HIV-1 cure after CCR5Δ32/Δ32 allogeneic hematopoietic stem cell transplantation

We describe a 53-year-old man with HIV-1 who received allogeneic CCR5Δ32/Δ32 hematopoietic stem cell transplantation (HSCT) in 2013 to treat acute myeloid leukemia. Four years after analytic treatment interruption (ATI), the absence of viral rebound and the lack of immunological correlates of HIV-1 antigen persistence provide convincing evidence for HIV-1 cure.

This is a summary of:
Jensen, B-E. O. et al. In-depth virological and immunological characterization of HIV-1 cure after 13 CCR5Δ32/Δ32 allogeneic hematopoietic stem cell transplantation. *Nat. Med.* https://doi.org/10.1038/s41591-023-02213-x (2023).

Published online: 27 February 2023

The question

HIV-1 can establish a persistent viral reservoir by integrating replication-competent proviruses into the DNA of long-lived immune cells. Latent HIV-1 reservoirs remain largely unaffected even after years of suppressive antiretroviral therapy and are not sufficiently reduced by 'shock and kill' strategies aimed at reversing latency and subsequently triggering death of HIV-infected cells[1]. However, it has been shown that HSCT can substantially reduce the viral reservoir, although rebound viremia still occurs after the interruption of antiretroviral therapy[2]. A homozygous deletion of 32 base pairs in the *CCR5* gene (CCR5Δ32/Δ32), which is present in only about 1% of the Caucasian population, confers extensive resistance to HIV-1 owing to the absence of a functional CCR5 co-receptor that is crucial for HIV-1 entry into immune cells. In the 'Berlin patient' described in 2009, the deliberate selection of a CCR5Δ32/Δ32 stem cell donor revealed that HIV-1 can indeed be cured[3]. In 2013, in the context of a man living with HIV-1 who needed HSCT to treat acute myeloid leukemia, the question emerged of whether this unique achievement of an HIV-1 cure could be replicated.

The observation

Pre-transplant analysis revealed that 99.86% of proviral sequences were predictive of CCR5 co-receptor usage. After a stable clinical situation had been achieved through HSCT, studies on the viral reservoir were initiated while continuing antiretroviral therapy. Analyses of the HIV-1 viral reservoir in peripheral blood including T cell subsets, as well as in lymph node and gut tissues, which are considered to be of particular importance for the HIV reservoir, were performed using highly sensitive methods such as digital droplet PCR, intact proviral DNA assay and in-situ hybridization (DNAscope/RNAscope). Furthermore, we performed cell culture-based quantitative outgrowth assays (qVOA) and in vivo outgrowth assays using two different humanized mouse models to detect residual replication-competent viruses. Extended immunologic profiling included activation and differentiation of $CD4^+$, $CD8^+$ and natural killer cells, as well as the longitudinal measurement of HIV-, CMV- and EBV-specific T cell responses and assays to quantify HIV-1-specific antibodies.

After HSCT, we were able to detect sporadic traces of HIV-1 DNA in peripheral T cell subsets and tissue-derived samples. Nonetheless, repeated ex vivo quantitative and in vivo outgrowth assays in humanized mice did not reveal replication-competent virus. Low levels of immune activation and waning HIV-1-specific humoral and cellular immune responses indicated antigen clearance (Fig. 1). After careful consideration, antiretroviral treatment was stopped at 69 months-post HSCT, which enabled us to determine whether cure of HIV-1 had been achieved. Four years later, no viral rebound had occurred, and there was an absence of immunologic correlates of ongoing HIV-1 antigen presentation.

The interpretation

Our results provide further evidence that modifying the immune system by CCR5Δ32/Δ32 HSCT is a powerful tool that can lead to HIV-1 cure. In-depth analyses of the viral reservoir are not only technically demanding but also challenging in their interpretation, as demonstrated by the sporadic detection of traces of HIV-1 DNA in the patient without being predictive of viral rebound. Although thorough analyses of the virologic reservoir in terms of intact proviral DNA and viral outgrowth assays will certainly remain of great importance, we believe that assessment of the humoral and, even more so, the cellular immune response to HIV-1 (ref. [4]) when monitoring patients undergoing curative strategies in the future will provide insights into the persistence of (replication-competent) virus (Fig. 1).

One limitation is the singularity of our observations. Numerous factors in the patient, such as the very low proportion of HIV-1 viruses capable of using CXCR4 – the alternative co-receptor used by HIV-1 for cell entry – cannot be generalized to all individuals with HIV-1. We hope that the knowledge generated through individual cases of HIV-1 cure within our IciStem collaboration will contribute to further elucidate the factors necessary for treatment success and to incorporate this knowledge into upcoming cure strategies[5] that would be both safer and more broadly applicable to people living with HIV-1.

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EXPERT OPINION

"The manuscript convincingly documents what appears to be a case of HIV cure, which will be of high interest in the field. Other strengths of the study include the long period of follow-up, the appropriately cautious approach taken to the performance of an ATI, and a strong effort by the study team to incorporate multiple measurements of virus reservoirs both before and after ATI". **Eli Boritz, National Institutes of Health, Bethesda, MD, USA.**

FIGURE

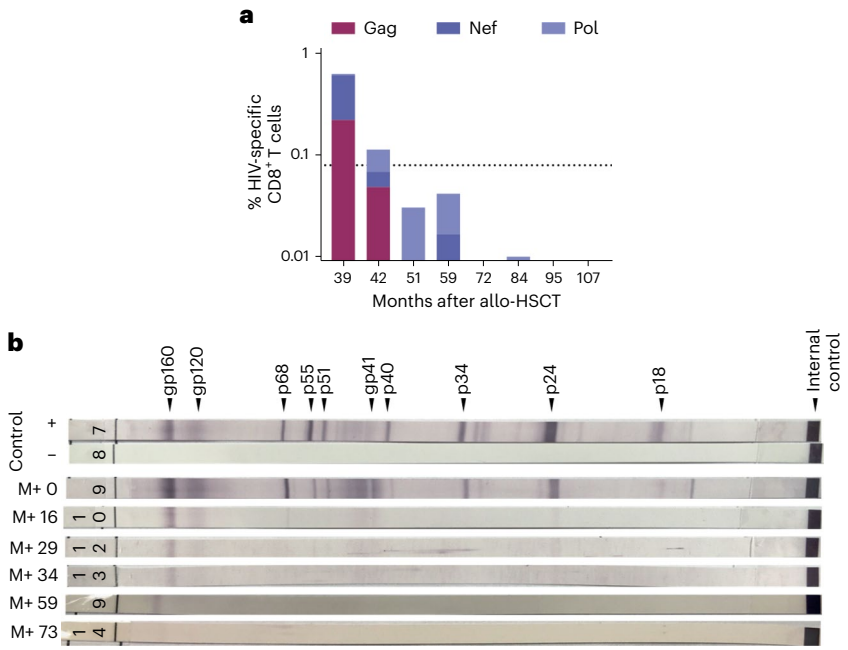

Fig. 1 | Waning HIV-1-specific cellular and humoral immune responses before and after ATI. **a**, HIV-1-specific CD8$^+$ T cell responses (production of interferon-γ (IFNγ), tumor necrosis factor (TNF), interleukin 2 (IL-2) and/or expression of CD107a) against HIV-1 Gag (burgundy), Nef (dark purple) and Pol (light purple) peptide pools waned after allogenic hematopoietic stem cell transplantation (allo-HSCT). Dotted line denotes average background signal. **b**, Full-virus lysate immunoblot assay for antibodies against HIV-1 antigens revealed waning HIV-1-specific antibody responses after HSCT and prolonged weakening of gp160- and gp120-specific antibodies. M+, months after HSCT. © 2023, Jensen, B-E. O. et al.

BEHIND THE PAPER

The 'Berlin patient' was inspiring proof that HIV-1 cure is possible. Identifying an HLA-identical CCR5Δ32/Δ32 donor, however, was a major challenge. When the donor search was started in 2012, there was only one female donor carrying CCR5Δ32/Δ32 among a total of 5 HLA-identical donors. A truly gratifying experience was the collaboration with all researchers involved, which eventually brought together researchers from 6 countries and 19 institutions. Combining different expertise allowed us to make a well-informed decision jointly with the patient to analytically interrupt antiretroviral therapy in the most careful way possible and to investigate whether this approach really cured HIV-1. We are extremely grateful to the patient for his outstanding willingness and commitment to participate in these strenuous investigations, not only to answer the question of HIV-1 cure for himself, but also to obtain as much knowledge as possible, hopefully contributing to the development of future HIV-1 cure strategies. **B-E.O.J.**

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FROM THE EDITOR

"The study goes to impressive lengths, waiting four years after interrupting antiviral treatment, to conclude durable cure of HIV-1 infection in a recipient of an allogeneic hematopoietic stem cell transplant." **Editorial Team, *Nature Medicine*.**