CCR5 Δ32 homozygous cord blood allogeneic transplantation in a patient with HIV: a case report

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Summary
Background Allogeneic donor CCR5 Δ32 homozygous haemopoietic cell transplantation (HCT) provides the only evidence to date of long-term control of HIV infection. However, availability of conventional CCR5 Δ32 homozygous donors is insufficient to develop this as a therapeutic strategy further.

Methods We present a 37-year-old patient with HIV-1 infection and aggressive lymphoma who had disease progression after five lines of radiochemotherapy including an autologous HCT, and in the absence of matched sibling donors, received an allogeneic HCT with four of six HLA-matched CCR5 Δ32 homozygous cord blood cells (StemCyte, Covina, CA), supported with purified CD34+ cells from a haploidentical sibling. Blood or tissue samples were obtained before and weekly after HCT to monitor transplant and HIV infection, including chimerism analysis, CCR5 genotyping and viral tropism, viral isolation and sequence, viral reservoir analysis, immune activation and proliferation, and ex-vivo cell infectivity assays. Combined antiretroviral therapy continued during the procedure.

Findings The patient’s HIV was CCR5-tropic by genotypic and phenotypic analyses. Baseline latent reservoir tests showed HIV DNA copies in bulk and resting CD4 T cells and in gut-associated lymphoid tissue, CD4 T-cell-associated HIV RNA, replication competent viral size of 2–1 copies per 10 7 CD4 T cells, and single copy assay of 303 copies per mL. After HCT, plasma HIV DNA load was undetectable by ultrasensitive analyses. Upon cord blood full chimerism, the patient’s CCR5 Δ32 homozygous CD4 T cells responded to proliferation and activation stimuli and became resistant to infection by the patient’s viral isolate and by laboratory-adapted HIV-1 strains. Death related to lymphoma progression regretfully prevented long-term monitoring of the patient’s viral reservoir.

Interpretation CCR5 Δ32 homozygous cord blood reconstitution can successfully eliminate HIV-1 and render the allogeneic graft recipient’s T lymphocytes resistant to HIV infection. Thus, they build on the evidence available to strongly support the use of cord blood as a strategic platform for a broader application of non-functional CCR5 transplantation to other infected individuals.

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Introduction A single case, the Berlin Patient reported by Hütter and colleagues,12 provides the only evidence to date of long-term control of HIV-1 infection after allogeneic haemopoietic cell transplantation (HCT) from a homozygous CCR5 Δ32 unrelated adult donor. Viral recurrence after HCT with heterozygous or wild-type variants makes a compelling argument for CCR5 Δ32 homozygous allogeneic reconstitution as the key mechanism driving resistance to infection in the Berlin Patient.14 Unfortunately, the search for another case remained unsuccessful for many years,2 and a recent case described by Kordelas and colleagues7 showed that in the presence of CXCR4-tropic HIV-1, CCR5-knockout strategies might not control HIV infection. The low prevalence of the genotype (<1% in white people and lower in other ethnic groups) and stringent HLA matching criteria make it unfeasible to obtain CCR5 Δ32 homozygous haemopoietic cells from conventional adult HCT donors for broader investigation of this strategy.9,10

Haemopoietic cells from cord blood allow more permissive HLA compatibility,13 leading recent efforts in this specialty towards the development of readily available inventories of CCR5-screened Δ32 homozygous units as a potential platform for allogeneic HCT in HIV-infected individuals.9,18 Here, we describe a case of allogeneic HCT with CCR5 Δ32 homozygous cord blood cells in a patient with HIV-1 infection and a diffuse large B-cell lymphoma (DLBCL).

Case report A 36-year-old man with HIV-1 infection identified in September, 2009, was referred to our hospital in March 2012 with a recent diagnosis of DLBCL. At the onset of lymphoma, the patient was on effective antiretroviral therapy (ART) with suppressed plasma viraemia (figure A) and no illnesses associated with the AIDS. ART combined the following drugs at various periods of the clinical course: emtricitabine 200 mg daily, tenofovir 300 mg daily, nevirapine 200 mg twice daily, lamivudine...
150 mg twice daily, abacavir 300 mg twice daily, and raltegravir 400 mg twice daily (figure A). He presented with a large abdominal mass expanding from the left kidney to the iliac lymph nodes and additional paraaortic and retrocrural involvement, stage II–A. At the onset of DLBCL, the patient’s CD4 count was 360 cells per μL, and he had an HIV RNA viral load of less than 50 copies per mL. Before allogeneic HCT, the patient received several lines of chemotherapy, including six courses of CHOP-R (rituximab 375 mg/m², vincristine 2 mg, doxorubicin 50 mg/m², cyclophosphamide 750 mg/m², and methylprednisolone 80 mg, all on day 1, and prednisone 100 mg on days 2–5), three courses of ESHAP-R (rituximab 375 mg/m² on day 1, methylprednisolone 500 mg, etoposide 40 mg/m², and cisplatin 25 mg/m² on days 1–4, and cytarabine 2000 mg/m² on day 5), an autologous HCT with BEAM conditioning (carmustine 300 mg/m² on day 1, etoposide 200 mg/m², and cyclophosphamide 200 mg/m²/12 h on days 2–5, and melphalan 140 mg/m² on day 6), three courses of GEMOX (gemcitabine 1000 mg/m² and oxaliplatin 100 mg/m² on day 1), and local abdominal radiotherapy (36 Gy, divided in 18 sessions of 2 Gy per session). At the time of allogeneic HCT the patient had a refractory lesion in the left psoas, no B symptoms, and had good clinical performance with adequate results on cardiac and pulmonary assessments.

No HLA-matched sibling donors were available. After approval by the Spanish Bone Marrow Donors Registry, a directed search identified two compatible CCR5 Δ32 homozygous cord blood units (StemCyte, Covina, CA, USA; table 1).2 A dual allogeneic HCT was done after myeloablative conditioning with fludarabine 120 mg/m², busulfan 12 mg/kg, cyclophosphamide 100 mg/kg, and thymoglobulin 4 mg/kg, graft-versus-host disease prophylaxis with ciclosporin plus short-course corticosteroids, and combining the single cord blood unit with higher cellularity (CBU 1) and purified CD34+ cells from a haploidentical sibling, as previously described.15–17 Blood or tissue samples were obtained before and weekly after HCT to monitor HIV infection. ART continued during the procedure (figure A). The patient provided informed consent and the institutional review board approved the transplant and investigational protocol (CEIC Bellvitge 2013-023449).

Early transplant complications, including ciclosporin-related toxicity, hepatic veno-occlusive disease and cytomegalovirus reactivation, all resolved with appropriate treatment. Neutrophil engraftment, 100% haploidential donor-derived, occurred 11 days after transplantation. However, donor switch from haploidential to cord blood origin did not follow as expected (figure B).15–17 High-resolution chimerism analysis detected low-level cord-blood-derived haemopoiesis below 5% for up to 7 weeks after transplant. Post-thawing analysis of CBU 1 showed a mild reduction in cell content and very poor clonogenic capacity (table 2). CBU 2 was subsequently infused on day 52 after 4 mg/kg thymoglobulin. Within 3 weeks from CBU 2 infusion, the chimerism from CBU 1 increased very rapidly to 20% on day 59, 83% on day 66, and 100% by day 73. In this process, the patient’s haemopoiesis, including CD4 T-cell subset analysis, changed from homozygous wild-type CCR5 (recipient) to heterozygous (haploidential sibling), and finally became homozygous (figure B). He did not develop any symptoms or signs of graft-versus-host disease. Regrettfully, the patient developed an aggressive progression of DLBCL. After very rapid clinical deterioration, he passed away from disease progression 3 months after transplant.
Methods

Chimerism analysis

Post-transplant chimerism was analysed on full peripheral blood mononuclear cells and on immuno-magnetically selected CD3+ and CD15+ cells with microsatellite markers fluorescent PCR (AmpFLSTR Identifier Plus, Applied Biosystems, Foster City, CA, USA), and indel markers high-resolution real-time PCR.
Table 2: Cell products content

<table>
<thead>
<tr>
<th>Recipient</th>
<th>CBU 1</th>
<th>CBU 2</th>
<th>TPHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) or year of collection</td>
<td>37</td>
<td>2008</td>
<td>2008</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Blood group</td>
<td>O positive</td>
<td>O positive</td>
<td>A positive</td>
</tr>
<tr>
<td>CCR5 genotype</td>
<td>WT, WT</td>
<td>Δ32, Δ32</td>
<td>Δ32, Δ32</td>
</tr>
<tr>
<td>HLA typing*</td>
<td>HLA A</td>
<td>01, 01, 02:02</td>
<td>01, 03, 02:02</td>
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<tr>
<td>HLA B</td>
<td>08, 08, 08, 08</td>
<td>08, 08, 08, 08</td>
<td>08, 08, 08, 08</td>
</tr>
<tr>
<td>HLA DR</td>
<td>03, 01, 13, 01</td>
<td>03, 01, 14, 01</td>
<td>03, 01, 14, 01</td>
</tr>
</tbody>
</table>

CBU=cord blood unit. TPHD=third-party haploidentical donor. WT=wild type. *Recipient had negative anti-HLA antibodies

Table 1: Characteristics of the patient and donors

<table>
<thead>
<tr>
<th>CBU 1 controls before HCT</th>
<th>CBU 1 after thawing*</th>
<th>CBU 2 controls before HCT</th>
<th>CBU 2 after thawing*</th>
<th>TPHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34 (×10⁴/kg)</td>
<td>0.7</td>
<td>0.52</td>
<td>0.73</td>
<td>0.22</td>
</tr>
<tr>
<td>TNC (×10⁴/kg)</td>
<td>2.53</td>
<td>2.05</td>
<td>1.98</td>
<td>1.64</td>
</tr>
<tr>
<td>CFU-GM (×10⁴/kg)</td>
<td>1.021</td>
<td>0.11</td>
<td>0.121</td>
<td>NG</td>
</tr>
<tr>
<td>Total CFU (×10⁴/kg)</td>
<td>2.131</td>
<td>0.21</td>
<td>0.421</td>
<td>NG</td>
</tr>
</tbody>
</table>

CBU=cord blood unit. HCT=haploidentical cell transplantation. CFU=colony forming units. GM=granulocyte-macrophages. HLA=human leucocyte antigens. NG=no growth. TNC=total nucleated cells. TPHD=third-party haploidentical donor. *Subsequent quality control results in the final cell product after thawing the CBU for infusion. †Purified by cell positive selection to include fewer than 10 000 CD3+ cells per kg patient’s bodyweight. Quality controls were done at the transplant centre’s processing laboratory before transplantation in aliquot samples from segments attached to the CBUs when data were not available in the original report from the cord blood bank.

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PCR (Mentype DIPquant, Biotype, Dresden, Germany) for selected samples.¹⁸

CCR5 genotyping and viral tropism assay
CCR5 genotyping was analysed as previously described.¹⁹

HIV full envelope was amplified from the patient and recombinant viruses were in MT-4 cells (in combination with a M143-Aenv plasmid), U87-CD4-CCR5 and U87-CD4-CXCR4 cells were infected with both env-recombinant virus and two different viral isolates from the patient, and tropism determined by HIV-1 replication (HIV-1 p24⁺⁺ ELISA, PerkinElmer). Furthermore, the patient’s virus envelope was sequenced and tropism predicted with Geno2Pheno software (GENAFORE, Bonn, Germany), which has a false positive rate of 37-7%.²⁰-²²

Virus isolation and sequence analysis
Isolation of replication-competent virus and full HIV-1 genome amplification from bulk CD4 T-cells were done as previously described.²³ Total CD4 and rCD4 T cells were isolated by negative selection and cultured with irradiated donor cells, 0.5 µg/mL phytohaemagglutinin in presence of interleukin 2, and T-cell growth factors. 2 days later, phytohaemagglutinin was removed and phytohaemagglutinin-activated donor blasts were added to the culture. HIV-1 p24⁺⁺ antigen was measured at day 14. Two different replication-competent isolates were obtained from total CD4 T cells. Resistance to ART was predicted from full viral genome sequences from the patient’s CD4 cells.²²

Reservoir measurement
HIV reservoir analyses included single copy assay and cell-associated HIV-1 DNA and RNA in CD4 cells measured by droplet digital PCR (ddPCR) as previously described.²³ Infectious units per million CD4 cells or resting CD4 cells were calculated after the viral outgrowth assay described above and estimated by maximum likelihood. Semiquantitative PCR-based detection of gag proviral DNA with ten-fold dilution of cell-associated DNA was done in ten replicates and expressed as a percentage of positive gag PCR amplification per total PCR wells.

Immune activation and proliferation
Phytohaemagglutinin-treated peripheral-blood mononuclear cells (5 µg/mL for 3 days) were tested for T-cell surface expression of activation markers (CD25, CD69, and HLA-DR) and for proliferation with flow cytometry. Data are presented as a percentage of marker expression and loss of membrane carboxyfluorescein diacetate succinimidyl ester expression (Vybrant Cell Tracer Kit; Life Technologies, Carlsbad, CA, USA) in CD4 cells before and after phytohaemagglutinin activation.

Cell infectivity assay
Viral fitness was analysed as described previously.²⁴ CD4 cells from the patient and from two healthy donors were activated for 3 days as described above and then infected by spinoculation (1200 g for 2 h) with the patient’s primary viral isolates, or with laboratory viral strains HIV-1Δenv (CCR5-tropic) and HIV-1NL4-3 (CXCR4-tropic). Supernatants were sampled over the course of 7 days. Viral replication was quantified with HIV-1 p24⁺⁺ ELISA.

Role of the funding source
The funders of this study had no role in the design of the study, data collection or analysis, or interpretation, writing of the report, or the decision to submit for publication. All authors had full access to all the data in the study and final responsibility for the decision to submit for publication.

Results
Full genome amplification of the patient’s virus from CD4 T cells showed no major deletions or mutations, suggesting infection with a full pathogenic virus. Genotypic analysis showed HIV-1 to be subtype B, to have no resistance mutations at the time of HCT, and to be CCR5-tropic. Additionally, we made a recombinant virus carrying the patient’s primary viral envelope gene sequences, obtained from bulk PCR, thus representing the viral quasispecies present in the patient’s T cells. This recombinant virus and two other independent viral culture isolates were used for phenotypic tropism determination. All three viruses were able to replicate...
in U87-CCR5 cells but not in U87-CXCR4 cells (appendix), confirming that residual CXCR4-tropic virus is very unlikely, and that the patient’s HIV is CCR5-tropic by genotypic and phenotypic analyses.

Sensitive assays were used to determine the latent reservoir of the patient before and after transplant (table 3). Before transplantation, HIV DNA could be detected in total and resting CD4 cells in peripheral blood and in gut-associated lymphoid tissue (GALT) biopsies. Additionally, we estimated that the replication competent viral size present in CD4 cells was 2·1 copies per 10 million cells. We also detected cell-associated HIV RNA in CD4 T cells and HIV RNA in plasma and cerebrospinal fluid. On day 76 after transplantation, with 100% CCR5 Δ32 homozygous cord blood chimerism, HIV DNA was not detectable in isolated CD4 T cells from peripheral blood with ddPCR quantification or semiquantitative test of amplification (table 3). Unfortunately, DLBCL progression limited our capacity to extend our measurements of the viral reservoir to other tissues and for a longer time after HCT.

Cord-blood-derived cells from the patient were assessed after HCT for activation, proliferation, and infectivity.

### Discussion

We describe a case of allogeneic HCT in an HIV-1-infected patient with CCR5 Δ32 homozygous haemopoietic cells from cord blood, a stem cell source with more permissive HLA-matching requirements, which might provide a broader allogeneic HCT platform for patients with HIV infection. Until now, two other attempted cases of CCR5 Δ32 homozygous cord blood HCT in HIV-infected patients, reported in preliminary online and in abstract format, showed no effect on infection.

Allogeneic HCT reduced the patient’s latent viral reservoir, which became undetectable in circulating CD4 T cells by both semiquantitative amplification and ddPCR quantification tests (table 3). Although myeloablative conditioning chemotherapy reduced viral levels before cord blood engraftment, evidence of HIV recurrence upon discontinuation of ART in cases of allogeneic HCT from CCR5 heterozygous or wild-type donors questions the potential contribution from conditioning chemo-radiotherapy, as well as from HCT alloreactive events, to long-term control of HIV infection. Despite the reduction of plasma viraemia, low HIV RNA copy numbers were detectable in the patient. Persistence of low-numbers of CCR5 wild-type cells below the detection level of chimerism analyses in tissues harboring infectious virus, long-lasting release of virus trapped in follicular dendritic cells in the lymph nodes, and the absence of long-term follow-up are among the potential explanations for this finding.

In addition to undetectable HIV DNA, upon achievement of full cord-blood CCR5 Δ32 homozygous chimerism, patient’s circulating CD4 T cells could be successfully stimulated to proliferate and express activation markers, but were refractory to infection by HIV. The patient’s CD4 T cells were resistant to infection by HIV isolates, by virus with the patient’s recombinant env, and by HIV-1ΔΔ32 laboratory strain (all CCR5-tropic); they were also refractory to infection by the CXCR4-tropic laboratory strain HIV-14NL. Agrawal and coworkers showed that CD4 T cells from CCR5 Δ32 homozygous people consistently express low levels of CXCR4 and are less susceptible to infection by CXCR4-tropic HIV than are those from individuals without the mutation. Petz and colleagues also found that peripheral-blood mononuclear cells from an HIV-negative patient with leukaemia who had received a CCR5 Δ32 homozygous cord-blood transplant were resistant ex vivo to HIV-1 infection with both CCR5-tropic and CXCR4-tropic strains. Beyond potential mechanisms of inhibition that could include scavenging of CXCR4 by endogenous expression of CCR5 Δ32, the lack of infection by CXCR4 tropic viruses of otherwise functional newly reconstituted CD4 T cells obtained shortly after achieving full chimerism is certainly intriguing and might be associated with maturation and the functional stage of lymphocytes derived from haemopoietic cells from cord blood, which would point towards potential unknown additional mechanisms that need further investigation.

<table>
<thead>
<tr>
<th>Total DNA (copies per million cells)</th>
<th>15 days before transplant</th>
<th>76 days after transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral CD4 T cells</td>
<td>372·9</td>
<td>Undetectable</td>
</tr>
<tr>
<td>Peripheral resting CD4 T cells</td>
<td>651·8</td>
<td>ND</td>
</tr>
<tr>
<td>GALT CD4 T cells</td>
<td>803·2</td>
<td>ND</td>
</tr>
<tr>
<td>Semiquantitative total DNA (amplification %)</td>
<td>100% (1.1, 1.10, 1.100) 20% (1-1000)</td>
<td>ND</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Single copy assay (copies per mL)</th>
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<th>76 days after transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>303</td>
<td>3</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>149</td>
<td>ND</td>
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<table>
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<th>Quantitative viral outgrowth (IUPM)</th>
<th>15 days before transplant</th>
<th>76 days after transplant</th>
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<tr>
<td>Peripheral CD4 T cells</td>
<td>0·211</td>
<td>ND</td>
</tr>
<tr>
<td>Peripheral rCD4 T cells</td>
<td>&lt;0·281</td>
<td>ND</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell-associated RNA (relative copies)</th>
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<th>76 days after transplant</th>
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</thead>
<tbody>
<tr>
<td>Peripheral CD4 T cells</td>
<td>2·5</td>
<td>ND</td>
</tr>
<tr>
<td>GALT</td>
<td>Undetectable</td>
<td>ND</td>
</tr>
</tbody>
</table>

Table 3: Latent HIV reservoir measurements
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Irrespective of experimental goals to eradicate HIV, the standards of care for the primary haematological disorder must be the top priority when considering allogeneic HCT for patients with HIV infection. However, although HIV-infected patients remain at an increased risk of haematological malignancies for which HCT is standard therapy, the outcome of allogeneic transplants in patients with HIV infection is largely unknown. The European Society for Bone Marrow Transplantation has recently communicated a large cohort study with 111 allogeneic HCT recipients with HIV infection each matched with three controls, which showed poor overall outcomes for patients with HIV infection. These results seem to be driven by higher non-relapse mortality and higher rates of severe graft-versus-host disease than in uninfected transplant recipients. Nevertheless, with a 47% overall survival at 2 years, allogeneic HCT is feasible in HIV-infected individuals, and the outcome is comparable between related and unrelated donor sources. Cord blood transplantation has become a standard source of alternative grafts for allogeneic HCT in patients without matched sibling donors. In combination with haploidentical haemopoietic cells, cord blood reliably leads to faster neutrophil engraftment than does alternative single or double-cord blood transplant protocols, and has similar overall outcomes while allowing the use of cord blood units with somewhat lower cellular content. This approach provides a major advantage in the setting of HIV infection, in which projections suggest that an inventory of 300 CCR5 A32 homozygous cord blood units would allow, with this protocol, an adequate HLA-matched unit for over 80% of HIV-1-infected adult and pediatric transplant candidates. The case we report shows that a directed cord blood search and CCR5 A32 homozygous cord blood transplantation is feasible in patients with HIV infection, can make viral DNA undetectable, and can render the patient’s cord-blood-derived CD4+ T lymphocytes refractory to infection by HIV. Despite lymphoma progression making long-term assessment of HIV infection unfeasible, in combination with the evidence available, our findings support the feasibility of cord blood transplantation as a therapeutic strategy and as a platform to investigate key patient-derived, HIV-dependent, and treatment-associated factors that might drive a broader application of CCR5-knockout strategies to patients with HIV.

Declaration of interests

During the study, MS, SM-L, and JM-P had a grant from the American Foundation for AIDS Research (amfAR). Additionally, MS reports personal fees through a Sara Borrell grant from the Spanish Ministry of Health, Social Services, and Equality, and SM-L reports personal fees from the Department of Economy and Knowledge of the Catalan Government. Outside the submitted work, BC reports grants from Gilead, Merck Sharp Dohme, and ViIV, as well as personal fees from AbbVie and Janssen. All other authors declare no competing interests.

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References