# Fully MHC-matched allogeneic HSCT in SIV-infected, ART-suppressed Mauritian cynomolgus macaques

Helen L. Wu[1], Benjamin J. Burwitz[1], Shaheed A. Abdulhaqq[1], Christine Shriver-Munsch[2], Tonya Swanson[2], Alfred W. Legasse[2], Katherine B. Hammond[1], Jason S. Reed[1], Mina Northrup[1], Stephanie L. Junell[4], Justin M. Greene[1], Gabriela M. Webb[1], Benjamin N. Bimber[2], Wolfram Laub[4], Paul Kievit[2], Rhonda MacAllister[2], Michael K. Axthelm[2], Rebecca Ducore[2], Anne Lewis[2], Lois Colgin[2], Theodore R. Hobbs[2], Lauren D. Martin[2], Charles R. Thomas[4], Angela Panoskaltsis-Mortari[5], Gabrielle Meyers[3], Richard T. Maziarz[3], Jeffrey J. Stanton[2], Jonah B. Sacha[1,2]

[1]Vaccine and Gene Therapy Institute, [2]Oregon National Primate Research Center, [3]Division of Hematology and Medical Oncology, [4]Division of Medical Physics, Department of Radiation Medicine, Oregon Health and Science University, Portland, Oregon, USA
[5]Division of Blood and Marrow Transplantation, University of Minnesota, Minneapolis, Minnesota, USA

## Abstract:

Timothy Brown remains in cART-free HIV remission following allogeneic hematopoietic stem cell transplant (HSCT), but attempts to recapitulate his cure have been unsuccessful. We recently established a nonhuman primate model of fully MHC-matched allogeneic HSCT to investigate the mechanism of cure in Timothy Brown. In this model, a reduced intensity conditioning (RIC) regimen consisting of chemotherapy, CD3 depletion, and total body irradiation (TBI) prior to HSCT results in durable, full multi-lineage donor chimerism in SIV-naïve HSCT recipients. Here, we sought to investigate (1) if similar results could be achieved without TBI, and (2) the impact of allogeneic HSCT on SIV reservoir size in cART-suppressed, SIV-infected recipients. Allogeneic HSCT was performed with fully MHC-matched Mauritian cynomolgus macaque (MCM) donor-recipient pairs, including two fully cART-suppressed, SIV-infected recipients. Mobilized peripheral stem cells collected from donors by leukapheresis were transplanted into recipients following RIC with or without TBI. Donor engraftment was monitored by Illumina sequencing of single nucleotide polymorphisms. Immune subset reconstitution was assessed longitudinally by flow cytometric phenotyping and complete blood counts. We performed two HSCTs without TBI, both resulting in low T cell donor chimerism (<15%). The first, SIV-naïve recipient experienced incomplete T cell rebound resulting in polyoma virus reactivation and euthanasia. The second, cART-suppressed, SIV-infected recipient stabilized post-HSCT, and experienced a reduction in lymph node-associated SIV DNA despite incomplete T cell donor chimerism. Adding back TBI for HSCT of a second cART-suppressed, SIV-infected recipient resulted in engraftment with high levels of donor chimerism in whole blood (>95%) and T cells (~80%) within 30 days of HSCT. Subsequent donor lymphocyte infusion increased blood and tissue T cell donor chimerism levels to nearly 100%, but led to development of clinical graft-versus-host disease (GVHD) and euthanasia. At necropsy, SIV DNA was below the limit of detection in lymph nodes, constituting a ~3 log reduction from pre-HSCT levels. These data demonstrate that TBI is critical to achieving high levels of T cell donor chimerism post-HSCT in MCM, and that while HSCT immune conditioning decreases the viral reservoir size, GVHD is associated with enhanced reservoir clearance. This model of fully MHC-matched allogeneic HSCT in cART-suppressed, SIV-infected MCM will facilitate future studies investigating mechanisms of HSCT-mediated HIV cure.

Project supported by NIH R21 #AI112433 and R01 #AI129703; amfAR grant #108832-55-RGRL

## Figure 1. Stable, multi-lineage donor chimerism in SIV-naive MCM after fully MHC-matched allogeneic HSCT.

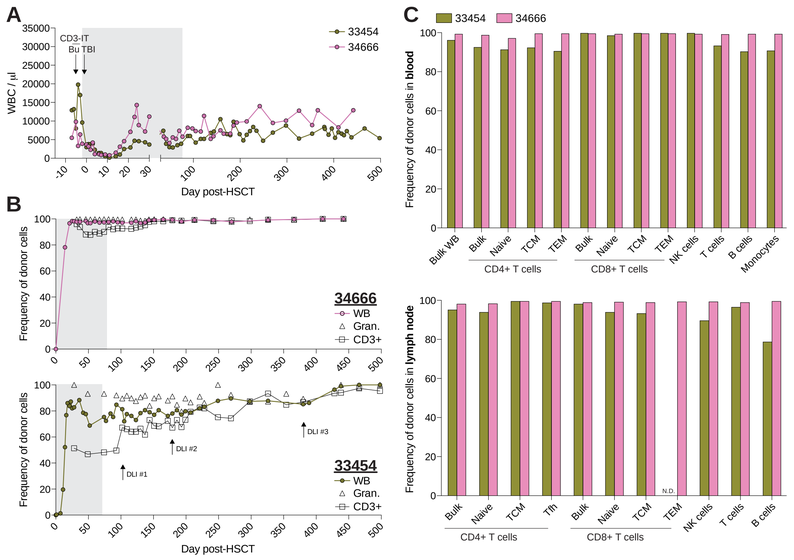



**Figure 1: (A)** Longitudinal white blood cell (WBC) absolute counts in recipient macaques 33454 and 34666. Immune conditioning regimens are indicated in top panels (Bu = busulfan, TBI = total body irradiation, CD3-IT = CD3-immunotoxin). Normal WBC reference range = 5,600-18,4000/µl; normal PLT reference range = 134,000-564,000/µl. Shaded gray boxes in (A,B) indicate tacrolimus treatment period. **(B,C)** Donor chimerism levels as measured by Illumina sequencing genomic DNA across SNPs differing between donor and recipient. Specific populations were isolated by flow cytometric cell sorting of ACK-treated whole blood prior to genomic DNA extraction. Longitudinal whole blood, granulocyte, and T cell donor chimerism levels shown in (B). Timepoints of donor lymphocyte infusions (DLIs) into 33454 are indicated with arrows in (B, bottom panel); DLI #1 = 1x107 CD3+ cells/kg, DLI #2 = 5x107 CD3+ cells/kg, DLI #3 = 5x107 CD3+ cells/kg. Comprehensive immune cell subset donor chimerism levels in whole blood (top) and lymph node (bottom) shown in (C), measured 428 days (33454) or 193 days (34666) post-HSCT. N.D. = not determined due to insufficient cell numbers.

## Figure 2. Timeline of HSCT in SIV-infected, cART-suppressed MCM.

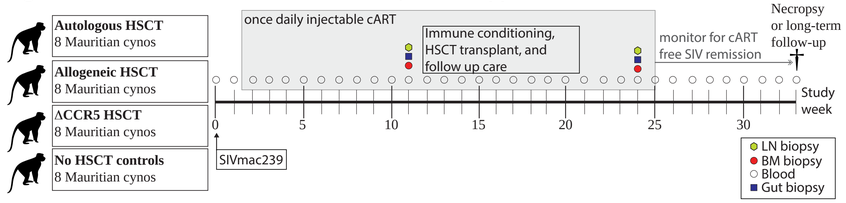



**Figure 2:** To investigate the mechanisms of HSCT-mediated HIV cure in Timothy Brown, we will conduct HSCT with three groups of MCM. Group 1 will receive myeloablative conditioning followed by autologous transplant. Group 2 will receive non-myeloablative reduced intensity conditioning followed by fully MHC-matched allogeneic transplant from an unrelated donor. Group 3 will receive fully MHC-matched allogeneic transplants from CCR5-knockout donors. A fourth group of MCM will serve as non-HSCT controls.

## Figure 3. Removing total body irradiation results in low levels of T cell donor chimerism post-HSCT: Case 1.

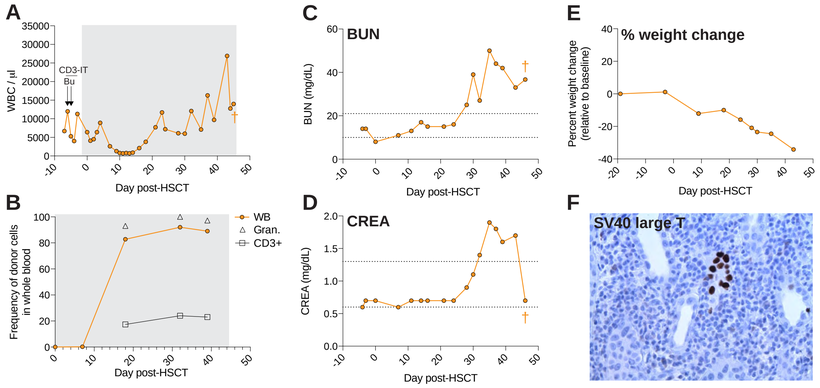



**Figure 3: (A)** Longitudinal white blood cell (WBC) absolute counts in recipient macaque 33460. Immune conditioning regimens are indicated in top panels (Bu = busulfan, CD3-IT = CD3-immunotoxin). **(B)** Donor chimerism levels as measured by Illumina sequencing genomic DNA across SNPs differing between donor and recipient. Specific populations were isolated by flow cytometric cell sorting of ACK-treated whole blood prior to genomic DNA extraction. Longitudinal whole blood, granulocyte, and T cell donor chimerism levels shown in (B). **(C)** Serum blood urea nitrogen levels, **(D)** Serum creatinine levels, and **(E)** Weight change in recipient macaque 33460. **(F)** Staining for SV40 large T antigen in renal tubule epithelial cell at time of necropsy. Recipient macaque 33460 developed the following symptoms post-HSCT: blood in urine, bladder inflammation, inappetence, weight loss, and lethargy.

## Figure 4. Removing total body irradiation results in low levels of T cell donor chimerism post-HSCT: Case 2.

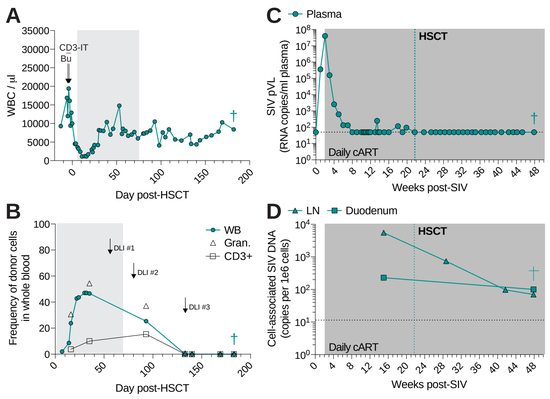



**Figure 4: (A)** Longitudinal white blood cell (WBC) absolute counts in recipient macaque 33461. Immune conditioning regimens are indicated in top panels (Bu = busulfan, CD3-IT = CD3-immunotoxin). **(B)** Donor chimerism levels as measured by Illumina sequencing genomic DNA across SNPs differing between donor and recipient. Specific populations were isolated by flow cytometric cell sorting of ACK-treated whole blood prior to genomic DNA extraction. Longitudinal whole blood, granulocyte, and T cell donor chimerism levels shown in (B). **(C)** Longitudinal SIV plasma viral loads. **(D)** Cell-associated SIV DNA levels pre- and post-HSCT.

## Figure 5. Adding back total body irradiation results in high levels of T cell donor chimerism post-HSCT. Development of GVHD associated with enhanced SIV reservoir clearance.

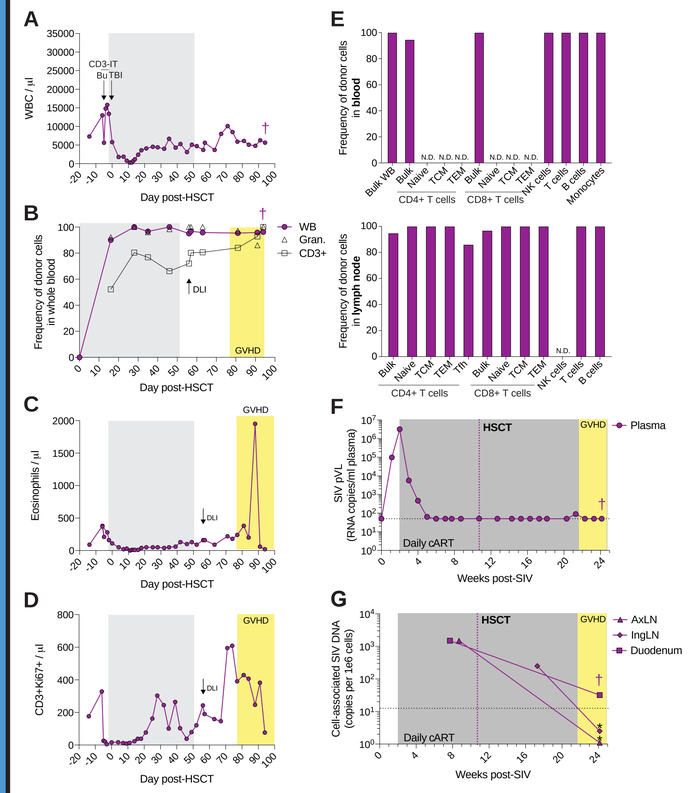



**Figure 5: (A)** Longitudinal white blood cell (WBC) absolute counts in recipient macaque 35133. Immune conditioning regimens are indicated in top panels (Bu = busulfan, TBI = total body irradiation, CD3-IT = CD3-immunotoxin). **(B, E)** Donor chimerism levels as measured by Illumina sequencing genomic DNA across SNPs differing between donor and recipient. Specific populations were isolated by flow cytometric cell sorting of ACK-treated whole blood prior to genomic DNA extraction. Longitudinal whole blood, granulocyte, and T cell donor chimerism levels shown in (B). Timepoints of donor lymphocyte infusions (DLIs) into 35133 are indicated with arrows in (B); DLI = 1x107 CD3+ cells/kg. Comprehensive immune cell subset donor chimerism levels in whole blood (top) and lymph node (bottom) shown in (E), measured 94 days post-HSCT. N.D. = not determined due to insufficient sample/cell numbers. **(C)** Eosinophil, and **(D)** Ki67+ T cell counts in recipient macaque 35133. **(F)** Longitudinal SIV plasma viral loads. **(G)** Cell-associated SIV DNA levels pre- and post-HSCT. Post-DLI, recipient macaque 35133 developed skin rash with histological hallmarks of GVHD and subsequently diarrhea. Asterisks (*) indicate only one replicate positive.

## Conclusions:

1. Total body irradiation is critical to achieving high levels of T cell donor chimerism post-HSCT.

2. Reduced-intensity HSCT immune conditioning decreases the SIV reservoir size, but GVHD is associated with enhanced reservoir clearance, suggesting graft-versus-host immunity can serve as a reservoir clearance mechanism.

This model of fully MHC-matched allogeneic HSCT in SIV-infected, cART-suppressed MCMs will facilitate studies investigating mechanisms of HSCT-mediated HIV cure:

- **Myeloablative conditioning**
- **Graft-versus-host (graft-versus-HIV) immunity**
- **CCR5Δ32/Δ32 graft**