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Mechanisms That Contribute to a Profound Reduction of the HIV-1 Reservoir After Allogeneic Stem Cell Transplant ^{FREE}

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Abstract

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Background: The multifactorial mechanisms associated with radical reductions in HIV-1 reservoirs after allogeneic hematopoietic stem cell transplant (allo-HSCT), including a case of HIV cure, are not fully understood.

Objective: To investigate the mechanism of HIV-1 eradication associated with allo-HSCT.

Design: Nested case series within the IciStem observational cohort.

Setting: Multicenter European study.

Participants: 6 HIV-infected, antiretroviral-treated participants who survived more than 2 years after allo-HSCT with CCR5 wild-type donor cells.

Measurements: HIV DNA analysis, HIV RNA analysis, and quantitative viral outgrowth assay were performed in blood, and HIV DNA was also measured in lymph nodes, ilea, bone marrow, and cerebrospinal fluid. A humanized mouse model was used for in vivo detection

of the replication-competent blood cell reservoir. HIV-specific antibodies were measured in plasma.

Results: Analysis of the viral reservoir showed that 5 of 6 participants had full donor chimera in T cells within the first year after transplant, undetectable proviral HIV DNA in blood and tissue, and undetectable replication-competent virus (<0.006 infectious unit per million cells). The only participant with detectable virus received cord blood stem cells with an antithymocyte globulin-containing conditioning regimen, did not develop graft-versus-host disease, and had delayed complete standard chimerism in T cells (18 months) with mixed ultrasensitive chimera. Adoptive transfer of peripheral $CD4^+$ T cells to immunosuppressed mice resulted in no viral rebound. HIV antibody levels decreased over time, with 1 case of seroreversion.

Limitation: Few participants.

Conclusion: Allo-HSCT resulted in a profound long-term reduction in the HIV reservoir. Such factors as stem cell source, conditioning, and a possible “graft-versus-HIV-reservoir” effect may have contributed. Understanding the mechanisms involved in HIV eradication after allo-HSCT can enable design of new curative strategies.

Primary Funding Source: The Foundation for AIDS Research (amfAR).

Combination antiretroviral therapy (cART) is unable to eliminate HIV-1 infection despite effective viremic control. This is attributable to a persistent latent HIV reservoir, which is responsible for rapid rebound of replication-competent virus after treatment interruption (1). Efforts to develop an effective curative strategy are needed to prevent long-term adverse effects of cART, improve patients' quality of life, and eradicate the HIV pandemic (2).

Allogeneic hematopoietic stem cell transplant (allo-HSCT) has contributed to the only known case of complete HIV-1 eradication (in the “Berlin patient”). The underlying biological mechanisms are not fully understood, although use of a donor with a homozygous mutation in the HIV coreceptor CCR5 seemed to be key to preventing HIV infection of the graft (3, 4). Other contributing factors may have been the conditioning regimen, which destroyed some or all reservoir T cells; an immunologic milieu favoring T-cell activation and reactivation of latent HIV; greater effectiveness at blocking reactivated

virus spread by CCR5-mutated donor cells compared with suppressive ART; and alloreactivity that could have eliminated infected cells in the recipient (5).

However, transplant using CCR5 wild-type donors also leads to a greater reduction in the latent reservoir than is obtained with any other clinical intervention (6–9). For example, despite the delayed viral rebound after interruption of cART that was observed in 2 HIV-infected patients undergoing allo-HSCT from CCR5 wild-type donors (the “Boston patients”) (10), these cases showed that allo-HSCT by itself was able to achieve large reductions in the viral reservoir. Transplant-associated mechanisms that reduce HIV latency and thus may play a role in eliminating the virus need to be understood to allow development of less invasive strategies to eradicate HIV-1 infection that may be applicable to the broader population of HIV-infected persons without hematologic disorders requiring stem cell transplant.

The scant experience with allo-HSCT in HIV-infected patients prevents definitive conclusions (11). To our knowledge, the IciStem Consortium (www.icistem.org) has assembled the largest and most exhaustive observational cohort for the study of HIV reservoir dynamics in HIV-positive persons who have hematologic disease and have undergone allo-HSCT. Its primary objective is to evaluate the mechanisms responsible for the dramatic reduction in HIV reservoirs associated with allo-HSCT.

In this study, we selected patients from the cohort with the longest survival and follow-up (>2 years after allo-HSCT). The study extends earlier reports—which involved single or few cases—by examining 6 patients with HIV-1 infection who underwent allo-HSCT from CCR5 wild-type donors and have been extensively studied. We analyzed reductions in HIV latency and viral-specific humoral responses with respect to factors associated with allo-HSCT in the absence of HIV resistance factors, such as CCR5 Δ 32 mutation.

Methods

Participants

At the time the study was designed, IciStem included 23 HIV-1-infected persons who had viral suppression due to cART and high-risk hematologic disease that required allo-HSCT. Thirteen died within 2 years after transplant. Seven of the remaining 10 patients survived more than 2 years after transplant, 1 of whom had a CCR5 Δ 32 donor. Therefore, the study included 6 participants (IciS-01 [12], IciS-03, IciS-06, IciS-17, IciS-27, and IciS-28) who

had survived more than 2 years after allo-HSCT with CCR5 wild-type cells, maintained use of cART, and achieved remission of their hematologic disease. All participants provided informed consent. The observational protocol (IciStem study) was approved by the institutional ethical review boards.

Chimerism Analysis

In 4 participants (IciS-01, IciS-03, IciS-06, and IciS-17), analyses were performed in whole bone marrow, peripheral blood, or both. In 3 participants (IciS-01, IciS-03, and IciS-06), T cells and myeloid cells were purified from peripheral blood by immunomagnetic means (autoMACS [Miltenyi Biotec]) using antibodies against CD3⁺ and CD13/CD33⁺, respectively. The minimum purity of isolated leukocyte subsets was 95%. In the other 2 participants (IciS-27 and IciS-28), mononuclear lymphocytes and monocytes were isolated, and the minimum purity was also 95%. In all participants, conventional chimerism analysis was performed with polymerase chain reaction of short tandem repeats (STR-PCR). In IciS-01, IciS-03, and IciS-06, when conventional chimerism analysis (with a sensitivity of 1%) was complete, ultrasensitive chimerism analysis in whole peripheral blood was also performed (Mentype DIPscreen and Mentype DIPquant [Biotype]), with a sensitivity of 0.01% to 0.001%, depending on the quality and quantity of purified DNA. Complete chimerism was defined as the absence of recipient-specific allelic patterns detectable by STR-PCR, with the level of sensitivity mentioned earlier.

Quantification of HIV Reservoir in Blood

HIV DNA in peripheral blood mononuclear cells or bulk CD4⁺ T cells was repeatedly measured after allo-HSCT in each participant, as previously described (13). Residual viremia (HIV RNA) was also measured from 9 mL of plasma (3). Leukaphereses were obtained from all participants in order to measure the number of infectious units in a large number of CD4⁺ T cells (range, 11 to 137 × 10⁶ CD4⁺ T cells [Appendix Table]) in accordance with previously described protocols (3), with the detection limit set at 0.005 infectious unit per million cells (IUPM).

Appendix Table. HIV Latent Reservoir in Blood and Tissues in All Samples Isolated From Each Patient*

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Quantification of HIV Reservoir in Anatomical Compartments

Per protocol, HIV was measured in tissue biopsy specimens only in participants who had undetectable viral reservoirs in peripheral blood. Target cells for HIV infection were isolated from different tissues to increase sensitivity for viral detection. CD45⁺ cells were isolated and processed from ileal biopsy specimens using the lamina propria leukocytes viral DNA assay (14). T-follicular helper CD4⁺ memory T cells, defined as CD3⁺CD4⁺CD45RA⁻PD1⁺CXCR5⁺, were sorted by flow cytometry from lymph node biopsy specimens obtained using fine-needle aspiration. Magnetic cell isolation of CD3⁺ or CD4⁺ T-cell populations was performed in bone marrow. In all cases, isolated cells were lysed, and viral DNA was quantified by using droplet digital PCR with 2 different sets of primers (14).

Lumbar puncture was performed to obtain 2 to 5 mL of cerebrospinal fluid (CSF), and residual viremia was quantified (3).

Quantification of HIV Antibodies

Specific HIV-1 antibodies in longitudinal plasma samples were measured using a qualitative Western blot assay (New LAV Blot I [Bio-Rad]) and the quantitative standard and low-sensitivity versions of the VITROS anti-HIV-1 assay (Ortho Clinical Diagnostics) (15).

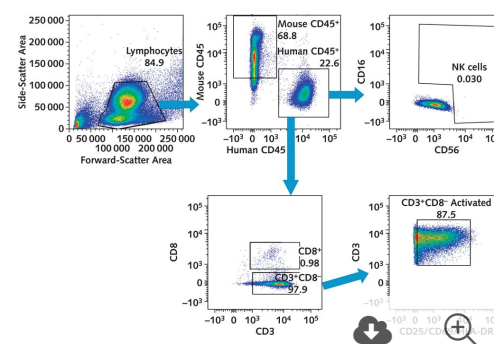
Humanized Mouse Viral Outgrowth Assay

As an *in vivo* measure of residual replication-competent reservoir cells in blood, we used a humanized mouse model modified to transfer CD4⁺ T cells instead of total peripheral blood mononuclear cells (16). All procedures were performed according to protocol 8927, which was reviewed by the Animal Experimentation Ethics Committee of the University Hospital Germans Trias i Pujol (registered as B9900005) and approved by the Catalan government according to current national and European Union legislation on the protection of

experimental animals. Mice were supervised daily according to a strict protocol to ensure their welfare and were euthanized, if required, with isoflurane (inhalation excess). Briefly, 50 to 250 million purified $CD4^+$ T cells were infused in 5 mice (10 to 50 million per mouse). Whole blood samples were collected every 2 weeks until week 12, when possible. Plasma was used for quantification of HIV RNA using the m2000 Abbott platform. Whole blood was stained to define human T-cell engraftment as the proportion of human $CD45^+$ cells in the total lymphocyte gate and activated $CD4^+$ T cells ($hCD45^+CD3^+CD8^-HLADR^+CD69^+CD25^+$) (Appendix Figure 1). We also lysed blood cells and quantified HIV DNA as previously described (14). Spleen samples were collected at the last time point and were mechanically disaggregated and used to quantify HIV DNA with droplet digital PCR (14) after lysis of erythrocytes.

APPENDIX FIGURE 1.

Gating strategy to quantify engraftment of human cells (proportion of human $CD45^+$ cells), $CD4$ (defined as $CD3^+CD8^-$) cell activation, and the event of any $CD8$ or NK contamination.
NK = natural killer.



Statistical Analysis

Given the small number of patients, no statistical analysis was performed.

Role of the Funding Source

This study was supported by the Foundation for AIDS Research (amfAR) through the amfAR Research Consortium on HIV Eradication (ARCHE) program (grants 108930-56-RGRL, 109293-59-RGRL, and 109552-61-RGRL) as well as Dutch Aidsfonds grants 2013034 and 2016026. Ms. Gálvez was supported by the PhD fellowship of the Spanish Ministry of Education, Culture and Sport (FPU15/03698). The funding sources had no role in the design or conduct of the study or the decision to submit the manuscript for publication.

Results

The 6 patients selected for this study survived more than 2 years after transplant with CCR5 wild-type donor cells. All of them showed complete remission of their hematologic disease, no longer had immunosuppression, and maintained cART during and after transplant; only participant IciS-06 interrupted cART from days 5 to 24 due to severe mucositis, with no evidence of viral rebound. Hematologic and virologic characteristics of the patients are shown in the [Table](#).

Table. Clinical, Hematologic, and Virologic Characteristics of the 6 Patients

Characteristic	IciS-01	IciS-03	IciS-06	IciS-17	IciS-27	IciS-28
Clinical characteristics						
Sex	Male	Male	Male	Male	Male	Male
Age, y	34	51	40	46	47	44
Country of origin	Spain	Spain	Spain	Italy	Spain	Spain
Hematologic characteristics						
Diagnosis	Burkitt NHL (stage IV)	NK-NHL (stage IV)	HL (stage IV)	NHL (DLBCL)	NHL (stage II)	HL (stage II)
Year of allo-HSCT	2012	2013	2014	2010	2013	2009
Source of transplant	CBS	CBS	CBS	CBS	CBS	CBS
Donor type/graft source	Cord blood T8 (mismatched DB1) + mismatched related PBCT	HLA-identical sibling/PBPC	HLA-haploidentical sibling/PBPC	HLA-identical sibling/PBPC	HLA-identical sibling/PBPC	HLA-identical unrelated/PBPC
Donor CCR5 type	CCR5 wt/wt	CCR5 wt/wt	CCR5 wt/wt	CCR5 wt/wt	CCR5 wt/wt	CCR5 wt/wt
Recipient HLA	A*02:02, B*44:01, Cw2:02, DB1*04:01, DB1*13:03, DB1*15:02	A*01:01, B*18:01, Cw2:02, DB1*13:03, DB1*15:02	A*02:03, B*44:01, Cw2:02, DB1*13:03, DB1*15:02	A*02:02, B*18:01, Cw2:02, DB1*13:03, DB1*15:02	A*01:01, B*18:01, Cw2:02, DB1*13:03, DB1*15:02	A*02:03, B*44:01, Cw2:02, DB1*13:03, DB1*15:02
Donor-recipient HLA	5/6 (DB1)B	10/10	6/8 (B*44:01 and DB1*15:02)	10/10	10/10	10/10
Conditioning	MAC, FLU, CY, busulfan, and ATG	RIC; FLU, CY, and melphalan	RIC; FLU, CY, and busulfan	RIC; Thiotepa, FLU, and CY	RIC; FLU and CY	RIC; FLU and melphalan
Transplant associated infections	Escherichia coli, BK virus, hemorrhagic cystitis	None	Cytosporidium difficile, CMV reactivation	EBV reactivation (treated with rituximab)	None	CMV reactivation
GvHD prophylaxis	CyA + steroids	CyA + Mtx	Posttransplant CY + CyA + MMF	CyA + Mtx	CyA + Mtx	Tacrolimus + sirolimus
GvHD	No	Acute: Mild (by month 8), affecting the skin	Acute: Severe (by month 3), affecting the skin and intestines	No	Chronic: Mild (by month 4)	Chronic: Mild (by day 12, grade I), affecting the skin and intestines
Day of neutrophil engraftment	15	18	22	15	11	21
Day of platelet engraftment	31	9	25	16	11	21
Time to complete chimerism, mo§	2	1	3	1	ND	ND
Peripheral blood T lymphocytes	18	1	3	ND	5.5	1
Bone marrow	12	6	5.5	ND	ND	ND
Immunosuppression at last follow-up	No	No	No	No	No	No
Status at last follow-up	Alive with CR	Alive with CR	Alive with CR	Alive with CR	Alive with CR	Alive with CR
Virologic characteristics						
Time from HIV diagnosis to allo-HSCT, y	1	27	2	16	8	11
Time from start of ART to allo-HSCT, y	1	19	2	13	8	11
HIV regimen	ABC + 3TC + RAL	Dual R5/R4 TDF, FTC, RAL	Dual R5/R4 TDF, FTC, RAL	ND	ND	ND
Posttransplant HIV ART	ABC + 3TC + RAL, maraviroc	TDF, FTC, RAL	TDF, FTC, RAL	TDF-FTC + DRVN + RAL	1. FTC + TDF + EFV 2. ABC + 3TC + RAL 3. ABC + 3TC + rilpivirine	1. FTC + TDF + RAL 2. ABC + 3TC + RAL 3. ABC + 3TC + DTG
Plasma VL before allo-HSCT, HIV-1 RNA copies/mL	65	<50	<50	<40	<1	<1
CD4 ⁺ T-cell count before allo-HSCT, × 10 ⁶ /cell	0.720	0.800	0.151	0.155	0.747	0.891
CD4 ⁺ T-cell count 3 mo after allo-HSCT, × 10 ⁶ /cell	0.410	0.558	0.324	0.320	0.160	0.390
Maximum CD4 ⁺ T-cell count after allo-HSCT, × 10 ⁶ /cell	0.891	0.640	0.759	0.773	0.815	2.550
Detectable plasma VL after allo-HSCT	Yes§	No	No	No	No	No

3TC = lamivudine; ABC = abacavir; allo-HSCT = allogeneic hematopoietic stem cell transplant; ART = antiretroviral therapy; ATG = antithymocyte globulin; CBS = cryopreserved; CR = complete remission; CyA = cyclosporine A; CY = cyclophosphamide; DLBCL = diffuse large B-cell lymphoma; DRVN = dolutegravir + rilpivirine; DTG = dolutegravir; EFV = Efavirenz; EBV = Epstein-Barr virus; EFV = efavirenz; FLU = flutamide; FTC = emtricitabine; GvHD = graft-versus-host disease; HLA = human leukocyte antigen; MAC = myeloablative conditioning; MMF = methotrexate; Mtx = methotrexate; ND = no data; NHL = non-Hodgkin lymphoma; NK = natural killer; PBPC = peripheral blood progenitor cells; RAL = raltegravir; RIC = reduced-intensity conditioning; TDF = tenofovir disoproxil fumarate; VL = viral load; y = years; cord blood transplant supported with third-party HLA-mismatched CD34⁺ cells (IciS-01 only); § Conventional chimerism (polymorphic chain reaction of short tandem repeats); ¶ Ultrasensitive VL with limit of detection of 0.5 copies/mL.

Different transplant strategies were used according to the decisions of the participants' hematologists. IciS-01 received a myeloablative single cord blood transplant supported with third-party HLA-mismatched CD34⁺ cells (haplo-cord HSCT) (12, 17). IciS-03, IciS-17, and IciS-27 underwent reduced-intensity, conditioned allo-HSCT from HLA-matched related donors. IciS-06 received a reduced-intensity, conditioned, nonmanipulated transplant from an HLA-haploidentical donor, with posttransplant cyclophosphamide for graft-versus-host disease (GvHD) prophylaxis (18). Finally, IciS-28 received a reduced-intensity, conditioned, HLA-matched transplant from an unrelated donor.

All participants achieved complete standard chimerism in peripheral blood and bone marrow in the first 12 months after allo-HSCT (Table). IciS-01 showed delayed achievement of complete T-lymphocyte chimerism (18 months) compared with patients with available data. Data on ultrasensitive chimerism in peripheral blood were available for IciS-01, IciS-03, and IciS-06; only IciS-01 showed mixed chimerism at the last follow-up. Four patients

had posttransplant GvHD; 3 of them had acute GvHD, and 2 had chronic GvHD that was treated with immunosuppression (Table).

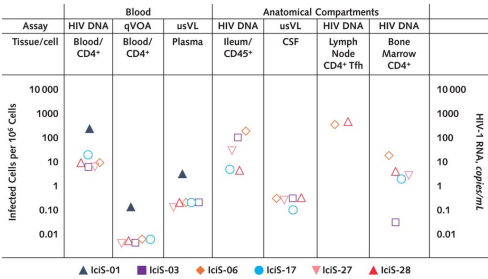
HIV Reservoir in Blood and Anatomical Compartments

Comprehensive virologic studies were performed in blood and tissue samples from the 6 participants (Figure 1 and Appendix Table). The blood HIV reservoir (proviral HIV DNA analysis and quantitative viral outgrowth assay [qVOA] in blood cells and HIV RNA analysis in plasma) was undetectable in 5 of 6 participants at the last follow-up. Of note, cell input for both HIV DNA analysis and qVOA was similar to that in previous reports of allo-HSCT and substantially higher than in other studies (7, 19). Conversely, low virus levels were consistently detected in blood samples from IciS-01 (453 HIV DNA copies per million CD4⁺ T cells, 3 HIV RNA copies per milliliter of plasma, and 0.13 IUPM [replication-competent virus]).

FIGURE 1.

HIV reservoirs measured in blood and tissues after transplant.

Data are from the last collected sample for each patient. Open symbols represent undetectable values (only IciS-01 had detectable values). In those cases, the limit of detection for the sample varied on the basis of cell/volume input, and that value is represented. CSF = cerebrospinal fluid; qVOA = quantitative viral outgrowth assay; Tfh = T-follicular helper cells; usVL = ultrasensitive viral load.



HIV was also undetectable in CSF and cells from bone marrow, lymph node, and ileal biopsy specimens in all participants, in line with previous observations in blood (Figure 1 and Appendix Table).

Longitudinal Correlation of Hematologic and HIV Reservoir Parameters

Five of 6 participants had undetectable HIV reservoirs (Appendix Figure 2). All 5 had peripheral blood progenitor cells as the graft source; 4 developed GvHD; and all 5 achieved

complete chimerism in peripheral blood, bone marrow, or T lymphocytes within the first year after transplant. Conversely, the only participant with a detectable HIV reservoir (IciS-01) received a cord blood transplant with a conditioning regimen that contained antithymocyte globulin (ATG). This participant did not develop GvHD and had mixed chimera in T cells up to posttransplant month 18, as measured by standard methods.

APPENDIX FIGURE 2.

Relationship between latency parameters measured in each participant and clinical conditions of each allogeneic stem cell transplant.

ND = not determined; PBPC = peripheral blood progenitor cells.

* Full donor chimera within a month.

Patient	HIV Reservoir	Stem Cell Donor	Time to Full Donor Chimera in T Lymphocytes	Graft-Versus-Host Disease
IciS-01	Detectable	Cord blood	>1 y	None
IciS-03	Undetectable	PBPC	<1 y*	Acute
IciS-06	Undetectable	PBPC	<1 y	Acute
IciS-17	Undetectable	PBPC	ND	None
IciS-27	Undetectable	PBPC	<1 y	Chronic
IciS-28	Undetectable	PBPC	<1 y*	Acute and Chronic

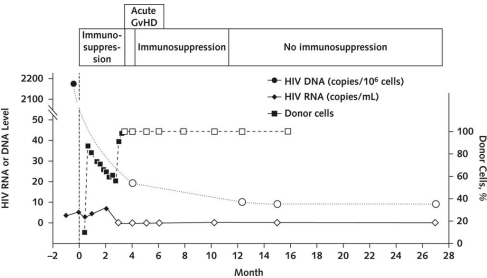


Longitudinal follow-up of IciS-06 is shown in Figure 2. This participant showed mixed chimerism in peripheral blood in the first few weeks after transplant, with concomitant detection of persistent reservoirs. By month 3, the patient developed acute grade III GvHD after withdrawal of immunosuppression coinciding with achievement of full donor chimerism. Coincidentally, residual viremia became undetectable in plasma. Cell-associated HIV DNA was also undetectable at that point, showing a 2-fold reduction in just 4 months. Clinical data suggest that similar phenomena may have occurred in the other 4 patients given that all had full chimerism within 6 months after allo-HSCT and/or GvHD (Table).

FIGURE 2.

Peripheral blood standard donor chimerism, proviral HIV DNA, and plasma HIV RNA evolution after transplant in IciS-06.

Open diamonds and circles indicate undetectable HIV RNA (ultrasensitive viral load) and proviral HIV DNA, respectively, and represent the limit of detection of each technique, which is based on cell/plasma input. For chimerism expressed as percentage of donor cells,



open squares indicate full donor chimera. GvHD = graft-versus-host disease.



HIV-Specific Humoral Response

We explored HIV humoral response dynamics in plasma samples after allo-HSCT ([Appendix Figure 3](#)). All participants lost the p18 band. We observed no other missing bands in IciS-01; however, IciS-03 and IciS-06 showed decreasing p31 antibody levels, and IciS-06 and IciS-17 lacked p55 and p24 bands. More important, we did not detect any viral antibodies in IciS-28 by month 88, suggesting that this patient experienced seroreversion. Overall, a longer interval after allo-HSCT seemed to be associated with greater antibody clearance among patients receiving cART. These data were confirmed with the low-sensitivity VITROS analysis, which showed decreased levels of HIV antibodies and a progressive loss over time after allo-HSCT ([Figure 3](#)). IciS-28 also showed antibody levels close to those of the HIV-negative donors. Overall, the data suggest limited de novo humoral responses that could sustain the HIV-specific immunoglobulin levels in the plasma of these patients.

APPENDIX FIGURE 3.

Western blot analysis from the 6 analyzed patients. Gray arrows indicate bands left or reduced intensity in each case.

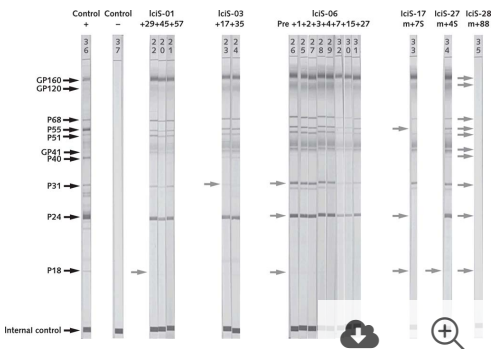
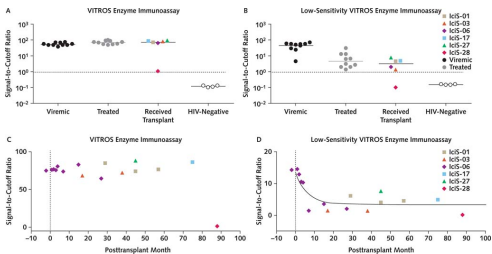


FIGURE 3.

HIV-specific antibody determination using the VITROS enzyme immunoassay.

A. Absolute antibody quantification in the last sample from each transplant recipient compared with viremic and treated HIV-positive patients and HIV-negative donors. B. Detuned low-sensitivity antibody quantification in the last sample from each transplant recipient compared with viremic and treated HIV-positive patients and HIV-negative donors. C. Absolute



antibody quantification in longitudinal plasma samples from all included patients. **D.** Detuned low-sensitivity antibody quantification in all included patients.



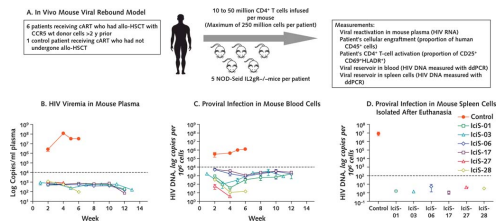
Humanized Mouse VOA

We transferred large numbers of CD4⁺ T cells purified from the participants' peripheral blood to immunosuppressed mice to detect any replication-competent blood cell reservoir ([Figure 4, A](#)). As a control, we also transferred cells from an HIV-infected person who had not undergone transplant, was receiving long-term cART, and had a standard HIV reservoir size (1.6 IUPM) ([20](#)).

FIGURE 4.

In vivo mouse viral rebound model.

All 6 patients had undetectable values. Open symbols represent undetectable values. Limit of detection relative to plasma volume input is shown. Error bars represent medians and interquartile ranges of the values from the 5 mice used for each patient. allo-HSCT = allogeneic hematopoietic stem cell transplant; cART = combination antiretroviral therapy; ddPCR = droplet digital polymerase chain reaction; wt = wild-type.

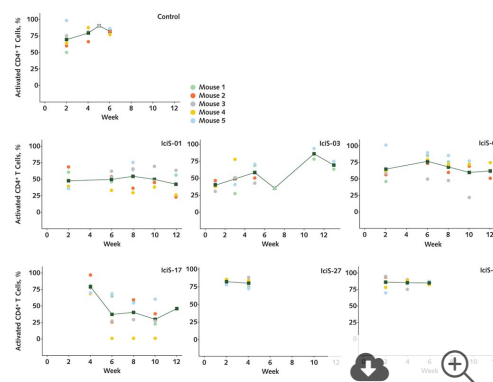


We detected high levels of HIV RNA in the plasma of humanized mice infused with control CD4⁺ T cells ([Figure 4, B](#)). Cell-associated HIV DNA was also detected in blood and spleen cells from the same infected mice ([Figure 4, C and D](#)). Conversely, none of the mice infused with cells from the 6 allo-HSCT recipients had detectable virus in plasma or cell-associated HIV DNA in the blood or spleen after 4 to 13 weeks of follow-up. Of note, median survival of the mice was 6 weeks (interquartile range, 5 to 12 weeks). Also, the median of maximum engraftment of human lymphocytes in the mice was 34% (interquartile range, 15% to 45%). Among engrafted human CD4⁺ T cells, activation levels reached a median of 95% (interquartile range, 80% to 97%), suggesting optimal conditions for eventual HIV reactivation ([Appendix Figure 4](#)).

APPENDIX FIGURE 4.

Activation profiles of CD4⁺ T cells from each mouse infused with human cells from each of the studied patients.

Activation is measured as expression of HLA-DR, CD25, and CD69 together.



Because ICI-01 did not show reactivation, we also tested cells from an HIV-infected person who did not undergo transplant, was receiving cART, and had a similarly small HIV reservoir (0.13 IUPM). HIV DNA (1000 copies per million cells) was detected in the spleen and blood of mice with human cells transferred from this person, proving the robustness of the technique.

This model suggested that immediate viral rebound was not likely after discontinuation of cART in the 6 transplant recipients. Moreover, the virus in ICI-01 might have low inducibility under in vivo physiologic conditions.

Discussion

Previous studies have shown that allo-HSCT can result in a significant reduction in the latent HIV reservoir (6–9) and, in a unique case linked to transplant of CCR5-mutated cells, even eradication of the virus (4, 19), making HIV cure a feasible target. However, the specific mechanisms that contributed to the decline in viral reservoirs in these persons are not fully understood, in part due to scant experience with allo-HSCT in HIV-infected persons. The IciStem consortium provides an opportunity to exhaustively study HIV remission in multiple HIV-infected persons who have undergone allo-HSCT, including the 6 long-term survivors described in this article. Not only have we confirmed the reduction of the HIV reservoir in blood (6, 7), but 5 of 6 participants eliminated any measurable HIV reservoir, as determined by highly sensitive techniques (10 to 100 times more sensitive than those used in previous studies [21]) in lymph nodes, ilea, bone marrow, and CSF.

The only patient who had a detectable reservoir underwent cord blood allo-HSCT with an ATG-containing conditioning regimen, did not develop GvHD, and had longer persistence of recipient cells in the T-cell compartment. All of the other participants, who did not have

a detectable reservoir, reached full donor chimerism within a year, and 4 of them developed GvHD, although we cannot confirm that those events converged in time for all of them. Exhaustive follow-up of 1 of the participants with complete viral clearance showed that HIV became undetectable coincidentally with achievement of complete donor chimerism and development of GvHD.

These results are in line with those of previous reports, where episodes of GvHD and achievement of complete chimerism also coincided with substantial reductions in the viral reservoir (4, 6, 7, 9). In contrast to the Boston patients, the IciStem participants included in our study were all free of immunosuppression at the last follow-up with T-cell immune reconstitution and had longer posttransplant survival. HIV-specific seroreversion at 8 years after transplant in IciS-28 suggests that longer time to remission might contribute to HIV clearance.

We postulate that replacement of recipient hematopoietic cells with donor cells (that is, achievement of complete chimerism in all compartments, with subsequent exertion of alloreactivity by the healthy donor immune system) might be a major factor in HIV remission after allo-HSCT in the setting of CCR5 wild-type donor transplantation, as previously suggested (7). After allo-HSCT, graft-versus-host immune responses against allelic variants of major and minor histocompatibility complex molecules contribute to a graft-versus-leukemia effect that is the basis of the therapeutic effect of allo-HSCT on hematologic disease (22). Similarly, this potent alloreactive immune effect may contribute to eradication of latently HIV-infected recipient cells through a “graft-versus-HIV-reservoir” effect.

Specific transplant-associated characteristics may explain why HIV persisted in IciS-01. For example, the immunosuppressive effect of ATG combined with use of a less mature graft source (cord blood cells) may have moderated the potential graft-versus-HIV-reservoir effect. Delayed immune T-cell reconstitution is the primary drawback of cord blood transplants because of the immature nature of the engrafted cells. In vivo profound T-cell depletion resulting from ATG-containing regimens further intensifies long-lasting impairment of immune reconstitution. Better T-cell recovery with lower incidence of virus reactivation and death from viral infection has been reported in cord blood transplant recipients not receiving ATG (23).

On the other hand, whether the absence of clinically significant GvHD in this setting also played a role is difficult to determine with certainty. Clinically evident GvHD is one of the

manifestations of alloreactivity when graft-versus-host immune responses target recipient tissues other than hematopoietic cells, and it occurs frequently after allo-HSCT. Of note, the ability of cord blood cells to exert potent antitumor activity has also been observed with low rates of GvHD (24). However, the long-term persistence of recipient cells in the T-cell compartment in IciS-01 clearly contrasts with the achievement of complete chimerism in the other participants. Evaluation of additional persons without GvHD is needed to better understand the role of GvHD among the other potential factors associated with allogeneic transplant in the eradication of HIV.

Finally, the specific posttransplant immunosuppressive regimen may also play a role in this setting. After infusion of donor hematopoietic cells, posttransplant immunosuppression exerts its inhibitory effects mainly in donor immune cells to prevent severe GvHD. This could have a dual effect in the setting of HIV infection depending on the mechanism of action, dose, and timing. On one hand, it could prevent or modulate alloreactivity against the residual HIV reservoir; on the other hand, it could also limit uninfected T-cell permissiveness for HIV replication, thus maximizing reservoir reduction. Posttransplant high-dose cyclophosphamide (participant IciS-06), which is commonly used for GvHD prophylaxis in unmanipulated haploidentical donor transplantation, eliminates rapidly proliferating alloreactive T cells of both donor and recipient origin and preserves resting memory T cells, which results in effective prevention of GvHD; this represents a potent graft-versus-leukemia effect together with relatively rapid immune reconstitution (25). Whether this strategy or other classic approaches could also enhance reservoir reduction deserves further investigation. Our series prevents definitive conclusions given the limited number of patients and their differing GvHD prophylaxis schemes. However, the fact that all participants had discontinued immunosuppressive therapy and 5 had an undetectable HIV reservoir at their last assessment suggests a positive effect of immunosuppression withdrawal in preserving alloreactivity against both the underlying hematologic disease and the HIV reservoir. The independent contribution of these factors is difficult to evaluate in the present study, but the results suggest a multifactorial interaction that promotes HIV remission.

We used an *in vivo* humanized mouse model that has proved highly sensitive in detecting replication-competent reservoir in HIV elite controllers (16). Infusion of CD4⁺ T cells from an HIV-infected control patient who had not undergone transplant led to virus reactivation in the plasma within 2 weeks, similar to what has been described elsewhere (26). In contrast, virus reactivation was not observed in mice infused with cells from participants who underwent allo-HSCT. This included IciS-01, who had shown low but detectable levels

of replication-competent virus when the ultrasensitive qVOA technique was used. The unusually high numbers of CD4⁺ T cells included in our qVOA (16, 27) with strong phytohemagglutinin-mediated stimulation enhanced virus reactivation in these cultures over physiologic conditions in mice. It seems reasonable that in the absence of cART, IciS-01 would have a longer HIV reactivation period than expected because this participant harbored a small replication-competent reservoir. Although we cannot rule out later HIV rebound after interruption of cART for any of the participants because of undetectable reservoir levels (as happened in previous reported cases [6–9]), our data contribute to mimicking of physiologic HIV reactivation dynamics.

Allo-HSCT is indicated for only a small subset of HIV-1-infected persons with underlying hematologic disease because of the high morbidity and mortality associated with the procedure. In those who survive in the long term, exhaustive consecutive studies using highly sensitive techniques could provide important information for better design of efficient, less toxic HIV cure strategies that could apply to the broader HIV-infected population.

In conclusion, our study shows that allo-HSCT yielded a profound long-term reduction in the HIV reservoir, including 1 case of seroreversion, in the CCR5 wild-type donor setting. Several transplant-associated factors may have contributed to this reduction. Further studies are needed to confirm that a graft-versus-HIV-reservoir effect might be key to achieving a sterilizing cure after allo-HSCT in HIV-infected persons. Detailed studies of chimerism dynamics at ultrasensitive levels in different reservoir compartments could further evaluate the elimination of HIV through a graft-versus-HIV-reservoir effect. Studies of monitored antiretroviral pause in selected patients will be needed to determine whether the observed absence of viral rebound in the humanized mouse model correlates with the in vivo experience.

Appendix: Members of the IciStem Consortium

The following members of the IciStem Consortium contributed to the article but did not author it: Asier Sáez-Cirión, Julian Schulze zur Wiesch, Johanna Maria Eberhard, Gero Hütter, Jürgen Kuball, Vanderson Rocha.

IciStem associated collaborators: Jorge Gayoso Cruz, Antonio Muscatello, Alessandro Soria, and Andrea Gori.

IciStem management team: Judith Dalmau, Antoinet van Kessel, and Susanne Loth.

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