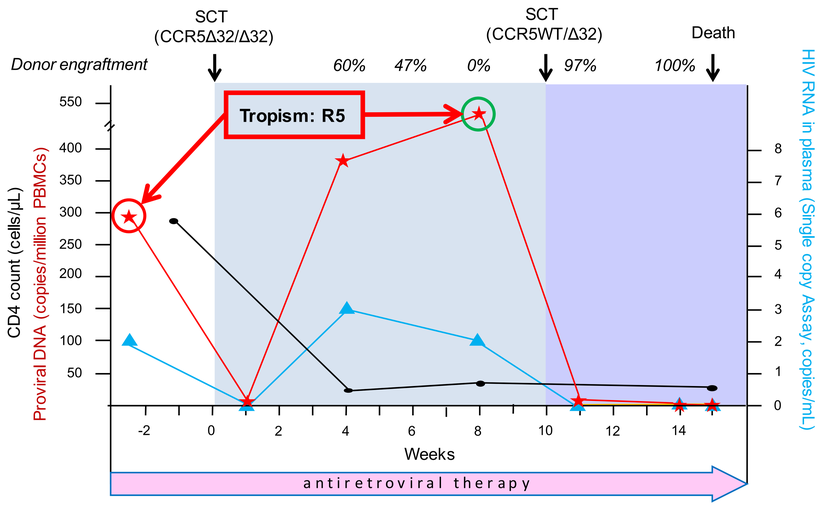


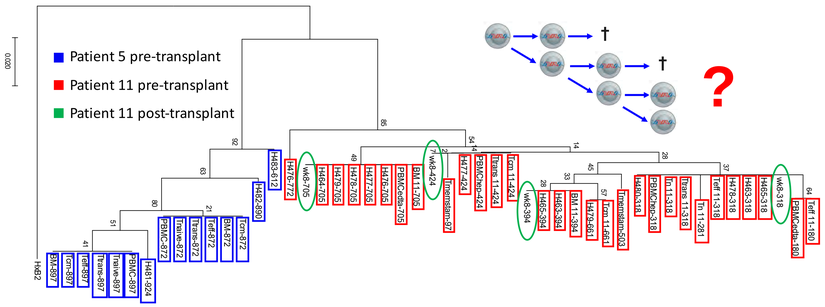
Results: patient 11 pre-SCT

Patient 11 harboured CCR5-tropic virus (FPR 33-49%), was treated with ART since 1996, and was effectively suppressed for 16 years prior to SCT. Before SCT, no viral RNA was detected in routine diagnostics while SCA in plasma detected 2 c/ml. HIV DNA was detected in PBMCs, bone marrow and T-cell subsets, showing increased HIV DNA abundance in the more differentiated T-cell subsets. Only one minor X4 sequence was predicted from bone marrow and one from virus culture. 8 Weeks after the first SCT, tropism prediction yielded exclusively R5 variants.







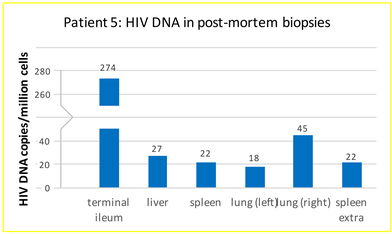


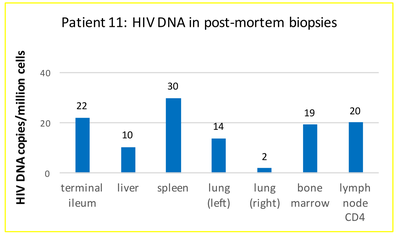
Results: Phylogenetics pre- and post-SCT

In patient 11, four and eight weeks after the first SCT an increase in proviral DNA was detected in PBMCs, whereas the number of CD4 cells in the PBMCs declined as compared to baseline. Deep-sequencing was performed on pre-SCT viral DNA from whole PBMCs and T-cell subsets of both patients and on 8 weeks post-SCT viral DNA from whole PBMCs of patient 11. Co-receptor tropism prediction and phylogenetic analysis revealed that the increase of post-SCT HIV DNA in patient 11 was unlikely to be the result of viral escape and replication, but rather due to clonal expansion of pre-SCT-existing variants.

Results: patients 5 and 11 post-mortem

Following the death of patients 5 and 11, 10-15 weeks after SCT, post-mortem analysis revealed that HIV DNA could be detected in ileum, liver, spleen, and lung , and additionally in patient 11 in bone marrow and in CD4 cells from lymph node.





Conclusion

Within IciStem, we show that after long-term effective ART HIV DNA can readily be detected in various T-cell subsets. In the neutropenic phase post-SCT, HIV DNA could no longer be detected in PBMCs. In contrast, early after SCT, HIV DNA was still found using multiple biopsies from ileum, liver, spleen, lung, bone marrow and lymph node, indicating that tissue reservoirs may play an important role as long-standing viral reservoirs.

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