Thresholds for post-rebound SHIV control after CCR5 gene-edited autologous hematopoietic cell transplantation

Short title: SHIV remission after ΔCCR5 HSPC transplantation

E. Fabian Cardozo¹, Elizabeth R. Duke¹,³, Christopher W. Peterson²,³, Daniel B. Reeves¹, Bryan T Mayer¹, Hans-Peter Kiem²,³,⁴, Joshua T. Schiffer¹,²,³,*

¹Vaccine and Infectious Disease Division,
²Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA;
³Department of Medicine and
⁴Department of Pathology, University of Washington, Seattle, WA, USA.

*Corresponding Author. Address: 1100 Fairview Ave. N. MS E4-100, Seattle, WA, 98119;
email: jschiffe@fredhutch.org; phone: (206)667-7359.

Text word count: 3896.
Abstract word count: 246.
Number of figures: 7.
Number of tables: 1.
Key Points

- Data-validated modeling suggest that loss of immune response after transplantation produces more depletion of CD4$^+$CCR5$^+$ T cells post-ATI.
- The minimum fraction of transplanted gene-edited cells for viral control linearly relates with the CD4$^+$CCR5$^+$ T cell nadir 10 weeks post-ATI.
Abstract (246/250 words)

The two recent cases of HIV cure/stable remission following allogeneic stem cell transplantation are difficult to reproduce because of the toxicity of the procedure and rarity of donors homozygous for the CCR5Δ32 deletion. One approach to overcome these barriers is the use of autologous, CCR5 gene-edited hematopoietic stem and progenitor cell (HSPC) products. Unlike allogeneic transplantation, in which the frequency of CCR5Δ32 donor cells approaches 100%, the CCR5 gene can currently only be edited ex vivo in a fraction of autologous HSPCs. Therefore, we sought to determine the minimum fraction required for viral control using mathematical modeling. We analyzed data from eight juvenile pigtail macaques infected intravenously with SHIV-1157ipd3N4, treated with combination antiretroviral therapy (cART), and infused with autologous HSPCs without CCR5 gene editing. We developed a mathematical model that simultaneously describes reconstitution of CD4⁺CCR5⁺, CD4⁺CCR5⁻, and CD8⁺ T cell counts, as well as SHIV plasma viral loads in control and transplanted macaques. The model predicts that transplantation decreases the immunologic response to SHIV to varying degrees in macaques. By modifying the model to hypothetically describe transplantation with a given fraction of protected CCR5-edited cells we found that loss of immunologic response correlated with a more profound depletion of CCR5⁺CD4⁺ T cells and a higher fractions of gene-edited cells required for cART-free viral remission. Our results provide a framework to predict the likelihood of post-rebound control in vivo using the percentage of CCR5-edited cells in peripheral blood and the loss of HIV-specific immunity following autologous HSPC.
INTRODUCTION

The major obstacle to HIV-1 eradication is a latent reservoir of long-lived infected cells. Cure strategies aim to eliminate all infected cells or permanently prevent viral reactivation from latency. The only known case of HIV cure and an additional, recently-reported case of prolonged remission, resulted from allogeneic hematopoietic stem cell transplant with homozygous CCR5Δ32 donor cells. The success of this procedure is likely multifactorial—in part attributable to HIV resistance of the transplant product, the conditioning regimen that facilitates engraftment and also eliminates infected cells, graft-versus-host effect, and immunosuppressive therapies for graft-versus-host disease.

A current research focus is to recapitulate this method of cure with minimal toxicity. One method is to perform autologous transplantation following ex vivo inactivation of the CCR5 gene with gene editing nucleases, eliminating the need for allogeneic CCR5-negative donors. While this procedure is safe and feasible in pigtail macaques infected with simian-human immunodeficiency virus (SHIV), only a fraction of HSPCs can be genetically modified ex vivo to be HIV-resistant.

To address this challenge, we developed a mathematical model to predict the minimum threshold of persisting, gene-modified cells necessary for functional cure. First, we modeled the kinetics of CD4+CCR5+, CD4+ CCR5−, and CD8+ T cell reconstitution after autologous transplantation. We then modeled SHIV rebound kinetics following analytical treatment interruption (ATI) and identified the degree of loss of anti-HIV cytolytic immunity following transplantation. Finally, we projected the proportion of gene-modified cells and the levels of SHIV-specific immunity required to eliminate viral replication following ATI.
METHODS

Experimental Data

Eight juvenile pigtail macaques were intravenously challenged with 9500 TCID50 SHIV-1157ipd3N4 (SHIV-C)\textsuperscript{14,17}. After 6 months, the macaques received combination antiretroviral therapy (cART: tenofovir [PMPA], emtricitabine [FTC], and raltegravir [RAL]). After \textasciitilde30 weeks on cART, four animals received total body irradiation (TBI) followed by transplantation of autologous HSPCs. After an additional 25 weeks following transplant, when viral load was fully suppressed, animals underwent analytical treatment interruption (ATI) of cART\textsuperscript{14}. A control group of four animals did not receive TBI or HSPC transplantation and underwent ATI after \textasciitilde50 weeks of treatment (Fig. 1A). Plasma viral loads and absolute quantified CD4\textsuperscript{+}CCR5\textsuperscript{−}, CD4\textsuperscript{+}CCR5\textsuperscript{+} and CD8\textsuperscript{+} total and subsets (naïve, central memory [TCM], and effector memory [TEM]) T cell counts from peripheral blood were measured as described previously\textsuperscript{14,17}. We analyzed peripheral T cell counts and plasma viral load from transplant until 43 weeks post-transplant (\textasciitilde25 weeks pre-ATI and \textasciitilde20 weeks post-ATI).

Mathematical modeling

We employed several series of ordinary differential equation models of cellular and viral dynamics after transplantation (Fig. 1B). First, we modeled T cell dynamics and reconstitution following transplant and before ATI, assuming that low viral loads on ART do not affect cell dynamics (Fig. 1C). After curation of that model, we introduced viral dynamics and fit those to the T cell and viral rebound dynamics from the animals pre- and post-ATI (Fig. 1D). Lastly, we applied our complete model to a transplant scenario with gene editing of CCR5 to predict the minimal threshold of editing for functional HIV cure (Fig. 1E).
We modeled the kinetics of CD4+ and CD8+ T cell subsets in blood, transplanted cells that home to the BM, and progenitor cells in the BM/thymus as shown in Fig. 1C. We included CD8+ T cells in the model because CD8+ and CD4+ T cells may arise from new naïve cells from the thymus and compete for resources that impact clonal expansion and cell survival. At the moment of HSPC infusion, transplanted animals are lymphopenic due to TBI. The control group did not have a transplanted-cell compartment, and all other compartments remained in steady state. We assumed that CD4+ and CD8+ T cell expansion may have two possible drivers: (1) lymphopenia-induced proliferation of mature cells that persist through myeloablative TBI, and (2) differentiation from naïve cells from progenitors in the thymus (from transplanted CD34+ HSPCs or CD34+ HSPCs that persist following TBI) and further differentiation to an activated effector state. We assumed that in a lymphopenic environment, factors that drive T cell proliferation are more accessible (i.e., self-MHC molecules on antigen-presenting cells and γ-chain cytokines such as IL-7 and IL-15). However, as they grow, cells compete for access to these resources, limiting clonal expansion such that logistic growth models are appropriate. We assume that new peripheral CD4+ and CD8+ T naïve cells come from a progenitor compartment in the BM/Thymus. For CD4+ T cells, we assume that naïve cells do not express CCR5, and subsequently up- and/or down-regulate expression of the CCR5 receptor. For CD8+ T cells, we included a single CD8+ memory precursor compartment of TN and TCM cells that differentiate linearly into TEM during lymphopenia. The details of the model are presented in the Supp. Material and in Fig. 1C with the notation described in Table 1. A parsimonious, curated version
of this model was selected from a series of models with varying mechanistic and statistical complexity as presented in the Supp. Materials.

T cell and viral dynamics: We next adapted the curated T cell reconstitution model by combining several adaptations of the canonical model of viral dynamics\(^4\)\(^{-5}\) as shown in Fig. 1D. The model assumes that SHIV infects only CD4\(^+\)CCR5\(^+\) T cells\(^1\)\(^\text{\cite{17}}\) and that a small fraction (~ 5\%) of those infected cells are able to produce infectious virus\(^5\)\(^1\),\(^5\)\(^4\),\(^5\)\(^5\). We modeled cART by reducing the infection rate to zero and modeled ATI by assuming infection is greater than zero after some time \(\Delta t\) after interruption. This model assumes that productively infected cells arise also from activation of a steady set of latently infected cells. The presence of both unproductively and productively infected cells leads to the expansion of CD8\(^+\) T\(_{\text{naive}}\) and T\(_{\text{CM}}\) cells, from which the majority of dividing cells differentiate into SHIV-specific effector cells\(^3\)\(^0\),\(^4\)\(^6\),\(^4\)\(^7\),\(^5\)\(^2\),\(^5\)\(^3\). The details of the model are presented in the Supp. Material and in Fig. 1D with the notation described in Table 1. A parsimonious version of this model was selected from a series of models with varying mechanistic and statistical complexity as presented in the Supp. Materials.

Viral and T cell dynamics in the setting of ΔCCR5 HSPC transplantation: We next adapted our full model to simulate scenarios in which autologous transplantation includes cells that are CCR5-edited (Fig 1E). We added variables representing CCR5-edited HSPCs in different compartments: (1) infused HSPCs in blood, (2) T cell progenitors in BM/thymus, and (3) CD4\(^+\)CCR5\(^-\) T cells in blood. These compartments have the same structure as CCR5-non-edited cells but with two differences. First, the value of gene-edited HSPCs at transplantation is a
fraction $f_p$ of the total number of infused cells. Second, mature, CCR5-edited CD4$^+$CCR5$^-$ cells do not upregulate CCR5 (see full model in Supp. Materials).

Fitting procedure and model selection

To fit our models (Fig. 1C-D) to the transplant data, we used a nonlinear mixed-effects modeling approach$^{56}$ described in detail in the Supp. materials. Briefly, we estimated the population mean and variance for each model parameter using the Stochastic Approximation Expectation Maximization (SAEM) algorithm embedded in the Monolix software (www.lixoft.eu). For a subset of parameters, random effects were specified, and those variances were estimated. Measurement error variance was also estimated assuming an additive error model for the logged outcome variables.

We first fit instances of models with varying statistical and parameter complexity in Fig. 1C to blood T cell counts during transplant and before ATI assuming that one or multiple mechanisms are absent, or that certain mechanisms have equal kinetics (Table S1 includes all 19 competing models). Then, we fit several instances of the model Fig. 1D to blood T cell counts and plasma viral load during the period after transplant including ATI using the best competing model (solid lines) for the model in Fig. 1C (Table S2 includes all 15 competing models including viral dynamics). To determine the best and most parsimonious model among the instances, we computed the log-likelihood ($\log L$) and the Akaike Information Criteria (AIC=$-2\log L+2m$, where $m$ is the number of parameters estimated)$^{57}$. We assumed a model has similar support from the data if the difference between its AIC and the best model (lowest) AIC is less than two$^{57}$ (see Supp. materials for details).
Simulations for each animal were computed using individual-level parameter estimates generated from the predicted random effects of the fitted population model.

**Data sharing statement.** Original data will be shared upon request.

**RESULTS**

**CD4⁺CCR5⁺ and CD8⁺ T cells recover more rapidly than CD4⁺CCR5⁻ T cells after HSPC transplantation.** We analyzed the kinetics of peripheral blood CD4⁺CCR5⁺ and CD4⁺CCR5⁻ T-cells, and total, T naïve, T CM, and T EM CD8⁺T-cells in macaques after HSPC transplantation. In controls, levels of CD4⁺ and CD8⁺ T cells oscillated around a persistent set point (Fig. S1). CD4⁺ CCR5⁺ T cell levels were ~100 cells/μl and were uniformly lower than the CD4⁺CCR5⁻ T cell counts (~1200 cells/μl) (p=0.01, paired t-test of the averaged post-transplant measures, Fig. 2A).

Total CD8⁺ T cell levels in the control group were ~1400 cells/μl with a greater contribution from T EM (73%) than T N+T CM (27%) (based on median values, Fig. 2B).

In the transplant group, CD4⁺ and CD8⁺ T cells expanded at different rates from the remaining levels post-TBI (Fig. 2C-D). The levels of CD4⁺CCR5⁺ T cells started at 1-10 cells/μl and reconstituted to levels similar to the control group over 5-10 weeks (Fig. 2D). After TBI, CD4⁺CCR5⁻ T cells remained at higher levels (~100 cells/μl) than CD4⁺CCR5⁺ T cells but expanded more slowly and did not reach the values of the control group after 25 weeks (Figs. 2C-D). The CD4⁺CCR5⁺ T cell compartment expanded 8-fold more rapidly than the CD4⁺CCR5⁻ compartment (p=0.01, paired t-test, Figs. 2C-D). CD8⁺ T cells decreased to levels between 10 and 100 cells/μl after TBI but recovered to levels below the control group in 5 weeks (Figs. 2C-D); CD8⁺ T cells recovered as rapidly as the CD4⁺CCR5⁺ population (Figs. 2C-D).
Overall, these results show that after transplantation there is a faster reconstitution of CD4^+CCR5^+ and CD8^+ T cells compared to CD4^+CCR5^- cells, suggesting each cell subset may have different mechanisms that drive their expansion. To explore these mechanisms, we analyzed the data in the context of mechanistic mathematical models of cell dynamics.

**Lymphopenia-induced proliferation drives early CD4^+CCR5^+ and CD8^+ T cell reconstitution after HPSC transplantation.** To identify the main drivers of T cell reconstitution after transplant, we developed a mathematical model that considered plausible mechanisms underlying reconstitution of distinct T cell subsets following autologous transplantation (Fig. 1C). We built 19 versions of the model by assuming that one or multiple mechanisms are absent, or by assuming certain mechanisms have equivalent or differing kinetics (Table S1). Using model selection theory based on AIC, we identified the model in Fig. 1C without the dashed lines which most parsimoniously reproduces the data (Table S1). The best fits of this model are presented in Fig. 2D and Fig. S1 with the respective parameter estimates in Tables S3-S4. The main features of this model are: (1) CD4^+CCR5^+ T cell reconstitution after transplant is driven by proliferation and upregulation of CCR5; (2) CD4^+CCR5^- T cell expansion is driven only by new naïve cells from the thymus and not proliferation; and (3), thymic export rates are equal between CD4^+ and CD8^+ naïve T cells.

The best fit model predicts that CD4^+CCR5^- T cells have a delayed reconstitution that occurs only when cells from the thymus (estimated with rate ~0.01/day) outnumber their loss due to death, trafficking to tissues, or upregulation of CCR5. Furthermore, the estimated CD4^+CCR5^+ T cell proliferation rate (~0.1/day) far exceeds the estimated CCR5 upregulation (~0.004/day) and thymic export rates (~0.01/day). Therefore, one month after transplantation, the
total concentration of CD4⁺CCR5⁺ T new cells generated by proliferation is predicted to be 40-fold higher than the concentration generated by up-regulation of CCR5 (Fig. 3).

Our model predicts that CD8⁺ T cells follow a similar pattern to CD4⁺CCR5⁺ T cells (Fig. 2D), as the CD8⁺ T EM proliferation rate is up to 10-fold higher than the CD8⁺ T cell differentiation rate (Fig. S2). Overall, these results suggest that following autologous HSPC transplant: (1) slow thymic export is the main driver of CD4⁺CCR5⁻ T cell growth, and (2) rapid lymphopenia-induced proliferation of remaining cells (rather than transplanted cells) after TBI is the main driver for CD4⁺CCR5⁺ and CD8⁺ T cell expansion.

Reduction of blood CD4⁺CCR5⁺ T cell counts correlates with plasma viral rebound after ATI in animals that underwent HSPC transplantation. We next aimed to extend previous analysis comparing plasma viral load rebound kinetics to CD4⁺CCR5⁺ and CCR5⁻ T cell subset dynamics after ATI. Fig. 4 and Fig. S3 presents the plasma viral loads and the blood CD4⁺CCR5⁺ and CD4⁺CCR5⁻ T cell kinetics before and after ATI in transplanted and control macaques respectively. Viral burden after ATI was more pronounced in the transplant group compared to the control group: median peak viral load was 10-fold higher (p=0.06, Mann-Whitney test. See Fig. 4A) and median final viral load measurements at necropsy were 2-log₁₀ higher (p=0.06, Mann-Whitney test. See Fig. 4B). CD4⁺CCR5⁺ T-cell counts decreased after ATI in the transplant group reaching a significantly lower nadir (~8-fold) than the control animals (p=0.01, Mann-Whitney test. Fig. 4C). The two animals with the largest reduction of CD4⁺CCR5⁺ T cells had the highest viral set points. There was no difference between control and transplant groups’ CD4⁺CCR5⁻ T cell nadir: both groups had an average reduction of ~200 cells/μL (Fig. 4D).
In the control group, individual plasma viral loads did not correlate with corresponding CD4⁺CCR5⁺ T-cell counts post-ATI. However, in three animals in the transplant group, viral load observations post-ATI correlated negatively with their corresponding CD4⁺CCR5⁺ T cell counts (Fig. S4).

Overall, these results show that transplanted animals had higher viral load that correlated with the reduction of CD4⁺CCR5⁺ T cells after ATI. This suggests that transplantation might affect macaques’ immunologic response to SHIV so that the presence of the virus leads to more depletion of CD4⁺CCR5⁺ T cells. We explore this possibility by simultaneously analyzing the viral and T cell subset observations using novel mechanistic mathematical models.

**Higher viral set points and CD4⁺CCR5⁺ T-cell depletion following transplantation and ATI are due to a reduction in SHIV-specific immunity.** To understand why transplantation may have an effect on plasma viral load and CD4⁺CCR5⁺ T cell kinetics during ATI, we modified our mathematical model to include SHIV infection as described in Fig. 1D (Methods). Using model selection theory based on AIC, we found that the most parsimonious model to explain the data was the one without the dashed lines in Fig. 1D (Table S2). The best fit model simultaneously recapitulates plasma viral rebound and the kinetics of CD4⁺ CCR5⁺ and CCR5⁻ T cells in each animal as shown in Fig. 4E and Fig. S3 with corresponding estimated parameters in Table 1 and Table S5-S6. In the best fit model, SHIV-specific CD8⁺ effector cells reduce virus production in a non-cytolytic manner⁵⁸-⁶⁰, possibly by secretion of HIV-antiviral factors⁶¹-⁶⁴—not included in the model. Additionally, the model suggests that infection leads to enhanced activation of CD4⁺CCR5⁻ T cells leading to replenishment of CD4⁺CCR5⁺ T cells, explaining the concentration reduction of the CD4⁺CCR5⁻ compartment after ATI⁶⁵-⁶⁸.
From the estimated parameters, only SHIV-based CD8⁺ proliferation rate, \( \omega_8 \), correlated positively with post-ATI CD4⁺CCR5⁺ T-cell nadir and negatively with viral load set point (Fig. 5A-B). We also found that the estimated SHIV-based CD8⁺ proliferation rate was significantly lower in the transplant group, and the estimated time to viral rebound (\( \Delta_t \)) was significantly higher in the transplant group (Fig. 5C-D). The projected fraction of SHIV-specific CD8⁺ T cells in the transplant group approached zero (Fig. S5). Overall, these results suggest that a lower nadir of CD4⁺CCR5⁺ T cells and a higher viral load after ATI in transplanted animals is due to a loss of the immune response to SHIV-infected cells.

**Greater loss of immunologic control during TBI/transplant requires higher numbers of CCR5-edited HSPCs to control viral rebound after ATI.** To calculate the minimum threshold of CCR5-edited cells necessary to induce cART-independent virus suppression, we added a population of transplanted, gene-edited CCR5 HSPCs to our complete, fitted model of T cell subset and viral dynamics. We assumed that in the infused product there is a fraction \( f_p \) of HSPCs that have a biallelically-modified CCR5 gene and do not express CCR5. In the model we added state variables for protected progenitors and CD4⁺CCR5⁻ T cells that cannot become CD4⁺CCR5⁺ T cells (Fig. 1E, full model in Supp. Materials).

We simulated the model using parameter values obtained from the best fit in the previous section for each animal in the transplant group using 100 values of \( f_p \) from zero to one (0-100% CCR5-edited HSPCs). The minimal initial fraction to achieve post-rebound viral control was dependent on the underlying viral and immune dynamics of the given animal. For example, Fig. 6A depicts projections of the model using the best estimates from the fits of the model to transplanted animal Z09144 using six values of \( f_p \): an initial fraction of protected cells smaller
than or equal to 40% will not lead to post-rebound viral control after ATI, even after a year. However, when $f_p$ is 60% or greater than 80% it is possible to have a spontaneous post-rebound viral control at ~40 weeks and 10 weeks after ATI respectively. In both cases, a period of high-level viremia occurs prior to control.

The heatmaps in Fig. 6B-E show plasma viral load projections over 2 years after the start of ATI for different values of $f_p$. The model predicts that the minimum $f_p$ to maintain post-rebound control for 2 years after ATI is higher for animals with lower estimated SHIV-specific immune response rates. For the two animals in the transplant group with lower viral setpoints, the minimum $f_p$ for viral control was 35% and 19% (Fig. 6D-E). In contrast, for the other two animals, the minimum $f_p$ for viral control was 56% and 97% (Fig. 6B-C). These model projections suggest that a larger loss of immunologic control during TBI/HSPC transplant results in a higher fraction of CCR5 gene-edited cells required for control of viral rebound after ATI.

The model also predicts that for some values of $f_p$ it is possible to have two viral load stages after ATI: a temporary high viral set point in the first weeks after ATI may be followed by a delayed ART-free viral remission stage (e.g. when $f_p$ is between 60% and 70% in Fig. 6B or between 5% and 20% in Fig. 6E). Therefore, in some cases the viral load set point determined during the initial weeks after ATI might not be a sufficient surrogate to predict viral control further in the future. On the other hand, when we project the CD4+CCR5+ T cell count for the same example in Fig. 6A we find that this cell subset does not undergo a significant change between weeks 2 and 10 after ATI for different scenarios of $f_p$ (Fig. 7A). Moreover, the maximum decrease of CD4+CCR5+ T cells observed during the first 10 weeks after ATI is predicted to have a linear relationship with the minimum initial fraction of protected cells required to obtain post-rebound control after 2 years (Fig. 7B). Therefore, the maximum initial
change in CD4^+CCR5^+ T cells 10 weeks after ATI, as well as the observed experimental value for \( f_p \), might predict late viral control.

**Discussion**

Here we introduce a data-validated mathematical model that to our knowledge is the first to simultaneously recapitulate SHIV viral loads, and CD4^+ and CD8^+ T cell subset counts during HIV or SHIV infection. We systematically selected from a series of models to arrive at a set of equations that most parsimoniously explains the available data. We recapitulated (1) peripheral CD4^+ and CD8^+ T-cell subset reconstitution dynamics following transplant, and (2) T-cell dynamics and SHIV viral rebound following ATI. Before ATI, all animals suppressed plasma viral load below the limit of detection, allowing analysis of T cell reconstitution dynamics independent of virus-mediated pressure. At each step, we applied model selection theory to select the simplest set of mechanisms capable of explaining the observed data. 

The model predicts that post-rebound viral control might be possible during autologous gene-edited HSPC transplantation if therapy achieves (1) a sufficient fraction of gene-protected, autologous HSPCs, and (2) maintenance or enhancement of SHIV-specific immune responses following transplantation. Specifically, the model predicts that increasing amounts of conditioning regimen-dependent depletion of the SHIV-specific immune response leads to a higher threshold of CCR5-gene-edited cells in the transplanted HSPC product that is required to obtain stable, ART-free viral control. These results are consistent with the cure achieved by the Berlin patient who received transplant with 100% HIV-resistant cells after intense conditioning. In the autologous setting where 100% CCR5 editing may not be feasible, adjunctive measures that augment virus-specific immunity, such as therapeutic vaccination,
infusion of HIV-specific CAR T cells or use of neutralizing antibodies, may synergize with
HSPC transplantation to achieve post-treatment control\textsuperscript{11,69}.

The best model predicts that the lack of complete elimination of lymphocytes by TBI
prevents CD4\textsuperscript{+}CCR5\textsuperscript{-} cells from predominating post-transplant: the rapid expansion of
CD4\textsuperscript{+}CCR5\textsuperscript{+} and CD8\textsuperscript{+} T cells during the first few weeks after HSPC transplantation is most
likely due to lymphopenia-induced proliferation of remaining cells after TBI via a thymus-
independent pathway; the slower expansion of CD4\textsuperscript{+}CCR5\textsuperscript{-} T cells is due to thymic export of
both transplanted and remaining cells. An important future research question will be to identify
anatomic sites and mechanisms that allow activated CD4\textsuperscript{+}CCR5\textsuperscript{+} to survive conditioning.

A challenge is that more intense conditioning may decrease remaining CD4\textsuperscript{+}CCR5\textsuperscript{+} cells
but will also lower SHIV specific immunity. We previously demonstrated the link between
disruption of the immune response during transplant and increased magnitude of viral rebound
during treatment interruption\textsuperscript{14,51}. Here we predict that the magnitude of the SHIV-specific
immune response is correlated not only with viral load set point, but also with the reduction of
CD4\textsuperscript{+}CCR5\textsuperscript{+} T cells after ATI. CD4\textsuperscript{+}CCR5\textsuperscript{+} T cell depletion might also be predictive of the loss
of depletion of virus-specific immunity following conditioning.

A final important observation from the model is that viral control may be delayed beyond
the first ten weeks after ATI, and instead occur many months after ATI. Thus, viral load levels
during the initial weeks after ATI may not completely define success (stable ART-free
remission), whereas CD4\textsuperscript{+}CCR5\textsuperscript{+} T-cell nadir should more strongly correlate with the degree of
depletion of virus-specific immunity. In this sense, minimal CD4\textsuperscript{+}CCR5\textsuperscript{+} T-cell nadir may
predict post-rebound viral control, if the starting fraction of protected cells is known.
Our results are limited by a small sample size of eight animals. For that reason, several model parameters were assumed to be the same among the population (i.e., without random effects). However, the number of observations for each animal was large enough to discriminate among different plausible model candidates. Therefore, we performed projections using only the individual estimated parameters. Reassuringly, our results align with prior mechanistic studies of cellular reconstitution after stem cell transplantation\(^\text{18,26,38,70,71}\). Our analysis also suggests that the majority of reconstituting CD4\(^+\)CCR5\(^-\) T cells do not proliferate and have a slow expansion that concurs with estimates of thymic export from previous studies\(^\text{26,70,71}\).

The interplay between reconstituting HIV susceptible CD4\(^+\) T cells, HIV-resistant CD4\(^+\) T cells, infected cells, virus-specific immune cells, and replicating virus following autologous, CCR5-edited HPSC transplantation is extremely complex. Our results illustrate the capabilities of mathematical models to glean insight from this system and highlight that modeling will be required to optimize strategies for HIV cure studies, both in the macaque model, as well as in HIV\(^+\) individuals.

**Acknowledgements**

This study was supported by grants from the National Institutes of Health, National Institute of Allergy and Infectious Diseases (UM1 AI126623). ERD is supported by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number KL2 TR002317. DBR is supported by a Washington Research Foundation postdoctoral fellowship, and a CFAR NIA P30 AI027757. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Washington Research Foundation.
Authorship Contributions

E.F.C. and J.T.S. conceived the study. C.W.P. and H.P.K. contributed ideas and data sources for the project. E.R.D. and D.B.R. contributed ideas for the development of mechanistic mathematical models. B.T.M. contributed ideas and support for statistical models and analyzes. E.F.C. assembled data, wrote all code, performed all calculations and derivations, ran the models, and analyzed output data. J.T.S. and E.F.C. wrote the manuscript with contributions from all other authors.

Disclosure of Conflicts of Interest

The authors declare no competing financial interests.

Animal Welfare.

The data used in this work were collected in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The study protocol was approved by the Institutional Animal Care and Use Committees (3235-03) of the Fred Hutchinson Cancer Research Center and the University of Washington.
Table 1. Parameters of the model. Values are from steady state equations, using population estimates from best model fits or assumed from the references as described. When assumed from steady state equations, population estimates were used. See Supp. Materials for more details.

| Parameter | Units | Description | Value | Source |
|-----------|-------|-------------|-------|--------|
| $T(t_0)$  | cells | Number of cells in the transplant product. | 0 | 4*10^7 | Fixed, assumed from reference 14. |
| $P(t_0)$  | cells | Number of cells in the BM/Thymus at the moment of transplant. | 4*10^8 | 0 | Control: Computed from the median of steady state equations. Transplant: Fixed, assumed. |
| $N(t_0)$  | cells/μL | Blood CD4⁺CCR5⁻ T cell concentration at the moment of transplant. | 1249 | 47 | Control: Computed from the median of steady state equations. Transplant: Fitted. |
| $S(t_0)$  | cells/μL | Blood CD4⁺CCR5⁺ T cell concentration at the moment of transplant. | 115 | 2 | Control: Computed from the median of steady state equations. Transplant: Fitted. |
| $M(t_0)$  | cells/μL | Blood CD8⁺ T_N + T_CM cell concentration at the moment of transplant. | 305 | 8 | Control: Computed from the median of steady state equations. Transplant: Fitted. |
| $E(t_0)$  | cells/μL | Blood CD8⁺ T_EM cell concentration at the moment of transplant. | 935 | 17 | Control: Computed from the median of steady state equations. Transplant: Fitted. |
| $E_h(t_0)$ | cells/μL | Blood SHIV-specific CD8⁺ T effector cell concentration at the moment of transplant. | 0 | 0 | Control: Computed from steady state equations. Transplant: Assumed. |
| $I_p(t_0)$ | cells/μL | Productively infected blood CD4⁺CCR5⁺ T cell | 2*10^6 | 2*10^6 | Computed from steady state equations. |
| Parameter | Description | Value | Notes |
|-----------|-------------|-------|-------|
| $I_u(t_0)$ | cells/$\mu$L | concentration at the moment of transplant. | 0 | Computed from steady state equations. |
| $V(t_0)$ | RNA copies/mL | Plasma viral load at the moment of transplant. | 0.5 | Computed from steady state equations. |
| $k_e$ | 1/day | Homing rate of transplanted cells into the bone marrow. | 1 | Fixed, assumed from references 72,73. |
| $\hat{r}_p = r_p - \lambda_p - d_p$ | 1/day | Renewal rate of stem and progenitor cells in the bone marrow/thymus. | 0.04 | Fitted. |
| $\hat{r}_s = r_s - d_s$ | 1/day | Proliferation rate of blood CD4$^+$CCR5$^+$ T cells. | 0.14 | Fitted. |
| $\hat{r}_m = r_m - \lambda_m - d_m$ | 1/day | Proliferation rate of blood CD8$^+$TN + TCM cells. | 0.003 | Fitted. |
| $\hat{r}_e = r_e - d_e$ | 1/day | Proliferation rate of CD8$^+$TEM cells. | 0.09 | Fitted. |
| $\bar{d}_n = \lambda_n + d_n$ | 1/day | Removal rate of blood CD4$^+$CCR5$^+$ T cells. | 0.01 | Fitted. |
| $\lambda_p = \lambda_e = \lambda_f$ | 1/day | Thymic output rate of T cells. | 0.01 | Fitted. |
| $\lambda_n$ | 1/day | CCR5 upregulation rate in CD4$^+$ T cells. | 0.004 | Fitted. |
| $\lambda_m$ | 1/day | Differentiation rate of CD8$^+$TN + TCM cells to CD8$^+$TEM cells. | 0.09 | Fitted. |
| $K_p = K_p$ | cells/$\mu$L | Effective carrying capacity of progenitor cells. | 1664 | Fitted. |
| $K_s = K_s$ | cells/$\mu$L | Effective carrying capacity of CD4$^+$CCR5$^+$ T cells. | 1328 | Fitted. |
| $K_m = K_m$ | cells/$\mu$L | Effective carrying capacity of CD8$^+$TN + TCM cells. | 49 | Fitted. |
\[ K_e = \frac{\bar{r}_e}{r_e} \]

| Parameter | Units | Description | Value | Source |
|-----------|-------|-------------|-------|--------|
| \( K_e \) | cells/\( \mu L \) | Effective carrying capacity of CD8\(^+\) TEM cells. | 1257 | Fitted. |
| \( \beta \) | \( \mu L/\) copies/day | Infectivity rate. | 0.0003 | Fitted. |
| \( \Delta_t \) | days | Time to rebound after ATI. | 7.5 | Fitted. |
| \( \delta_l \) | 1/day | Death rate of infected CD4\(^+\)CCR5\(^+\) T cells. | 1 | Fixed, assumed using estimates from references 74,75. |
| \( \tau \) | - | Fraction of infected cell that produce infectious virus. | 0.05 | Fixed, assumed from reference 54. |
| \( \xi L \) | cells/\( \mu L/day \) | Number of latent cells that activate per day. | \( 2*10^{-7} \) | Fixed, assumed to have a viral load of ~0.5 copies/mL during cART. |
| \( \pi \) | 1/day | Viral production rate. | \( 5*10^4 \) | Fixed, assumed using estimates from reference 76. |
| \( \gamma \) | 1/day | Virus clearance rate. | 23 | Fixed, assumed using estimates from reference 77. |
| \( \omega_4 \) | \( \mu L/\) cells/day | SHIV-dependent replenishment of CD4\(^+\)CCR5\(^+\) T cells. | 0.19 | Fitted. |
| \( \omega_8 \) | \( \mu L/\) cells/day | SHIV-dependent proliferation rate of CD8\(^+\) T cells. | 0.002 | Fitted. |
| \( I_{50} \) | cells/\( \mu L \) | 50\% maximum value of adaptive infected cells, allows bounded growth. | 0.20 | Fitted. |
| \( f \) | - | Fraction of SHIV-CD8\(^-\)-responding cells that become SHIV-specific effectors. | 0.9 | Fixed, assumed from reference 46. |
| \( d_h \) | 1/day | Death rate of SHIV-specific effector CD8\(^+\) T cells. | 0.05 | Fitted. |
| \( \frac{1}{\theta} \) | \( \mu L/\) cells | 50\% maximum value of SHIV-specific immune cells to block virus production. | 1 | Fixed. |
1. Chun T-W, Carruth L, Finzi D, et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature*. 1997;387(6629):183.

2. Chun T-W, Finzi D, Margolick J, Chadwick K, Schwartz D, Siliciano RF. In vivo fate of HIV-1-infected T cells: Quantitative analysis of the transition to stable latency. *Nature Medicine*. 1995;1(12):1284-1290.

3. Finzi D, Hermankova M, Pierson T, et al. Identification of a Reservoir for HIV-1 in Patients on Highly Active Antiretroviral Therapy. *Science*. 1997;278(5341):1295-1300.

4. Allers K, Hütter G, Hofmann J, et al. Evidence for the cure of HIV infection by CCR5Δ32/Δ32 stem cell transplantation. *Blood*. 2011;117(10):2791-2799.

5. Hütter G, Nowak D, Mossner M, et al. Long-Term Control of HIV by CCR5 Delta32/Delta32 Stem-Cell Transplantation. *New England Journal of Medicine*. 2009;360(7):692-698.

6. Gupta RK, Abdul-jawad S, McCoy LE, et al. HIV-1 remission following CCR5Δ32/Δ32 haematopoietic stem-cell transplantation. *Nature*. 2019:1.

7. Henrich TJ, Hanhauser E, Harrison LJ, et al. CCR5-Δ32 Heterozygosity, HIV-1 Reservoir Size, and Lymphocyte Activation in Individuals Receiving Long-term Suppressive Antiretroviral Therapy. *The Journal of Infectious Diseases*. 2016;213(5):766-770.

8. Henrich TJ, Hanhauser E, Marty FM, et al. Antiretroviral-Free HIV-1 Remission and Viral Rebound After Allogeneic Stem Cell Transplantation: Report of 2 Cases. *Annals of Internal Medicine*. 2014;161(5):319.

9. Henrich TJ, Hu Z, Li JZ, et al. Long-Term Reduction in Peripheral Blood HIV Type 1 Reservoirs Following Reduced-Intensity Conditioning Allogeneic Stem Cell Transplantation. *The Journal of Infectious Diseases*. 2013;207(11):1694-1702.

10. Salgado M, Kwon M, Gálvez C, et al. Mechanisms That Contribute to a Profound Reduction of the HIV-1 Reservoir After Allogeneic Stem Cell Transplant. *Annals of Internal Medicine*. 2018.

11. Haworth KG, Peterson CW, Kiem H-P. CCR5-edited gene therapies for HIV cure: Closing the door to viral entry. *Cytotherapy*. 2017;19(11):1325-1338.

12. Tebas P, Stein D, Tang WW, et al. Gene Editing of CCR5 in Autologous CD4 T Cells of Persons Infected with HIV. *New England Journal of Medicine*. 2014;370(10):901-910.

13. Peterson CW, Wang J, Norman KK, et al. Long-term multilineage engraftment of autologous genome-edited hematopoietic stem cells in nonhuman primates. *Blood*. 2016;127(20):2416-2426.

14. Peterson CW, Benne C, Polacino P, et al. Loss of immune homeostasis dictates SHIV rebound after stem-cell transplantation. *JCI Insight*. 2017;2(4).

15. Peterson CW, Haworth KG, Polacino P, et al. Lack of viral control and development of combination antiretroviral therapy escape mutations in macaques after bone marrow transplantation. *Aids*. 2015;29(13):1597-1606.

16. Peterson CW, Wang J, Deleage C, et al. Differential impact of transplantation on peripheral and tissue-associated viral reservoirs: Implications for HIV gene therapy. *PLOS Pathogens*. 2018;14(4):e1006956.

17. Ho O, Larsen K, Polacino P, et al. Pathogenic infection of Macaca nemestrinawith a CCR5-tropic subtype-C simian-human immunodeficiency virus. *Retrovirology*. 2009;6(1):65.
18. Jameson SC. Maintaining the norm: T-cell homeostasis. Nature Reviews Immunology. 2002;2(8):547-556.
19. Mehr R, Perelson AS. Blind T-cell homeostasis and the CD4/CD8 ratio in the thymus and peripheral blood. Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology: Official Publication of the International Retrovirology Association. 1997;14(5):387-398.
20. Margolick JB, Donnenberg AD. T-cell homeostasis in HIV-1 infection. Seminars in Immunology. 1997;9(6):381-388.
21. Schluns KS, Williams K, Ma A, Zheng XX, Lefrançois L. Cutting Edge: Requirement for IL-15 in the Generation of Primary and Memory Antigen-Specific CD8 T Cells. The Journal of Immunology. 2002;168(10):4827-4831.
22. Schluns KS, Kieper WC, Jameson SC, Lefrançois L. Interleukin-7 mediates the homeostasis of naïve and memory CD8 T cells in vivo. Nature Immunology. 2000;1(5):426-432.
23. Goldrath AW, Luckey CJ, Park R, Benoist C, Mathis D. The molecular program induced in T cells undergoing homeostatic proliferation. Proceedings of the National Academy of Sciences. 2004;101(48):16885-16890.
24. Voehringer D, Liang H-E, Locksley RM. Homeostasis and Effector Function of Lymphopenia-Induced “Memory-Like” T Cells in Constitutively T Cell-Depleted Mice. The Journal of Immunology. 2008;180(7):4742-4753.
25. Boyman O, Létourneau S, Krieg C, Sprent J. Homeostatic proliferation and survival of naïve and memory T cells. European Journal of Immunology. 2009;39(8):2088-2094.
26. Douek DC, Vescio RA, Betts MR, et al. Assessment of thymic output in adults after hematopoietic stemcell transplantation and prediction of T-cell reconstitution. The Lancet. 2000;355(9218):1875-1881.
27. Douek DC, McFarland RD, Keiser PH, et al. Changes in thymic function with age and during the treatment of HIV infection. Nature. 1998;396(6712):690-695.
28. Bender J, Mitchell T, Kappler J, Marrack P. Cd4+ T Cell Division in Irradiated Mice Requires Peptides Distinct from Those Responsible for Thymic Selection. Journal of Experimental Medicine. 1999;190(3):367-374.
29. Kieper WC, Jameson SC. Homeostatic expansion and phenotypic conversion of naïve T cells in response to self peptide/MHC ligands. Proceedings of the National Academy of Sciences. 1999;96(23):13306-13311.
30. Sallusto F, Geginat J, Lanzavecchia A. Central Memory and Effector Memory T Cell Subsets: Function, Generation, and Maintenance. Annual Review of Immunology. 2004;22(1):745-763.
31. Saout CL, Mennechet S, Taylor N, Hernandez J. Memory-like CD8+ and CD4+ T cells cooperate to break peripheral tolerance under lymphopenic conditions. Proceedings of the National Academy of Sciences. 2008;105(49):19414-19419.
32. Sprent J, Surh CD. Normal T cell homeostasis: the conversion of naïve cells into memory-phenotype cells. Nature Immunology. 2011;12(6):478-484.
33. Takeda S, Rodewald H-R, Arakawa H, Bluethmann H, Shimizu T. MHC Class II Molecules Are Not Required for Survival of Newly Generated CD4+ T Cells, but Affect Their Long-Term Life Span. Immunity. 1996;5(3):217-228.
34. Tanchot C, Lemonnier FA, Pérarnau B, Freitas AA, Rocha B. Differential Requirements for Survival and Proliferation of CD8 Naïve or Memory T Cells. Science. 1997;276(5321):2057-2062.
35. Fry TJ, Mackall CL. Interleukin-7: master regulator of peripheral T-cell homeostasis? *Trends in Immunology*. 2001;22(10):564-571.

36. Tan JT, Dudl E, LeRoy E, et al. IL-7 is critical for homeostatic proliferation and survival of naïve T cells. *Proceedings of the National Academy of Sciences*. 2001;98(15):8732-8737.

37. Yamaki S, Ine S, Kawabe T, et al. OX40 and IL-7 play synergistic roles in the homeostatic proliferation of effector memory CD4+ T cells. *European Journal of Immunology*. 2014;44(10):3015-3025.

38. Guillaume T, Rubinstein DB, Symann M. Immune Reconstitution and Immunotherapy After Autologous Hematopoietic Stem Cell Transplantation. *Blood*. 1998;92(5):1471-1490.

39. Spits H. Development of αβ T cells in the human thymus. *Nature Reviews Immunology*. 2002;2(10):760.

40. Berkowitz RD, Beckerman KP, Schall TJ, McCune JM. CXCR4 and CCR5 Expression Delineates Targets for HIV-1 Disruption of T Cell Differentiation. *The Journal of Immunology*. 1998;161(7):3702-3710.

41. Bleul CC, Wu L, Hoxie JA, Springer TA, Mackay CR. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. *Proceedings of the National Academy of Sciences*. 1997;94(5):1925-1930.

42. Zaitseva MB, Lee S, Rabin RL, et al. CXCR4 and CCR5 on Human Thymocytes: Biological Function and Role in HIV-1 Infection. *The Journal of Immunology*. 1998;161(6):3103-3113.

43. Buchholz VR, Flossdorf M, Hensel I, et al. Disparate Individual Fates Compose Robust CD8+ T Cell Immunity. *Science*. 2013;340(6132):630-635.

44. Farber DL, Yudanin NA, Restifo NP. Human memory T cells: generation, compartmentalization and homeostasis. *Nature Reviews Immunology*. 2014;14(1):24-35.

45. Kaech SM, Wherry EJ, Ahmed R. Effector and memory T-cell differentiation: implications for vaccine development. *Nature Reviews Immunology*. 2002;2(4):251-262.

46. Borducchi EN, Cabral C, Stephenson KE, et al. Ad26/MVA therapeutic vaccination with TLR7 stimulation in SIV-infected rhesus monkeys. *Nature*. 2016;540(7632):284.

47. De Boer RJ. Understanding the Failure of CD8+ T-Cell Vaccination against Simian/Human Immunodeficiency Virus. *Journal of Virology*. 2007;81(6):2838-2848.

48. Hill AL, Rosenbloom DIS, Nowak MA, Siliciano RF. Insight into treatment of HIV infection from viral dynamics models. *Immunological Reviews*. 2018;285(1):9-25.

49. Perelson AS. Modelling viral and immune system dynamics. *Nature Reviews Immunology*. 2002;2(1):28-36.

50. Perelson AS, Essunger P, Cao Y, et al. Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature*. 1997;387(6629):188.

51. Reeves DB, Peterson CW, Kiem H-P, Schiffer JT. Autologous Stem Cell Transplantation Disrupts Adaptive Immune Responses during Rebound Simian/Human Immunodeficiency Virus Viremia. *Journal of Virology*. 2017;91(13):e00095-00017.

52. Wodarz D, Garg N, Komarova NL, et al. Kinetics of CLL cells in tissues and blood during therapy with the BTK inhibitor ibrutinib. *Blood*. 2014;123(26):4132-4135.

53. Wodarz D, Nowak MA. Specific therapy regimes could lead to long-term immunological control of HIV. *Proceedings of the National Academy of Sciences*. 1999;96(25):14464-14469.

54. Doitsh G, Cavrois M, Lassen KG, et al. Abortive HIV Infection Mediates CD4 T Cell Depletion and Inflammation in Human Lymphoid Tissue. *Cell*. 2010;143(5):789-801.
520 55. Matrajt L, Younan PM, Kiem H-P, Schiffer JT. The Majority of CD4+ T-Cell Depletion
during Acute Simian-Human Immunodeficiency Virus SHIV89.6P Infection Occurs in
Uninfected Cells. Journal of Virology. 2014;88(6):3202-3212.
522 56. Lavielle M. Mixed Effects Models for the Population Approach: Models, Tasks, Methods
and Tools (ed 1 edition). Boca Raton: Chapman and Hall/CRC; 2014.
524 57. Burnham KP, Anderson DR. Model Selection and Multimodel Inference: A Practical
Information-Theoretic Approach (ed 2). New York: Springer-Verlag; 2002.
526 58. Elemans M, Basatena N-KSa, Klatt NR, Gkekas C, Silvestri G, Asquith B. Why Don’t
CD8+ T Cells Reduce the Lifespan of SIV-Infected Cells In Vivo? PLOS Computational
Biology. 2011;7(9):e1002200.
528 59. Klatt NR, Shudo E, Ortiz AM, et al. CD8+ Lymphocytes Control Viral Replication in
SIVmac239-Infected Rhesus Macaques without Decreasing the Lifespan of Productively
Infected Cells. PLOS Pathogens. 2010;6(1):e1000747.
530 60. Wong JK, Strain MC, Porrata R, et al. In Vivo CD8+ T-Cell Suppression of SIV Viremia
Is Not Mediated by CTL Clearance of Productively Infected Cells. PLOS Pathogens.
2010;6(1):e1000748.
532 61. Shridhar V, Chen Y, Gupta P. The CD8 Antiviral Factor (CAF) can suppress HIV-1
transcription from the Long Terminal Repeat (LTR) promoter in the absence of elements
upstream of the CATATAA box. Virology Journal. 2014;11(1):130.
534 62. Blazek D, Teque F, Mackewicz C, Peterlin M, Levy JA. The CD8+ cell non-cytotoxic
antiviral response affects RNA polymerase II-mediated human immunodeficiency virus
transcription in infected CD4+ cells. Journal of General Virology. 2016;97(1):220-224.
536 63. Zhang L, Yu W, He T, et al. Contribution of Human α-Defensin 1, 2, and 3 to the Anti-
HIV-1 Activity of CD8 Antiviral Factor. Science. 2002;298(5595):995-1000.
538 64. Levy JA, Mackewicz CE, Barker E. Controlling HIV pathogenesis: the role of the
noncytotoxic anti-HIV response of CD8+ T cells. Immunology Today. 1996;17(5):217-224.
540 65. A. OA, J. PL. CD4+ T cell depletion in HIV infection: mechanisms of immunological
failure. Immunological Reviews. 2013;254(1):54-64.
542 66. Douek DC, Picker LJ, Koup RA. T Cell Dynamics in HIV-1 Infection. Annual Review of
Immunology. 2003;21(1):265-304.
544 67. Okoye A, Meier-Schellersheim M, Brenchley JM, et al. Progressive CD4+ central–
memory T cell decline results in CD4+ effector–memory insufficiency and overt disease in
chronic SIV infection. Journal of Experimental Medicine. 2007;204(9):2171-2185.
546 68. Okoye AA, Rohankhedkar M, Abana C, et al. Naive T cells are dispensable for memory
CD4+ T cell homeostasis in progressive simian immunodeficiency virus infection. Journal of
Experimental Medicine. 2012;209(4):641-651.
548 69. Zhen A, Peterson CW, Carrillo MA, et al. Long-term persistence and function of
hematopoietic stem cell-derived chimeric antigen receptor T cells in a nonhuman primate model
of HIV/AIDS. PLOS Pathogens. 2017;13(12):e1006753.
550 70. Krenger W, Blazar BR, Holländer GA. Thymic T-cell development in allogeneic stem
cell transplantation. Blood. 2011;117(25):6768-6776.
552 71. Roux E, Dumont-Girard F, Starobinski M, et al. Recovery of immune reactivity after T-
cell–depleted bone marrow transplantation depends on thymic activity. Blood. 2000;96(6):2299-
2303.
554 72. Lapidot T, Dar A, Kollet O. How do stem cells find their way home? Blood.
2005;106(6):1901-1910.
73. Chute J. Stem cell homing. *Current Opinion in Hematology*. 2006;13(6):399-406.
74. Markowitz M, Louie M, Hurley A, et al. A Novel Antiviral Intervention Results in More Accurate Assessment of Human Immunodeficiency Virus Type 1 Replication Dynamics and T-Cell Decay In Vivo. *Journal of Virology*. 2003;77(8):5037-5038.
75. Cardozo EF, Andrade A, Mellors JW, Kuritzkes DR, Perelson AS, Ribeiro RM. Treatment with integrase inhibitor suggests a new interpretation of HIV RNA decay curves that reveals a subset of cells with slow integration. *PLOS Pathogens*. 2017;13(7):e1006478.
76. Chen HY, Mascio MD, Perelson AS, Ho DD, Zhang L. Determination of virus burst size in vivo using a single-cycle SIV in rhesus macaques. *Proceedings of the National Academy of Sciences*. 2007;104(48):19079-19084.
77. Ramratnam B, Bonhoeffer S, Binley J, et al. Rapid production and clearance of HIV-1 and hepatitis C virus assessed by large volume plasma apheresis. *The Lancet*. 1999;354(9192):1782-1785.
Figure Legends

Figure 1. Study design and mathematical modeling. A. Four animals were infected with SHIV, suppressed with cART and then underwent TBI/HSPC transplantation without editing of CCR5 (Transplant group). A control group of four animals did not receive TBI or HSPC transplantation. Both groups underwent ATI approximately one year after cART initiation. B. Mathematical modeling approach. C. Mathematical model for T cell reconstitution. Each circle represents a cell compartment: T represents the HSPCs from the transplant; P, the progenitor cells in bone marrow (BM) and Thymus; S and N, CD4+CCR5+ and CD4+CCR5− T cells, respectively; M and E, the CD8+ T cells with naïve and central memory phenotypes, and effector memory phenotypes, respectively. D. Mathematical model for virus dynamics. We adapted the previous model by including the following assumptions. Susceptible cells, S, are infected by the virus, V. Ip and Iu represent a fraction τ of the infected cells produce virus, Ip, and the other fraction become unproductively infected, Iu. Total CD4+CCR5+ T cell count is given by the sum of S, Ip and Iu. All infected cells die at rate δI. Ip cells arise from activation of latently infected cells at rate ω2 and produce virus at a rate π, that is cleared at rate γ. CD8+ M cells proliferate in the presence of infection with rate ω8 from which a fraction f become SHIV-specific CD8+ effector T cells, Eh, that are removed at a rate dh. These effector cells reduce virus production or infectivity by 1/(1+θEh), or 1/(1+φEh), respectively. Non-susceptible CD4+ T cells upregulate CCR5 in the presence of infection and replenish the susceptible pool with rate ω4. E. Schematic of the extended mathematical model that includes CCR5-edited, protected cells. Protected cells from transplant: Tp, protected progenitor cells in bone marrow/thymus: Pp, and protected CD4+CCR5− T cells: Np are included. The initial fraction of protected cells is represented by the parameter fp.
Figure 2. **CD4+ and CD8+ T cell dynamics post-transplantation, pre-ATI.** Range of blood A. CD4+ and B. CD8+ T cell counts using all data points for the period before ATI in control animals (p-value calculated with a paired t-test for averaged measurements post-transplant). C. Distribution of the growth rate estimates of CD4+CCR5+, CD4+CCR5-, and CD8+ T cells using all data points from time of transplant until their levels reached set point in transplanted animals (p-value calculated using a paired t-test). We assumed set point as the data point after which the sum of consecutive changes from the moment of transplant in T cell counts was smaller or equal to zero. D. Empirical data for peripheral subset counts (colored data points) and best fits of the model (solid lines) to all blood T cell subsets before ATI for all animals in the transplant group. Each row is one animal.

Figure 3. **Model predictions of the CD4+CCR5+ T cell turnover.** Model prediction of the total concentration of CD4+CCR5+ T new cells generated by proliferation (solid line) and the concentration generated by up-regulation of CCR5 (dashed line) over time using the best estimates for all 4 transplanted animals.

Figure 4. **Plasma viral load and CD4+ T cell kinetics after ATI.** A-D: Distributions of A. peak viral load post-ATI, B. viral load at endpoint necropsy, C. CD4+CCR5+ T-cell normalized nadir post-ATI relative to the CCR5 concentration at ATI, D. CD4+CCR5- T-cell nadir post-ATI. P-values were calculated using Mann-Whitney test. E. Best fits of the model (black lines) to SHIV RNA, and blood CD4+CCR5+ T, CD4+CCR5- T, total CD8+ T, CD8+ T_EM, and CD8+ T_n and T_CM cell counts (colored data points) for all 4 transplanted animals.
Figure 5. Loss of SHIV-specific CD8 response after transplantation. Scatterplots of the SHIV-dependent CD8 proliferation rate ($\omega_8$) vs. A. CD4$^+$CCR5$^+$ normalized nadir post-ATI relative to the CCR5 concentration at ATI, and B. final observed viral load from all animals; (p-values calculated using Spearman’s rank test). C-D: Individual parameter estimates of C. the SHIV-dependent CD8 proliferation rate ($\omega_8$) and D. the time of rebound after ATI (see text). Blue: control, and red: transplant groups (p-values calculated by Mann-Whitney test).

Figure 6. Model predictions for post-rebound viral control after CCR5 gene-edited HSPC transplant. A. Predictions for plasma viral load post-ATI using the adapted model for varying values of $f_p$ (using parameter estimates from animal Z09144). B-E. Predictions for plasma viral load (heat-map color) for each animal at a given time post-ATI (x-axis) and a given $f_p$ (y-axis).

Figure 7. CD4$^+$CCR5$^+$ T cell nadir as a predictor for necessary minimum initial fraction of protected cells to achieve post ATI control. A. Predictions for the normalized CD4$^+$CCR5$^+$ T cell counts relative to their concentration at ATI using the adapted model for varying values of $f_p$ (using parameter estimates from animal Z09144). B. Predicted CD4$^+$CCR5$^+$ T cell normalized nadir during the first 10 weeks after ATI for the minimum fraction of protected cells required to obtain post-rebound control after 2 years using parameter estimates for each animal. Dashed line describes a linear regression of the computed minimum fraction of protected cells ($f_p$) and the CCR5 normalized nadir.
Predicted maximum decrease of CD4^+CCR5^+ T cells during the first 10 weeks after ATI for the minimum fraction of protected cells required to obtain post-rebound control after 2 years using parameter estimates for each animal.
A. CD4+ subsets in control group

B. CD8+ subsets in control group

C. Growth rate in transplant group

D. Growth rate in transplant group

Weeks after transplant
Predicted CD4\(^+\)CCR5\(^+\) T cell turnover

- New CCR5\(^+\) clones
- Up regulation of CCR5

Weeks after transplant

0 5 10 15 20 25

0 5 10 15

0 1 2 3 4 5

0 5 10 15

0 1 2 3 4 5

0 5 10 15 20 25

0 1 2 3 4 5

0 5 10 15 20 25

0 1 2 3 4 5

0 5 10 15 20 25

0 1 2 3 4 5

0 5 10 15 20 25

0 1 2 3 4 5

0 5 10 15 20 25

0 1 2 3 4 5

0 5 10 15 20 25

0 1 2 3 4 5

0 5 10 15 20 25

0 1 2 3 4 5
SHIV-dependent CD8 Prol. (ω8) nadir post-ATI

A. CCR5 norm.

$\text{p} = 0.002$

$r = 0.93$

B. Final plasma viral load

$\text{p} = 0.02$

$r = -0.81$

SHIV-dependent CD8 Prol. (ω8)

C. Days to rebound ($\Delta t$)

$\text{p} = 0.03$

D. $\text{p} = 0.01$

SHIV-dependent CD8 Prol. (ω8)

Control Transplant

Control Transplant
A. Weeks after ATI

B. Z09144

C. Z08214

D. A11200

E. Z09196

Viral load (copies/ml)

Viral control

Initial % protected cells

Weeks after ATI
Weeks after ATI

\[ f_p = 0 \]
\[ f_p = 0.2 \]
\[ f_p = 0.4 \]
\[ f_p = 0.6 \]
\[ f_p = 0.8 \]
\[ f_p = 1 \]

Min. \( f_p \) for viral control

Z09144
Z08214
A11200
Z09196

Z09144