

Failure of BMT to Eradicate HIV Reservoir despite Effective HAART

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Background: We explored the impact of Bone Marrow Transplantation (BMT) on HIV reservoir in a Romanian 17-year-old HIV-1 infected man presenting a leukemia. This is the first case of BMT performed on a patient on effective HAART for 18 months.

Methods: HIV infection occurred at 8 years of age by transfusion. He developed a Burkitt's lymphoma in June 2003 and received effective HAART. He developed leukemia in April 2005 and a BMT was proposed in October. HAART was maintained except a short interruption due to toxicity between day +114 and +134. We explored HIV-DNA and HIV-RNA by a sensitive real-time PCR (*LTR* region - ANRS) before and after BMT. The lowest threshold possible for each sample was obtained even in cases with few cells, as the maximum tests per sample were realized to explore all the available cells.

Results: Before BMT, HIV-DNA was at 2.76 Log copies/10⁶ PBMC and 1.77 Log cp/10⁶ bone marrow cells. After BMT, HIV-RNA in plasma remained <1.7 Log cp/mL; HIV-DNA was undetectable in PBMC during more than 4 months (<2 Log cp/10⁶ PBMC) although more than 1 million PBMC was explored. Chimerism study showed a 100% donor phenotype. However, after HAART cessation, rebounds of HIV-RNA (4.61 Log cp/mL) and HIV-DNA (2.50 Log) in blood were observed. Resumption of HAART at day +134 returned viral loads to undetectable level at day +152. HIV-DNA was still detectable <1.5 to 2.5 Log cp/10⁶ PBMC but not detectable in oesophageal, antral, duodenal and rectal biopsies realized 12 days after HAART resumption. A phylogenetic analysis based on the gp120 C2-V3 sequences on 4 plasma viral strains (2 in 2003 and 2 in 2006 after BMT) confirmed that the viral reservoir was not eradicated and was able to produce quasiespecies in 2006 different from those in 2003. The patient died in multivisceral failure at day +191.

Conclusion: Despite BMT associated with the powerful cytotoxic conditioning regimen, eradication was not observed in this patient with long-term controlled viral replication. A short HAART interruption revealed the persistence of an infectious archived viral reservoir previously constituted in profound tissues. We hypothesized that some recipient antigen-presenting cells can survive and play a critical role as a virus reservoir. Moreover, totipotent hematopoietic progenitors may possibly survive intensive pre-transplant conditioning regimen and support the failure of eradication.

Introduction

One of the challenges in human immunodeficiency virus (HIV) infection is viral eradication, as underlined by recent studies. Allogeneic Bone Marrow Transplantation (BMT) has been suggested to be able to reconstitute patients' haematopoietic systems after the clearance of HIV-infected cells with intensive chemotherapy and radiation. In 1999, Huzicka reviewed 32 allogeneic BMT in HIV-infected patients between 1982 and 1996: in two cases, HIV seemed to be eradicated as judged by negative HIV-DNA and HIV-RNA levels using the classical polymerase chain reaction (PCR).

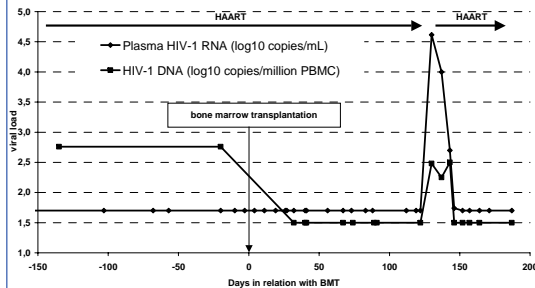
We report here the first case of BMT in a HIV-1 infected patient treated by HAART. To explore HIV reservoirs, we used a sensitive method to quantify HIV-DNA levels in peripheral blood mononuclear cells (PBMC) and biopsies.

A Romanian 17-year-old man had a transfusion-related HIV-1 infection diagnosed at 8 years of age. He was asymptomatic with CD4 cell count greater than 350/μl until he developed a Burkitt's lymphoma in June 2003 and then received effective HAART. He developed monocytic acute myeloid leukemia in April 2005. After transiently effective chemotherapy (ELAM02), a relapse occurred in September and a second line of chemotherapy followed by a human leukocyte antigen phenotypical BMT was proposed in October. Marrow cytoablation consisted of a conditioning regimen including idarubicin, Fludarabine and Aracytine. Horse anti-lymphocytic serum and cyclosporine were used. Haematological restoration was observed at day 19 posttransplant and chimerism study showed a 100% donor phenotype at days 30 and 119 posttransplant. Cytogenetic remission was confirmed on bone marrow at day 124. The patient suffered of an early and prolonged cutaneous and digestive graft-versus-host disease (grade III) partly controlled with a combination of immunosuppressive therapy. He presented several infectious complications and died in multivisceral failure at day 191 posttransplant. During all the procedure, HAART was maintained except a short interruption for a suspected toxicity between days 114 and 134 posttransplant.

HIV-RNA and HIV-DNA quantifications

HIV-DNA was quantified in PBMC, bone marrow cells and biopsies, using a real-time PCR assay amplifying the *LTR* region according to a previously described technique. The lowest threshold possible was obtained and adapted to each sample even in cases with few cells during the aplastic and immunosuppressive phases, because the maximum tests per sample were performed to explore all available cells. Before transplantation, HIV-DNA was at 2.76 log₁₀ copies/10⁶ PBMC. The medullar sample was at 1.77 log₁₀ copies/10⁶ bone marrow cells before BMT, it contained 60% monoclonal blasts, suggesting that blasts were not infected otherwise the HIV-DNA load would have been dramatically higher. The plasma HIV-RNA load (Cobas Monitor; ROCHE, France) was undetectable for 18 months before BMT. After BMT, HIV-RNA in the plasma remained at less than 1.7 log₁₀ cp/mL. HIV-DNA levels in PBMC became undetectable during more over 4 months (< 2 log₁₀ cp/10⁶ PBMC), although more than one million PBMC was explored (Fig.).

Sixteen days after HAART cessation, however, rebounds of HIV-RNA (4.61 log₁₀ cp/mL) and HIV-DNA (2.50 log₁₀ cp/10⁶ PBMC) were detected (Fig.). Resumption of HAART at day 134 returned viral loads to undetectable levels at day 152 (Fig.). HIV-DNA was still detectable (<1.5 to 2.5 log₁₀ cp/10⁶ PBMC) (Fig.) but undetectable in oesophageal, antral, duodenal and rectal biopsies performed 12 days after HAART resumption (< 2.3 - 3.3 log₁₀ copies/10⁶ cells).

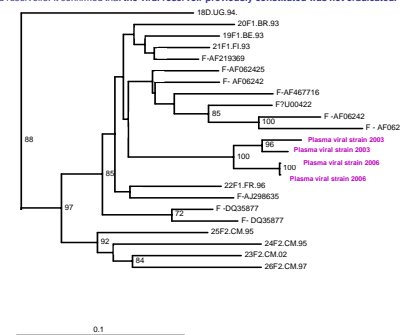


HIV-1 DNA in peripheral blood mononuclear cells (PBMC) and HIV-1 RNA in plasma before and after bone marrow transplantation (BMT).

The sensitivity threshold was 1.7 Log for HIV-1 RNA and it was arbitrary fixed at 1.5 Log for HIV-1 DNA. The thresholds for HIV-1 DNA were dependent on the number of available cells in each experiment.

Phylogenetic analysis

We performed a phylogenetic analysis based on the gp120 C2-V3 sequences on four plasma viral strains (two in 2003 and two in 2006 after BMT). They clustered together but two separate lineages were identified (one for the strains of 2003 and one of 2006) with high bootstrap value (> 95), suggesting that quasiespecies which circulated before BMT were different than those observed after. They may come from profound reservoirs. It confirmed that the viral reservoir previously constituted was not eradicated.



Conclusion

Negative results on HIV-DNA after BMT gave hope of a significant reduction of the reservoir, indeed HIV remission. The organism was repopulated by donor-derived cells that could mount a successful antiviral response through cytotoxic T lymphocytes. Moreover, graft-versus-host disease could have destroyed residual haematopoietic cells potentially harbouring virus including infected macrophages. We did not, however, observe HIV-1 eradication. Unfortunately, the HIV-1 replication recovery a few days after HAART interruption unambiguously showed the persistence of an infectious viral residual reservoir, may be in profound tissues. Despite the use of intensive pretransplant cytoablative conditioning with chemotherapy combined with 18 months of potent effective HAART before BMT (contrary to zidovudine alone used in cases described before 1996), the goal of HIV eradication was not achieved for this patient with a relatively high HIV-DNA level in PBMC.

We hypothesized that recipient antigen-presenting cells as well as totipotent hematopoietic progenitors can survive and play a critical role as a virus reservoir. Finally, this case emphasizes that the establishment of an "eradication concept" needs to be evaluated after HAART cessation.

