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Incomplete T cell reconstitution and vulnerability to reservoir reseeded after allogeneic hematopoietic stem cell transplantation in individuals with HIV-1

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41 post allo-HSCT in HIV-infected individuals .

42

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Abstract:

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only medical intervention which has led to an HIV cure. Whereas the size of 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 5 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 was slow and heterogeneous with an initial expansion of activated CD4⁺ T-cells that preceded the expansion of CD8⁺ T-cells; full immune reconstitution was not achieved after allo-HSCT. Although HIV-specific CD8⁺ T-cells disappeared immediately after allo-HSCT, weak *ex vivo* 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 to 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 an HLA-identical donor who was CCR5 Δ 32 homozygous (1). CCR5 Δ 32 homozygosity impairs cell surface expression of CCR5 and confers protection towards HIV-1 strains using CCR5 as entry co-receptor (2, 3). Recently, a second case of durable HIV remission after allo-HSCT with a CCR5 Δ 32 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 drastic decrease of the frequency of HIV-infected cells in blood and different tissues often to undetectable amounts (7), independent of engraftment with CCR5 Δ 32 or CCR5 wildtype (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 CCR5 Δ 32 heterozygous (9), homozygous (10) or wildtype (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 CCR5 Δ 32 homozygous transplants to impose a strong barrier for HIV dissemination upon engraftment (13). At this point,

it is not clear whether a CCR5 Δ 32 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), 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-vs-host disease, delayed immune reconstitution) 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 following 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 participants 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 participants who underwent allo-HSCT.

Results

Study population

Sixteen IciStem participants from eight medical centers in six countries were included. A summary of the participants' clinical and hematologic, as well as virologic details are shown in **Table 1** and **Table S1**, respectively. The most frequent hematologic malignancies of the participants were non-Hodgkin lymphoma (n=6) and acute myeloid leukemia (n=5). The majority 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 following allo-HSCT. Individual HSCT conditioning and ART regimens are described in **Table 1** and **Table S1** respectively. The HLA-alleles of every participant and their respective donor are depicted in **Table S2**.

Nine out of 16 (56%) participants (including two who received cells from CCR5 Δ 32/ Δ 32 donors) were alive and in active follow-up at the time of this report (**Fig. S1**). Seven participants (44%, including three who received hematopoietic stem cells from CCR5 Δ 32/ Δ 32) died due to 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 significantly 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 LTR copies/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/million CD4⁺ T-cells 45 months after allo-HSCT (7) and IciS-20 who had 186 HIV LTR copies/million CD4⁺ T-cells 8 months after allo-HSCT) at the last follow up post allo-HSCT (range: 2-88 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 ultrasensitive viral loads (usVL) 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 1 out of 9 patients tested at this time point, undetectable for the others). However, during the initial 6 months 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 (PBMC) were available for immunological analysis of bulk and HIV-specific T-cells in nine participants before allo-HSCT and up to month 27 after allo-HSCT; and in 7 other participants samples were collected at various time points between month 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/ μ l of

blood) (**Table S3**) and an inverted CD4/CD8 ratio <1 just before allo-HSCT (**Fig. 1A**). As expected, a generalized severe lymphopenia was observed during the initial 2-6 weeks post-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 subjects after allo-HSCT (27, 28). As a consequence, an inverse CD4/CD8 T-cell ratio was again observed for most patients around two months after allo-HSCT. In general, T-cell reconstitution and normalization of the CD4/CD8 T-cell ratio usually takes 1-2 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 following allo-HSCT (**Fig. 1A-B, lower panels**). The increase of the CD4/CD8 ratio observed in IciS-20 between month 4 and 9 followed donor lymphocyte infusions this patient received in month 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 vs 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 figure S3. Reconstitution of naïve and memory T-cell populations showed high inter-individual differences in this group of individuals (**Fig. S2 and S4**). In general, early time points after HSCT were characterized by a drop in frequency of

naïve T-cells; this held especially true for the CD4⁺ T-cell compartment (**Fig. 1C, left panel and S4**) (24.5% [8.1-42.3] at baseline vs. 8.2% [2.6-15.7] one month post-HSCT, median and interquartile range (IQR)). In contrast, central memory CD4⁺ T-cells were more frequent early after HSCT (28.6% [16.3-43.2] at baseline vs. 51.6% [33.9-67.9] one-month post-HSCT, median and IQR). Overall, naïve CD4⁺ T-cells remained low until almost 2 years post-HSCT, before some recovery could be noted (**Fig. 1C left panel, and Fig. S4**). This is in agreement with the strong reduction of naïve T cells that is initially observed after allo-HSCT in non-infected individuals. However, the recovery of naïve T cells appeared somewhat delayed with regards 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 post-HSCT in some cases) in frequency of terminally differentiated effector CD8⁺ T-cells (TemRA) after the initial drop observed post-HSCT. In contrast, CD8⁺ effector memory (Tem) and transitional memory (Ttm) T-cell subsets peaked at one and five months respectively and then constantly declined (**Fig. 1C right panel, and Fig. S4**).

In order to better understand the mechanisms of T-cell expansion, we also analyzed the expression of CD127 expression (IL-7 receptor- α) on memory CD4⁺ and CD8⁺ T-cells (**Fig. 1D-E**). The frequency of CD127⁺ cells was extremely low immediately post-HSCT, even among central memory (Tcm) and Ttm subsets, confirming high homeostatic expansion during this period (**Fig. 1D-E, fig. S5**). The frequency of CD127⁺ memory T-cells, in particular among CD4⁺ T-cells, started to increase four months post-HSCT in most patients, which plateaued around 20 months post-HSCT (**Fig. S5**). This pattern likely indicates expansion of memory T cells related to new thymic generation during this phase (27), since 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 memory CD8⁺ T-cells (**Fig. S5**).

High T-cell activation early after allo-HSCT: window of vulnerability for HIV reseed

We then studied the evolution of immune activation in CD4⁺ and CD8⁺ T-cells following allo-HSCT. Strong CD4⁺ and CD8⁺ T-cell activation (as measured by co-expression of CD38 and HLA-DR) followed allo-HSCT and peaked between months 2 and 3 post-HSCT (**Fig. 2A-B**). T-cell activation decreased, and reached a plateau around 20 months post-HSCT. An exception was observed in IciS-20, who showed a strong increase of T-cell activation 9 months post allo-HSCT, following a relapse of his AML in month 8. A similar pattern was found for T-cell proliferation as measured by Ki-67 expression (**Fig. 2C-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 PD-1, which has been shown to be upregulated 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 month 2 and 4 post-HSCT for both CD4⁺ and CD8⁺ T-cells, decreasing afterwards to baseline frequencies (**Fig. 2F-G**). Overall, this period of strong T-cell activation preceded the establishment of full donor chimerism, which was achieved in median 4.25 months post-HSCT. IciS-20 again was an exception with an increase of PD-1⁺CD8⁺ T-cells 9 months post allo-HSCT after relapsing AML. Interestingly, IciS-01 was the only patient who maintained high PD-1 expression in CD8⁺ T-cells (**Fig. 2F, lower panel**), which may have impaired graft-vs-host reaction (34), and 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 co-receptors 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 following allo-HSCT and peaked 1-3 months after allo-HSCT, including among CCR5 Δ 32/ Δ 32 recipients (i.e. IciS-20, IciS-11, IciS-04), even though 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 CCR5⁻ CD4⁺ T-cells from IciS-20 three months after allo-HSCT (**Fig. S6**). As expected, CCR5 expression was lost in all of the patients who received a CCR5 Δ 32 homozygous graft several months after allo-HSCT and after achieving a full T-cell chimerism (**Fig. 3A, right panel**). The frequency of CCR5⁺CD4⁺ T-cells decreased to baseline for the individuals who did not receive CCR5 Δ 32/ Δ 32 cells.

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 α 4 β 7-integrin, which was also described to bind HIV virions (35), early after allo-HSCT (**Fig. 3C-D**) and only regained baseline frequencies several months after allo-HSCT.

Overall, our results show that initial weeks following 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 impacted the HIV-specific CD8⁺ T-cell response. We monitored the frequency of CD8⁺ T-cells that were able to produce cytokines (IL-2, IFN γ , 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 due to 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 month 18. In most cases, responses could be detected against several HIV-1 antigens (**Fig. 4C**). It is worth noting 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 1.5-2 years after allo-HSCT, although significant HIV-specific CD8⁺ T-cells still persist 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. 5A-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, while HIV-specific CD8⁺ T-cells had limited functionality. Both CMV-specific and HIV-specific CD8⁺ T-cells could not be detected one month after allo-HSCT, but responses against both HIV and CMV were detected again 2 months post-HSCT. At this time point, the frequency and functionality of both responses was limited and did not differ much. However, while 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 month 110 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 viral markers were undetectable 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 due to 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 (cART, from the ANRS TRANSBioHIV study) or controlling HIV spontaneously (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 subjects 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 following 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 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, pre-transplant 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 post-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 due to conditioning, immunosuppression, graft-vs-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. Interestingly, 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 CCR5 Δ 32/ Δ 32 donors and survived over a year. However, we cannot determine if there is a direct relationship between immune reconstitution and engraftment with CCR5 Δ 32/ Δ 32 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 immune activation was observed before full donor chimerism was typically reached, and lasted for 6-9 months after allo-HSCT. Such an activation phase is consistent with the observations after allo-HSCT in non-HIV-infected recipients, 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). It is worth noting 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 CCR5 Δ 32 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 reseeded of the reservoir in engrafted cells if 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 patients during the initial 6 months following allo-HSCT, becoming undetectable later. This might represent a burst in HIV production during this period by infected cells that were being rapidly depleted.

Low frequency HIV-specific CD8⁺ T-cell responses appeared weeks after HSCT, expanded for several months and were still detectable even when full donor T-cell chimerism was achieved, as previously shown in another case (47). This is consistent with the generation of *de novo* HIV-specific CD8⁺ T-cells in response to HIV antigens potentially released during the initial expansion of T-cells following 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, as allo-HSCT from CMV⁺ donors to CMV⁺ recipients has been repeatedly reported to limit CMV-associated disease

in recipients (49). Interestingly, while 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 following allo-HSCT, and immune control of diverse viral reactivations at this time appears only achieved upon transfer of donor derived pre-existing immunity (19). Indeed, the period following allo-HSCT is not supportive for the development of efficient T-cell immunity (38, 39, 52) due to severe lymphopenia, limited capacity to generate new effector cells, and restricted TCR repertoire. All these defects are palliated much later when/if *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^{neg} haploidentical sibling, both CMV-specific and HIV-specific CD8⁺ T-cell responses had similar limited functionality two months after allo-HSCT, but CMV-specific cells, contrary to HIV-specific cells, strongly increased their polyfunctionality three months later. This suggests that there are additional particular constraints in the development of HIV-specific responses in this patient. This might be due to 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. Indeed, 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 if a few infected cells persist (55).

Interestingly, 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 due to the long half-life of certain memory T-cell populations, before they are diluted out. Indeed, 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 post-HSCT in patient IciS-28 who, at this time point,

411 had sero-reverted and had undetectable infection in our exhaustive analyses (7). However, IciS-28
412 has recently experienced viral rebound after spontaneously discontinuing ART. Several clinical
413 cases of HIV-infected patients with undetectable HIV reservoir after HSCT, who received CCR5
414 wildtype and heterozygous grafts, have reported viral rebound upon analytical treatment
415 interruption (9, 12, 20). This exposes the current limits of available virologic tools to predict
416 rebound post-treatment interruption. Further analysis of additional cases will be needed to
417 determine whether the persistence of HIV-specific T-cells might be a sensitive indicator of the
418 presence of HIV reservoir with potential replicative capacity. In contrast, IciS-19 who received a
419 CCR5 Δ 32/ Δ 32 graft underwent a structured ART interruption and had not shown detectable
420 viremia one year later (56, 57). In this patient, while most HIV markers were negative in blood
421 and tissues, a few positive HIV DNA signals were detected by *in situ* hybridization in lymph nodes.
422 It is therefore possible that a few infected cells are still present several years after allo-HSCT even
423 in individuals who received CCR5 Δ 32/ Δ 32 transplants. Therefore, despite the strong diminution
424 in the viral reservoir associated with allo-HSCT, the presence of additional effective barriers
425 appears determinant to achieve HIV remission (13). In the case of the individuals not receiving
426 cells from CCR5 Δ 32/ Δ 32 donors, this might require the genetic modification of the cells to induce
427 HIV resistance or the implementation of immunotherapies to boost immune responses. In the
428 context of allo-HSCT several strategies, such as infusion of CAR T cells or adoptive transfer of
429 antigen-specific cells derived *ex vivo* from naïve T cells from negative donors are being explored
430 to control relapse of chronic infections (19, 58, 59). Adoptive transfer of antigen-specific cells
431 expanded *ex vivo* has shown clinical efficacy against CMV, EBV and adenovirus (60) and might
432 constitute a suitable strategy to improve CD8⁺ T-cell immunity against HIV after allo-HSCT. In
433 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 pre-transplant conditioning, transplant protocols (including the presence of immunosuppressive drugs) and CCR5 donor genotype, and also differed in the timing and extent of graft-vs-host reactions and delay to full donor chimerism; ii) PBMCs were not available for each patient at the same timepoints and discussion has been sometimes extrapolated to observed general trends; iii) due to cell number limitations, we had to focus our analyses on limited phenotypical markers and antigen-specific responses; iv) finally, 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, in particular 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 post-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

456 reconstituted T-cell compartment may limit the capacity of CD8⁺ T-cells to contain the virus if
457 treatment is discontinued.

458

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 participants 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 participants were compared to those from other individuals with HIV analyzed at the same period of time. HIV controllers (~~HIC~~) included in this study participated to the ANRS CO21 Codex cohort and were defined as individuals naïve of antiretroviral treatment and whose last 5 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.

Residual viremia by ultra-sensitive 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 Abbot 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, 10–103 absolute copies) (63), which had previously been validated using a standard HIV-1 DNA control from the WHO in the range of 128–0.5 copies per 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 alloHSCT in each participant, as previously described (64). Total DNA from CD4⁺ T-cells was isolated using DNeasy Blood & Tissue kit (Qiagen). Ultra-sensitive HIV DNA quantification was performed using primers in the very conserved HIV-LTR region and the pol region and total cellular DNA was quantified using an RNaseP (RPP30, Ribonuclease P/MRP 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 peripheral blood mononuclear cells (PBMCs) as previously described(65). At least 1×10^6 PBMCs were stained with a fixable viability dye (Zombie

NIR Fixable Viability Kit, BioLegend). This was followed by incubation with unlabeled mouse anti-human $\alpha 4\beta 7$ antibody (obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: Anti-Human $\alpha 4\beta 7$ integrin Monoclonal (Act-1) (cat#111718) from Dr. 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), CXCR4 (12G5, BioLegend), CD45RA (HI100, BioLegend), CCR7 (G043H7, BioLegend), CD27 (M-T271, BD), HLA-DR (L243, BioLegend), CD38 (HB-7, BioLegend), CD25 (M-A251, BioLegend), PD-1 (EH12.2H7, BioLegend), CD127 (A019D5, BioLegend), CD19 (HIB19, BioLegend), CD14 (M5E2, 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 LSR Fortessa flow cytometer (BD Biosciences). Raw data were analysed with Diva (BD Biosciences) and FlowJo software version 10.4.2 (TreeStar Inc.).

The differentiation into naïve, central memory (T_{cm}), early (T_{emRO}) and late effector memory (T_{emRA}) T-cells over time was analyzed via expression of CCR7 and CD45RA (**Figure S2.**)(67). T_{emRO} cells were further subdivided into CD27 positive, transitional memory T-cells (T_{tm}) and CD27 negative effector memory T-cells (T_{em})(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 L-glutamine and antibiotics) with 20% heat-inactivated FCS. Cells were then incubated with overlapping peptide pools encompassing HIV-1 consensus subtype B Gag, Pol, and Nef or HCMV pp65 (all obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH, cat #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 myristate acetate (80 ng/mL) together with ionomycin (1 µg/mL; Sigma-Aldrich) were used as positive control. Anti-CD107a V450 (BD Bioscience) 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-APC and anti-CD8a APC-Cy7 antibodies (BD Bioscience). Cytofix/Cytoperm (BD Biosciences) was used for cell permeabilization prior to staining for intracellular markers. Intracellular staining used anti-IFN γ PE-Cy7, anti-IL-2 FITC, anti-TNF α PE-CF594 (BD Biosciences). Cell staining was then measured with a LSRII flow cytometer (BD Bioscience). Results were analyzed with FlowJo v10.5. Due to 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

549 Medians, means and interquartile ranges were calculated using Graph Pad Prism 7 software and
550 Microsoft Excel 15. Comparisons were done with non-parametric ANOVA analysis.

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References and Notes

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Supplementary materials:

Fig. S1: Participant sampling

Fig S2: Longitudinal characterization of naïve and memory CD4+ and CD8+ T-cell populations for 3 IciStem 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 pre and post allo-HSCT in an HIV infected cohort.

Table S1: Virologic characteristics of 16 IciStem participants included in this study

Table S2: HLA-types of 16 IciStem 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

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Author contributions:

J.M.E., J.S.z.W., J.M.P. and A.S.C. designed the study, J.M.E., M.A., C.P. and V.M. performed experiments, analyzed and interpreted the data. J.M.E., J.S.z.W. and A.S.C. interpreted the data and drafted the manuscript. B.J., E.K., L.V., J.B., A.B., K.R., J.H.E.K., P.B., M.K., J.L.D.M. provided patient 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.M. critically revised the manuscript for important intellectual content. M.N., A.W., J.M.P. and A.S.C. obtained funding. M.S., J.v.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 IciStem consortium and provided constant support during the discussion and assembly of the data.

Competing interests:

B.J. 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, CLJI, and Virology Education outside the submitted work. J.M.P. reports institutional grant and educational/consultancy fees outside the submitted work from Astra-Zeneca, Gilead Sciences, Grifols, Janssen, Merck and ViiV Healthcare. J.S.z.W. reports speaker honoraria from Gilead and MSD outside the submitted work. A.S.C. reports institutional grants and educational/consultancy fees outside the submitted work from MSD, Gilead, ViiV Healthcare, Janssen and BMS. J.v.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 Supplemental Materials.

Table 1: Clinical and hematologic characteristics of 16 IciStem participants included in this study

Patient	Site	Gender	Age at HSCT	Hematological malignancy	Graft	CCR5 status donor	HSCT conditioning regimen	GvHD prophylaxis	GvHD	Complete Chimerism [month]	HSCT associated infections	Engraftment [month]	Outcome [months post-HSCT] (cause of death)
IciS-01	ES	M	34	Burkitt lymphoma	Haploidentical and cord blood transplant	wt/wt	MAC: ATG, FLU, BU, CY	CsA and corticosteroids	no	PB: 2 T cells: 18 BM: 12	BK virus	≤1	alive [87]
IciS-02	ES	M	33	Lymphohistiocytosis maligna	HLA-matched sibling	wt/wt	RIC: ATG, FLU, melphalan	CsA and MTX	yes (severe)	PB: 1 BM: 3.5		<1	deceased [26] (infection, GVHD)
IciS-03	ES	M	51	NK-Non-Hodgkin lymphoma	HLA-identical sibling	wt/wt	RIC: FLU, melphalan	CsA and MTX	yes (mild)	PB: 1 T cells: 1 BM: 6.5	no	<1	alive [75]
IciS-04	ES	M	37	Diffuse large B-cell lymphoma	Haploidentical and cord blood transplant	Δ32/Δ32	ATG, FLU, BU, CY	CsA and short course corticosteroids	no	PB?: 73 days (T1)	CMV reactivation (resolved soon post-HSCT)	<1	deceased [2] (relapse)
IciS-05	NL	M	52	Myelodysplastic syndrome	Haploidentical and cord blood transplant	Δ32/Δ32	not disclosed	CsA, MMF and corticosteroids	yes (acute)	not reached	not disclosed	not reached	deceased [14] (relapse, pneumonia)
IciS-06	ES	M	40	Relapsing Hodgkin lymphoma	HLA-haploidentical sibling	wt/wt	RIC: FLU, BU, CY	CY, CsA, MMF	yes (severe)	PB: 3 T cells: 3 BM: 6	CMV reactivation	<1	alive [57]
IciS-08	ES	M	57	Myelofibrosis	HLA-haploidentical sibling	wt/wt	RIC: FLU, BU, CY	MMF, CsA, CY	no	n.a.	Fungal infection	not reached	deceased [2] (infection)
IciS-11	NL	M	60	Acute myeloid leukemia	HLA-matched unrelated donor	Δ32/Δ32	not disclosed	MMF, CsA (T1 and 2)	no	not reached	not disclosed	not reached	deceased [3] (respiratory insufficient)
IciS-12	ES	M	31	Relapsing lymphoma	Haploidentical mother	wt/wt	RIC: FLU, BU	MMF, CsA, CY	yes	PB: 0.5 T cells: 1	unknown	<1	deceased [2]

BM: 1											(relapse)		
IciS-17	IT	M	46	Diffuse large B cell lymphoma	HLA-haploidentical sibling	wt/wt	RIC: FLU, Thiotepa, CY	CsA, MTX	no	PB: 1	EBV reactivation	<1	alive [107]
IciS-19	DE	M	43	Acute myeloid leukemia	HLA-matched unrelated donor	$\Delta 32/\Delta 32$	RIC: FLU, treosulfan	TAC and corticosteroids	yes (severe)	PB: 23 BM: 7	HSV-reactivation, HHV8-reactivation	<1	alive [79]
IciS-20	GB	M		Acute myeloid leukemia from CMML	HLA-matched unrelated donor	$\Delta 32/\Delta 32$	FLU, BU, campath	CsA	no	T cells: 10	CMV reactivation; EBV reactivation	<1	alive [42]
IciS-23	BE	M	59	Acute myeloid leukemia	Haploidentical sibling	wt/wt	BU, cytarabine, idarubicin	MMF and TAC	no	PB: 6 T cells: 6 BM: 6	Neutropenic fever; CMV reactivation	<1	alive [35]
IciS-27	ES	m	47	Non-Hodgkin lymphoma	HLA-matched related donor	wt/wt	RIC: FLU, CY	CsA and MTX	yes (mild)	T cells: 5.5 month	no	<1	alive [77]
IciS-28	ES	m	44	Hodgkin lymphoma	HLA-matched unrelated donor	wt/wt	RIC: FLU, melphalan	TAC and Sirolimus	yes (acute)	T cells: 1 month	CMV reactivation	<1	alive [120]
IciS-29	ES	m	44	Acute leukemia	HLA-matched unrelated donor	wt/wt	FLU, BU	MMF, CsA, CY	yes (acute)	PB: 1 BM: 1	no	<1	deceased [6] (relapse)

ATG = antithymocyte globulin; BE= Belgium; BU= busulfan; 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 = metho-trexate; NK= Natural killer cell; NL= Netherlands; RIC = reduced-intensity conditioning; TAC = Tacrolimus

FIGURE LEGENDS

Figure 1: Reconstitution of the T-cell compartment in an HIV-positive cohort after allo-HSCT. (A) Frequency of CD4⁺ and CD8⁺ T-cells as % of CD3⁺ T-cells and CD4⁺/CD8⁺ T-cell ratio. Symbols and lines represent one patient over time. Grey 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 (upper panel) and median CD4⁺/CD8⁺ ratios (lower panel). (C) Proportions of naive (CCR7⁺CD45RA⁺), central memory (T_{cm}, CCR7⁺CD45RA⁻), transitional memory (T_{tm}, CCR7⁻CD45RA⁻CD27⁺), early effector memory (T_{em}, CCR7⁻CD45RA⁻CD27⁻), and late effector memory (T_{emRA}, CCR7⁻CD45RA⁺) populations of 16 patients total. Mean values from 2-10 patients per time point were summarized. (D) Frequencies of CD127⁺CD4⁺ (upper panel) and CD8⁺ T-cells (lower panel). (E) Median frequencies of CD127⁺ CD4⁺ and CD8⁺ T cells.

Figure 2: CD4⁺ and CD8⁺ T-cell activation is high in the early months post allo-HSCT. (A) Frequency of activated (CD38⁺HLA-DR⁺) CD4⁺ and CD8⁺ T-cells, measured as % of CD4⁺ or CD8⁺ T-cells, respectively. Symbols and lines represent one patient over time (B). Median frequencies of CD38⁺HLA-DR⁺ CD4⁺ and CD8⁺ T-cells. Medians from 2-10 patients per time point were summarized. (C) Frequency of proliferating (Ki-67⁺) CD4⁺ and CD8⁺ T-cells. (D) Median frequencies of Ki-67⁺ CD4⁺ and CD8⁺ T-cells. (E) Correlation analysis between Ki-67 expression and HLA-DR/ CD38 co-expression of CD4⁺ T-cells and CD8⁺ T-cells. (F) Frequency of PD-1⁺CD4⁺ (upper panel) and CD8⁺ T-cells (lower panel) Symbols and lines represent one patient over time. (G) Median frequencies of PD-1⁺ CD4⁺ and CD8⁺ T-cells.

Figure 3: HIV co-receptor expression on CD4⁺ T-cells and gut migration marker $\alpha 4\beta 7$ integrin on CD4⁺ and CD8⁺ T-cells. (A) Frequency of CCR5⁺CD4⁺ T-cells in CCR5 wt/wt and CCR5 Δ 32/wt (left panel) and CCR5 Δ 32/ Δ 32 (right panel) transplanted patients. (B) Frequency of CXCR4⁺CD4⁺ T-cells in CCR5 wt/wt and CCR5 Δ 32/wt (left panel) and CCR5 Δ 32/ Δ 32 (right panel) transplanted patients. (C) Frequencies of $\alpha 4\beta 7$ ⁺CD4⁺ (upper panel) and CD8⁺ T-cells (lower panel). (D) Median frequencies of $\alpha 4\beta 7$ ⁺CD4⁺ and CD8⁺ T-cells.

Figure 4: HIV-specific immune responses are weak but recur after HSCT. (A) Representative flow cytometry plots of CD107a, IL-2, IFN γ and TNF α staining after HIV-specific stimulation. (B) Frequency of HIV-specific CD8⁺T cells at different times after allo-HSCT. Symbols and lines represent evolution of values over time for each patient. Open symbols (between M1-M3 after HSCT) represent time points where CD8⁺ T-cell numbers were <100 cells per analysis. (C) Frequencies of CD8⁺T cells exerting at least one function against HIV-1 Pol (green), Nef (red) and Gag (blue) derived peptide pools.

Figure 5: HIV-specific CD8⁺ T-cell responses are less polyfunctional compared to CMV-specific CD8⁺ T-cell responses. (A) Proportion of CD8⁺ T-cells exerting simultaneous functions (blue= 1 function, red= 2 functions, green= 3 functions, purple= 4 functions, among IL2, IFN γ , TNF α and CD107) in response to stimulation with HIV-1 or CMV peptides. The labels in the donut plots indicate the total frequency of the response. (B) Polyfunctionality of HIV- and CMV-specific CD8⁺ T-cells in Icis23 patient before (baseline) and at different months after allo-HSCT. (C) Frequency of total (left panel) and polyfunctional (exerting at least three functions, center

panel) HIV-specific CD8⁺ T-cells (Gag+Pol responses) in Icistem patients (n=12, only samples beyond M3 post allo-HSCT were considered), regular HIV-infected individuals with undetectable viral loads on cART (n=7) and natural HIV controllers (HIC, n=21). The proportion of polyfunctional cells among the overall response is shown in the right panel. Symbols represent individual values at last time of follow up. Box plots represent 25%-75% quartile range. Median, maximum and minimum values are indicated. Comparisons were done with non-parametric ANOVA analysis.

A

B

C

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IciS-01 IciS-02 IciS-03 IciS-04 IciS-05 IciS-06 IciS-08 IciS-11
IciS-12 IciS-17 IciS-19 IciS-20 IciS-23 IciS-27 IciS-28 IciS-29

C

Figure S1: Participant sampling. Bars indicate months of follow-up after allo-HSCT. Red triangles indicate time point of blood draw and PBMC collection.

Figure S2: Longitudinal characterization of naïve and memory CD4⁺ and CD8⁺ T-cell populations for 3 IciStem participants included in this study. (A) IciS-06, (B) IciS-20, (C) IciS-23. Representative flow cytometry plots showing T-cell memory gating based on CCR7 and CD45RA expression are depicted.

Figure S3: Gating strategy of naïve and memory T-cell populations. Shown are representative flow cytometry plots of a non-infected donor. Bold titles indicate the parent populations, descriptions in the plot indicate the populations that result from the gating. In the “dump”, dead cells (Zombie NIR), monocytes (CD14) and B-cells (CD19) were excluded from further analysis.

A **B**

Figure S4: Reconstitution of naïve and memory T-cell populations. (A) CD4⁺ and (B) CD8⁺ T-cell populations.

Shown are medians and IQR for each time period before and after HSCT. Circles represent the T-cell frequency per participant. Means were derived for measurements with more than one data point for each participant within the respective time period.

Figure S5: CD127 expression on memory T-cells. (A) Frequency of CD127⁺CD4⁺ memory and (B) CD127⁺CD8⁺ memory T-cells. Symbols and lines represent individual participants over time.

CCR5⁺ T-cells

CCR5⁻ T-cells

Figure S6: Frequencies of activated CCR5⁺ and CCR5⁻CD4⁺ T-cells during mixed chimerism. Flow cytometry plots depicting HLA-DR and CD38 expression of CCR5⁺ and CCR5⁻CD4⁺T-cells of patient IciS-20.

Figure S7: Sequence of hematological events and T-cell reconstitution pre and post allo-HSCT in an HIV-infected cohort.

Table S1: Virologic characteristics of 16 IciStem participants included in this study

Patient	HIV infection pre-HSCT [years]	ART pre-HSCT[years]	HIV tropism	Post-HSCT ART regimens	HIV plasma load [date and copies/ml]	CMV status of patient before HSCT	CMV status of donor
IciS-01	1	1	R5	ABC + 3TC + RAL, month 29 post-HSCT: 5 copies /ml; month 45 post-HSCT:3 copies /ml		negative	Donor 1: not evaluated Donor 2: positive
IciS-02	8 months	8 months	R5	1) ABC + 3TC , MVC 2) DRV/r, MVC 3) DRV/r 4) ABC + 3TC , MVC 5) ABC + 3TC , RAL 6) ABC + 3TC , MVC	n.a.	positive	Positive
IciS-03	27	19	R5 and X4	TDF, FTC, RAL	Pre-SCT:<50, no detectable usVL post-HSCT	positive	Positive
IciS-04	4	n.a.	R5	ABC + 3TC, RAL	continuous viral suppression	n.a.	n.a.
IciS-05	14	14	R5	TDF, FTC, RAL, MVC, T20, ETR	14 years pre HSCT: 4800 copies/ml 2 month post HSCT <50 copies/ml	negative	Donor 1: not evaluated Donor 2: positive
IciS-06	2	2	R5 and X4	TDF, FTC, RAL	Pre-SCT:<50, no detectable usVL post-HSCT	positive	Positive
IciS-08	5	7 months	R5	DTG, ABC + 3TC	n.a.	positive	Positive
IciS-11	22	19	R5	1) ABC + 3TC, DTG 2) ABC substituted with TDF	continuous viral suppression	positive	Donor1: positive Donor 2: negative
IciS-12	2	1	R5	ABC + 3TC, MVC	n.a.	positive	Positive
IciS-17	16	13	n.a.	TDF, FTC + DRV/r + RAL	1 month pre-HSCT: 155 copies/ml 6 years post-HSCT:0	IgM negative IgG positive	Positive
IciS-19	2y and 4 months	2y and 4 months	R5 (minority X4)	1) TDF, FTC, RAL 2) ABC, 3TC, DTG	2 years before HSCT: 95 copies/ml plasma ; a week later: 44 copies/ml plasma, since then <50 copies/ml	IgM negative IgG positive	Positive
IciS-20	10	10	R5 (minority X4)	ABC + 3TC, DTG	n.a.	n.a.	n.a.
IciS-23	24	12	X4	ABC + 3TC, RAL	12 years pre-HSCT: >100000 4 month post-HSCT: <20	positive	Negative
IciS-27	8	8	X4	1) TDF + FTC + EFV 2) ABC + 3TC + EFV 3) ABC + 3TC, rilpivirine	continuous viral suppression pre-HSCT, last positive viremia 6 years before	IgM negative IgG positive	Positive
IciS-28	11	11	n.a.	1) FTC + TDF + RAL 2) ABC + 3TC + RAL 3) ABC + 3TC + DTG	continuous viral suppression pre-HSCT, last positive viremia 6 years before (14700 copies/ml)	IgM negative IgG positive	Negative
IciS-29	6	6	R5	ABC + 3TC	n.a.	positive	Positive

3TC = lamivudine; ABC = abacavir; ART = antiretroviral therapy; CMV = cytomegalovirus; DRV/r = darunavir + ritonavir; DTG = dolutegravir; EFV = efavirenz; FLU = fludarabine; FTC = emtricitabine; HSCT = hematopoietic stem cell transplant; MVC = maraviroc; n.a. = not available; RAL = raltegravir; TDF = tenofovir disoproxil fumarate

Table S2: HLA-types of 16 IciStem participants included in this study

Patient	HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DQB1	Donor	Donor	Donor	Donor	Donor
						HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DQB1
IciS-01	02/02	44/51	w02/w05	04/07	03/03	02/- (CBU)	44/51 (CBU)		07/- (CBU)	
IciS-02	02/02	44/50	05/06	04/11	03:02/03	02/02	44/50	05/06	04:03:01/11:03	03:02:01/03:01:01
IciS-03	25/01	18/15	w12/w03	13:02:01/06:04:01G	06:04:01G/02:01:01G	25/01	18/15	w12/w03	13:02:01/03:01:01G	06:04:01G/02:01:01G
IciS-04	01:01/68:01	08:01/07:02	n.a.	03:01/13:01	n.a.	01:01/03:01 (CBU1) 01:01/02:XX (CBU2)	08:01/07:02	n.a.	03:01/14:01	n.a.
IciS-05	03:01:01/24:02	07:02:01/35:01:01	w04:01:01/w07:02:01	01:01:01/04:04:01	03:02:01/05:01:01	24:02/26:01:01	07:02:01/14:01:01	w07:02:01/08:02:01	04:04:01/14:54	03:02:01/05:03:01
IciS-06	02/03	44/51	w05/w07	07/04	02/03	02/03	44/57	05/06	07/07	02/03
IciS-08	02:01:01:1/03:01:01:01	27:05:02/14:02:01	02:02:02/15:05:01	08:01:01G/01:02:01	04:02:01G:05:01:01G	02/23	27/35	02/04	08/03	04/02
IciS-11	01:01:01:01/02:01:01:01	07:02:01/-	w07:02:01:03/-	15:01:01/-	06:02:01/-	01:01:01:01/02:01:01:01	07:02:01/-	07:02:01:03/-	15:01/-	06:02:01/-
IciS-12	01:01:01:01/01:01:01:01	08/35	07/04	07/04	02/04	01/23	08/51	07/15	07/11	002/06
IciS-17	03/24	18/51	w12/w14	07/11	02/03	03/24	18/51	w12/w14	07/11	02/03
IciS-19	2:ANGA/24:02	07:02/-	07:02/-	04:01/15:01	03:02/ 06:02	2:ANGA/ 24:02	07:02/-	07:02/-	04:01/?	03:02/ 06:02
IciS-20	02:01/ 32:01	07:02/15:01	03:03/07:02	15:01/04:01	06:02/ 03:01	02:01/ 32:01	07:02/15:01	03:03/07:02	15:01/04:01	06:02/ 03:02
IciS-23	03:01/ 03:01	08:01/50:01	w06:02/w07:01	03:01/03:01	02:01/ 02:01	03:01/ 03:01	08:01/50:01	06/07	03:01/03:01	02/02
IciS-27	01/34	08/18	07/12	01/11	03/05	01/34	08/18	07/12	01/11	03/05
IciS-28	26/29	44/49	07/16	01/07	02/05	26/29	44/49	07/16	01/07	02/05
IciS-29	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.a. = not available

Table S3: Longitudinal CD4⁺ T-cell counts of 16 IciStem participants included in this study

Patients	CD4 at start of ART	Pre-HSCT visit CD4 cells/ μ l	1 month post-HSCT CD4 cells/ μ l	2-3 month post first HSCT CD4 cells/ μ l	6-8 month post-HSCT CD4 cells/ μ l	1year post-HSCT CD4 cells/ μ l	Maximum post-HSCT CD4 cells/ μ l
IciS-01		720		55			891
IciS-02			1001	1512	2132	918	
IciS-03		800	251	289	381		660
IciS-04							
IciS-05	46	392	28 (day16)				28 (day16)
IciS-06		151		127		835	759
IciS-08			3				
IciS-11	190	283	21 (day33)	1 (day80/day7 after 2nd transplant)			25
IciS-12							
IciS-17	unknown	155	unknown	320	351	688	773
IciS-19		750		251			495
IciS-20		490		142			415
IciS-23	172	258	unknown	304	140	362	688
IciS-27	203	747		160	394	553	815
IciS-28	45	891		390			2550
IciS-29							

Table S4: Summary of main clinical events observed for IciS-28

Date	Event	Log HIV RNA copies/ml plasma Median (range)
30/Aug/1998	Primary infection/ART initiation	5.37
30/Aug/1998-1/Sept/2018	ART	<1.69 (<1.28-4.17)
15/Oct/2005	Hodgkin Lymphoma (Stage III)	<1.28
14/May/2008	Autologous HSCT	<1.69
10/Sept/2009	Allogeneic HCST	<1.69
1/Sept/2018	ART discontinuation	<1.69
20/Jan/2019	Viral rebound	5