Evidence for HIV-1 cure after CCR5Δ32/Δ32 allogeneic haemopoietic stem-cell transplantation 30 months post analytical treatment interruption: a case report

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Summary

Background The London patient (participant 36 in the IciStem cohort) underwent allogeneic stem-cell transplantation with cells that did not express CCR5 (CCR5Δ32/Δ32); remission was reported at 18 months after analytical treatment interruption (ATI). Here, we present longer term data for this patient (up to 30 months after ATI), including sampling from diverse HIV-1 reservoir sites.

Methods We used ultrasensitive viral load assays of plasma, semen, and cerebrospinal fluid (CSF) samples to detect HIV-1 RNA. In gut biopsy samples and lymph-node tissue, cell-copy number and total HIV-1 DNA levels were quantified in multiple replicates, using droplet digital PCR (ddPCR) and quantitative real-time PCR. We also analysed the presence of intact proviral DNA using multiplex ddPCR targeting the packaging signal (ψ) and envelope (env). We did intracellular cytokine staining to measure HIV-1-specific T-cell responses. We used low-sensitive and low-avidity antibody assays to measure the humoral response to HIV-1. We predicted the probability of rebound using a mathematical model and inference approach.

Findings HIV-1 viral load in plasma remained undetectable in the London patient up to 30 months (last tested on March 4, 2020), using an assay with a detection limit of 1 copy per mL. The patient’s CD4 count was 430 cells per µL (23·5% of total T cells) at 28 months. A very low-level positive signal for HIV-1 DNA was recorded in peripheral CD4 memory cells at 28 months. The viral load in semen was undetectable in both plasma (lower limit of detection [LLD] <12 copies per mL) and cells (LLD 10 copies per 10⁶ cells) at 21 months. CSF was within normal parameters at 25 months, with HIV-1 RNA below the detection limit (LLD 1 copy per mL). HIV-1 DNA by ddPCR was negative in rectum, caecum, and sigmoid colon and terminal ileum tissue samples at 22 months. Lymph-node tissue from axilla was positive for the long-terminal repeat (33 copies per 10⁶ cells) and env (26·1 copies per 10⁶ cells), negative for ψ and integrase, and negative by the intact proviral DNA assay, at 27 months. HIV-1-specific CD4 and CD8 T-cell responses have remained absent at 27 months. Low-avidity Env antibodies have continued to decline. Mathematical modelling suggests that the probability of remission for life (cure) is 98% in the context of 80% donor chimerism in total HIV target cells and greater than 99% probability of remission for life with 90% donor chimerism.

Interpretation The London patient has been in HIV-1 remission for 30 months with no detectable replication-competent virus in blood, CSF, intestinal tissue, or lymphoid tissue. Donor chimerism has been maintained at 99% in peripheral T cells. We propose that these findings represent HIV-1 cure.

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Introduction HIV cure, exemplified by extended periods of antiretroviral therapy (ART) with negative results on testing for HIV nucleic acid and loss of adaptive immune responses, has been elusive. For a decade, only one such case, referred to as the Berlin patient, was recognised. This individual underwent two rounds of total body irradiation and allogeneic haemopoietic stem-cell transplantation (allo-HSCT) with donor cells that did not express CCR5 (CCR5Δ32/Δ32) for treatment of acute myelogenous leukaemia. In 2019 we reported HIV-1 remission in the London patient (participant 36 in the IciStem cohort [IciS-36]), who underwent one HSCT procedure with CCR5Δ32/Δ32 donor cells for treatment of refractory Hodgkin lymphoma (stage IVb). Remission in the London patient, shown by testing of plasma and circulating peripheral CD4 T cells for traces of HIV-1, was reported 18 months after analytical treatment interruption (ATI) of ART. Here, we present longer term clinical, laboratory, and mathematical modelling data to make the case for long-term remission of HIV-1 (cure) in this individual.
**Research in context**

**Evidence before this study**

We searched PubMed on Feb 1, 2020, with the terms “allogeneic transplantation”, “CCR5”, and “HIV”, and either “cure” or “remission”. We did not restrict our search by date or language.

To date, the only reported case of long-term remission (cure) of HIV is the Berlin patient, who underwent two allogeneic haemopoietic stem-cell transplantation (allo-HSCT) procedures using cells from a homozygous CCR5Δ32 (CCR5Δ32/Δ32) donor. Despite reports showing unsuccessful allo-HSCT with CCR5Δ32/Δ32 tissue, in 2019 we reported an individual (the London patient) who achieved long-term suppression of HIV-1 after such a procedure. We did not report data for HIV-1 testing of reservoirs other than blood.

**Added value of this study**

We have shown in the London patient an absence of HIV-1 replication in samples of blood, cerebrospinal fluid, semen, intestinal tissue, and lymphoid tissue up to 30 months after analytical treatment interruption of antiretroviral therapy. Remnants of integrated HIV-1 DNA sequences that are unlikely to be capable of producing virus remain in tissue samples and can be regarded as so-called fossils. Antibodies to HIV-1 envelope protein remain positive.

**Implications of all the available evidence**

Long-term remission of HIV-1 can be achieved using reduced-intensity drug regimens with one CCR5Δ32/Δ32 allo-HSCT procedure without total body irradiation. Evidence of past HIV infection might persist analogous to antibody responses to other viral infections that have been cleared.

**Methods**

**Viral load testing**

All testing was done at University College Hospitals NHS Trust (London, UK). We used standard clinically validated hospital laboratory assays to measure HIV-1 viral load in plasma and semen samples. Further, we used an in-house ultrasensitive plasma and cerebrospinal fluid (CSF) viral load assay to detect HIV-1 RNA. Seminal plasma was separated from the cellular fraction by centrifugation. We centrifuged 8 mL plasma or CSF at 21 000 g for 2 h at 4°C before removing the supernatant and resuspending the pellet in 700 µL of residual plasma. We then tested the suspension using the Hologic Aptima HTV RNA Quant Dx assay (Marlborough, MA, USA).

**Blood and tissue processing**

We isolated peripheral blood mononuclear cells (PBMCs) from 60 mL EDTA (edetic acid) blood by centrifugation on a Ficoll gradient, and did magnetic activated cell sorting to retrieve naïve T cells and memory T cells (CD4+ T-cell and Th1ve CD4+ Cell Isolation Kit; Miltenyi Biotec, Cologne, Germany). DNA was isolated using a DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). Biopsy samples of gut tissue (rectum, caecum, and sigmoid colon and terminal ileum) were obtained by colonoscopy and targeted and ddPCR controls were used. The DNA was isolated using a QiaGen AllPrep DNA/RNA Mini kit. Increased uptake of [18F]fluorodeoxyglucose in an axillary lymph node was seen on PET-CT and a diagnostic lymph-node biopsy sample was taken. The biopsy specimen was processed the same way as were the gut biopsy samples.

**Quantitative real-time PCR**

For each quantitative real-time PCR (rtPCR), standards were tested in triplicate, in addition to two positive control wells in triplicate and six negative control wells. We used 25 000 cells’ worth of DNA (based on albumin rtPCR) per well for the London patient. The packaging site (ψ) rtPCR amplifies a fragment between positions 692 and 797. The envelope (env) rtPCR amplifies a fragment between positions 7736 and 7851.

**Droplet digital PCR**

We quantified HIV-1 DNA using droplet digital PCR (ddPCR; Bio-Rad, Hercules, CA, USA) targeting the long terminal repeat (LTR), gag, and integrase regions, shown as target copies per 10⁶ cells tested. We measured in duplicate amounts of the human gene for RNase P (RPP30), which is present twice in diploid cells, to ascertain the input cell number. In all ddPCR runs, water and donor PBMCs were tested in duplicate as negative controls for HIV target regions and U1 cells were tested as a positive control. Samples generating one positive droplet were interpreted as negative based on the occurrence of a sporadic positive droplet in the negative controls (one in 40 reactions). We further analysed samples showing at least two positive droplets in an intact proviral DNA assay (IPDA), essentially as described. The IPDA assay is based on a duplex ddPCR targeting two regions in the viral genome that are present in most intact proviruses, ψ and the Rev response element (RRE) in env. The env ddPCR also contains a probe without a fluorescent label that can discriminate G-A hypermutated proviruses. Again, to ascertain cell number and DNA shearing, RPP30 was targeted and ddPCR controls were used. The DNA shearing index was calculated to correct the number of intact proviruses (double positives for ψ and env).
HIV-1, cytomegalovirus, and Epstein-Barr virus specific CD4 and CD8 T-cell responses

For intracellular cytokine staining and peptide stimulation, PBMCs were thawed and resuspended in RPMI complete media. After overnight rest at 37°C and 5% carbon dioxide, PBMCs were stimulated for 6 h with 2 μg/mL HIV-1 Gag pools or cytomegalovirus (CMV) pp65 (JPT Peptide Technologies, Berlin, Germany) or the PepTivator Epstein-Barr virus (EBV) consensus pool (Miltenyi Biotec) containing 43 peptides of between eight and 20 amino acids in length from the proteins LMP2A, BRLF1, BMFL1 (EB2), BNLFL1 (LMP1), BERF3 (EBNA6), BERF1 (EBNA3), BERF2 (EBNA4), BALF2 (DNBI), BMFRF1, BZLF1, BNRF1 (MTP), EBNA1, BLLFL1 (pp350), and BARF in the presence of 1 μg/mL anti-CD28 and anti-CD49d CoStim antibodies (BD Biosciences, Cowley, UK), 2 μmol/L Monensin (BD Biosciences), 10 μg/mL Brefeldin A (Sigma, St Louis, MO, USA), and anti-CD107a APC-H7 antibody (BD Biosciences). Stimulation with costimulatory antibodies, protein transport inhibitors, and (eBioscience, Waltham, MA, USA). Stimulation with 0–005% dimethyl sulphoxide in the presence of costimulatory antibodies, protein transport inhibitors, and CD107a was done as a negative control. Samples were acquired on a BD Fortessa X20 using BD FACSDiva 8.0 (BD, Ashland, OR, USA).

HIV-1 antibody responses

Specific HIV-1 antibodies in longitudinal serum samples diluted one part in two parts were tested in a qualitative western blot assay (New Lav Blot 1; Bio-Rad). Standard and low-sensitive versions of the Vitros anti-HIV-1 assay (Ortho-Clinical Diagnostics, High Wycombe, UK) and the low-sensitive version of the low-sensitive Vitros assay uses a one part in two parts. First, we used a mathematical model8 to simulate the expected distribution of rebound times as a function of reservoir size and target cell fraction (ie, chimerism). Second, we used this model with a previously developed Bayesian inference framework9,10 to interpret the outcome of the London patient. In particular, we estimated the posterior probability of a rebound sometime in the future (vs lifetime remission) as a function of both the degree of target cell...
susceptibility (CCR5Δ32/Δ32 chimerism) and the current time off ART without rebound (appendix pp 1–5).

Ethics and consent

The London patient was registered to the IciStem consortium as IciS-36. We obtained ethics approval from the UK National Health Service Health Research Authority Research Ethics Committee in April, 2017 (reference 17/SW/0021, protocol no 16/0594). The patient provided full written informed consent in July, 2017. The patient also reviewed the final version of the updated Article and provided written consent to its publication.

Role of the funding source

The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Since 2019, the London patient has shown somewhat slow CD4 reconstitution (figure 1). At 28 months after ATI, the CD4 count reached near pretransplant levels (430 cells per µL [23·5% of total T cells]), with CD4:CD8 of 0·86 (normal range 1·5–2·5). However, no opportunistic infections were reported and peripheral T-cell chimerism was maintained at 99% (figure 1). HIV-1 plasma viral load was monitored up to 30 months after ATI, using a hospital-based assay with a lower limit of detection (LLD) of 20 copies per mL, and viral load was below the LLD (<1 copy per mL) up to 30 months after ATI using an ultrasensitive assay ( viral load on March 4, 2020, <1 copy per mL). Reactivation of EBV at around 21 months after ATI, with peak viral load of 35 000 copies per mL plasma, was managed conservatively without specific treatment and viral load fell to 3500 copies per mL at 23 months. No clinically significant CMV reactivation was seen from 18 months to 28 months post ATI (figure 1). Of note, no further episodes of graft versus host disease (GvHD) were reported since gut GvHD at 2 months post-transplant, and no immunosuppressive drugs were taken after ATI. The London patient did not use ART for pre-exposure prophylaxis.

HIV-1 LTR ddPCR analyses were negative in bulk CD4 T cells (<2·4 LTR copies per 10⁶ cells) and in the naïve CD4 T-cell subset (<3·5 LTR copies per 10⁶ cells) 28 months after ATI. In the memory subset, two positive droplets were detected in the LTR ddPCR in one of the eight replicates. IPDA showed one droplet positive in six replicates for both ψ and HIV env. HIV env was also detected as one positive droplet in two other wells (appendix p 7). The DNA shearing index was 0·24. No correction for shearing was applied in view of the uncertainty of a false-positive signal with one double-positive droplet.

A semen sample was obtained from the individual at 21 months after ATI. Semen viraemia was below the LLD (<12 copies per mL) and cells (<10 copies per 10⁶ cells). It was not possible to quantify the input number of cells in this assay, however.

Because of neurological symptoms, the London patient underwent MRI and lumbar puncture 25 months after ATI. CSF contained normal protein with no cells (<5 cells per µL) detected. HIV-1 viral load in CSF was negative (LLD 1 copy per mL).

Colonoscopy was done 22 months after ATI and tissue biopsy samples were taken. No evidence was seen of recurrence of gut GvHD. HIV-1 LTR and integrase ddPCR analyses were negative in all gut biopsy specimens (sigmoid colon and ileum >0·2 million cells tested; caecum >0·3 million cells tested; rectum >0·15 million cells tested; appendix p 8). HIV-1 LTR DNA by rtPCR was also negative in rectum, caecum, and sigmoid colon and terminal ileum (appendix p 9).

The London patient had increased metabolic activity in an axillary lymph node on PET-CT and underwent diagnostic lymph node biopsy 27 months after ATI. Histology...
was reported as showing follicular hyperplasia with follicle lysis and large numbers of EBV-positive cells within the follicles, most likely a reactive lymphadenopathy. The lymph node was positive for HIV-1 env. The absence of rebound after ART interruption could be due to two mechanisms: a reduction in the latent reservoir after conditioning and transplantation, and a reduction in the fraction of susceptible target cells for CCR5 tropic virus because of engraftment of donor CCR5Δ32/Δ32 cells. To understand the effect of reservoir size on rebound time and in combination, we used a mathematical model of latent and active HIV-1 dynamics. This model is based on previously developed tools to understand the effect of reservoir size on rebound time and is adapted to account for the effect of a reduced target cell pool on viral growth (appendix pp 1–5). Although donor cell chimerism was measured in PBMCs to be approximately 99%, we postulated that this fraction could be lower in tissue sites vital for HIV-1 replication. Therefore, we considered a range of scenarios for the fraction of cells susceptible to HIV-1 infection. For each scenario, we simulated the distribution of expected rebound times and the probability of long-term remission (cure) as a function of reservoir size at the time of ART interruption (appendix p 3). These results were then used to calculate the probability that the patient would remain in remission for life, in view of observations of 29 months without rebound and no viral outgrowth seen in 24 million CD4 T cells, as previously reported at 18 months (figure 5). If target cells remain susceptible to infection...
(and reservoir reduction is the main mechanism for delayed rebound), the model predicts there is only a 38% chance the patient will be in remission for life. With 50% chimerism, the likelihood of full remission jumps to 87%; with 80% chimerism, the chance of remission rises to 98%. For 90% or higher reduction in susceptible cells, we estimate a negligible chance of future rebound.

**Discussion**

After extensive tissue sampling completed by 3·5 years after ATI, the Berlin patient was reported as being cured from HIV-1. The London patient has been in HIV remission for 30 months (as of March 4, 2020), with plasma negative for HIV-1 RNA (<1 copy per mL) and semen negative at less than 12 copies per mL at 21 months. HIV-1 replication can occur in the CNS even during suppressive ART and is frequently associated with symptoms and MRI abnormalities. However, no detectable virus was recorded in CSF. Similarly, intestinal lymphoid tissue is an important HIV reservoir and HIV-1 DNA was negative in gut biopsy samples by ddPCR and rtPCR.

Similar to the Berlin patient, highly sensitive tests showed very low levels of so-called fossilised HIV-1 DNA in some tissue samples from the London patient. Residual HIV-1 DNA in axillary lymph node tissue could represent a defective clone that expanded during hyperplasia within the lymph node sample. In support of this hypothesis, we recorded EBV-specific CD4 and CD8 responses in peripheral blood, although we were unable to separate lymph node-associated T cells for testing. Sequence analysis of HIV-1 DNA in lymph nodes was not successful, probably because of the very low copy number; therefore, we cannot fully rule out the possibility that very low levels of intact HIV-1 provirus have persisted. The signal could also be attributable to contamination, although sequencing would be needed to investigate this possibility further.

Myeloid cells (eg, macrophages) are also present in lymph nodes and are susceptible to both T-cell dependent and independent infection with HIV-1, and they survive chemotherapy with drugs such as etoposide. Myeloid cells can also proliferate and could, therefore, have contributed to the noted HIV-1 DNA signal in lymph node tissue.

Moreover, a low-level signal in peripheral memory CD4 T cells was recorded by ddPCR. Such occasional low-level positive signals were also seen in the Berlin patient and, previously, in CD4 T cells in the London patient, which could reflect a false ddPCR signal, potential contamination, or evidence of very low levels of persistent HIV-1 DNA-positive cells. HIV-1-specific CD4 and CD8 T-cell responses also remained below detection, by contrast with maintenance of CMV-specific responses. We saw declining...
responses in HIV-1 specific antibodies up to 27 months after ATI using detuned (low-sensitive) and low-avidity assays, despite positive ELISA. Western blot results showed similar appearances at 27 months compared with at 18 months after ATI, with only gp160 antibodies remaining. These results are consistent with a positive standard ELISA against HIV-1 env reported previously both in the London patient and the Berlin patient. We believe that persistent HIV-1 Env-specific plasma cells could have survived allo-HSCT and that the low-level env PCR signal in lymph nodes could be related to this observation. Standard ELISA testing, therefore, cannot be used as a marker for cure, although more work needs to be done to assess the role of detuned and low-avidity antibody assays in defining cure.

The slow recovery in CD4 count in the London patient could be the result of the lengthy duration of untreated HIV infection between 2003 and 2013 or to the effects of aternutuzumab (anti-CD52 monoclonal antibody used for lymphodepletion in the setting of allo-HSCT). The noted reactivation of EBV at 21 months after ATI could have been related to this continued relative immunosuppression, although CMV reactivation did not occur at that time and no opportunistic infections arose. Importantly, the London patient did not receive any cytotoxic agents or GVHD prophylaxis beyond 6 months after allo-HSCT.

Mathematical modelling suggested that if more than 80% of target cells were donor-derived then remission for life (cure) was 98% likely in view of no rebound at 29 months. With chimerism higher than 90%, cure is almost certain in the model. These results can be understood along the same principle as the vaccination threshold in epidemiology. As long as a critical fraction of target cells are protected (eg, a critical percentage of chimerism is reached), the infection cannot grow exponentially. This critical fraction depends on the value of the basic reproduction ratio $(R_0)$ for the infection, and a distribution for this value across patients can be estimated from viral growth rates during acute infection combined with estimates of the HIV generation time (appendix pp 1–5). We plan to do viral load testing twice a year up to 60 months after ATI before testing yearly to 120 months.

In conclusion, we believe these findings probably represent the second recorded HIV-1 cure after CCR5Δ32/Δ32 allo-HSCT, with evidence of residual low-level HIV-1 DNA. Despite showing (in both the London patient and the Berlin patient) that CCR5-directed approaches can lead to long-term remission of HIV-1, several barriers remain to be overcome (eg, gene editing efficiency and robust safety data) before CCR5 gene editing can be used as a scalable cure strategy for HIV-1.

**Declaration of interests**

RKG has received consultancy fees from ViV Healthcare and Gilead Sciences, outside of the submitted work. JM-P holds institutional grants or has received educational or consultancy fees from AbiVax, AstraZeneca, Gilead Sciences, Grifols, Janssen, Merck, and ViV Healthcare, outside of the submitted work. MN has received consultancy fees from Gilead Sciences, outside of the submitted work. AMJW reports grants paid to his institution from CLJ, Gilead Sciences, Janssen, Merck, and ViV Healthcare, outside of the submitted work; financial support paid to her institution from Virology Education (conference organiser), outside of the submitted work; and non-financial support from ARK diagnostics, outside of the submitted work. EO has received grants from the National Institute for Health Research and GlaxoSmithKline. DP, ALJ, CG, MS, MP, LEM, SAG, JT, AA, LEPH, PG, SGE, AJI, JF, and EO declare no competing interests.

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**References**


