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A second patient cured of HIV infection: hopes and limitations

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On March 5th, 2019, Dr. Ravindra Gupta from University College London gave a presentation at the Conference on Retroviruses and Opportunistic Infections (CROI) in Seattle, Washington, USA, on a second patient that has potentially been cured of human immunodeficiency virus (HIV) type 1 infection. On the same day, the corresponding article preview was released in *Nature* [1]. The patient remained anonymous and was called the "London patient". This comes 10 years after Timothy Brown, initially referred to as the "Berlin patient", was reported to be potentially cured of HIV [2]. Since no virus has been detected 12 years after Mr. Brown stopped combination antiretroviral therapy (cART), he is now considered to be cured and, based on his experience, there is a good chance that the "London patient" has also been cured. These two patients certainly bring a great hope to all HIV-infected individuals in the world. From those successes, several questions remain to determine why only two patients have been cured 10 years apart, whether similar procedures are feasible for all infected individuals and what are their limitations.

Allogeneic bone marrow transplants to cure HIV infection

Timothy Brown contracted HIV and was diagnosed in 1995. He was successfully treated with cART, but developed acute myeloid leukemia in 2006. The "London patient" contracted HIV in 2003, was treated by cART, but developed Hodgkin's lymphoma in 2013. The only option to treat both cancers was a bone marrow transplant from another individual (allogeneic). This is a risky procedure with possible fatal complications that requires finding the best human leukocyte antigen (HLA)-compatible donor to avoid graft *versus* host disease (GVHD) and an intensive pre-transplant treatment to remove the recipient's bone marrow [3]. To cure HIV, one possibility was to find a compatible donor who is naturally resistant to HIV. HIV uses CD4 as a receptor for entry together with a coreceptor, which is a chemokine receptor, mostly CCR5 (R5 viruses) or CXCR4 (X4 viruses). About 1% of individuals of Caucasian origin have a homozygous mutation on the *CCR5* gene, called CCR5Δ32/Δ32, and cannot be infected with R5 viruses. Both patients received an allogeneic hematopoietic stem cell transplant (HSCT) from an HLA-compatible individual with the CCR5Δ32/Δ32 deletion. Timothy Brown stopped cART one day before his transplant in 2007 and has since had no detectable virus in blood and tissue samples. He had a relapse in his leukemia 332 days after his transplant and received a second transplant from the same donor. Since he was HIV free in the absence of cART before his second transplant, it is not known whether this event contributed to his cure. Prior to both transplants, he received total body irradiation and chemotherapy. The "London patient" had no irradiation, received a reduced intensity conditioning treatment and had a single HSCT in 2016. He discontinued cART in September 2017, 510 days after his transplant and had no rebound of viremia nor detectable DNA for 18 months at the time of publication. Timothy Brown is considered cured while the "London patient" is in remission, but similarities

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between both cases indicate that a cure may be achieved in this second case if HIV RNA and DNA remain undetectable for several years [1, 4].

Allogeneic HSCTs have been performed for several years on HIV$^+$ patients with cancer. In these cases, donors had wild type *CCR5* genes, and although several patients were cured from their cancer, none were cured from HIV. Two individuals, called the "Boston patients", had cART free HIV remissions following transplant, but both eventually experienced viral rebound, confirming the requirement for homozygous CCR5Δ32/Δ32 deletion to cure HIV infection [5].

Several other HIV$^+$ individuals received allogeneic HSCTs with CCR5Δ32/Δ32 cells but did not survive, due to either a cancer relapse, severe GVHD, or other causes [4, 6, 7]. The only individual who survived long enough to make conclusions about the success of the transplant in curing HIV infection was the "Essen patient" who survived for 1 year following his transplant but had viral rebound 27 days after stopping cART. This was linked to the emergence of X4 viruses (instead of R5) that can infect and replicate in CCR5Δ32/Δ32 cells [8]. The different reported attempts are summarized in *table 1*. Overall, the "Berlin and the London patients" cases remain exceptional. They may be followed soon by the "Düsseldorf patient" who also has undergone allogeneic HSCT from a CCR5Δ32/Δ32 donor and has been off cART since November 2018 [9]. All these examples increase our awareness of the limitations of the procedure, but also teach us lessons about how to apply this knowledge to new treatment options.

Limitations to apply the procedure as a large scale treatment

The next question is now about the possibility of obtaining a similar treatment for all HIV-infected individuals. In addition to the high cost of allogeneic HSCT and associated treatment, it is a very risky procedure and the fatality rate has been estimated to 10-12% at the time of the transplant, but 40-45% after one year [3, 10]. For an HIV$^+$ individual with a well-suppressed viremia, the risk is much higher than taking pills every day. Therefore, the general consensus is to reserve this procedure for patients with hematologic malignancies that are refractory to standard chemotherapy and autologous HSCT.

To be successful, all HSCTs require an HLA-compatible donor to avoid GVHD. The "London patient" was compatible with 9/10 HLA subtypes from the donor, which provided a high chance of success [1]. In addition, the only known natural resistance to HIV entry is in individuals with homozygous CCR5Δ32/Δ32 mutation. These patients are rare and finding HLA-compatible donors homozygous for this mutation has been extremely difficult [11, 12].

Sequencing of the viral envelope gives a good prediction of the coreceptor usage, but X4 viruses may not be detected when they are present in low amount in the patient's virus stock. This is exemplified by the report about the "Essen patient" who had a rebound of X4 viruses that were amplified by the selection against R5 viruses. Viremia was effectively suppressed by re-initiation of cART, but this case demonstrates that another limitation of allogeneic CCR5Δ32/Δ32 transplant is its potential to select for X4 capable viruses [7, 8] (*table 1*).

Strategies to reproduce a similar procedure using the patient's own cells

The "Berlin and London patients" represent proof of concept that a cure for HIV can be achieved, but the steps to have a therapy that can be widely applied may be long. Different strategies to mimic this procedure and overcome the obstacles have been developed. Activating and killing all HIV-infected reservoir cells is a method that has been tried but has been unsuccessful so far, due to the difficulty to identify and reach all cellular and anatomical reservoirs. Obtaining long-term non-progression after early treatment and interruption is another approach but has been obtained only in a limited number of infected individuals. Locking the virus in an inactive form to prevent reactivation by using specific compounds has also been proposed and identifying molecules that can achieve deep latency is the subject of intense research. The use of clustered regularly interspaced short palindromic repeats (CRISPR) nucleases to remove HIV DNA from the cells is very attractive and can be achieved in cultured cells, but with current delivery methods it will be very difficult to find a safe and effective method to reach all HIV reservoir cells. The closest and most achievable methods to reproduce the cure obtained with the "Berlin and the London patients" are autologous transplants, which use the patient's own cells and modify them by the mutation of the CCR5 gene and the expression of antiviral molecules by gene therapy.

Although CCR5 has some function in cells, individuals having a mutation seem to live normal lives. Therefore, modifying the CCR5 gene in targeted cells (lymphocytes or HSCs) followed by autologous reinfusion should be safe. Several nucleases have been used to mutate the CCR5 gene including Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and more recently CRISPR nucleases with associated guide RNA (gRNA). Ongoing clinical trials include patient-ZFN modified CD4$^+$ T cells and HSCs (SB-728-T and SB-728-HSPC)

Table 1 HIV positive allogeneic hematopoietic stem cell transplant (HSCT) recipients from CCR5Δ32/Δ32 donors (all male).

Patient/transplant location	Reason for transplant	Age[1]	Outcome
Timothy Brown/Berlin	Acute myeloid leukemia	40	No viremia for >12 years following transplant and discontinuation of cART [2]
NR/Utrecht	Myelodysplastic syndrome	53	Relapse of myelodysplastic syndrome and death 2 months after transplant [7]
NR/Münster	Non-Hodgkin's lymphoma	51	Infection and death 4 months after transplant [7]
NR/Minneapolis	Acute lymphoblastic leukemia	12	GVHD and death 3 months after transplant [7]
NR/Santiago	Non-Hodgkin's lymphoma	46	Pneumonia and death shortly after transplant [7]
NR/Barcelona	Non-Hodgkin's lymphoma	37	Relapse of lymphoma and death 3 months after transplant [6]
NR/Essen	Non-Hodgkin's lymphoma	27	Viral rebound with CXCR4 using virus 27 days after discontinuation of cART (20 days after transplant). Relapse of lymphoma and death 12 months after transplant [8]
NR/Halifax	Chronic myeloid leukemia	58	Decreased viral reservoir 9 months after transplant. Death from myocardial infarction 1.5 years after transplant [L. Barrett, personal communication]
NR/London	Hodgkin's lymphoma	NR	No viremia for 18 months following discontinuation of cART [1]
NR/Düsseldorf	Acute myeloid leukemia	49	No viremia for 4 months following discontinuation of cART [9]

NR: not reported.
[1] Age at time of transplant.

and more recently patient CRISPR modified HSCs (ClinicalTrials.gov Identifier: NCT03164135) [13, 14]. Although this approach to engineer HIV resistance most closely mimics the cases of the "Berlin and London patients", results from the "Essen patient" demonstrate that CCR5 modification alone will not be successful for individuals that harbor even very small amounts of X4 viruses, which may necessitate alternative strategies, such as the insertion of antiviral genes into patient cells.

Another strategy to reach a cure and to circumvent the problems of HSCT from a resistant donor would be an autologous transplant, after engineering the cells to make them HIV-resistant by gene therapy. The first clinical trials were performed by transplant of $CD4^+$ T cells, which were transduced with retroviral vectors that harbour genes producing anti-HIV RNAs, peptides or proteins. Since then, several clinical trials using both $CD4^+$ T cell transplants and HSCTs have been conducted. So far, the procedure has proven to be safe but a cure has not yet been achieved. The problems encountered include a low transduction efficiency by the retroviral vectors used to deliver the genes, poor long-term engraftment of the modified cells, and weak efficacy of the molecules used [15-17]. Therefore, the future of these studies will be to find combinations of safe and effective genes or gene editors using an effective delivery method to HSCs or T cells so that HIV replication is completely inhibited and no resistant virus emerges. Given the diversity of both HIV and HIV-infected individuals, it is likely that several different combinations will be needed to ensure that successful cures can be obtained for all HIV-infected individuals. Improvements will be made over time to decrease the costs and facilitate the use in low-income countries.

Conclusions

The "Berlin and London patients" represent a proof of concept that a cure can be obtained with cells homozygous for CCR5Δ32, but the compatible donors remain rare and low amount of R4 strains can result in a rebound in viremia. Finally, autologous transplant with modified cells remains the safest and most promising procedure that could be applied on a large scale once optimized. It could be done either by mutating CCR5 using gene-editing nucleases or by gene therapies that will induce a permanent production of small antiviral RNAs or peptides. A combination of both methods will probably become the most efficacious. Our

path to the elimination of HIV from the human body has gone one step further and gives us great hope and encouragement to continue our efforts towards a cure for HIV infection.

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Declaration of interests : A.G., R.J.S. and McGill University hold the US patent 9,932,364 issued April 3rd, 2018 for one antiviral RNA that could be used in gene therapy. We have no affiliation with a financial or commercial entity for this patent.

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