HIV-1 Host Reservoir Reactivation after a CCR5Δ32/32 Allogeneic Hematopoietic Stem Cell Transplant

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BACKGROUND

To date, four persons living with HIV infection (PLWH) have achieved sustained antiretroviral-free remission through a CCR5Δ32/32 homozygous allogeneic stem cell transplantation (SCT). Post-SCT events leading to remission in this setting are uncertain, and no reports of HIV reactivation have been reported. Here we conducted virologic and immunologic analyses of HIV reactivation after a CCR5Δ32/32 SCT; in a 67 year old with high risk acute myeloid leukemia with reduced intensity conditioning with a 10/10 matched unrelated donor.

METHODS

The HEME-17 protocol evaluates HIV persistence in PLWH before and after allogeneic SCT utilizing CCR5Δ32/32 donors. Pre- and post-SCT blood samples were analyzed by single-copy (scPCR) quantification of CCR5/CCR5Δ32 (digital PCR, dPCR) and of HIV *gag* DNA and RNA (HMMC-*gag*), and by single-genome sequencing (SGS) of HIV *env*. Plasma antibodies to HIV Gag, Env, RT and Nef were quantified by luciferase immunoprecipitation system (LIPS). Control samples from PLWH and uninfected individuals were also studied. Prior to ART interruption (ATI), peripheral blood mononuclear cells (PBMC) had to demonstrate no cell-associated HIV DNA or RNA, undetected plasma HIV RNA, for 1 year and no copies of wt CCR5 identified by dPCR.

Results

1) We report the first known case of HIV reactivation during ATI following a CCR5Δ32/32 Allogenic SCT.
2) Residual R5-tropic HIV can persist *in vivo* even >1 year after SCT.
3) R5 tropic reactivation occurs without increases in plasma HIV-associated antibodies and without evidence of graft infection to date.

Figure 2: (A) Two months after ATI, Viral Load rebound was identified at 780 copies/ml utilizing quantitative HIV-1 PCR (qPCR). Repeat qPCR 1 week later showed a decrease in HIV viral load to 300 copies/ml (A). ART was re-initiated. (B) Chimerism at viral rebound remained 100% and scPCR for cell-associated HIV-1 DNA and RNA remained Undetectable. dPCR demonstrated 100% CCR5d32 DNA in the PBMCs at time of HIV relapse (data not shown).

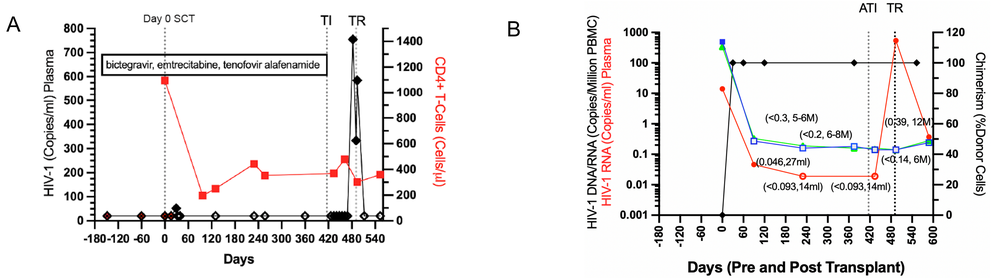

Figure 1:(A)PRE-ATI dPCR of CCR5Δ32 of PBMCs at 1 year post SCT, demonstrating 100% CCR5Δ32. (B) Demonstration of 100% Chimerism, single copy PCR of Plasma HIV-1 RNA and Cell associated RNA/DNA below level of detection.

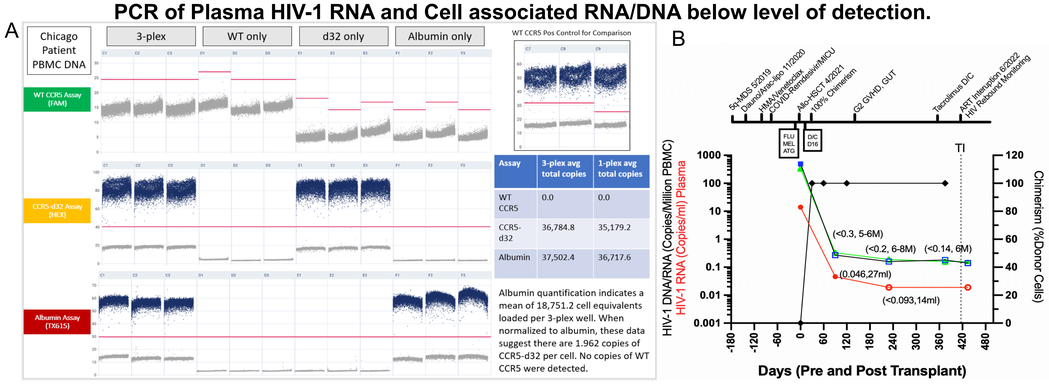

Figure 1: (A) Demonstrates DNA amplification via dPCR of CCR5(wt) and CCR5d32 1 year post SCT compared to controls. The red Line demonstrates the limit of detection. **(B)** AML and transplant treatment course are noted, top. scPCR of cell-associated HIV-1 DNA (▮), RNA (▲), and plasma HIV-1 RNA(●) are noted with respect to days pre and post stem cell transplant. Open symbols represent below the limit of detection. Chimerism (◆) described as % donor cells is also noted over time.

Figure 3:Plasma anti-MA, p24, GP120, and RT antibody levels over time. At ATI were similar to HIV-uninfected controls.

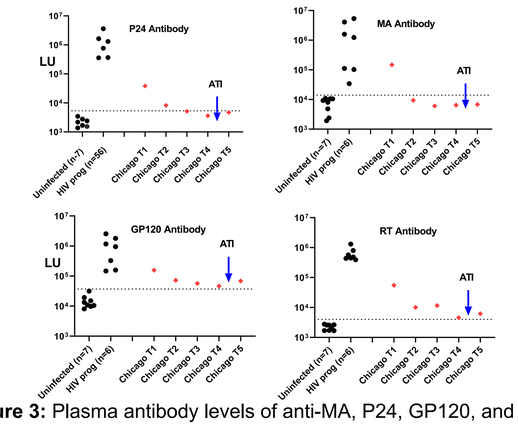

Figure 3: Plasma antibody levels of anti-MA, P24, GP120, and anti-RT of the Chicago patient over time compared to levels of uninfected controls and HIV progressors. The blue arrow represents time of ATI. The T5 time point represents antibody levels 3 weeks after HIV rebound.

Figure 4: Phylogenetic tree of the seven HIV *env* sequences obtained at time of HIV Reactivation. All predicted to be CCR5-tropic by Geno2pheno coreceptor analysis.

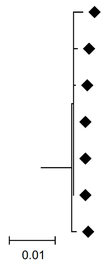

Table 1:Types of Conditioning, ATI initiation, and years of HIV prior SCT of the previous 4 reported cures utilizing CCR5Δ32/32 SCT compared to the Chicago patient

Berlin Patient[1]
Myeloablative SCT, ATI day 0, HIV dx >10 years prior to SCT
London Patient[2]
Non-Myeloablative, ATI 16 months HIV dx 9 years prior to SCT
City of Hope Patient[3]
Non-myeloablative, ATI 17 months, HIV dx >30 years prior to SCT
Impact P1107 Patient[4]
Myeloablative, ATI 37 months, HIV dx 4 years prior to SCT
Chicago Patient
Non-myeloablative, ATI 15 months, HIV dx 10 years prior to SCT

CONCLUSIONS

- We report a successful CCR5Δ32/32 homozygous allogenic reduced intensity SCT in a 67yo with high risk AML, in remission for 20 months.
- ATI was initiated after 15 months after undetectable HIV Plasma RNA and cell-associated DNA and RNA for over 12 months and no detectable wt CCR5 DNA by dPCR in the PBMCs.
- HIV plasma RNA was detected by qPCR 2 months after ATI at 780 copies/ml, with repeat viral load 1 week later at 300 copies/ml, prior to resumption of ART.
- At rebound: 1) the HIV was found to be 100% R5 tropic; 2) only CCR5Δ32 DNA was identified in the PBMCs via dPCR; and 3) to date no cell-associated HIV RNA/DNA has been identified by scPCR.
- Data implies that the virus arose from virally infected wt CCR5 cells prior to transplant, secondary to ATI, and that the graft (PBMCs) to date remains HIV free despite rebound.
- 40 millions PBMCs and 9 million CD4+ T-cell have been assayed for HIV cell-associated DNA/RNA
- Reactivation occurred wo elevations in plasma HIV-associated antibodies. Antibody levels remain close to uninfected controls, even after HIV reactivation.
- Seven HIV *env* sequences were identified, genetically diverse, all predicted to be R5 tropic, implying a non-clonal HIV reactivation

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[1]Hütter et al. N Engl J Med 2009; 360:692-698, [2]Gupta et al Nature 2019; **568:** 244–248, [3] Dickter et al. 24th international AIDS Conference, 2022; [4] JingMei Hsu et al. CROI 2022, Abstract Number 65, Oral 05