HIV

Vulnerability to reservoir reseeding due to high immune activation after allogeneic hematopoietic stem cell transplantation in individuals with HIV-1

Johanna M. Eberhard1,2, Mathieu Angin3, Caroline Passaes3, Maria Salgado3, Valerie Monceaux3, Elena Knops3, Guido Kobbé3, Björn Jensen4, Maximilian Christopect5, Nicolaus Kröger2, Linos Van der Kerckhove9, Jon Badiola10, Alessandra Bandera11, Kavita Raj12, Jan van Lunzen1,13, Gero Hütter1, Jürgen H. E. Kuball15, Carolina Martínez-Laperche16, Pascual Balsalobre16, Mi Kwon16, José L. Diez-Martín16, Monique Nijhuis15, Annemarie Wensing15, Javier Martínez-Picado4,17,18, Julian Schulze zur Wiesch1,2a1, Asier Sáez-Cirión3a†

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only medical intervention that has led to an HIV cure. Whereas the HIV reservoir sharply decreases after allo-HSCT, the dynamics of the T cell reconstitution has not been comprehensively described. We analyzed the activation and differentiation of CD4+ and CD8+ T cells, and the breadth and quality of HIV- and CMV-specific CD8+ T cell responses in 16 patients with HIV who underwent allo-HSCT (including five individuals who received cells from CCR5Δ32/Δ32 donors) to treat their underlying hematological malignancy and who remained on antiretroviral therapy (ART). We found that reconstitution of the T cell compartment after allo-HSCT was slow and heterogeneous with an initial expansion of activated CD4+ T cells that preceded the expansion of CD8+ T cells. Although HIV-specific CD8+ T cells disappeared immediately after allo-HSCT, weak HIV-specific CD8+ T cell responses were detectable several weeks after transplant and could still be detected at the time of full T cell chimerism, indicating that de novo priming, and hence antigen exposure, occurred during the time of T cell expansion. These HIV-specific T cells had limited functionality compared with CMV-specific CD8+ T cells and persisted years after allo-HSCT. In conclusion, immune reconstitution was slow, heterogeneous, and incomplete and coincided with de novo detection of weak HIV-specific T cell responses. The initial short phase of high T cell activation, in which HIV antigens were present, may constitute a window of vulnerability for the reseeding of viral reservoirs, emphasizing the importance of maintaining ART directly after allo-HSCT.

INTRODUCTION

The Berlin patient is the only HIV-infected individual who is considered to be HIV cured after he underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) with cells of a human leukocyte antigen (HLA)–identical donor who was CCR5Δ32 homozygous (1). CCR5Δ32 homozygosity impairs cell surface expression of CCR5 and confers protection toward HIV-1 strains using CCR5 as entry coreceptor (2, 3). Recently, a second case of durable HIV remission after allo-HSCT with a CCR5Δ32 homozygous graft has been described in a patient from London who did not show signs of HIV rebound 30 months after stopping antiretroviral therapy (ART) (4, 5). Whereas HIV reservoirs stably persist even after decades of efficient ART (6), allo-HSCT in people with HIV is accompanied by a rapid and marked decrease of HIV-infected cells in blood and different tissues often to undetectable amounts (7), independent of engraftment with CCR5Δ32 or CCR5 wild-type (WT) stem cells (8). Therefore, allo-HSCT, although not scalable, theoretically offers an opportunity to achieve HIV cure in HIV-infected patients who require such intervention to treat their underlying hematological cancer.

However, other HIV-infected individuals who interrupted their ART after receiving CCR5Δ32 heterozygous (9), homozygous (10), or wt (9, 11, 12) grafts have shown an eventual resurgence of HIV replication despite having undetectable virologic reservoir markers while on ART and being able to transiently control HIV replication after treatment discontinuation. Therefore, the clinical, immunological, and virologic factors associated with durable HIV remission in HIV-infected individuals undergoing allo-HSCT remain unclear. The cases of the Berlin and London patients emphasize the critical importance of CCR5Δ32 homozygous transplants to impose a strong barrier for HIV dissemination upon engraftment (13). At this point, it is not clear whether a CCR5Δ32 homozygous transplant status is an essential condition to achieve HIV cure, but immune responses in allo-HSCT HIV-infected individuals may not be sufficient to counteract...
the spread of HIV from any remaining residual viral reservoir. Reactivation of different latent viral infections [e.g., cytomegalovirus (CMV), hepatitis B virus (HBV), and Epstein-Barr virus (EBV)] are common among individuals receiving allo-HSCT (14–16), and different aspects of the allo-HSCT [e.g., conditioning, immunosuppressive drugs, graft-versus-host disease (GVHD), and delayed immune reconstruction] are associated with flawed development of virus-specific immune responses and enhanced morbidity and mortality related to viral infections (17–19). Despite their evident interest in the search for an HIV cure, the exceptionality of cases of HIV-infected individuals undergoing allo-HSCT (1, 4, 7, 10, 12, 20–22) has precluded detailed comprehensive immunological studies during and after allo-HSCT in groups of such individuals.

Since 2014, the IciStem consortium (International Collaboration to guide and investigate the potential for HIV cure by stem cell transplantation) has followed HIV-infected individuals who receive allo-HSCT for hematologic conditions in an observational study (www.icistem.org) (7). IciStem longitudinally collects samples and clinical data of the individuals and generates comprehensive immunological and virologic data to elucidate correlates of HIV persistence. In the current study, we describe the immunological reconstitution of the T cell compartment and the breadth and functionality of HIV-specific T cell responses of 16 IciStem-enrolled individuals who underwent allo-HSCT.

RESULTS

Study population

Sixteen individuals enrolled in IciStem from eight medical centers in six countries were included. A summary of clinical and hematologic, as well as virologic, details are shown in Table 1 and table S1, respectively. The most frequent hematologic malignancies were non-Hodgkin’s lymphoma (n = 6) and acute myeloid leukemia (n = 5). Most of the participants received an allogeneic hematopoietic stem cell graft from the peripheral blood of HLA-matched unrelated or related donors (n = 8) or HLA-haploidentical siblings or relatives (n = 5). IciS-01, IciS-04, and IciS-05 received a cord blood graft. All patients were maintained on ART after allo-HSCT. Individual HSCT conditioning and ART regimens are described in Table 1 and table S1, respectively. The HLA alleles of every subject and their respective donor are depicted in table S2.

Nine of 16 (56%) individuals (including two who received cells from CCR5A32/A32 donors) were alive and in active follow-up at the time of this report (fig. S1). Seven individuals (44%, including three who received hematopoietic stem cells from CCR5A32/A32) died because of relapse of their primary disease or complications associated with allo-HSCT (median, 3 months after allo-HSCT). Overall, the mortality rate of the patients in this cohort was not substantially different from that observed in uninfected individuals with similar malignancies undergoing allo-HSCT (23, 24). Although this high mortality rate is in agreement with previous reports (25, 26), some of the surviving patients in our study were included retrospectively, which may affect our estimations.

All the patients had detectable cell-associated HIV DNA at baseline [median of 1458 HIV long terminal repeat (LTR) copies per million CD4+ T cells]. Independently of the donor-CCR5 genotype, the cell-associated HIV DNA dropped and could not be detected for all but two patients [IciS-01 who had 453 HIV LTR copies per million CD4+ T cells 45 months after allo-HSCT (7) and IciS-20 who had 186 HIV LTR copies per million CD4+ T cells 8 months after allo-HSCT] at the last follow up after allo-HSCT (range, 2 to 88 months), in agreement with the profound decrease in viral reservoir markers that we have previously reported in a subset of the patients analyzed here (8).

All study participants had undetectable viral loads by clinical standards before and after allo-HSCT. However, eight of the nine patients with samples available at the baseline had detectable ultrasound-guided viral loads before allo-HSCT (median, 38 HIV-1 RNA copies/ml of plasma). The frequency progressively decreased until virtually nothing could be detected 7 months after allo-HSCT (0.8 HIV RNA copies/ml of plasma for one of nine patients tested at this time point, undetectable for the others). However, during the initial 6-month low-level viremia (median, 3 HIV RNA copies/ml of plasma) could be detected in 57 to 67% of patients with available samples.

Partial reconstitution of the T cell compartment after allo-HSCT

We aimed to characterize the kinetics of T cell immune reconstitution and T cell activation and differentiation in our cohort before and after allo-HSCT. Frozen peripheral blood mononuclear cells (PBMCs) were available for immunological analysis of bulk and HIV-specific T cells in nine participants before allo-HSCT and up to 27 months after allo-HSCT; and in seven other participants, samples were collected at various time points between months 6 and 88 after allo-HSCT (fig. S1).

The study participants were treated with chemotherapy regimens for their respective underlying hematological malignancy and showed relatively low CD4+ T cell counts (median, 490 cells/μL of blood) (table S3) and an inverted CD4/CD8 ratio of <1 just before allo-HSCT (fig. 1A). As expected, a generalized severe lymphopenia was observed during the initial 2 to 6 weeks after HSCT, with some prevailing CD4+ T cells in circulation in almost total absence of CD8+ T cells (fig. 1 and fig. S2). Despite their initial lower frequency, a more rapid expansion of CD8+ T cells was observed in later weeks, similarly to what has been described for non–HIV-infected individuals after allo-HSCT (27, 28). As a consequence, an inverse CD4/CD8 T cell ratio was again observed for most patients around 2 months after allo-HSCT. In general, T cell reconstitution and normalization of the CD4/CD8 T cell ratio usually takes 1 to 2 years after allo-HSCT in HIV-negative individuals (29). In contrast, inverse CD4/CD8 T cell ratios (<1) were observed for IciStem participants up to 7 years after allo-HSCT (Fig. 1, A and B, bottom panels). The increase of the CD4/CD8 ratio observed in IciS-20 between months 4 and 9 followed donor lymphocyte infusions this patient received in months 4 and 8.

Immune reconstitution after allo-HSCT occurs in two phases. The initial phase is characterized by homeostatic and antigen-driven expansion of patient’s naïve and memory T cells that resisted conditioning regimens (before they are eliminated by graft-versus-host alloreactivity) and T cells from the donor that were present in the allograft or were adoptively transferred. Complete immune reconstitution is only achieved when mature naïve T cells from donor origin are produced by the thymus (27, 30, 31). Therefore, we longitudinally analyzed the distribution of the different T cell subsets after allo-HSCT to assess cellular turn-over and T cell reconstitution in the study participants. The gating strategy used is shown in fig. S3. Reconstitution of naïve and memory T cell populations showed high interindividual differences in this group of individuals (figs. S2 and S4). In general, early time points after HSCT were
Table 1. Clinical and hematologic characteristics of 16 IciStem-enrolled participants included in this study. ATG, antithymocyte globulin; BE, Belgium; BU, busulfan; CMML, chronic myelomonocytic leukemia; CsA, cyclosporine A; CY, cyclophosphamide; DE, Germany; EBV, Epstein-Barr virus; ES, Spain; FLU, fludarabine; GB, England; GvHD, graft-versus-host disease; HHV8, human herpesvirus 8; HSV, herpes simplex virus; IT, Italy; MAC, myeloablative conditioning; MMF, mycophenolate mofetil; MTX, methotrexate; NK, natural killer cell; NL, Netherlands; RIC, reduced-intensity conditioning; TAC, tacrolimus; TBI, total body irradiation; n.a., not applicable.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Site</th>
<th>Gender</th>
<th>Age (at HSCT)</th>
<th>Hematological malignancy</th>
<th>Graft</th>
<th>CCRS status</th>
<th>Hematopoietic conditioning regimen</th>
<th>GVHD prophylaxis</th>
<th>GVHD</th>
<th>Complete chimerism (months)</th>
<th>Engraftment (months)</th>
<th>HSCT associated infections</th>
<th>Outcome (months after HSCT)</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>IciS-01</td>
<td>ES</td>
<td>M</td>
<td>34</td>
<td>Burkitt lymphoma</td>
<td>Haploidentical and cord blood transplant</td>
<td>wt/wt</td>
<td>MAC, ATG, FLU, BU, CY</td>
<td>CsA and corticosteroids</td>
<td>No</td>
<td>PB: 2; T cells: 18; BM: 12</td>
<td>Yes (severe)</td>
<td>BK virus</td>
<td>&lt;1</td>
<td>Alive (87)</td>
</tr>
<tr>
<td>IciS-02</td>
<td>ES</td>
<td>M</td>
<td>33</td>
<td>Lymphohistiocytosis</td>
<td>HLA-matched sibling</td>
<td>wt/wt</td>
<td>RIC, ATG, FLU, melphalan</td>
<td>CsA and MTX</td>
<td>Yes (severe)</td>
<td>PB: 1; BM: 3.5</td>
<td>&lt;1</td>
<td>Deceased</td>
<td>26 (infection, GvHD)</td>
<td>Alive (75)</td>
</tr>
<tr>
<td>IciS-03</td>
<td>ES</td>
<td>M</td>
<td>51</td>
<td>Non-Hodgkin's lymphoma</td>
<td>HLA-identical sibling</td>
<td>wt/wt</td>
<td>RIC, FLU, melphalan</td>
<td>CsA and MTX</td>
<td>Yes (mild)</td>
<td>PB: 1; BM: 6.5</td>
<td>No</td>
<td>&lt;1</td>
<td>Alive</td>
<td>75</td>
</tr>
<tr>
<td>IciS-04</td>
<td>ES</td>
<td>M</td>
<td>37</td>
<td>Diffuse large B cell</td>
<td>Haploidentical and cord blood transplant</td>
<td>Δ32/Δ32</td>
<td>ATG, FLU, BU, CY</td>
<td>CsA and short course corticosteroids</td>
<td>No</td>
<td>PB: 73 days (T1)</td>
<td>CMV reactivation (resolved soon after HSCT)</td>
<td>&lt;1</td>
<td>Deceased</td>
<td>2 (relapse)</td>
</tr>
<tr>
<td>IciS-05</td>
<td>NL</td>
<td>M</td>
<td>52</td>
<td>Myelodysplastic syndrome</td>
<td>Haploidentical and cord blood transplant</td>
<td>Δ32/Δ32</td>
<td>ATG, FLU, BU</td>
<td>CsA, MMF and corticosteroids</td>
<td>Yes (acute)</td>
<td>Not reached</td>
<td>Fungal infection</td>
<td>Not reached</td>
<td>Deceased</td>
<td>14 (relapse, pneumonia)</td>
</tr>
<tr>
<td>IciS-06</td>
<td>ES</td>
<td>M</td>
<td>40</td>
<td>Relapsing lymphoma</td>
<td>HLA-matched sibling</td>
<td>wt/wt</td>
<td>RIC, FLU, BU, CY</td>
<td>CsA, CsA, MMF</td>
<td>No</td>
<td>PB: 3; T cells: 3; BM: 6</td>
<td>CMV reactivation</td>
<td>&lt;1</td>
<td>Alive</td>
<td>57</td>
</tr>
<tr>
<td>IciS-08</td>
<td>ES</td>
<td>M</td>
<td>57</td>
<td>Myelofibrosis</td>
<td>HLA-matched sibling</td>
<td>wt/wt</td>
<td>RIC, FLU, BU, CY</td>
<td>MMF, CsA, CY</td>
<td>No</td>
<td>n.a.</td>
<td>Fungal infection</td>
<td>Not reached</td>
<td>Deceased</td>
<td>2 (infection)</td>
</tr>
<tr>
<td>IciS-11</td>
<td>NL</td>
<td>M</td>
<td>60</td>
<td>Acute myeloid leukemia</td>
<td>HLA-matched unrelated donor</td>
<td>Δ32/Δ32</td>
<td>1: ATG, FLU, TBI; 2: ATG, FLU, treosulfan</td>
<td>MMF, CsA (T1 and 2)</td>
<td>No</td>
<td>Not reached</td>
<td>CMV reactivation, bacterial infections</td>
<td>Not reached</td>
<td>Deceased</td>
<td>3 (respiratory insufficent)</td>
</tr>
<tr>
<td>IciS-12</td>
<td>ES</td>
<td>M</td>
<td>31</td>
<td>Relapsing lymphoma</td>
<td>Haploidentical sibling</td>
<td>wt/wt</td>
<td>RIC, FLU, BU</td>
<td>MMF, CsA, CY</td>
<td>Yes</td>
<td>PB: 0.5; T cells: 1; BM: 1</td>
<td>Unknown</td>
<td>&lt;1</td>
<td>Deceased</td>
<td>2 (relapse)</td>
</tr>
<tr>
<td>IciS-17</td>
<td>IT</td>
<td>M</td>
<td>46</td>
<td>Diffuse large B cell lymphoma</td>
<td>HLA-matched sibling</td>
<td>wt/wt</td>
<td>RIC, FLU, Thiopeta, CY</td>
<td>CsA, MTX</td>
<td>No</td>
<td>PB: 1</td>
<td>EBV reactivation</td>
<td>&lt;1</td>
<td>Alive</td>
<td>107</td>
</tr>
<tr>
<td>IciS-19</td>
<td>DE</td>
<td>M</td>
<td>43</td>
<td>Acute myeloid leukemia</td>
<td>HLA-matched unrelated donor</td>
<td>Δ32/Δ32</td>
<td>RIC, FLU, treosulfan</td>
<td>TAC and corticosteroids</td>
<td>Yes (severe)</td>
<td>PB: 23; BM: 7</td>
<td>CMV reactivation, HHV8 reactivation</td>
<td>&lt;1</td>
<td>Alive</td>
<td>79</td>
</tr>
<tr>
<td>IciS-20</td>
<td>GB</td>
<td>M</td>
<td>59</td>
<td>Acute myeloid leukemia from CMML</td>
<td>HLA-matched unrelated donor</td>
<td>Δ32/Δ32</td>
<td>FLU, BU, campath</td>
<td>CsA</td>
<td>No</td>
<td>T cells: 10</td>
<td>CMV reactivation, EBV reactivation</td>
<td>&lt;1</td>
<td>Alive</td>
<td>42</td>
</tr>
<tr>
<td>IciS-23</td>
<td>BE</td>
<td>M</td>
<td>59</td>
<td>Acute myeloid leukemia</td>
<td>Haploidentical sibling</td>
<td>wt/wt</td>
<td>BU, cytarabine, idarubicin</td>
<td>MMF and TAC</td>
<td>No</td>
<td>PB: 6; T cells: 6; BM: 6</td>
<td>Neutropenic fever, CMV reactivation</td>
<td>&lt;1</td>
<td>Alive</td>
<td>35</td>
</tr>
<tr>
<td>IciS-27</td>
<td>ES</td>
<td>M</td>
<td>47</td>
<td>Non-Hodgkin’s lymphoma</td>
<td>HLA-matched related donor</td>
<td>wt/wt</td>
<td>RIC, FLU, CY</td>
<td>CsA and MTX</td>
<td>Yes (mild)</td>
<td>T cells: 5.5</td>
<td>No</td>
<td>&lt;1</td>
<td>Alive</td>
<td>77</td>
</tr>
<tr>
<td>IciS-28</td>
<td>ES</td>
<td>M</td>
<td>44</td>
<td>Hodgkin’s lymphoma</td>
<td>HLA-matched unrelated donor</td>
<td>wt/wt</td>
<td>RIC, FLU, melphalan</td>
<td>TAC and sirolimus</td>
<td>Yes (acute)</td>
<td>T cells: 1</td>
<td>CMV reactivation</td>
<td>&lt;1</td>
<td>Alive</td>
<td>120</td>
</tr>
<tr>
<td>IciS-29</td>
<td>ES</td>
<td>M</td>
<td>44</td>
<td>Acute leukemia</td>
<td>HLA-matched unrelated donor</td>
<td>wt/wt</td>
<td>FLU, BU</td>
<td>MMF, CsA, CY</td>
<td>Yes (acute)</td>
<td>PB: 1; BM: 1</td>
<td>No</td>
<td>&lt;1</td>
<td>Deceased</td>
<td>6 (relapse)</td>
</tr>
</tbody>
</table>
memory CD4+ T cells were more frequent early after HSCT (28.6% after HSCT, median, and interquartile range (IQR)). In contrast, central memory CD4+ T cells as percentage of CD3+ T cells and CD4+/CD8+ T cell ratio. Symbols and lines represent one patient over time. Gray lines indicate median and 25 and 75 percentiles of T cell frequencies in a cohort of healthy controls (n = 30). Open symbols represent time points where T cell numbers were very low (<100 T cells per analysis).

To better understand the mechanisms of T cell expansion, we also analyzed the expression of CD127 expression [interleukin-7 (IL-7) receptor-α] on memory CD4+ and CD8+ T cells (Fig. 1, D and E). The frequency of CD127+ cells was extremely low immediately after HSCT, even among central memory (Tcm) and Ttm subsets, confirming high homeostatic expansion during this period (Fig. 1, D and E, and fig. S4). The frequency of CD127+ memory T cells, in particular among CD4+ T cells, started to increase 4 months after HSCT in most patients, which plateaued around 20 months after HSCT (Fig. S5). This pattern likely indicates expansion of memory T cells related to new thymic generation during this phase (27) because CD127+ is highly expressed on thymic progenitors and on mature T cells in peripheral lymphoid tissues before they expand in response to antigens (32). Only one participant, IciS-01, maintained very low frequencies of CD127+ among CD4+ Tem cells and among CD8+ T cells (fig. S5).

Fig. 1. Reconstitution of the T cell compartment in an HIV-positive cohort after allo-HSCT. (A) Frequency of CD4+ and CD8+ T cells as percentage of CD3+ T cells and CD4+/CD8+ T cell ratio. Symbols and lines represent one patient over time. Gray lines indicate median and 25 and 75 percentiles of T cell frequencies in a cohort of healthy controls (n = 30). Open symbols represent time points where T cell numbers were very low (<100 T cells per analysis). (B) Median frequencies of CD4+ and CD8+ T cell frequencies (top) and median CD4+/CD8+ ratios (bottom). (C) Proportions of naïve (CCR7+CD45RA+), central memory (Tcm, CCR7+CD45RA+), transitional memory (Ttm, CCR7+CD45RA+), early effector memory (Tem, CCR7+CD45RA+CD27+), and late effector memory (TemRA, CCR7+CD45RA+CD27+) populations of 16 patients in total. Mean values from 2 to 10 patients per time point were summarized. (D) Frequencies of CD127+CD4+ (top) and CD8+ T cells (bottom). (E) Median frequencies of CD127+CD4+ and CD8+ T cells.

characterized by a drop in frequency of naïve T cells; this held especially true for the CD4+ T cell compartment (Fig. 1C, left, and fig. S4) [24.5% (8.1 to 42.3) at baseline versus 8.2% (2.6 to 15.7) 1 month after HSCT, median, and interquartile range (IQR)]. In contrast, central memory CD4+ T cells were more frequent early after HSCT (28.6% (16.3 to 43.2) at baseline versus 51.6% (33.9 to 67.9) 1 month after HSCT, median, and IQR). Overall, naïve CD4+ T cells remained low until almost 2 years after HSCT, before some recovery could be noted (Fig. 1C, left, and fig. S4). This is in agreement with the strong reduction of naïve T cells that is initially observed after allo-HSCT in noninfected individuals. However, the recovery of naïve T cells appeared somewhat delayed with regard to what has been reported in non–HIV-infected patients in whom recovery is detected a few months after allo-HSCT (31). In the case of CD8+ T cells, the most notable change was a progressive increase (until 6 years after HSCT in some cases) in frequency of terminally differentiated effector CD8+ T cells (TemRA) after the initial drop observed after HSCT. In contrast, CD8+ effector memory (Tem) and transitional memory (Ttm) T cell subsets peaked at 1 and 5 months, respectively, and then constantly declined (Fig. 1C, right, and fig. S4).

High T cell activation early after allo-HSCT: Window of vulnerability for HIV reseeding

We then studied the evolution of immune activation in CD4+ and CD8+ T cells after allo-HSCT. Strong CD4+ and CD8+ T cell activation (as measured by coexpression of CD38 and HLA-DR) followed allo-HSCT and peaked between months 2 and 3 after HSCT (Fig. 2, A and B). T cell activation decreased and reached a plateau around 20 months after HSCT. An exception was observed in IciS-20 who showed a strong increase of T cell activation 9 months after allo-HSCT, after a relapse of his acute myeloid leukemia (AML) in month 8. A similar pattern was found for T cell proliferation as measured by Ki-67 expression (Fig. 2, C and D). Overall, the magnitude of immune activation of all patients’ T cells was directly correlated to Ki-67 expression,
consistent again with homeostatic/antigen-driven expansion of the cells during this period (Fig. 2E). We also analyzed the expression of programmed cell death protein 1 (PD-1), which has been shown to be up-regulated during reconstitution in lymphopenic environments to limit the reactivity of acutely expanding cells (33). PD-1 expression was higher in the CD4+ T cell than in the CD8+ T cell compartment and peaked between months 2 and 4 after HSCT for both CD4+ and CD8+ T cells, decreasing afterward to baseline frequencies (Fig. 2, F and G). Overall, this period of strong T cell activation preceded the establishment of full donor chimerism, which was achieved in median 4.25 months after HSCT. IciS-20 again was an exception with an increase of PD-1+CD8+ T cells 9 months after allo-HSCT after relapsing AML. IciS-01 was the only patient who maintained high PD-1 expression in CD8+ T cells (Fig. 2F, bottom), which may have impaired graft-versus-host reaction (34), and has been associated with delayed T cell chimerism and the persistence of detectable infected cells in this patient (7).

Next, we analyzed the expression of HIV coreceptors CCR5 and CXCR4 in the CD4+ T cell compartment. A general increase in the frequency of CCR5 expressing CD4+ T cells was observed during the first weeks after allo-HSCT and peaked 1 to 3 months after allo-HSCT, including among CCR5Δ32/Δ32 recipients (i.e., IciS-20, IciS-11, and IciS-04), although cell numbers were very low in the latter (Fig. 3A). This suggests that during the early homeostatic expansion period that followed allo-HSCT, immune activation occurred in both the remaining host cells and the donor cells. A strong increase in HLA-DR and CD38 expression was indeed observed in both CCR5+ and CCR5Δ32Δ32 expressing CD4+ T cells from IciS-20 3 months after allo-HSCT (fig. S6). As expected, CCR5 expression was lost in all of the patients who received a CCR5Δ32 homozygous graft several months after allo-HSCT and after achieving full T cell chimerism (Fig. 3A, right). The frequency of CCR5Δ32Δ32 cells decreased to baseline for the individuals who did not receive CCR5Δ32Δ32.

The frequency of CXCR4+CD4+ T cells also increased early after allo-HSCT (Fig. 3B). In contrast, we detected a drop of CD4+ T cells that expressed the gut migration marker α4β7 integrin, which was also described to bind HIV virions (35), early after allo-HSCT (Fig. 3, C and D) and only regained baseline frequencies several months after allo-HSCT.

Overall, our results show that the initial weeks after allo-HSCT are characterized by the presence of few but highly activated CD4+ T cells from both donor and recipient individuals. This supposes a risk for HIV reservoir reseeding in the absence of complete pharmacological or genetic protection of donor cells.

**HIV-specific CD8+ T cell responses primed after HSCT**

Next, we evaluated how the depletion and replenishment of the T cell compartment that accompanied allo-HSCT affected the
HIV-specific CD8+ T cell response. We monitored the frequency of CD8+ T cells that were able to produce cytokines [interleukin-2 (IL-2), interferon-γ (IFN-γ), tumor necrosis factor–α (TNF-α)] and/or degranulate (assessed by CD107a expression) in response to stimulation with pools of overlapping peptides spanning HIV-1 Pol, Gag, and Nef (Fig. 4A). As expected, HIV-specific CD8+ T cell responses, which were readily detected at baseline, could not be detected during the initial weeks that followed allo-HSCT in most cases because of the virtual absence of circulating CD8+ T cells at those early time points (Fig. 4B). However, HIV-specific CD8+ T cells could be detected several months after allo-HSCT, and their frequency increased coinciding with the expansion of the CD8+ T cell compartment (Fig. 4B), reaching a peak around 18 months. In most cases, responses could be detected against several HIV-1 antigens (Fig. 4C). Note that these responses were detectable even when full donor T cell chimerism was achieved, indicating de novo responses primed after allo-HSCT, which implies that HIV antigens were produced and presented during the expansion of donor CD8+ T cells, despite the presence of ART in these individuals. HIV-specific CD8+ T cell responses appear to start to wane in most IciStem participants 1.5 to 2 years after allo-HSCT, although HIV-specific CD8+ T cells still persisted at low frequencies in IciS-17 and IciS-28 at 6.3 and 7.3 years after allo-HSCT, respectively.

We then compared the quality of the HIV-specific and CMV-specific CD8+ T cells that expanded in the same individuals after allo-HSCT. Overall, the ex vivo HIV-specific CD8+ T cell responses exhibited limited functionality [as defined by their capacity to produce two or more simultaneous functions among degranulation, IL-2, IFN-γ, or TNF-α secretion (36)] when compared to CMV-specific CD8+ T cell responses (Fig. 5, A and B). This may reflect a skewed priming of HIV-specific CD8+ T cells as observed during natural HIV history (37). One possible difference is that, in most cases IciStem participants received cells from CMV+ donors (table S2), and this has been shown to favor the rapid expansion of preexisting functional CMV-specific CD8+ T cells and enhanced control of CMV reactivation (38). In contrast, IciS-23 and IciS-28 who were CMV-positive received transplants from CMV-negative donors. As depicted in Fig. 5B, highly polyfunctional CMV-specific CD8+ T cells were found in IciS-23 at baseline, whereas HIV-specific CD8+ T cells had limited functionality.
Both CMV-specific and HIV-specific CD8+ T cells could not be detected 1 month after allo-HSCT, but responses against both HIV and CMV were detected again 2 months after HSCT. At this time point, the frequency and functionality of both responses were limited and did not differ much. However, whereas CMV-specific CD8+ T cells kept expanding and enhanced their functionality (albeit below baseline), HIV-specific CD8+ T cells did not expand and remained of limited functionality (Fig. 5B). Similar differences in the polyfunctionality of CMV- and HIV-specific cells were found for IciS-28 88 months after transplantation. Of note, during the writing of this manuscript, we learned that IciS-28 spontaneously interrupted the treatment at 110 months after allo-HSCT. A retroviral syndrome began 3 months after interruption of ART, and viral rebound was detected 4 months later (table S4), revealing the presence of replication-competent virus in this individual despite undetectable viral markers in multiple analyses performed after allo-HSCT (7). Our results therefore indicated that the limited functionality of de novo–primed HIV-specific CD8+ T cells was not solely because of the conditions inherent to allo-HSCT. Overall, the frequencies of HIV-specific CD8+ T cell responses and their functionality in the IciStem cohort were significantly smaller compared to other HIV-infected cohorts without detectable plasma viremia, either receiving ART [ART, from the ANRS (French Agency for Research on AIDS and Viral Hepatitis) TRANSBioHIV study] or controlling HIV spontaneously [HIV controllers (HIC), from the ANRS CO21 CODEX cohort] (Fig. 5C).

**DISCUSSION**

The present study provides detailed and standardized analysis of the reconstitution of the T cell compartment and HIV-specific T cell responses in 16 HIV-infected individuals who underwent allo-HSCT. Our results indicate that immune reconstitution remains incomplete in HIV-infected individuals several years after allo-HSCT. Weakly functional de novo HIV-specific CD8+ T cell responses were primed during the initial months after allo-HSCT, suggesting the presence of HIV antigens during this period. Moreover, although allo-HSCT was accompanied by a drop of HIV-infected cells in the peripheral blood below detectable levels (7), we identified an initial phase of high T cell activation after allo-HSCT, which may constitute a window of risk of infection of engrafted cells in deep tissues.

Despite the great heterogeneity of the cohort in terms of underlying hematological disease, pretransplant conditioning and allo-HSCT protocol, CCR5 donor genotype, and clinical course after transplantation (e.g., occurrence and severity of GvHD or opportunistic infections), some common patterns could be observed in the participants (fig. S6). One common observation in our study is that most IciStem participants did not show restoration of the T cell compartment to normal magnitudes despite several years of follow-up after HSCT. Initial homeostatic expansion of the T cell compartment involved in particular CD8+ T cell memory cells, resulting in inverted CD4/CD8+ T cell ratios. This is consistent with similar observations in non–HIV-infected patients undergoing allo-HSCT (27, 28). However, full T cell reconstitution requires de novo production of naïve T cells in the thymus of the transplant recipient. T cell reconstitution can be hampered by thymus damage during allo-HSCT because of conditioning, immunosuppression, graft-versus-host reactions, or CMV reactivation (39). In the case of HIV recipients, this may be aggravated by previous HIV-induced thymic damage (40), which was not evaluated here. We observed normalization of the CD4/CD8+ T cell ratios and increased frequencies of naïve T cells in the two patients in our study who received cells from CCR5Δ32/Δ32 donors and survived over a year. However, we cannot determine whether there is a direct relationship between immune reconstitution and engraftment with CCR5Δ32/Δ32 cells.

Another clear finding was the occurrence of a generalized strong immune activation of the circulating T cell compartment as evidenced by high HLA-DR, CD38, and PD-1 expression and low CD127 expression in CD4+ and CD8+ T cells. This state of immune activation was observed before full donor chimerism was typically reached and lasted for 6 to 9 months after allo-HSCT. Such activation phase is consistent with the observations after allo-HSCT in non–HIV-infected individuals, in particular in patients who developed acute and/or chronic GvHD (41–43), and is largely related to the expansion of T cells in response to cytokines and antigens, two forms of proliferation that have also been implicated in the persistence of the HIV reservoir size (44, 45). Note that we observed an increase in the frequency of CCR5+ cells in all IciStem patients during this period, either receiving cells from CCR5WT donors or from CCR5Δ32 donors. Enhanced CCR5 expression after allo-HSCT has been associated
with increased probability of GvHD in non–HIV-infected patients undergoing allo-HSCT, which can be mitigated by maraviroc (anti-CCR5) treatment (46). It is unknown whether maraviroc containing regimens might offer additional benefits to HIV-infected patients undergoing transplantation.

During this time period, expanded T cells are characterized by qualitative defects and impaired functionality (30), and patients undergoing allo-HSCT are prone to experience severe viral reactivations such as CMV or EBV (16, 29). In the case of HIV-infected patients, the strong activation of both donor and host CD4+ T cells may prompt the production of HIV particles from persistently infected cells. This viral production could lead to the reseeding of the reservoir in engrafted cells whether or where the pressure of the ART might not be fully effective. We did not detect an increase in plasma viremia during this period in the study participants who remained on ART before, during, and after HSCT. Nevertheless, HIV RNA could still be detected at extremely low copy numbers in a fraction of the participants during the initial expansion of T cells after HSCT or at a later time by infected cells in the tissues (48) that we could not detect in our analyses. We cannot totally exclude that some of these responses correspond to cross-reactive donor CD8+ T cells that may also expand after HSCT (31). However, we found responses against different HIV antigens maintained for several years. CMV responses followed similar dynamics than HIV responses in the study participants. Despite similar magnitudes, CMV T cell responses had superior polyfunctionality than HIV-specific T cell responses, which were predominantly monofunctional. Most of the IciStem participants studied here were CMV+ before allo-HSCT and received cells from CMV+ donors. This is common practice, since allo-HSCT from CMV+ donors to CMV+ recipients has been repeatedly reported to limit CMV-associated disease in recipients (49). Although monofunctional CMV responses are expanded after allo-HSCT from CMV+ donors, highly polyfunctional CMV responses are detected when the allograft proceeds from CMV+ donors (38), which is associated with more rapid control of CMV reactivation in these individuals. Along these lines, clearance of HBV infection after transplantation in HBV antigen-positive recipients is favored when donors had immunity from natural HBV infection (50, 51). Suboptimal T cell responses are developed during the initial period after allo-HSCT, and immune control of diverse viral reactivations at this time appears only when achieved upon transfer of donor-derived preexisting immunity (19). The period after allo-HSCT is not supportive for the development of efficient T cell immunity (38, 39, 52) because of severe lymphopenia, limited capacity to generate new effector cells, and restricted T cell receptor repertoire. All these defects are palliated much later when/whether de novo T cell production by the thymus is satisfactorily reestablished. This unfavorable context is consistent with the poor functionality of HIV-specific CD8+ T cells that we detected in IciStem patients after allo-HSCT. Moreover, as previously mentioned, damage of lymphoid organs associated with HIV-1 infection may further hinder the development of de novo responses in IciStem patients.

It is interesting to notice that for patient IciS-23 who received cells from a CMV+ haploidentical sibling, both CMV-specific and HIV-specific CD8+ T cell responses had similar limited functionality 2 months after allo-HSCT, but CMV-specific cells, contrary to HIV-specific cells, strongly increased their polyfunctionality 3 months later. This suggests that there are additional particular constraints in the development of HIV-specific responses in this patient. This might be because of not yet identified intrinsic defects in the priming of the HIV-specific CD8+ T cell responses when compared to CMV. It is also likely that although some HIV antigens were produced in IciStem patients, sufficient to prime de novo responses, exposure to HIV antigens, contrary to CMV, was very much limited by the presence of ART and the rapidly decreasing numbers of infected cells potentially producing these antigens. The maturation of the T cell response and the antigen-specific repertoire appears determined by repeated encounter with antigens (53, 54). Overall, such deficiency in the development of HIV-specific CD8+ T cells may explain the inability of HIV-infected patients to control viral relapse upon treatment discontinuation whether a few infected cells persist (55).
HIV-specific CD8+ T cells were also detectable in the patients who received CCR5Δ32/Δ32 cells. We assume that these cells were primed during the time of mixed chimerism and persisted for a few years as seen in patient IciS-19 because of the long half-life of certain memory T cell populations before they are diluted out. In most cases, the HIV-specific responses in IciStem patients tended to wane over time, which is suggestive of lack of continuous antigenic stimulation. The frequency of HIV-specific T cell responses observed in the IciStem cohort was low when compared to the size of the HIV-specific T cell response detectable in other chronically HIV-infected patients. The allo-HSCT–transplanted HIV+ patients described in this manuscript are unique from a virologic perspective with respect to other cohorts of HIV+ individuals. With the exception of IciS-01 and IciS-20 who had detectable HIV DNA at the time of the analyses, the frequency of infected cells dropped to undetectable levels after allo-HSCT in all the other participants (7). However, in a few cases, HIV-specific T cell responses were detected many years after HSCT, and it is unclear whether this may reveal persistent infection and antigen stimulation in tissues in these individuals. In particular, a relatively high frequency of HIV-specific CD8+ T cells could be detected more than 7 years after HSCT in patient IciS-28 who, at this time point, had sero-reverted and had undetectable infection in our exhaustive analyses (7). However, IciS-28 has recently experienced viral rebound after spontaneously discontinuing ART. Several clinical cases of HIV-infected patients with undetectable HIV reservoir after HSCT who received CCR5 wt and heterozygous grafts have reported viral rebound upon analytical treatment interruption (9, 12, 20). This exposes the current limits of available virologic tools to predict rebound posttreatment interruption. Further analysis of additional cases will be needed to determine whether the persistence of HIV-specific T cells might be a sensitive indicator of the presence of HIV reservoir with potential replicative capacity. In contrast, IciS-19 who received a CCR5Δ32/Δ32 graft underwent a structured ART interruption and had not shown detectable viremia 1 year later (56, 57). In this patient, although most HIV markers were negative in blood and tissues, a few positive HIV DNA signals were detected by in situ hybridization in lymph nodes. It is therefore possible that a few infected cells are still present several years after allo-HSCT even in individuals who received CCR5Δ32/Δ32 transplants. Therefore, despite the strong diminution in the viral reservoir associated with allo-HSCT, the presence of additional effective barriers appears determinant to achieve HIV remission (13). In the case of the individuals not receiving cells from CCR5Δ32/Δ32 donors, this might require the genetic modification of the cells to induce HIV resistance or the implementation of immunotherapies to boost immune responses. In the context of allo-HSCT, several strategies, such as infusion of CAR T cells or adoptive transfer of antigen-specific cells derived ex vivo from naïve T cells from negative donors, are being explored to control relapse of chronic infections (19, 58, 59). Adoptive transfer of antigen-specific cells expanded ex vivo has shown clinical efficacy against CMV, EBV, and adenovirus (60) and might constitute a suitable strategy to improve CD8+ T cell immunity against HIV after allo-HSCT. In addition, infusion of broadly neutralizing antibodies at the time of treatment interruption might not only limit relapse of HIV infection but also favor the development of new autologous responses against HIV (61).

This study has certain limitations, most of them inherent to the observational nature of diverse clinical cases: (i) Patients were heterogeneous in terms of pretransplant conditioning, transplant protocols (including the presence of immunosuppressive drugs), and CCR5 donor genotype and also differed in the timing and extent of graft-versus-host reactions and delay to full donor chimerism; (ii) PBMCs were not available for each patient at the same time points and discussion has been sometimes extrapolated to observed general trends; (iii) because of cell number limitations, we had to focus our analyses on limited phenotypical markers and antigen-specific responses; (iv) and last, we could not analyze in parallel non–HIV-infected individuals undergoing allo-HSCT, although T cell reconstitution has been extensively studied and reported in this population, offering solid reference for comparison with our own results.

The IciStem consortium aims to find correlates for the persistence of an HIV reservoir and for the outcome after allo-HSCT. At this point, when most analyzed participants are still on ART, no conclusion can be drawn on the divergences between patients for any of the parameters described here, particularly regarding their potential to identify those individuals who could be safely taken off ART. However, this study provides a detailed outline of particularities of the T cell compartment in this cohort after HSCT that might be helpful to guide future interventions aiming at HIV cure in allo-HSCT and other patients. Our results suggest that there is a risk of reseeding of the HIV reservoir during the first weeks that follow allo-HSCT, and that dysfunctions in the reconstituted T cell compartment may limit the capacity of CD8+ T cells to contain the virus if treatment is discontinued.

MATERIALS AND METHODS

Study design

This was an observational study nested in the IciStem collaborative (http://icistem.org), which investigates the potential for HIV cure by allogeneic stem cell transplantation. The purpose of the study was to analyze the T cell compartment in HIV-infected individuals receiving allo-HSCT for diverse hematological malignancies. Individuals enrolled in the IciStem program are included either prospectively starting at baseline preceding their allo-HSCT or months or years after having received allo-HSCT. All samples are stored in a centralized manner. The present study describes the immune responses of the first 16 HIV-positive individuals with severe hematological diseases who underwent allo-HSCT transplantation recruited within the observational IciStem cohort. These individuals were clinically monitored at eight medical centers in six countries. All individuals gave written consent in this study that was approved in the local ethic boards. The samples were thawed in the respective research laboratories where analyses were performed retrospectively.

HIV-specific CD8+ T cell responses of IciStem-enrolled participants were compared to those from other individuals with HIV analyzed at the same period of time. HIV controllers included in this study participated to the ANRS CO21 CODEX cohort and were defined as individuals naïve of antiretroviral treatment and whose last five consecutive plasma HIV RNA values were below 400 copies/ml. HIV-infected individuals on antiretroviral treatment were included in the ANRS TRANSbio HIV study. The TRANSbioHIV study and CODEX CO21 cohort were approved by the Ethics Review Committee (Comité de protection des personnes) of Île-de-France VII. Samples were obtained and analyzed after obtaining written informed consent in accordance with the Declaration of Helsinki. Primary data are reported in data file S1.
Residual viremia by ultrasensitive viral load assay
Residual viremia (HIV RNA) was measured by ultracentrifugation of up to 9 ml of plasma at 170,000g at 4°C for 30 min, followed by viral RNA extraction using the m2000sp Abbott RealTime HIV-1 Assay device and laboratory-defined applications software from the instrument (62). HIV-1 RNA copies in the low range were determined by an in-house calibration curve set (range, 10 to 103 absolute copies) (63), which had previously been validated using a standard HIV-1 DNA control from the World Health Organization in the range of 128 to 0.5 copies/ml. Limit of detection was calculated relative to the plasma volume used in each sample (down to 0.5 HIV-1 RNA copies/ml when using 9 ml of plasma).

Cell-associated HIV DNA quantities
HIV DNA in CD4+ T cells was repeatedly measured before and after allo-HSCT in each participant, as previously described (64). Total DNA from CD4+ T cells was isolated using a DNeasy Blood and Tissue kit (Qiagen). Ultrasensitive HIV DNA quantification was performed using primers in the very conserved HIV LTR region, and the pol region and total cellular DNA were quantified using an RNAseP [RPP30 (ribonuclease P/MPR subunit P30)] primer and probe set. All assays were performed on the QX200 Droplet Digital PCR System (Bio-Rad).

Flow cytometry phenotyping
All analyses were performed with thawed PBMCs as previously described (65). At least 1 x 10^6 PBMCs were stained using a fixable viability dye (Zombie NIR Fixable Viability Kit, BioLegend). This was followed by incubation with unlabeled mouse anti-human α4β7 antibody [obtained through the National Institutes of Health (NIH) AIDS Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases (NIAID), NIH; anti-human α4β7 integrin monoclonal (Act-1) (catalog no. 11718) from A. A. Ansari.” (Act-1) (66)], a secondary staining with fluorochrome-labeled rat anti-mouse antibody (BD Biosciences), and an Fc-blocking step compatible with CD16 and CD32 staining (Human TruStain FcX, BioLegend). Then, fluorochrome-conjugated antibodies (clone, company) directed against the following cell surface markers were added directly: CD3 (UCHT1, BioLegend), CD4 (SK3, BioLegend), CD8 (RPA-T8, BioLegend), CCR5 (2D7, BD Biosciences), CXC4 (12G5, BioLegend), CD45RA (H100, BioLegend), CCR7 (G043H7, BioLegend), CD27 (M-T271, BD Biosciences), HLA-DR (L243, BioLegend), CD38 (HB-7, BioLegend), CD25 (M-A251, BioLegend), PD-1 (EH12.2H7, BioLegend), CD27 (A019D5, BioLegend), CD19 (HB19, BioLegend), and CD14 (5M2E, BioLegend). The proliferation capacity was measured via intranuclear staining of Ki-67 using a fixation and permeabilization kit optimized for staining of transcription factors and nuclear proteins (Foxp3/ transcription factor staining buffer set, eBioscience) and anti–Ki-67 antibody (Ki-67, BioLegend), according to the manufacturer’s protocol. All samples were acquired on an LSRFortessa flow cytometer (BD Biosciences). Raw data were analyzed with Diva (BD Biosciences) and FlowJo software version 10.4.2 (Tree Star Inc.).

The differentiation into naïve, Tem, early (TemRO), and late effector memory (TmnRA) T cells over time was analyzed via expression of CCR7 and CD45RA (fig. S2.) (67). TemRO cells were further subdivided into CD27-positive Tmn and CD27-negative Tem (68).

T cell stimulation and intracellular cytokine staining
Purified PBMC were thawed and rested overnight at 37°C in RPMI medium (RPMI 1640 supplemented with 1-glutamine and antibiotics) with 20% heat-inactivated fluorescence correlation spectroscopy. Cells were then incubated with overlapping peptide pools encompassing HIV-1 consensus subtype B Gag, Pol, and Nef or human cytomegalovirus pp65 (all obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH, catalog nos. 12425, 12438, 12545, and 11549, respectively) (2 μg/ml) and anti-CD28/anti-CD49d co-stimulation (1 μl/ml; BD Biosciences). No peptides were added in the negative controls. Phorbol 12-myristate 13-acetate (80 ng/ml) together with ionomycin (1 μg/ml; Sigma-Aldrich) were used as positive control. Anti-CD107a V450 (BD Biosciences) was added to all conditions. Golgi stop (1 μg/ml; BD Biosciences) and brefeldin A (10 μg/ml; Sigma-Aldrich) were added 30 min after the start of all incubations. PBMCs were stimulated for 6 hours. Cells were then stained with the LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific) and with anti–CD3-Alexa700, anti–CD4-allophycocyanin (APC), and anti–CD8a APC-Cy7 antibodies (BD Biosciences). Cytokine/Cytoperm (BD Biosciences) was used for cell permeabilization before staining for intracellular markers. Intracellular staining used anti–IFN-γ PE-Cy7, anti–IL-2 fluorescein isothiocyanate, and anti–TNF-α PE-CF594 (BD Biosciences). Cell staining was then measured with a LSRII flow cytometer (BD Biosciences). Results were analyzed with FlowJo v10.5. Because of limited number of circulating T cells at some time points after allo-HSCT, results were only considered when at least 1000 CD8+ T cells could be analyzed and the number of positive events was at least 50% higher than the negative control.

Statistical analysis
Medians, means, and IQRs were calculated using GraphPad Prism 7 software and Microsoft Excel 15. Comparisons were done using nonparametric analysis of variance (ANOVA) analysis.

SUPPLEMENTARY MATERIALS
stm.sciencemag.org/cgi/content/full/12/542/eaay9355/DC1
Fig. S1. Participant sampling.
Fig. S2. Longitudinal characterization of naïve and memory CD4+ and CD8+ T cell populations for three IciStem-enrolled participants included in this study.
Fig. S3. Gating strategy of naïve and memory T cell populations.
Fig. S4. Reconstitution of naïve and memory T cell populations.
Fig. S5. CD127 expression on memory T cells.
Fig. S6. Frequencies of activated CCR5+ and CCR5+CD4+ T cells during mixed chimerism.
Fig. S7. Sequence of hematological events and T cell reconstitution before and after allo-HSCT in an HIV-infected cohort.
Table S1. Virologic characteristics of 16 IciStem-enrolled participants included in this study.
Table S2. HLA-types of 16 IciStem-enrolled participants included in this study.
Table S3. Longitudinal CD4+ T cell counts of 16 IciStem participants included in this study.
Table S4. Summary of main clinical events observed for IciS-28.
Data file S1. Primary data.

View/request a protocol for this paper from Bio-protocol.

REFERENCES AND NOTES
15. transplantation with

10. frequent event after allogeneic stem cell transplantation (SCT) and mortality in

18. Olavarria, HIV-1 remission following CCR5Delta32/Delta32 haematopoietic stem-cell

7. Wietgrefe, X.

3 HIV-infected individuals.

P. Mikhailova, J.

B. Marty, M.

M. Mikhailova, J.

S. Rasmussen, W.

H. van Boehm, J.


11 of 12

Downloaded from http://stm.sciencemag.org/ by guest on June 30, 2021


Acknowledgments: We thank all those who participated in this study and the lCStem study group (www.lcstem.org) for constant support and discussion of our results. We also thank the participants and investigators of the ANRS CODEX cohort and ANRS TRANSBio HIV study. Funding: This study was funded by the amfAR (The Foundation for AIDS Research) through the amfAR Research Consortium on HIV Eradication (ARCHE) program (grants 108930-56-R3G1, 109293-59-R3G1, and 109552-61-R3G1). J.M.E. and J.S.W. were supported by the German Center for Infection Research (DZIF) and the European HIV Alliance (EHA). J.S.W. got additional funding from the German Research Agency (DFG SFB1328 A12). Author contributions: J.M.E., J.S.W., J.M.P., and A.S.-C. designed the study. J.M.E., M.A., C.P., and V.M. performed the experiments and analyzed and interpreted the data. J.M.E., J.S.W., and A.S.-C. interpreted the data and drafted the manuscript. B.J., E.K., G.K., C.M.L., L.V., J.B., A.K., R.K., J.H.E.K., P.B., M.K., and J.L.-D. provided patients samples and clinical information and assisted in their interpretation. M.C. and N.K. helped in the interpretation and revision of HSCT-related data. M.S., J.M.P., and J.L.-D. critically revised the manuscript for important intellectual content. M.N., A.W., J.M.P., and A.S.-C. obtained funding. M.S., J.L., G.H., P.B., M.K., J.L.-D.M., M.N., A.W., J.M.P., and A.S.-C. were initiating partners of the ICStem consortium and provided constant support during the discussion and assembly of the data. Competing interests: BJL reports speaker honoraria and consultancy fees from ViiV Healthcare, Gilead, Janssen, Merck, and Bristol-Myers Squibb outside the submitted work. L.V. reports that his institution received grants and consultancy fees from ViiV Healthcare, Gilead, Janssen, and Merck outside the submitted work. A.W. reports grants and consultancy fees from ViiV Healthcare, Gilead, Janssen, Merck, and Gilead outside the submitted work. J.M.P.-L. reports institutional grants and educational/consultancy fees outside the submitted work from MSD, Gilead, ViiV Healthcare, Janssen, and BMS. B.J.L. is a full-time employee of ViiV Healthcare and holds shares from GSK. Data and materials availability: All data associated with this study are present in the paper or the Supplementary Materials.

Submitted 11 September 2019
Accepted 7 April 2020
Published 6 May 2020
10.1126/scitranslmed.aay9355

Vulnerability to reservoir reseeding due to high immune activation after allogeneic hematopoietic stem cell transplantation in individuals with HIV-1

Johanna M. Eberhard, Mathieu Angin, Caroline Passaes, Maria Salgado, Valerie Monceaux, Elena Knops, Guido Kobbe, Björn Jensen, Maximilian Christopeit, Nicolaus Kröger, Linos Vandekerckhove, Jon Badiola, Alessandra Bandera, Kavita Raj, Jan van Lunzen, Gero Höuter, Jürgen H. E. Kuball, Carolina Martinez-Laperche, Pascual Balsalobre, Mi Kwon, José L. Diez-Martín, Monique Nijhuis, Annemarie Wensing, Javier Martinez-Picado, Julian Schulze zur Wiesch and Asier Sáez-Cirión

Sci Transl Med 12, eaay9355.
DOI: 10.1126/scitranslmed.aay9355

Closing a door, opening a window
Some persons living with HIV also have hematologic malignancies, which require treatment by hematopoietic stem cell transplant (HSCT). Eberhard et al. collected clinical, virologic, and immunologic data on 16 such individuals before and after transplant. They observed incomplete T cell reconstitution after HSCT, although there was de novo priming of HIV-specific T cells. A period of high immune activation indicated a window of vulnerability for HIV reservoir re-seeding. These valuable data may hold clues for helping other individuals in a similar situation, and more generally for curing HIV.