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

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

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One sentence summary: Heterogenous immune reconstitution and priming of HIV-specific cells post allo-HSCT in HIV-infected individuals .

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 CCR5Δ32 homozygous (*1*). CCR5Δ32 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 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 drastic decrease of the frequency of HIV-infected cells in blood and different tissues often to undetectable amounts (*7*), independent of engraftment with CCR5Δ32 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 CCR5Δ32 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 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), 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 CCR5Δ32/Δ32 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 CCR5Δ32/Δ32) 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/µl 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 naive 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 reseeding

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 $PD\text{-}1^+CD8^+$ 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 CCR5Δ32/Δ32 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 CCR5Δ32 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 CCR5Δ32/Δ32 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 α4β7-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, IFNγ, TNFα) 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, IFNγ or TNFα 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

289 functionality of *de novo* primed HIV-specific $CD8^+$ T-cells was not solely due to the conditions
290 inherent to allo-HSCT. Overall, the frequencies of HIV-specific $CD8^+$ T-cell responses and their
291 functionality in the IciStem cohort were significantly smaller compared to other HIV-infected
292 cohorts without detectable plasma viremia, either receiving ART (cART, from the ANRS
293 TRANSBioHIV study) or controlling HIV spontaneously (HIC, from the ANRS CO21 CODEX
294 cohort)(**Fig. 5C**).
295

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 CCR5Δ32/Δ32 donors and survived over a year. However, we cannot determine if 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 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 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

342 reactivations such as CMV or EBV (*16, 29*). In the case of HIV-infected patients, the strong
343 activation of both donor and host $CD4^+$ T-cells may prompt the production of HIV particles from
344 persistently infected cells. This viral production could lead to the reseeding of the reservoir in
345 engrafted cells if or where the pressure of the ART might not be fully effective. We did not detect
346 an increase in plasma viremia during this period in the study participants, who remained on ART
347 before, during and after HSCT. Nevertheless, HIV RNA could still be detected at extremely low
348 copy numbers in a fraction of the patients during the initial 6 months following allo-HSCT,
349 becoming undetectable later. This might represent a burst in HIV production during this period by
350 infected cells that were being rapidly depleted.

351

352 Low frequency HIV-specific $CD8^+$ T-cell responses appeared weeks after HSCT, expanded for
353 several months and were still detectable even when full donor T-cell chimerism was achieved, as
354 previously shown in another case (*47*). This is consistent with the generation of *de novo* HIV-
355 specific $CD8^+$ T-cells in response to HIV antigens potentially released during the initial expansion
356 of T-cells following HSCT or at a later time by infected cells in the tissues (*48*) that we could not
357 detect in our analyses. We cannot totally exclude that some of these responses correspond to cross-
358 reactive donor $CD8^+$ T-cells that may also expand after HSCT (*31*). However, we found responses
359 against different HIV antigens maintained for several years. CMV responses followed similar
360 dynamics than HIV responses in the study participants. Despite similar magnitudes, CMV T-cell
361 responses had superior polyfunctionality than HIV-specific T-cell responses, which were
362 predominantly monofunctional. Most of the IciStem participants studied here were CMV^+ before
363 allo-HSCT and received cells from CMV^+ donors. This is common practice, as allo-HSCT from
364 CMV^+ donors to CMV^+ recipients has been repeatedly reported to limit CMV-associated disease

365 in recipients (*49*). Interestingly, while monofunctional CMV responses are expanded after allo-
366 HSCT from CMV^- donors, highly polyfunctional CMV responses are detected when the allograft
367 proceeds from CMV^+ donors (*38*), which is associated with more rapid control of CMV
368 reactivation in these individuals. Along these lines, clearance of HBV infection after
369 transplantation in HBV antigen-positive recipients is favored when donors had immunity from
370 natural HBV infection (*50, 51*). Suboptimal T-cell responses are developed during the initial period
371 following allo-HSCT, and immune control of diverse viral reactivations at this time appears only
372 achieved upon transfer of donor derived pre-existing immunity (*19*). Indeed, the period following
373 allo-HSCT is not supportive for the development of efficient T-cell immunity (*38, 39, 52*) due to
374 severe lymphopenia, limited capacity to generate new effector cells, and restricted TCR repertoire.
375 All these defects are palliated much later when/if de novo T-cell production by the thymus is
376 satisfactorily reestablished. This unfavorable context is consistent with the poor functionality of
377 HIV-specific $CD8^+$ T-cells that we detected in IciStem patients after allo-HSCT. Moreover, as
378 previously mentioned, damage of lymphoid organs associated with HIV-1 infection may further
379 hinder the development of *de novo* responses in IciStem patients.

380

381 It is interesting to notice that for patient IciS-23, who received cells from a CMV^{neg} haploidentical
382 sibling, both CMV-specific and HIV-specific $CD8^+$ T-cell responses had similar limited
383 functionality two months after allo-HSCT, but CMV-specific cells, contrary to HIV-specific cells,
384 strongly increased their polyfunctionality three months later. This suggests that there are additional
385 particular constraints in the development of HIV-specific responses in this patient. This might be
386 due to not yet identified intrinsic defects in the priming of the HIV-specific $CD8^+$ T-cell responses
387 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,

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 wildtype 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 post-treatment 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 one year later (*56, 57*). In this patient, while 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

434 only limit relapse of HIV infection but also favor the development of new autologous responses
435 against HIV (*61*).

436

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

447

448 The IciStem consortium aims to find correlates for the persistence of an HIV reservoir and for the
449 outcome after allo-HSCT. At this point, when most analyzed participants are still on ART, no
450 conclusion can be drawn on the divergences between patients for any of the parameters described
451 here, in particular regarding their potential to identify those individuals who could be safely taken
452 off ART. However, this study provides a detailed outline of particularities of the T-cell
453 compartment in this cohort post-HSCT that might be helpful to guide future interventions aiming
454 at HIV cure in allo-HCST and other patients. Our results suggest that there is a risk of reseeding
455 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 170,000*g* 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 $1x10^6$ PBMCs were stained with a fixable viability dye (Zombie

NIR Fixable Viability Kit, BioLegend). This was followed by incubation with unlabeled mouse anti-human α4β7 antibody (obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: Anti-Human α4-β7 integrin Monoclonal (Act-1) (cat#11718) 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 (Tcm), early (TemRO) and late effector memory (TemRA) T-cells over time was analyzed via expression of CCR7 and CD45RA (**Figure S2.**)(*67*). TemRO cells were further subdivided into CD27 positive, transitional memory T-cells (Ttm) and CD27 negative effector memory T-cells (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 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-IFNγ PE-Cy7, anti-IL-2 FITC, anti-TNFα 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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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	Δ32/Δ32	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	Δ32/Δ32	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 (Tcm, $CCR7^+CD45RA^-$), transitional memory (Ttm, $CCR7^-CD45RA^-CD27^+$), early effector memory (Tem, $CCR7^-CD45RA^-CD27^-$), and late effector memory (TemRA, $CCR7^-CD45RA^+$) 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.

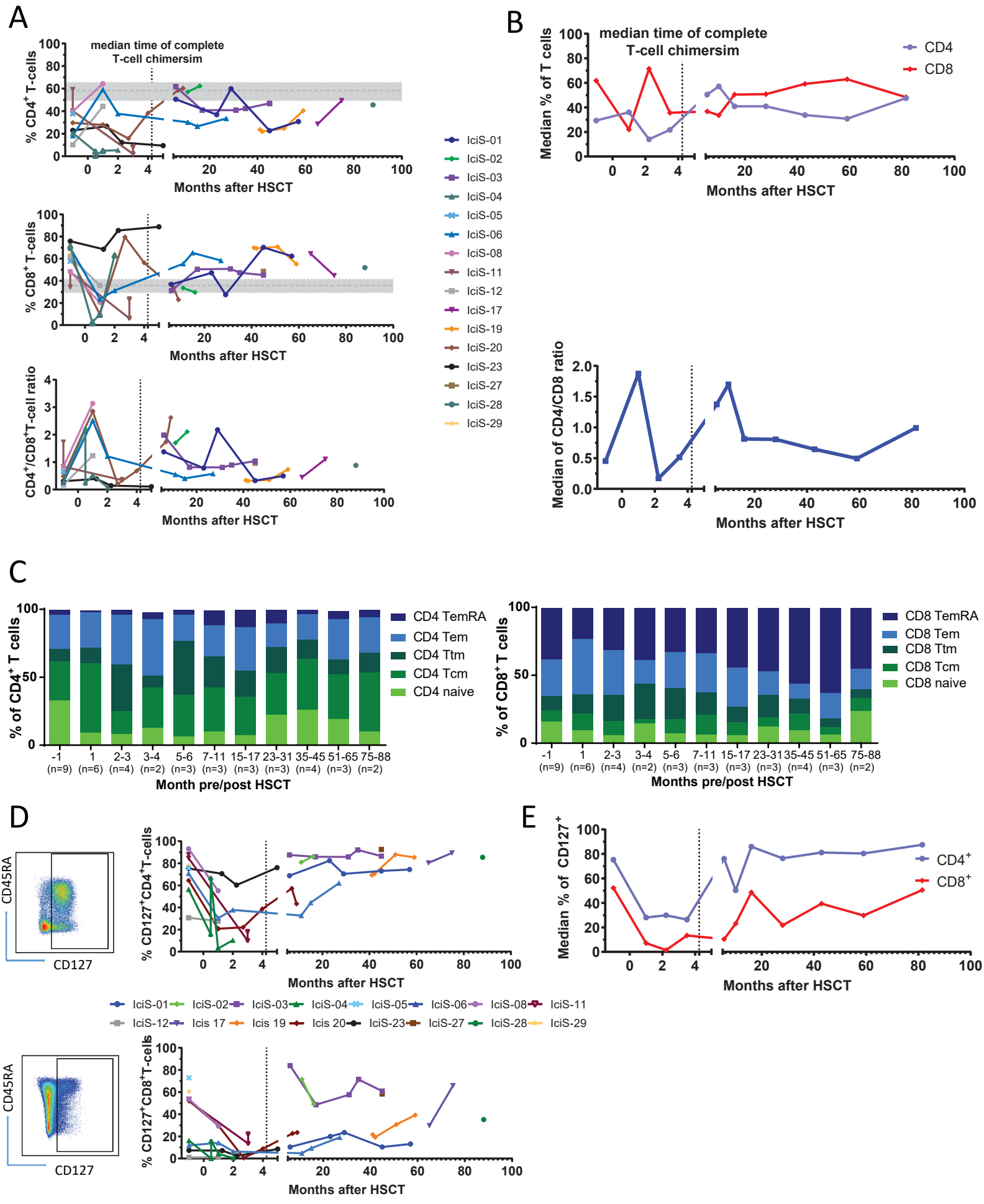
Figure 2: $CD4^+$ and $CD8^+$ T-cell activation is high in the early months post allo-HSCT. (A) Frequency of activated ($CD38^+HLA\text{-}DR^+$) $CD4^+$ and $CD8^+$ T-cells, measured as % of $CD4^+$ or $CD8^+$ T-cells, respectively. Symbols and lines represent one patient over time (B). Median frequencies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

Figure 3: HIV co-receptor expression on $CD4^+$ T-cells and gut migration marker α4β7 integrin on $CD4^+$ and $CD8^+$ T-cells. (A) Frequency of $CCR5^+CD4+$ T-cells in CCR5 wt/wt and CCR5Δ32/wt (left panel) and CCR5Δ32/Δ32 (right panel) transplanted patients. (B) Frequency of $CXCR4^+CD4^+$ T-cells in CCR5 wt/wt and CCR5Δ32/wt (left panel) and CCR5Δ32/Δ32 (right panel) transplanted patients. (C) Frequencies of $\alpha4\beta7^+CD4^+$ (upper panel) and $CD8^+$ T-cells (lower panel). (D) Median frequencies of $\alpha4\beta7^+CD4^+$ 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, IFNγ and TNFα 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, IFNγ, TNFα 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.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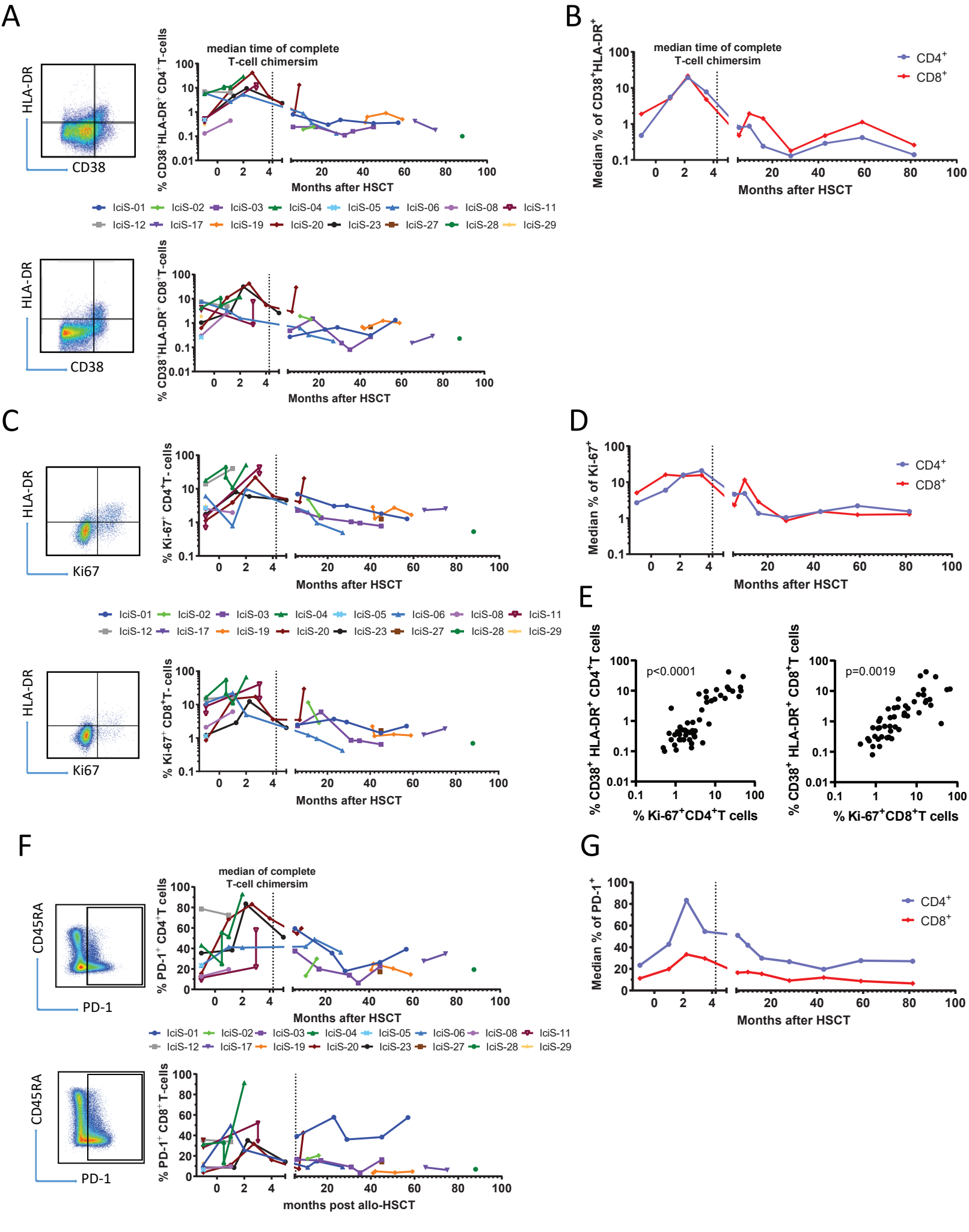
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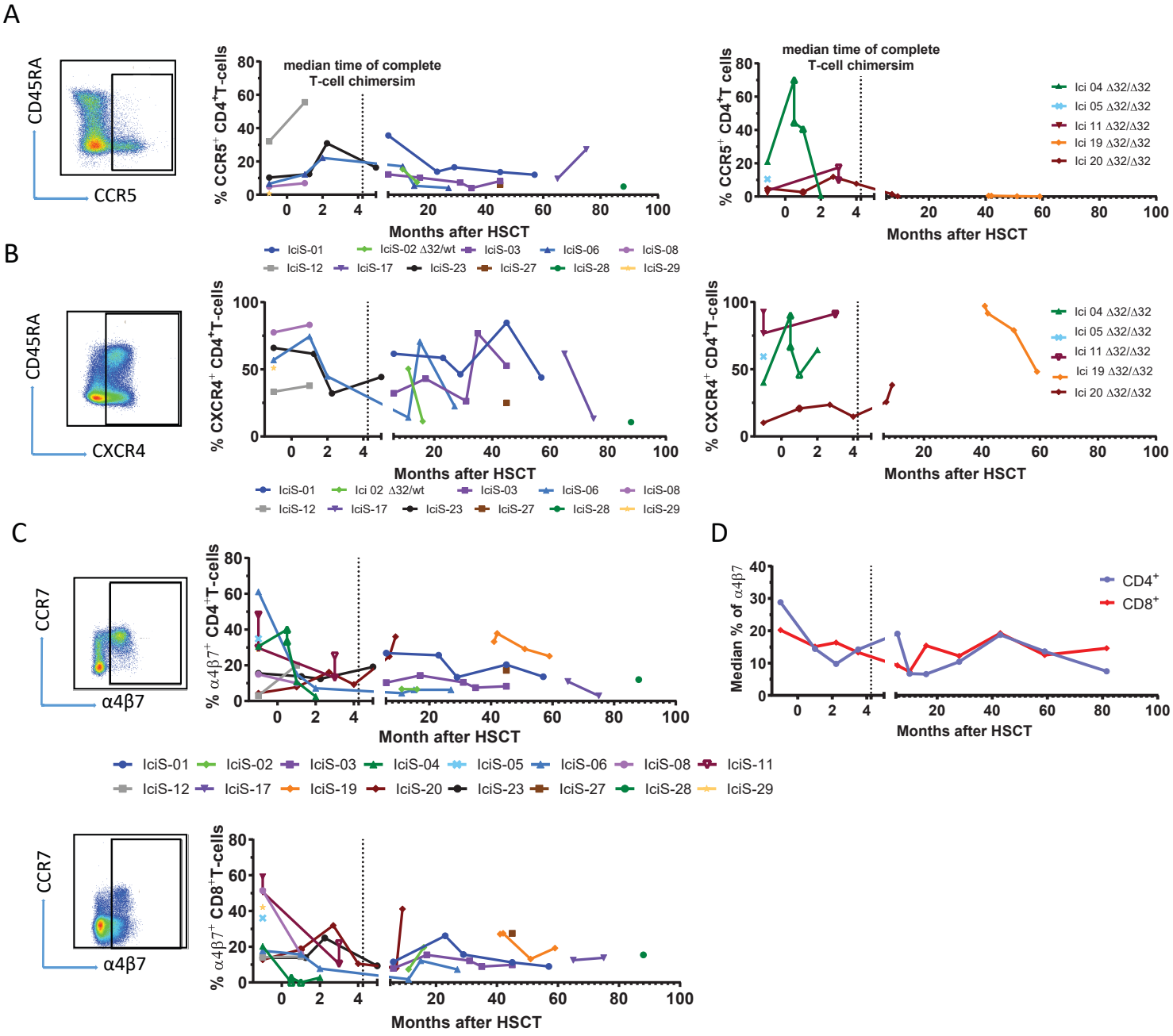
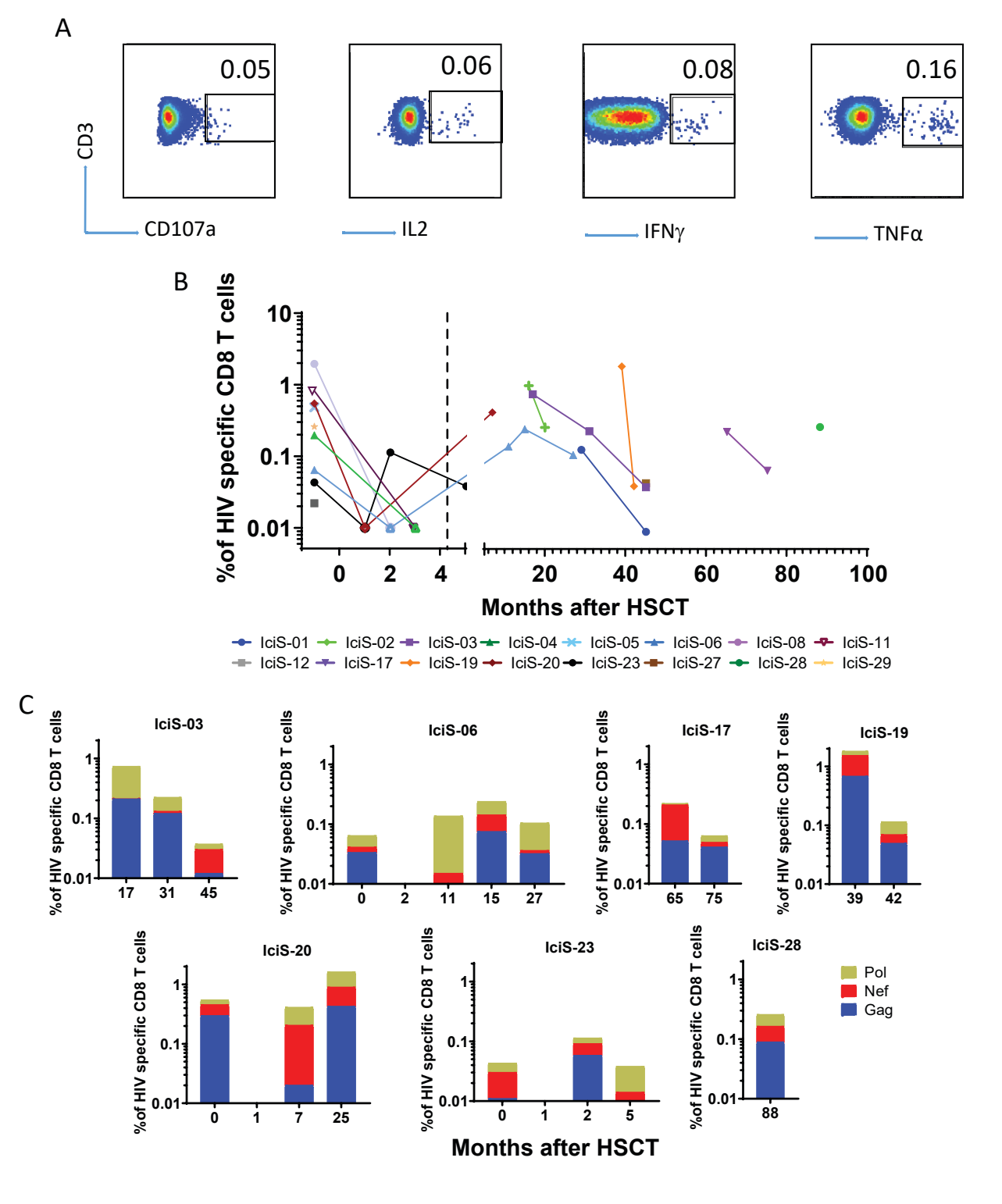
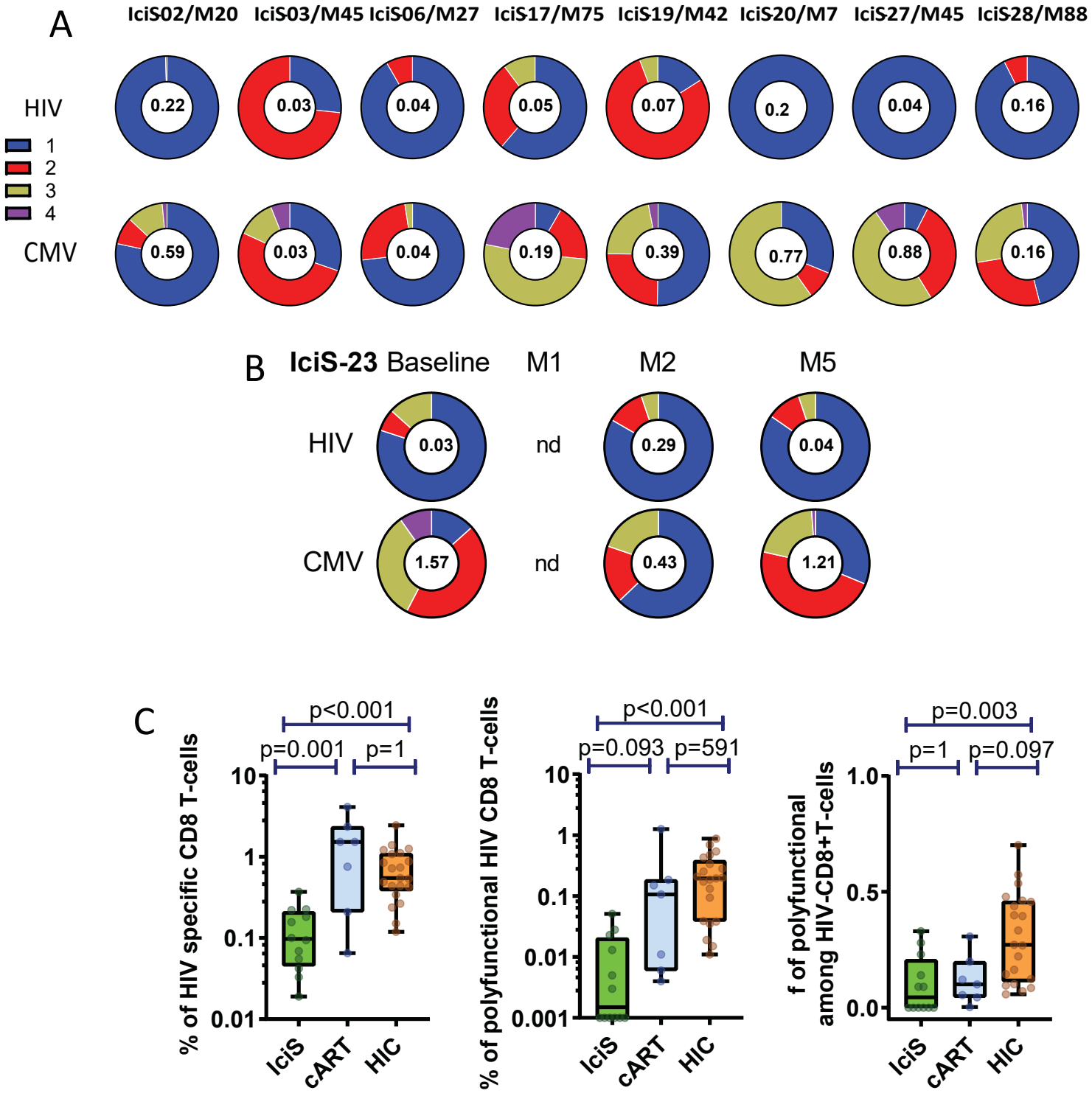
% Ki-67+CD8+T cells
F
CD45RA
PD-1
median of complete T-cell chimersim
% PD-1+ CD4+T cells
% PD-1+ CD8+ T-cells
months post allo-HSCT
G
Median % of PD-1+


A
CD45RA
CCR5
% CCR5+ CD4+T-cells
median time of complete T-cell chimersim
Months after HSCT
IciS-01
IciS-02 Δ32/wt
IciS-03
IciS-06
IciS-08
IciS-12
IciS-17
IciS-23
IciS-27
IciS-28
IciS-29
% CCR5+ CD4+T cells
Ici 04 Δ32/Δ32
Ici 05 Δ32/Δ32
Ici 11 Δ32/Δ32
Ici 19 Δ32/Δ32
Ici 20 Δ32/Δ32
B
CD45RA
CXCR4
% CXCR4+ CD4+T-cells
Ici 02 Δ32/wt
C
CCR7
α4β7
% α4β7+ CD4+T-cells
Month after HSCT
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
D
Median % of α4β7
CD4+
CD8+
% α4β7+ CD8+T-cells


A
0.05
0.06
0.08
0.16
CD3
CD107a
IL2
IFNγ
TNFα
B
%of HIV specific CD8 T cells
10
1
0.1
0.01
0
2
4
20
40
60
80
100
Months after HSCT
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
IciS-03
17
31
45
IciS-06
0
2
11
15
27
IciS-17
65
75
IciS-19
39
42
IciS-20
0
1
7
25
IciS-23
0
1
2
5
IciS-28
88
Pol
Nef
Gag
Months after HSCT


A
IciS02/M20
IciS03/M45
IciS06/M27
IciS17/M75
IciS19/M42
IciS20/M7
IciS27/M45
IciS28/M88
HIV
1
2
3
4
CMV
0.22
0.03
0.04
0.05
0.07
0.2
0.04
0.16
0.59
0.03
0.04
0.19
0.39
0.77
0.88
0.16
B
IciS-23 Baseline
M1
M2
M5
HIV
CMV
0.03
nd
0.29
0.04
1.57
nd
0.43
1.21
C
% of HIV specific CD8 T-cells
p<0.001
p=0.001
p=1
10
1
0.1
0.01
IciS
cART
HIC
% of polyfunctional HIV CD8 T-cells
p<0.001
p=0.093
p=591
10
1
0.1
0.01
0.001
IciS
cART
HIC
f of polyfunctional among HIV-CD8+T-cells
p=0.003
p=1
p=0.097
1.0
0.5
0.0
IciS
cART
HIC

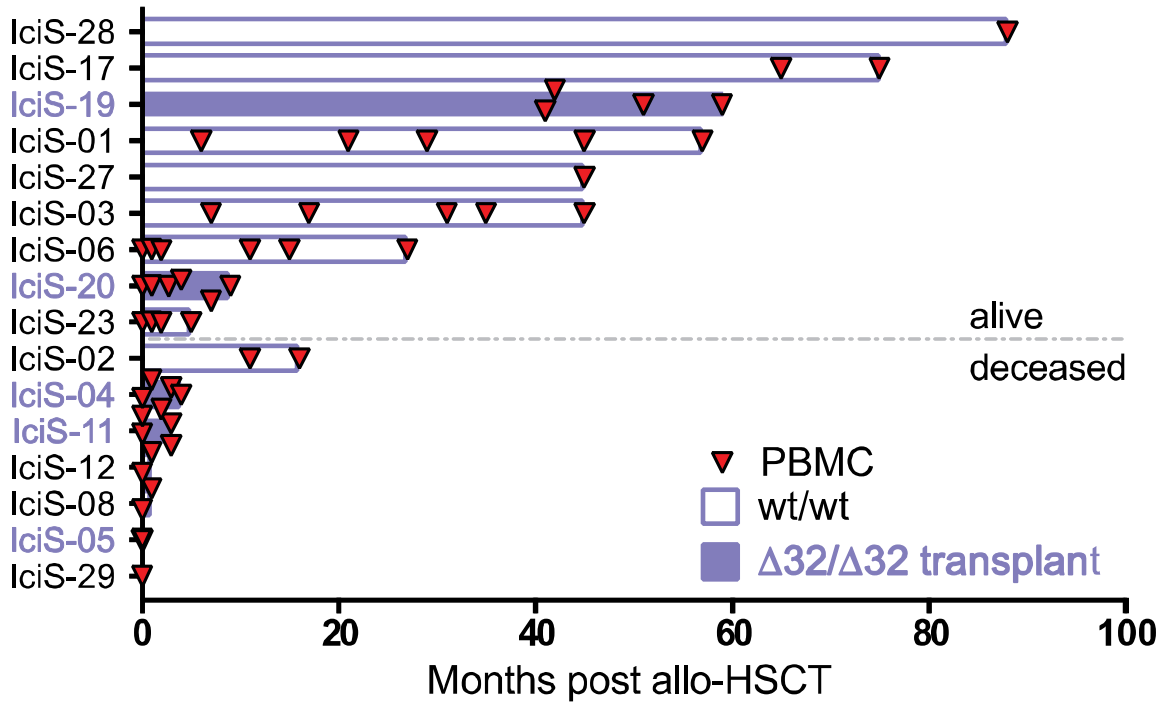


Figure S1: Participant sampling. Bars indicate months of follow-up after allo-HSCT. Red triangles indicate time point of blood draw and PBMC collection.

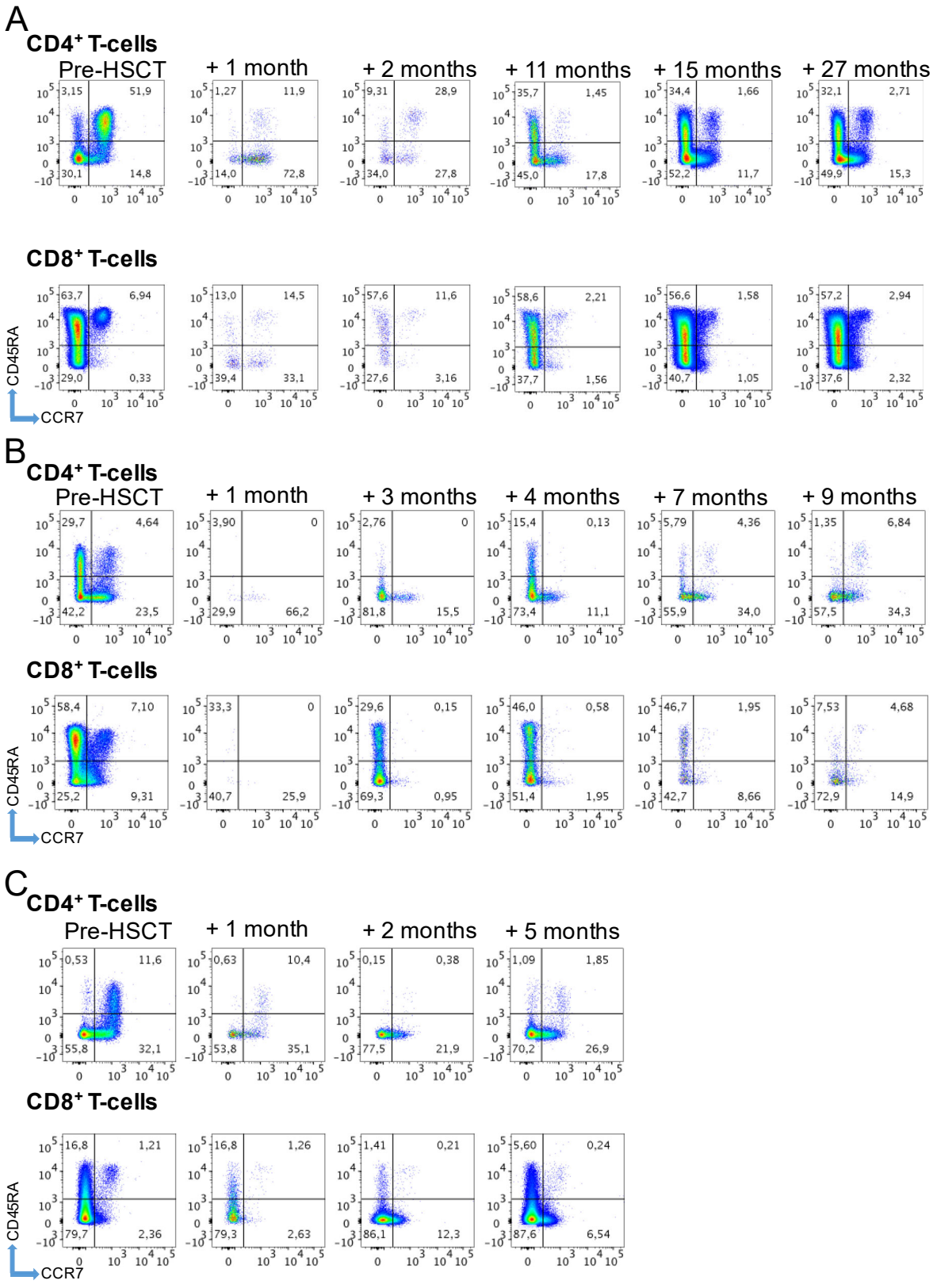

A
CD4+ T-cells
Pre-HSCT
+ 1 month
+ 2 months
+ 11 months
+ 15 months
+ 27 months
3,15
51,9
30,1
14,8
1,27
11,9
14,0
72,8
9,31
28,9
34,0
27,8
35,7
1,45
45,0
17,8
34,4
1,66
52,2
11,7
32,1
2,71
49,9
15,3
CD8+ T-cells
63,7
6,94
29,0
0,33
13,0
14,5
39,4
33,1
57,6
11,6
27,6
3,16
58,6
2,21
37,7
1,56
56,6
1,58
40,7
1,05
57,2
2,94
37,6
2,32
CD45RA
CCR7
B
CD4+ T-cells
Pre-HSCT
+ 1 month
+ 3 months
+ 4 months
+ 7 months
+ 9 months
29,7
4,64
42,2
23,5
3,90
0
29,9
66,2
2,76
0
81,8
15,5
15,4
0,13
73,4
11,1
5,79
4,36
55,9
34,0
1,35
6,84
57,5
34,3
CD8+ T-cells
58,4
7,10
25,2
9,31
33,3
0
40,7
25,9
29,6
0,15
69,3
0,95
46,0
0,58
51,4
1,95
46,7
1,95
42,7
8,66
7,53
4,68
72,9
14,9
CD45RA
CCR7
C
CD4+ T-cells
Pre-HSCT
+ 1 month
+ 2 months
+ 5 months
0,53
11,6
55,8
32,1
0,63
10,4
53,8
35,1
0,15
0,38
77,5
21,9
1,09
1,85
70,2
26,9
CD8+ T-cells
16,8
1,21
79,7
2,36
16,8
1,26
79,3
2,63
1,41
0,21
86,1
12,3
5,60
0,24
87,6
6,54
CD45RA
CCR7

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.

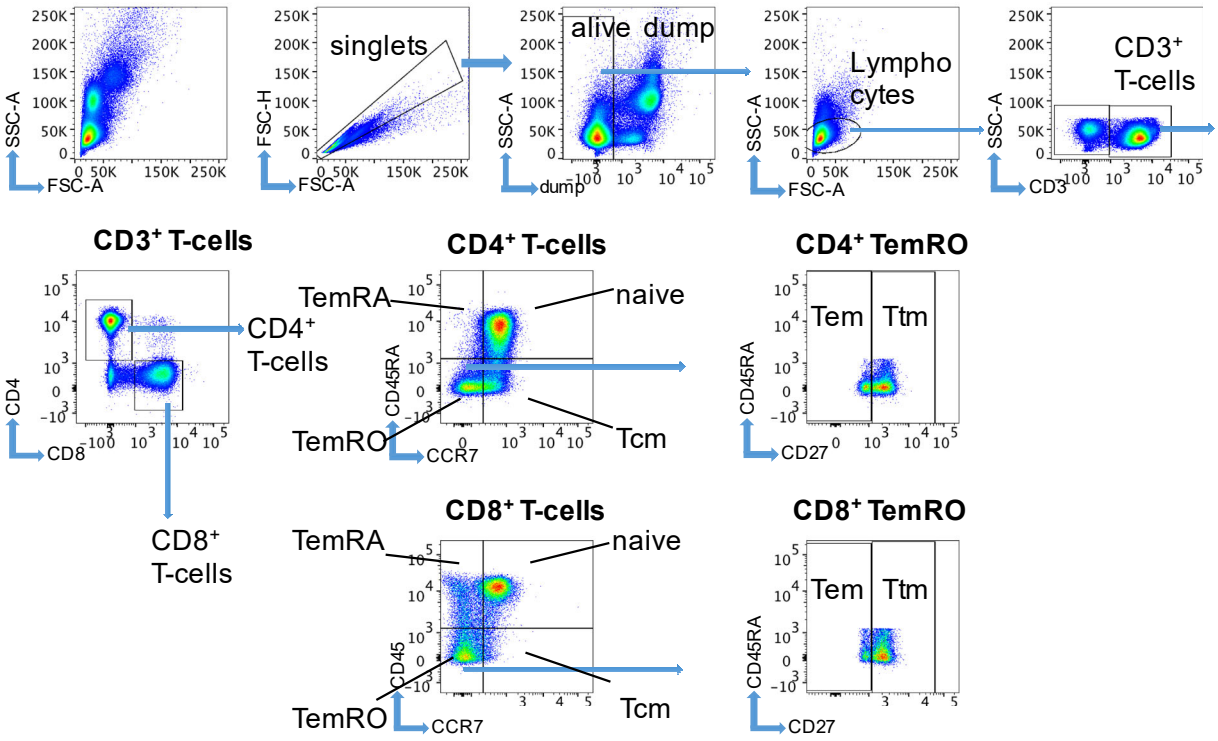


Figure S3: Gating strategy of naïve and memory T-cell populations. Shown are representative flow cytometry plots of a non-infected donor. Bold titles indicate the parent populations, descriptions in the plot indicate the populations that result from the gating. In the "dump", dead cells (Zombie NIR),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

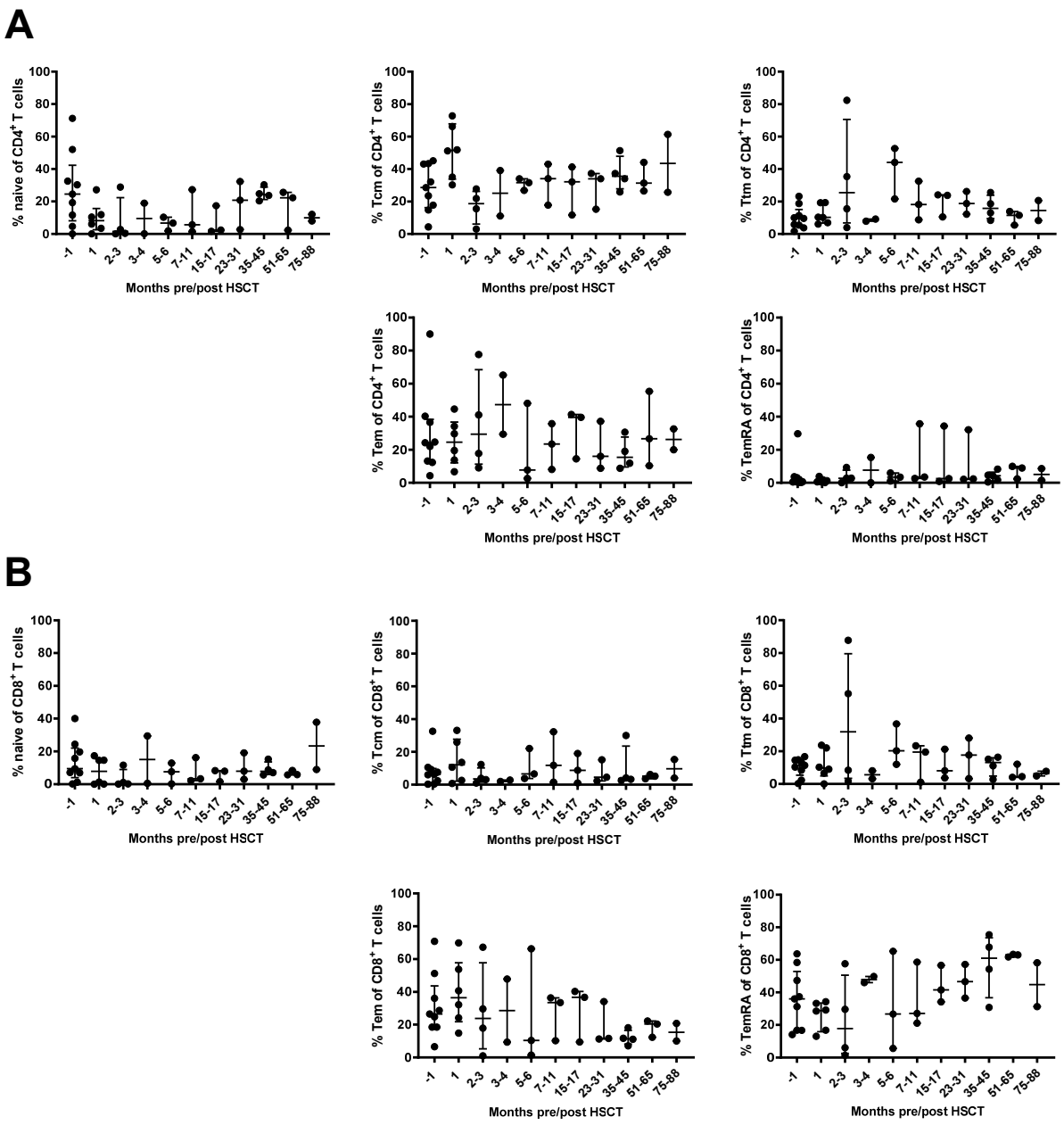


Figure S4: Reconstitution of naïve and memory T-cell populations. (A) $CD4^+$ and (B) $CD8^+$ T-cell populations**.** Shown are medians and IQR for each time period before and after HSCT. Circles represent the T-cell frequency per participant. Means were derived for measurements with more than one data point for each participant within the respective time period.

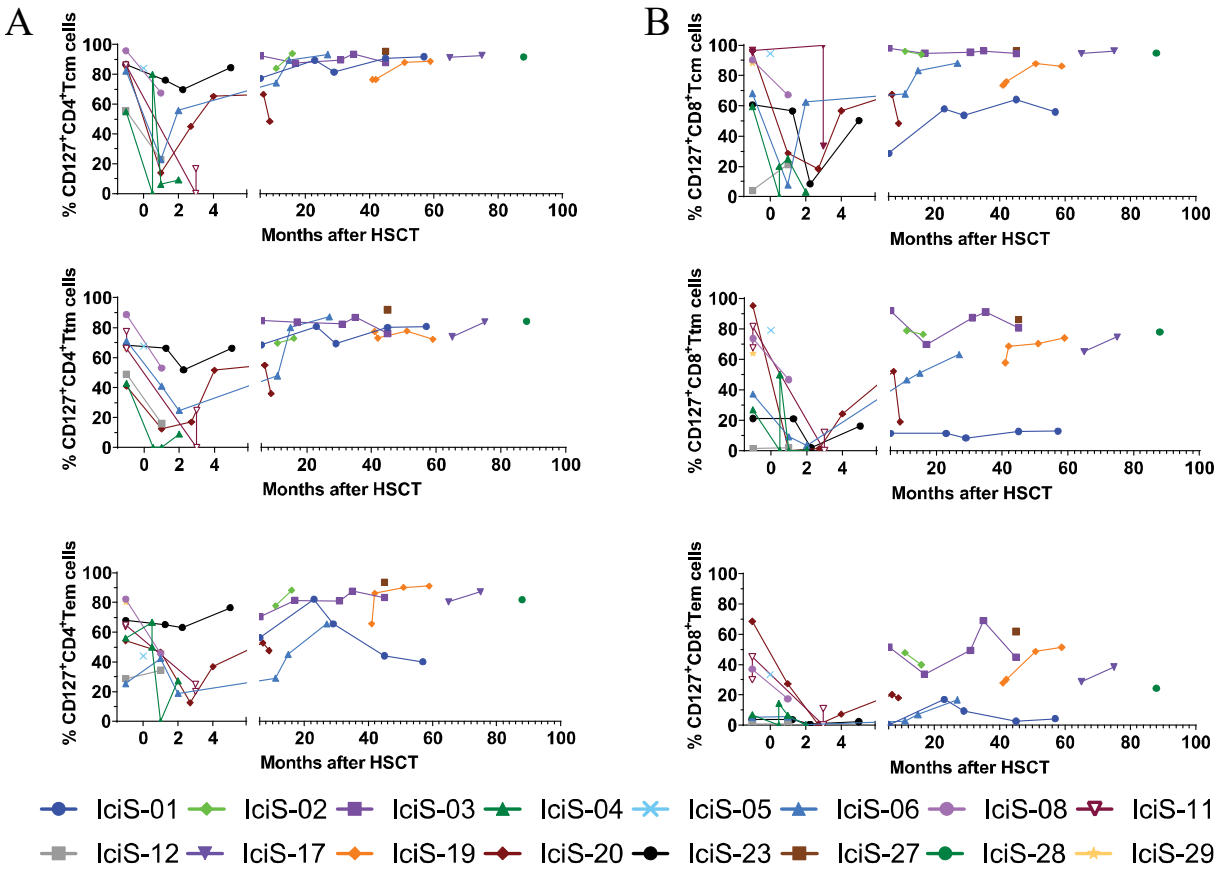


Figure S5: CD127 expression on memory T-cells. **(A)** Frequency of $CD127^{+}CD4^{+}$ memory and **(B)** $CD127^{+}CD8^{+}$ memory T-cells. Symbols and lines represent individual participants over time.

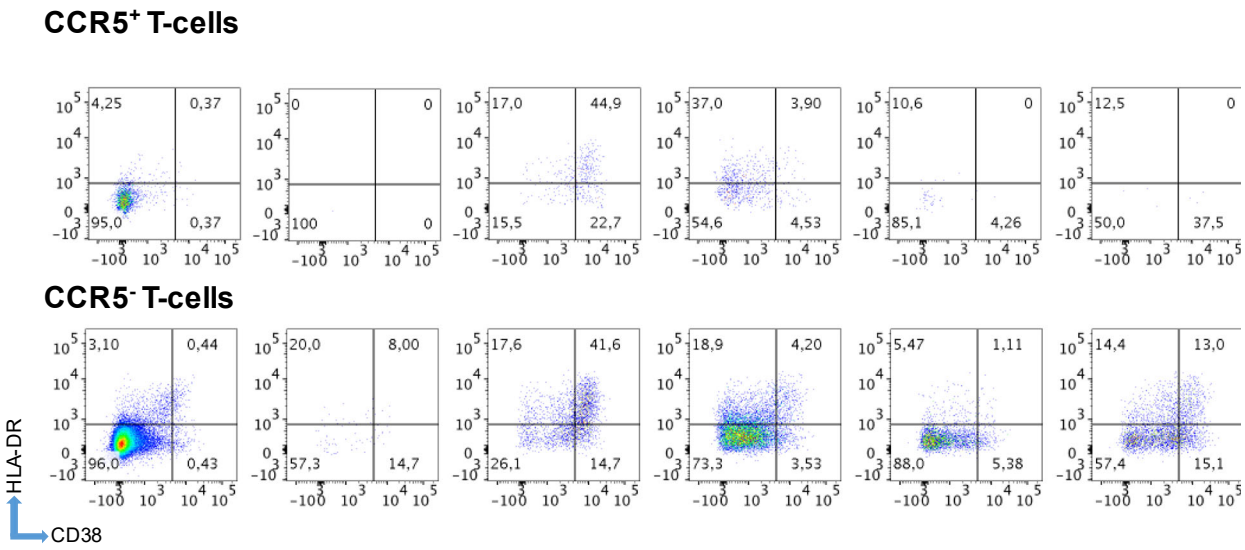


Figure S6: Frequencies of activated CCR5^{+} and CCR5^{-}CD4^{+} T-cells during mixed chimerism. Flow cytometry plots depicting HLA-DR and CD38 expression of CCR5^{+} and CCR5^{-}CD4^{+}T-cells of patient IciS-20.

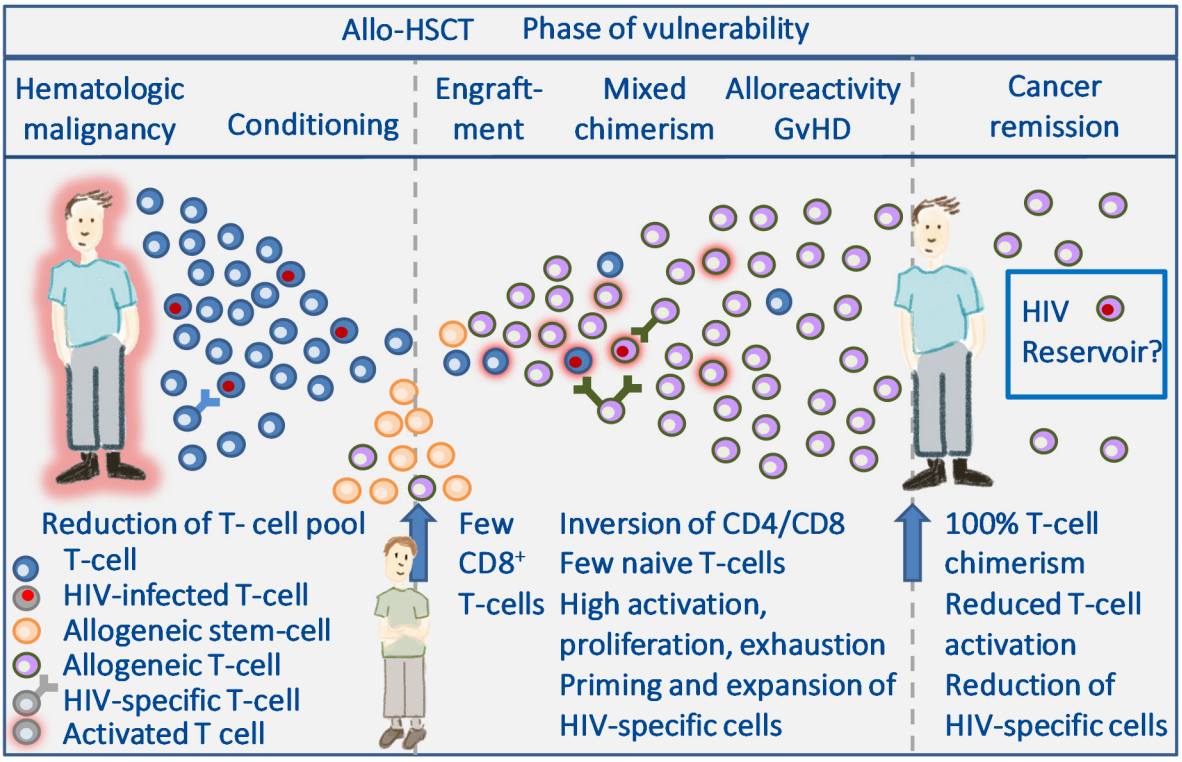


Figure S7: Sequence of hematological events and T-cell reconstitution pre and post allo-HSCT in an HIV-infected cohort.

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, MVC	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	continous 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: positvie
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 HLA-A	Donor HLA-B	Donor HLA-C	Donor HLA-DRB1	Donor 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/-	w07:02:01:0 3/-	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/µl	1 month post-HSCT CD4 cells/µl	2-3 month post first HSCT CD4 cells/µl	6-8 month post-HSCT CD4 cells/µl	1year post-HSCT CD4 cells/µl	Maximum post-HSCT CD4 cells/µl
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