MHC-matched allogeneic bone marrow transplant fails to eliminate SHIV-infected cells from ART-suppressed Mauritian cynomolgus macaques

Jason T. WEINFURTER^{a*}, Saritha S. D'SOUZA^{b*}, Lea M. MATSCHKE^a, Sarah BENNETT^a, Laurel E. KELNHOFER-MILLEVOLTE^a, Kran SUKNUNTHA^b, Akhilesh KUMAR^b, Jennifer COONEN^c, Christian M. CAPITINI^{d,e}, Peiman HEMATTI^{e,f}, Thaddeus G. GOLOS^{c,g,h}, Igor I. SLUKVIN^{b,c,i,**}, and Matthew R. REYNOLDS^{a,c,**}

^a*Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison WI*

^b*Department of Pathology and Laboratory Medicine, School of Medicine, University of Wisconsin, Madison WI*

^c*Wisconsin National Primate Research Center, University of Wisconsin, Madison WI*

^d*Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, WI*

^e*Carbone Cancer Center, University of Wisconsin School of Medicine and Public Health, Madison, WI.*

^f*Division of Hematology/Oncology/Bone Marrow Transplantation, Department of Medicine, University of Wisconsin Hospital and Clinics, Madison, WI.*

^g*Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin, Madison WI*

^h*Department of Obstetrics and Gynecology, University of Wisconsin-Madison, Madison, WI, USA*

ⁱ*Department of Cell and Regenerative Biology, University of Wisconsin School of Medicine and Public Health, Madison, WI*

*Jason Weinfurter and Saritha D'Souza contributed equally to this study.

**Matthew R. Reynolds and Igor I. Slukvin should be considered co-senior authors.

Corresponding author:

Matthew Reynolds

555 Science Dr., Madison, WI USA 53711

matt.reynolds@wisc.edu

Abstract

Objective: Allogeneic hematopoietic stem cell transplants (allo-HSCTs) in antiretroviral therapy (ART) suppressed individuals can significantly reduce human immunodeficiency virus (HIV) latent reservoirs and lead to prolonged ART-free remission. The mechanisms reducing the reservoir size are not fully understood but may include pre-transplant conditioning regimens, ART-mediated protection of donor cells, and graft-versus-host responses.

Design: We modeled allo-HSCT in four ART-suppressed simian-human immunodeficiency virus (SHIV)-infected Mauritian cynomolgus macaques (MCMs) to examine the role of transplant-mediated factors in eliminating SHIV latently infected cells.

Methods: SHIV-infected MCMs started ART 6-16 weeks post-infection. After 3-6 weeks on ART, the MCMs received myeloablative conditioning and MHC-matched bone marrow depleted of α/β T cells. The MCMs were treated with GvH disease (GvHD) prophylaxis consisting of cyclophosphamide and tacrolimus and maintained daily ART post-transplant. One MCM was removed from ART 74 days post-transplant, while the remaining three MCMs continued ART until their necropsies. Viral reservoirs were measured in the peripheral blood and lymph nodes pre- and post-transplant and tissues at necropsy.

Results: The treatment regimen induced profound lymphocyte depletion without causing severe GvHD, producing undetectable viral loads post-transplant. However, SHIV-harboring cells persisted in lymphoid and non-lymphoid tissues, resulting in a rapid viral rebound in the ART-withdrawn MCM.

Conclusions: Our results indicate that myeloablative conditioning and maintaining ART through the peri-transplant period alone are insufficient for eradicating latent reservoirs early after transplant. They also suggest that GvH responses mediated by α/β T cells are likely necessary to kill HIV latently infected cells following allo-HSCTs.

Keywords: HIV/AIDS, SHIV, macaque, allogeneic hematopoietic stem cell transplant, GvHD

Introduction

Allogeneic hematopoietic stem cell transplants (allo-HSCTs) are essential curative therapies for high-risk hematologic malignancies [1–3]. Additionally, allo-HSCTs can dramatically reduce viral reservoirs and promote prolonged ART-free remission in persons living with HIV [4–10]. While allo-HSCT can be a potent cellular immunotherapy, allo-HSCTs carry considerable safety risks and are not scalable to the broader HIV community without underlying hematologic cancer. Understanding the mechanism(s) of allo-HSCT-mediated reservoir decay can help design safer and more efficacious treatment regimens. Allo-HSCT components postulated to curtail reservoir size include pre-transplant conditioning regimens, ART-mediated protection of donor CD4⁺ T cells, graft-versus-host (GvH) responses, and the absence of the HIV coreceptor CCR5 on donor HSCs [11, 12].

In two widely publicized cases, the Berlin and London patients received allo-HSCTs from donors homozygous for the *CCR5*Δ32 (Δ32/Δ32) mutation, "curing" them of HIV [5, 8]. The transplants profoundly depleted their viral reservoirs, allowing them to stop ART without viral rebound [6, 13]. Undoubtedly, transplantation with donor cells lacking functional CCR5 contributed significantly to their ART-free remissions [14]. However, CCR5 deficiency only protects against CCR5-tropic viruses, making them susceptible to CXCR4 utilizing variants, which frequently arise during chronic infection [15]. Indeed, CXCR4-tropic viruses rapidly emerged from a third Δ32/Δ32 transplant recipient after stopping ART [16, 17].

Permanent ART-free remission may require allo-HSCTs with Δ32/Δ32 donors, but transplants with HSCs expressing wild-type CCR5 also substantially reduce viral reservoirs [4, 7, 10]. The Boston patients received allo-HSCTs from CCR5 wild-type donors and delayed viral rebound for up to 8 months after stopping ART [7]. A key feature of their treatment regimen was maintaining ART during the peri-transplant period, potentially protecting donor cells from HIV, and preventing viral reservoir reseeding. The duration of ART-free remission suggests that few virally infected cells remained after the allo-HSCTs [18].

An intuitive explanation for allo-HSCT-mediated reservoir declines is pre-transplant conditioning regimens. These cytoreductive treatments create space for HSC engraftment and ablate host immune cells that mediate graft rejection, while possibly reducing the viral reservoir burden. However, autologous HSCT studies in humans and nonhuman primates show that conditioning regimens result in only transient viral reservoir decay [19–23]. In these studies, viral reservoirs were refilled by latently infected cells contaminating autologous HSC infusions and antigen-driven proliferation of virally infected cells.

A more likely mechanism of allo-HSCT-mediated viral reservoir decay is the beneficial effects of GvH responses [7, 24, 25]. After allo-HSCT, major histocompatibility complex (MHC)-matched donor T cells recognize minor alloantigens expressed by recipient leukocytes, eliminating malignant and HIV-infected cells simultaneously, thereby mediating graft-versus-tumor (GvT) and graft-versus-viral reservoir (GvVR) effects, respectively [26, 27]. However, donor cells recognizing alloantigens expressed on non-immune cells can result in tissue destruction and potentially life-threatening graft-versus-host disease (GvHD). For allo-HSCTs in persons living with HIV, declines in viral DNA (vDNA) harboring cells are associated with transient GvHD [4, 6, 7, 9, 28]. Disentangling harmful GvHD from beneficial GvVR effects may lead to improved therapies and increased allo-HSCT-mediated viral reservoir destruction.

In humans, treatment regimen variability makes it challenging to evaluate the influence of allo-HSCT on HIV reservoirs. However, transplant conditions can be standardized across nonhuman primate cohorts, permitting an examination of each component's impact on reservoir depletion. Cynomolgus macaques from the island of Mauritius are particularly suited to model allo-HSCT studies. Mauritian cynomolgus macaques (MCM) are descended from a small founder population [29], resulting in limited genetic diversity, even at polymorphic loci. As a result, only seven MHC haplotypes (termed M1-M7) have been identified, facilitating the assembly of MHC-matched transplant pairs [30–32].

This study modeled allo-HSCTs from CCR5 wild-type donors in four SHIV-infected and ART-suppressed MCMs to examine viral reservoir decay using our recently established TCR α / β -depleted MHC-matched bone marrow (BM) transplant model, which enables donor chimerism without GvHD [33]. As anticipated, the myeloablative conditioning regimen substantially depleted circulating leukocytes. However, SHIV-harboring cells persisted in lymphoid and non-lymphoid tissues early after transplantation, demonstrating that ART-suppression, allo-HSCs, and cytoreductive conditioning alone do not eliminate virally infected cells. Our findings suggest that extended ART treatments and GvH responses are needed to significantly deplete viral reservoirs to achieve ART-free remission.

Methods

Ethics statement and animal care

Cynomolgus macaques (*Macaca fascicularis*) were cared for by the staff at the Wisconsin National Primate Research Center (WNPRC) according to the regulations and guidelines of the University of Wisconsin Institutional Animal Care and Use Committee, which approved this study (protocol g005424) following the recommendations of the Weatherall Report and the principles described in the National Research Council's Guide for the Care and Use of Laboratory Animals. Allo-HSCT recipients were closely monitored for signs of stress or pain. In consultation with the WNPRC veterinarians, euthanasia was employed in the event of an untreatable opportunistic infection, inappetence, and/or progressive decline in condition.

MHC typing

MHC genotyping was performed by Genetic Services at WNPRC as previously described [32].

Simian-human immunodeficiency virus (SHIV) infection and viral loads

The MCMs were inoculated intravenously with 500 median tissue culture infectious doses (TCID₅₀) of SHIV162P3 [34]. Plasma viral loads were measured via quantitative real-time polymerase chain reactions (qRT-PCRs) as previously described [35, 36].

Antiretroviral treatment

SHIV-infected MCMs received a combination of reverse transcriptase inhibitors tenofovir (PMPA) and emtricitabine (FTC), and integrase inhibitor raltegravir (RAL). PMPA and FTC were pre-formulated at concentrations of 20 mg/ml and 40 mg/ml in water containing sodium hydroxide (NaOH) and administered subcutaneously once daily at 1 ml/kg of body weight. 100 mg of RAL was mixed twice daily with food [37]. Gilead Sciences kindly provided PMPA and FTC, and Merck kindly provided RAL through material transfer agreements.

Donor bone marrow collection and TCRα/β cell depletion

HSCs were prepared as previously described [33]. Briefly, we collected BM from sex and MHC-matched MCMs by aspirating up to 5 mL from four sites, 20 mL total, and removed red blood cells using ACK lysis buffer (Thermo Fisher Scientific). The cells were then washed twice with phosphate-buffered saline and incubated with anti-rat TCRα/β allophycocyanin (APC) antibody (clone R73, BioLegend) for 20 min, followed by staining with anti-APC microbeads (Miltenyi Biotec) for another 20 min at 4°C. The stained cells were passed

through two LS columns (Miltenyi) stacked one on top of the other, and both the negative and positive cell fractions were collected. These cell fractions were counted and cryopreserved at concentrations of 30 million/mL in SFEM (Stem Cell Technologies) with 5% FBS and 10% DMSO until further use.

Pre-transplant conditioning and post-transplant care

Six days before transplant (day -6), allo-HSCT recipients were given trimethoprim/sulfamethoxazole for gut decontamination, continuing until the absolute neutrophil count surpassed >500 cells/ μ L. Systemic bacterial prophylaxis with ceftriaxone (50mg/kg) was started at day -2, cy0930 was switched to cefazolin (25mg/kg twice daily) at day -1, maintaining treatment until the absolute neutrophil count were stabilized. On day -2, the MCMs were administered total body irradiation (TBI) of five separate 1 Gy fractions on 2 consecutive days (10 Gy total), sparing the lungs and eyes as previously described [33]. One day before transplantation, cryopreserved TCR α/β -depleted BM fractions were thawed at 37°C and cultured overnight in serum-free expansion medium containing 100 ng/mL stem cell factor (SCF, Peprotech), 100 ng/mL Fms-related tyrosine kinase 3 (FL3T) ligand (Peprotech), and 50 ng/mL thrombopoietin (TPO, Peprotech). On the transplant day, BM cells were washed with Plasma-Lyte, resuspended in 15 mL of Plasma-Lyte containing 5 U/mL heparin and 2% autologous serum, and administered intravenously. GvHD prophylaxis consisted of cyclophosphamide (50 mg/kg) on days 4 and 5 post-transplant, followed by twice-daily tacrolimus (0.01 mg/kg) starting at day 5, maintaining tacrolimus serum levels between 5–15 ng/mL. We administered fluconazole (5 mg/kg) once daily for fungal prophylaxis starting on day 0. Lastly, cy0915 and cy0930 received oral eltrombopag (1.5mg/kg) and N-acetyl L-cysteine (50mg/kg), respectively, to support platelet engraftment. See Supplementary Table 1 for details.

Measuring peripheral leukocyte populations and GvHD

Following transplantation, we collected peripheral blood from recipient MCMs twice per week for the first month, followed by bi-weekly blood draws for the next 2 months and monthly thereafter. We used an XS-1000i automated hematology analyzer to measure platelets and leukocyte populations. GvHD was evaluated as previously described [38].

Measuring donor chimerism

We identified single-nucleotide polymorphisms (SNPs) that distinguish donor and recipient MCMs from a panel of 12 SNPs (see Supplementary Table 2) using the rhAMP SNP

Genotyping System (Integrated DNA Technologies) as described previously [33, 39], preferentially using homozygous/homozygous mismatches.

We quantified donor chimerism in whole PBMC or immune subsets by sequencing diagnostic SNPs with an Illumina MiSeq using previously described primer sets, PCR conditions, and analysis pipeline (see also Supplementary Table 2) [33]. To isolate specific immune subsets, we stained PBMC with anti-CD3 FITC (clone SP34, BD Pharmingen), anti-CD14 PerCP-Cy5.5 (clone M5E2, BioLegend), anti-CD20 APC (clone 2H7, BioLegend), anti-CD45 PE (clone D058-1283, BD Pharmingen), and Live/Dead Fixable Near-IR dead cell stain (ThermoFisher Scientific) and sorted into myeloid (singlets, live, lymphocytes, CD45⁻, CD14⁺ cells), T lymphocyte (singlets, live, lymphocytes, CD14⁻, CD45⁺, CD3⁺), or B lymphocyte (singlets, live, lymphocytes, CD14⁻, CD45⁺, CD20⁺) populations using a BD FACSJazz cell sorter. For each sorted population, we used between 179-61,251 cells for gDNA extraction and sequencing.

Lymphocyte isolation from tissues

As previously described, we isolated lymphocytes from the blood, lymph nodes, spleen, and bone marrow [40].

Quantifying total cell-associated vDNA by digital droplet PCR

We extracted DNA from tissues or PBMCs using the DNeasy Blood & Tissue Kit (Qiagen) per the manufacturer's instructions, except for eluting the DNA twice with 75 uL molecular grade water. DNA concentrations were determined using a Nanodrop spectrophotometer, digesting up to 3 µg of DNA with 3 µl of the EcoRI-HF restriction enzyme per 50µl. We performed two digital droplet PCR (ddPCR) reactions for each sample: one quantifying vDNA and the other determining total cell number.

First, we quantified vDNA using the primers, probes, and cycling conditions described by Gama *et al.* [41]. Second, we normalized vDNA copies to cell numbers by quantifying the RNase P (RPP30) gene, as previously described [42]. We generated droplets and read the samples with a QX200 ddPCR system (Bio-Rad). For each sample, we quantified each target gene in duplicate across at least two independent runs.

Results

SHIV infection and ART suppression

We inoculated four MCMs intravenously with the CCR5-tropic chimeric virus clone SHIV162P3 (Fig. 1). Infections progressed untreated for at least six weeks to establish robust viral reservoirs. As anticipated, virus replication peaked in the first three weeks post-infection (p.i.) and declined afterward (Fig. 2). The MCMs started an ART regimen consisting of tenofovir, emtricitabine, and raltegravir, between 6-16 weeks p.i., suppressing plasma viremia within three weeks to undetectable levels (<100 copy/eq per ml; Fig. 2). ART was maintained for the study's duration, except where noted below, keeping plasma viremia undetectable, save for viral blips in cy0905 and cy0930.

Allo-HSCT in SHIV-infected, ART-suppressed MCM

Our goal was to model key features of allo-HSCT with CCR5 wild-type donors in ART-suppressed HIV+ individuals while minimizing the likelihood of severe GvHD. We collected BM aspirates from sex and MHC-matched MCM and depleted cells expressing α/β T-cell receptors (TCR), removing potentially alloreactive T cells [33]. Approximately one month after starting ART, SHIV-infected MCMs received myeloablative conditioning using TBI (two consecutive days of 5 Gray (Gy), 10 Gy total) and were infused with 2×10^8 /kg α/β T cell-depleted HSCs. Post-transplant GvHD prophylaxis included cyclophosphamide and tacrolimus treatments (Supplementary Table 1).

Myeloablative TBI resulted in expected rapid declines in whole blood counts (WBC) and platelets (Fig. 3a and b). Post-transplant, all four MCMs experienced chronic thrombocytopenia and received supportive irradiated whole blood transfusions from SHIV-negative MCMs. Neutrophils reemerged in the peripheral blood of the recipient MCMs by 21 days post-transplant, with absolute counts in cy0930 spiking above pre-transplant levels before rapidly declining (Fig. 3c). The remaining three allo-HSCT recipients experienced persistent neutropenia after an initial burst of circulating neutrophils. Likewise, monocytes reappeared in the peripheral blood by 21 days post-transplant, with cy0930 again having the most robust recovery (Fig. 3d). Absolute monocyte counts were more variable than neutrophil counts, gradually declining in cy0905 and cy0915, while rapidly increasing before death in cy0906 and cy0930.

Lymphocytes recovered slowly after the allo-HSCTs (Fig.3e). In cy0930, lymphocytes reappeared in the peripheral blood approximately three weeks post-transplant and returned to near pre-transplant levels by day 38 post-transplant. Cy0905 exhibited a slow but steady

increase in peripheral lymphocytes, reaching ~3,500 cells/ μ l by day 71 post-transplant. Conversely, lymphocyte recovery was stunted in cy0906 and cy0915, with absolute lymphocyte counts failing to reach 1,000 cells/ μ l of blood by 5- and 7-weeks post-transplant, respectively. Similarly, CD4+ and CD8+ T cells failed to fully recover in the blood post-transplant, with cy0915 and cy0930 having CD4+ T cell counts below 300 cells/ μ l pre-transplant (Fig. 3f and 3g).

We measured post-transplant donor engraftment by deep SNPs unique to the donor and recipient MCMs. Despite low absolute leukocyte counts, the allo-HSCT recipients exhibited greater than 85% whole blood donor chimerism by three weeks post-transplant, followed by modest declines to approximately 80% donor chimerism. Notably, cy0905, the longest surviving transplant recipient, maintained 70-90% donor chimerism for two months until euthanasia (Fig. 4a). We also measured donor chimerism of circulating cells representing the myeloid and lymphoid (T cells and B cells) lineages at necropsy for cy0905, cy0915, and cy0930. The frequencies of donor-derived myeloid and lymphoid cells in cy0915 and cy0930 ranged between 65-93% of total cells (Fig. 4b), consistent with the whole blood chimerism results (Fig. 4a). In cy0905, 78% of the B and T cells were of donor origin (Fig. 4b), similar to the 83% of donor cells detected in the whole blood. However, only 45% of the myeloid cells were of donor origin. These observations collectively suggest that donor leukocytes replaced recipient cells early after transplant, with notable donor lymphocyte engraftment despite usage of α/β TCR-depleted grafts.

Pre- and post-ART viral reservoirs

We designed our GvHD prophylaxis regimen of cyclophosphamide/tacrolimus to minimize high-grade GvHD. As anticipated, GvHD was limited post-transplant with cy0905 exhibiting transient skin lesions (white flaking/dry skin) that resolved with minimal intervention, and cy0915 and cy0930 had diarrhea, which may have been a TBI side effect. None of the animals showed significant abnormalities in liver function and total bilirubin elevation.

To determine the effect of ART, myeloablative conditioning, and allo-HSCT on viral reservoirs in the absence of severe GvHD, we measured cell-associated vDNA in PBMCs and inguinal lymph nodes. Before starting ART, more SHIV-harboring cells were present in the inguinal lymph nodes (range 743-2,680 copy Eq/ 1×10^6 cells) than the PBMC (range 255-512 copy Eq/ 1×10^6 cells), consistent with lymph nodes being a primary viral reservoir [43, 44]. The higher number of SHIV-infected cells in the lymph nodes of cy0905 and cy0906 compared to cy0930 may be the result of starting ART later in infection (14-16 weeks versus 6 weeks). All

MCMs exhibited declines in SHIV-infected cells in the blood post-transplant, with cy0905, cy0906, and cy0930 having undetectable vDNA (Fig. 5a).

Nevertheless, virally infected cells persisted in other tissues. Three recipient MCMs remained on ART until their necropsies, enabling a more thorough examination of residual post-transplant viral reservoirs. We observed increased vDNA in cy0915's inguinal lymph nodes at necropsy compared to pre-transplant samples (1,151 vs. 4,807 vDNA copy Eq/million cells; Fig. 5b). This animal developed a viral-induced colitis with ulcers post-transplant, potentially leading to the expansion of latently infected cells or localized virus replication, seeding secondary lymphoid tissues with SHIV-harboring cells. Although tissue analysis was more limited, we detected fewer SHIV-infected cells in the lymphoid tissues of cy0906 and cy0930 compared to cy0915. We also discovered vDNA in the bone marrow and non-lymphoid tissues, including the lung and jejunum (Fig. 5c). Collectively these results demonstrate persistent viral reservoirs in lymphoid and non-lymphoid tissues early after allo-HSCTs in the absence of high-grade GvHD.

SHIV rapidly rebounds after removing ART

HIV cure studies often use analytical treatment interruptions (ATIs) to determine treatment efficacy. We modeled ATI in cy0905, an animal with a single viral blip but otherwise suppressed plasma viremia while on ART and had undetectable vDNA in PBMCs post-transplant. However, we detected 271 vDNA copies/million cells in axillary lymph nodes collected one day before stopping ART, day 63 post-transplant, a slight decrease from pre-transplant inguinal lymph nodes (359 vDNA copies/million cells; Fig. 5b). Surprisingly, plasma viral loads were 1×10^7 copy eq/ml seven days post-ART, roughly equivalent to peak acute viremia and spiked to nearly 1×10^8 copy eq/ml plasma at 10 days post-ART. It is possible that the presence of wild-type CCR5 on donor cells helped amplify virus replication, which transplants with modified CCR5 may ameliorate. These results demonstrate that viral reservoirs can rebound rapidly following ART cessation early after allo-HSCTs.

Discussion

Allo-HSCT's ability to shrink HIV reservoirs likely results from parallel processes protecting donor cells from infection and eliminating latently infected recipient cells [10, 11].

Here, we employed the clinically relevant SHIV/MCM model to assess the impact of allo-HSCT on viral reservoirs in the absence of severe GvHD while maintaining continuous ART. We recently reported that depleting TCR α / β^+ cells from MHC-matched MCM HSCs and post-transplant cyclophosphamide with tacrolimus prophylaxis prevented GvHD [33]. Using this

strategy with SHIV-infected MCMs, we observed only a transient skin lesion in one MCM post-transplant. These results contrast with GvHD developing shortly after transplant with T-cell replete HSCs in ART-suppressed SHIV-infected rhesus macaques [45] or uninfected MCMs [46]. Although we performed transplants with cells from sex and MHC-matched donors, our strategy to limit post-transplant GvHD could broaden donor pools, allowing for more extensive MHC-mismatches, similar to a recent human study [47].

One advantage of performing TCR α/β -depletions is that it not only reduces the risk of GvHD, but it also enriches TCR γ/δ^+ T cells and natural killer cells that may mediate GvVR. Although we prevented GvHD, removing alloreactive TCR α/β^+ T cells from the bone marrow grafts may have inhibited more effective viral reservoir clearance. SHIV-harboring cells decreased in the peripheral blood post-transplant, reaching undetectable levels in three of the four allo-HSCT recipients. Nevertheless, viral reservoir depletion was incomplete, with latently infected cells remaining in lymphoid and non-lymphoid tissues. However, reductions in the number of vDNA-harboring cells in the lymph nodes of three recipient MCMs indicate that the treatment regimen can reduce viral reservoirs early after transplant without inducing pronounced GvHD.

Supplementing allo-HSCTs with cellular therapies may improve viral reservoir clearance [48, 49]. Donor lymphocyte infusions (DLIs) can drive relapsing hematologic tumors into remission [50]. Conceivably, incorporating DLIs into HIV+ patient's allo-HSCT treatment plans could similarly augment GvVR responses [25]. However, since DLIs involve transferring T cells with polyclonal specificities, they also carry significant risks of inducing GvHD [51]. Targeted cellular immunotherapies maximizing GvVR effects while minimizing GvHD side effects are preferred. Therefore, adoptive cell therapies designed to fight cancer may be repurposed to combat latent HIV [52–54]. Chimeric antigen receptors (CAR) hold considerable promise as cancer therapies, stimulating the development of CAR strategies to treat HIV [55], though they have had limited impact on HIV reservoirs to date [56–59]. Alternatively, focusing T cells on minor histocompatibility antigens (mHAg) exclusively expressed by recipient leukocytes may promote GvVR effects without causing GvHD [60]. Recently, we identified a mHAg in MCMs that is expressed preferentially by immune cells [61]. This epitope may serve as a model antigen for testing mHAg-targeted cellular immunotherapies to eliminate SIV/SHIV reservoirs in allo-HSCT models.

Our study has several drawbacks. First, the limited animal numbers make it challenging to draw firm conclusions. Second, ART was started 3-6 weeks before allo-HSCT. While this was sufficient time to suppress SHIV replication and mediate vDNA declines, it does not reflect

prolonged pre- and post-transplant ART typical of HIV⁺ allo-HSCT recipients [4, 5, 8, 24, 28]. Longer treatment windows likely replicate HIV reservoir dynamics more effectively. Third, we were unable to distinguish whether virally-infected cells were of the donor or recipient origin post-transplant. A study in rhesus macaques exploited haploidentical allo-HSCT to sort donor and recipient cells post-transplant based on differential MHC expression, finding that most of the SHIV reservoir remained in recipient cells early after transplant [45]. We were unable to perform a similar analysis because MHC-matched MCMs lack distinct surface markers. Lastly, we observed poor engraftment post-transplant, which hindered analyzing anti-viral immune responses and viral reservoirs and may have limited SIV reservoir depletion. A less toxic conditioning regimen, higher infused HSC doses or T-cell replete allo-HSCT may promote more sustainable donor cell engraftment. Burwitz *et al.* established an allo-HSCT model in MHC-matched MCMs, achieving durable post-transplant donor chimerism with reduced-intensity conditioning but with an increased risk of GvHD [46]. Allo-HSCT regimens in NHP models will likely need to balance full donor engraftment with the suppression of severe GvHD to dissect the mechanisms of GvVR effects.

In conclusion, MHC-haploidentical bone marrow transplants in ART-suppressed SHIV-infected MCMs reduced but did not eliminate latent viral reservoirs early after transplantation. However, depleting TCR α/β T cells from the HSCs and post-transplant prophylaxis with cyclophosphamide and tacrolimus prevented high-grade GvHD, highlighting the careful balance that must be considered when engineering donor HSC grafts. Our results indicate that GvVR responses and sustained ART after allo-HSCTs are necessary for depleting viral reservoirs. The study also suggests that augmenting allo-HSCTs with cellular therapies could promote viral reservoir eradication.

Acknowledgments

We thank the veterinary staff at the Wisconsin National Primate Research Center (WNPRC) for their assistance.

Author contributions: M.R.R., T. G., and I.I.S.: Conceptualization and study design; J.T.W and S.S.D.: coordinated the animal experiments; J.T.W.: processed samples and performed flow cytometry; S.S.D., K.S., and A.K.: prepared TCR α/β -depleted HSCs; L.M.M.: performed SHIV DNA measurements; S.B. and L.E.K.: performed chimerism analysis; J.C.: contributed to treatment design and handled animals; C.M.C. and P.H.: advised on the allo-HSCT platform,

GvHD prophylaxis, and critically reviewed the manuscript; M.R.R.: wrote the manuscript. All authors revised the manuscript and approved its submission.

This study was funded by the National Institutes of Health (NIH) via grants R24 OD021322 awarded to I.I.S., T.G., and M.R.R. Additional support was provided by the Office of Research Infrastructure Programs/ OD via grant P51 OD011106 awarded to the WNPRC at the University of Wisconsin-Madison, and by St. Baldrick's-Stand up to Cancer Dream Team Translational Research Grant SU2C-AACR-DT-27-17 to C.M.C and P.H., NIH/National Cancer Institute (NCI) Grant K08 CA174750 and NIH/NCI Grant R01 CA215461 to C.M.C.

References:

1. Dickinson AM, Norden J, Li S, Hromadnikova I, Schmid C, Schmetzer H et al. **Graft-versus-Leukemia Effect Following Hematopoietic Stem Cell Transplantation for Leukemia.** *Front Immunol.* 2017;**8**:496.
2. Falkenburg JH, Warren EH. **Graft versus leukemia reactivity after allogeneic stem cell transplantation.** *Biol Blood Marrow Transplant.* 2011;**17**:S33-S38.
3. Warren EH, Fujii N, Akatsuka Y, Chaney CN, Mito JK, Loeb KR et al. **Therapy of relapsed leukemia after allogeneic hematopoietic cell transplantation with T cells specific for minor histocompatibility antigens.** *Blood.* 2010;**115**:3869-3878.
4. Cummins NW, Rizza S, Litzow MR, Hua S, Lee GQ, Einkauf K et al. **Extensive virologic and immunologic characterization in an HIV-infected individual following allogeneic stem cell transplant and analytic cessation of antiretroviral therapy: A case study.** *PLoS Med.* 2017;**14**:e1002461.
5. Gupta RK, Abdul-Jawad S, McCoy LE, Mok HP, Peppas D, Salgado M et al. **HIV-1 remission following CCR5Δ32/Δ32 haematopoietic stem-cell transplantation.** *Nature.* 2019
6. Gupta RK, Peppas D, Hill AL, Gálvez C, Salgado M, Pace M et al. **Evidence for HIV-1 cure after CCR5Δ32/Δ32 allogeneic haemopoietic stem-cell transplantation 30 months post analytical treatment interruption: a case report.** *Lancet HIV.* 2020
7. Henrich TJ, Hu Z, Li JZ, Sciaranghella G, Busch MP, Keating SM et al. **Long-term reduction in peripheral blood HIV type 1 reservoirs following reduced-intensity conditioning allogeneic stem cell transplantation.** *J Infect Dis.* 2013;**207**:1694-1702.
8. Hutter G, Nowak D, Mossner M, Ganepola S, Mussig A, Allers K et al. **Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation.** *N Engl J Med.* 2009;**360**:692-698.
9. Koelsch KK, Rasmussen TA, Hey-Nguyen WJ, Pearson C, Xu Y, Bailey M et al. **Impact of Allogeneic Hematopoietic Stem Cell Transplantation on the HIV Reservoir and Immune Response in 3 HIV-Infected Individuals.** *J Acquir Immune Defic Syndr.* 2017;**75**:328-337.
10. Prator CA, Donatelli J, Henrich TJ. **From Berlin to London: HIV-1 Reservoir Reduction Following Stem Cell Transplantation.** *Curr HIV/AIDS Rep.* 2020;**17**:385-393.
11. Smiley ST, Singh A, Read SW, Sharma OK, Finzi D, Lane C et al. **Progress toward curing HIV infections with hematopoietic stem cell transplantation.** *Clin Infect Dis.* 2015;**60**:292-297.
12. Hutter G. **Stem cell transplantation in strategies for curing HIV/AIDS.** *AIDS Res Ther.* 2016;**13**:31.
13. Allers K, Hutter G, Hofmann J, Loddenkemper C, Rieger K, Thiel E et al. **Evidence for the cure of HIV infection by CCR5Delta32/Delta32 stem cell transplantation.** *Blood.* 2011;**117**:2791-2799.
14. Symons J, Vandekerckhove L, Hütter G, Wensing AM, van Ham PM, Deeks SG et al. **Dependence on the CCR5 coreceptor for viral replication explains the lack of rebound of CXCR4-predicted HIV variants in the Berlin patient.** *Clin Infect Dis.* 2014;**59**:596-600.

15. Ribeiro RM, Hazenberg MD, Perelson AS, Davenport MP. **Naïve and memory cell turnover as drivers of CCR5-to-CXCR4 tropism switch in human immunodeficiency virus type 1: implications for therapy.** *J Virol.* 2006;**80**:802-809.
16. Kordelas L, Verheyen J, Beelen DW, Horn PA, Heinold A, Kaiser R et al. **Shift of HIV tropism in stem-cell transplantation with CCR5 Delta32 mutation.** *N Engl J Med.* 2014;**371**:880-882.
17. Verheyen J, Thielen A, Lübke N, Dirks M, Widera M, Dittmer U et al. **Rapid Rebound of a Preexisting CXCR4-tropic Human Immunodeficiency Virus Variant After Allogeneic Transplantation With CCR5 Δ32 Homozygous Stem Cells.** *Clin Infect Dis.* 2019;**68**:684-687.
18. Hill AL, Rosenbloom DI, Goldstein E, Hanhauser E, Kuritzkes DR, Siliciano RF et al. **Real-Time Predictions of Reservoir Size and Rebound Time during Antiretroviral Therapy Interruption Trials for HIV.** *PLoS Pathog.* 2016;**12**:e1005535.
19. Cillo AR, Krishnan S, McMahon DK, Mitsuyasu RT, Para MF, Mellors JW. **Impact of chemotherapy for HIV-1 related lymphoma on residual viremia and cellular HIV-1 DNA in patients on suppressive antiretroviral therapy.** *PLoS One.* 2014;**9**:e92118.
20. Gabarre J, Marcelin AG, Azar N, Choquet S, Lévy V, Lévy Y et al. **High-dose therapy plus autologous hematopoietic stem cell transplantation for human immunodeficiency virus (HIV)-related lymphoma: results and impact on HIV disease.** *Haematologica.* 2004;**89**:1100-1108.
21. Henrich TJ, Hobbs KS, Hanhauser E, Scully E, Hogan LE, Robles YP et al. **Human Immunodeficiency Virus Type 1 Persistence Following Systemic Chemotherapy for Malignancy.** *J Infect Dis.* 2017;**216**:254-262.
22. Mavigner M, Watkins B, Lawson B, Lee ST, Chahroudi A, Kean L et al. **Persistence of virus reservoirs in ART-treated SHIV-infected rhesus macaques after autologous hematopoietic stem cell transplant.** *PLoS Pathog.* 2014;**10**:e1004406.
23. Simonelli C, Zanussi S, Pratesi C, Rupolo M, Talamini R, Caffau C et al. **Immune recovery after autologous stem cell transplantation is not different for HIV-infected versus HIV-uninfected patients with relapsed or refractory lymphoma.** *Clin Infect Dis.* 2010;**50**:1672-1679.
24. Henrich TJ, Hanhauser E, Marty FM, Sirignano MN, Keating S, Lee TH et al. **Antiretroviral-free HIV-1 remission and viral rebound after allogeneic stem cell transplantation: report of 2 cases.** *Ann Intern Med.* 2014;**161**:319-327.
25. Serrano D, Miralles P, Balsalobre P, Kwon M, Rodriguez-Macias G, Gayoso J et al. **Graft-versus-tumor effect after allogeneic stem cell transplantation in HIV-positive patients with high-risk hematologic malignancies.** *AIDS Res Hum Retroviruses.* 2013;**29**:1340-1345.
26. Griffioen M, van Bergen CA, Falkenburg JH. **Autosomal Minor Histocompatibility Antigens: How Genetic Variants Create Diversity in Immune Targets.** *Front Immunol.* 2016;**7**:100.
27. Warren EH, Deeg HJ. **Dissecting graft-versus-leukemia from graft-versus-host-disease using novel strategies.** *Tissue Antigens.* 2013;**81**:183-193.
28. Salgado M, Kwon M, Gálvez C, Badiola J, Nijhuis M, Bandera A et al. **Mechanisms that contribute to a profound reduction of the HIV-1 reservoir after allogeneic stem cell transplant.** *Ann Intern Med.* 2018;**169**:674-683.
29. Lawler SH, Sussman RW, Taylor LL. **Mitochondrial DNA of the Mauritian macaques (*Macaca fascicularis*): an example of the founder effect.** *Am J Phys Anthropol.* 1995;**96**:133-141.
30. Budde ML, Wiseman RW, Karl JA, Hanczaruk B, Simen BB, O'Connor DH. **Characterization of Mauritian cynomolgus macaque major histocompatibility complex class I haplotypes by high-resolution pyrosequencing.** *Immunogenetics.* 2010;**62**:773-780.
31. O'Connor SL, Blasky AJ, Pendley CJ, Becker EA, Wiseman RW, Karl JA et al. **Comprehensive characterization of MHC class II haplotypes in Mauritian cynomolgus macaques.** *Immunogenetics.* 2007;**59**:449-462.
32. Wiseman RW, Wojcechowskyj JA, Greene JM, Blasky AJ, Goon T, Soma T et al. **Simian immunodeficiency virus SIVmac239 infection of major histocompatibility complex-identical cynomolgus macaques from Mauritius.** *J Virol.* 2007;**81**:349-361.
33. D'Souza SS, Bennett S, Kumar A, Kelnhofer LE, Weinfurter J, Suknuntha K et al. **Transplantation of T-cell receptor α/β-depleted allogeneic bone marrow in nonhuman primates.** *Exp Hematol.* 2021;**93**:44-51.

34. Harouse JM, Gettie A, Eshetu T, Tan RC, Bohm R, Blanchard J et al. **Mucosal transmission and induction of simian AIDS by CCR5-specific simian/human immunodeficiency virus SHIV(SF162P3).** *J Virol.* 2001;**75**:1990-1995.
35. Cline AN, Bess JW, Piatak M, Jr., Lifson JD. **Highly sensitive SIV plasma viral load assay: practical considerations, realistic performance expectations, and application to reverse engineering of vaccines for AIDS.** *J Med Primatol.* 2005;**34**:303-312.
36. Friedrich TC, Valentine LE, Yant LJ, Rakasz EG, Piaskowski SM, Furlott JR et al. **Subdominant CD8+ T-cell responses are involved in durable control of AIDS virus replication.** *J Virol.* 2007;**81**:3465-3476.
37. Lewis MG, Norelli S, Collins M, Barreca ML, Iraci N, Chirullo B et al. **Response of a simian immunodeficiency virus (SIVmac251) to raltegravir: a basis for a new treatment for simian AIDS and an animal model for studying lentiviral persistence during antiretroviral therapy.** *Retrovirology.* 2010;**7**:21.
38. Miller WP, Srinivasan S, Panoskaltsis-Mortari A, Singh K, Sen S, Hamby K et al. **GVHD after haploidentical transplantation: a novel, MHC-defined rhesus macaque model identifies CD28- CD8+ T cells as a reservoir of breakthrough T-cell proliferation during costimulation blockade and sirolimus-based immunosuppression.** *Blood.* 2010;**116**:5403-5418.
39. Maufort JP, Israel JS, Brown ME, Kempton SJ, Albano NJ, Zeng W et al. **Major Histocompatibility Complex-Matched Arteries Have Similar Patency to Autologous Arteries in a Mauritian Cynomolgus Macaque Major Histocompatibility Complex-Defined Transplant Model.** *J Am Heart Assoc.* 2019;**8**:e012135.
40. Reynolds MR, Rakasz E, Skinner PJ, White C, Abel K, Ma ZM et al. **CD8+ T-lymphocyte response to major immunodominant epitopes after vaginal exposure to simian immunodeficiency virus: too late and too little.** *J Virol.* 2005;**79**:9228-9235.
41. Gama L, Abreu CM, Shirk EN, Price SL, Li M, Laird GM et al. **Reactivation of simian immunodeficiency virus reservoirs in the brain of virally suppressed macaques.** *AIDS.* 2017;**31**:5-14.
42. Dyavar SR, Ye Z, Byrareddy SN, Scarsi KK, Winchester LC, Weinhold JA et al. **Normalization of cell associated antiretroviral drug concentrations with a novel RPP30 droplet digital PCR assay.** *Sci Rep.* 2018;**8**:3626.
43. Cadena AM, Ventura JD, Abbink P, Borducchi EN, Tuyishime H, Mercado NB et al. **Persistence of viral RNA in lymph nodes in ART-suppressed SIV/SHIV-infected Rhesus Macaques.** *Nat Commun.* 2021;**12**:1474.
44. Wong JK, Yukl SA. **Tissue reservoirs of HIV.** *Curr Opin HIV AIDS.* 2016;**11**:362-370.
45. Colonna L, Peterson CW, Schell JB, Carlson JM, Tkachev V, Brown M et al. **Evidence for persistence of the SHIV reservoir early after MHC haploidentical hematopoietic stem cell transplantation.** *Nat Commun.* 2018;**9**:4438.
46. Burwitz BJ, Wu HL, Abdulhaqq S, Shriver-Munsch C, Swanson T, Legasse AW et al. **Allogeneic stem cell transplantation in fully MHC-matched Mauritian cynomolgus macaques recapitulates diverse human clinical outcomes.** *Nat Commun.* 2017;**8**:1418.
47. Durand CM, Capoferri AA, Redd AD, Zahurak M, Rosenbloom DIS, Cash A et al. **Allogeneic bone marrow transplantation with post-transplant cyclophosphamide for patients with HIV and haematological malignancies: a feasibility study.** *Lancet HIV.* 2020;**7**:e602-e610.
48. Ward AR, Mota TM, Jones RB. **Immunological approaches to HIV cure.** *Semin Immunol.* 2020;101412.
49. Zhou Y, Maldini CR, Jadowsky J, Riley JL. **Challenges and Opportunities of Using Adoptive T-Cell Therapy as Part of an HIV Cure Strategy.** *J Infect Dis.* 2021;**223**:38-45.
50. Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, Arcese W et al. **Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients.** *Blood.* 1995;**86**:2041-2050.
51. Frey NV, Porter DL. **Graft-versus-host disease after donor leukocyte infusions: presentation and management.** *Best Pract Res Clin Haematol.* 2008;**21**:205-222.
52. Mylvaganam G, Yanez AG, Maus M, Walker BD. **Toward T Cell-Mediated Control or Elimination of HIV Reservoirs: Lessons From Cancer Immunology.** *Front Immunol.* 2019;**10**:2109.

53. Rust BJ, Kiem HP, Uldrick TS. **CAR T-cell therapy for cancer and HIV through novel approaches to HIV-associated haematological malignancies.** *Lancet Haematol.* 2020;**7**:e690-e696.
54. Yang H, Wallace Z, Dorrell L. **Therapeutic Targeting of HIV Reservoirs: How to Give T Cells a New Direction.** *Front Immunol.* 2018;**9**:2861.
55. Mu W, Carrillo MA, Kitchen SG. **Engineering CAR T Cells to Target the HIV Reservoir.** *Front Cell Infect Microbiol.* 2020;**10**:410.
56. Deeks SG, Wagner B, Anton PA, Mitsuyasu RT, Scadden DT, Huang C et al. **A phase II randomized study of HIV-specific T-cell gene therapy in subjects with undetectable plasma viremia on combination antiretroviral therapy.** *Mol Ther.* 2002;**5**:788-797.
57. Mitsuyasu RT, Anton PA, Deeks SG, Scadden DT, Connick E, Downs MT et al. **Prolonged survival and tissue trafficking following adoptive transfer of CD4zeta gene-modified autologous CD4(+) and CD8(+) T cells in human immunodeficiency virus-infected subjects.** *Blood.* 2000;**96**:785-793.
58. Scholler J, Brady TL, Binder-Scholl G, Hwang WT, Plesa G, Hege KM et al. **Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells.** *Sci Transl Med.* 2012;**4**:132ra53.
59. Walker RE, Bechtel CM, Natarajan V, Baseler M, Hege KM, Metcalf JA et al. **Long-term in vivo survival of receptor-modified syngeneic T cells in patients with human immunodeficiency virus infection.** *Blood.* 2000;**96**:467-474.
60. Summers C, Sheth V, Bleakley M. **Minor histocompatibility antigen-specific T cells.** *Frontiers in Pediatrics.* 2020
61. Weinfurter JT, Graham ME, Ericson AJ, Matschke LM, Llewellyn-Lacey S, Price DA et al. **Identifying a Minor Histocompatibility Antigen in Mauritian Cynomolgus Macaques Encoded by APOBEC3C.** *Front Immunol.* 2020;**11**:586251.

Figure 1. Study overview. Four Mauritian cynomolgus macaques (MCMs) were infected intravenously with SHIV162P3. The infections progressed for between 6 and 16 weeks before starting antiretroviral drug therapy (ART). Three to four weeks later, the MCMs underwent myeloablative conditioning and received MHC-matched T-cell receptor (TCR) α/β -depleted hematopoietic stem cells (HSCs). The MCMs received GvHD prophylaxis and daily maintained ART post-transplant.

Fig. 2. Plasma viral loads after SHIV162P3 infection. Plasma viral loads were measured by qRT-PCR for the duration of the study. The gray boxes indicate the periods of ART treatment. The red arrow indicates the infusion of allo-HSCs. The dashed line represents the qRT-PCR limit of detection (100 RNA copy Eq/ml plasma).

Figure 3. Effect of allo-HSCTs on cell populations and platelets. We monitored major cell populations and platelets in the blood after allo-HSCTs. Counts are displayed as $\times 10^3/\mu\text{l}$.

Figure 4. Donor chimerism after allo-HSCT. Diagnostic SNPs were sequenced to distinguish cells of donor or recipient origin. (a) Longitudinal percent donor chimerism in the whole blood. (b) At necropsy, peripheral blood was flow sorted to determine the percent donor chimerism among myeloid cells (CD45⁻CD14⁺ lymphocytes), T cells (CD14⁻CD45⁺CD3⁺ lymphocytes), and B cells (CD14⁻CD45⁺CD20⁺).

Figure 5. SHIV DNA in allo-HSC transplanted MCMs. Copies of viral DNA were measured in the PBMC (a) and (b) peripheral lymph nodes before ART initiation, after ART but before allo-HSCTs, and post-transplant. SHIV DNA levels were also measured in the tissues of cy0906, cy0915, and cy0930 that were on ART when they died (c). Total SHIV DNA was measured by digital droplet PCR using Gag-specific primers and normalized to 10⁷ cells by measuring macaque RNaseP p30, RPP30 in companion ddPCR assays.