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

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Short title: Post-rebound SHIV control after ΔCCR5 HSPC transplantation

Abstract (248/250 words)

Two recent cases of HIV cure/stable remission following allogeneic stem cell transplantation are difficult to reproduce because of inherent toxicities and the rarity of donors homozygous for the CCR5Δ32 deletion. One approach to overcome these barriers and improve safety 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 in a fraction of autologous HSPCs. Therefore, we sought to determine the minimum fraction required for post-rebound 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 described reconstitution of $CD4^+$ $CCR5^+$, $CD4^+$ $CCR5^-$, and $CD8^+$ T cell counts, as well as SHIV plasma viral loads in control and transplanted macaques. By modifying the model to hypothetically describe transplant with some fraction of protected CCR5-edited cells, we found that transplantation had differential effects on the macaques' immunologic control of SHIV: those with a loss of immunologic control had a more profound depletion of $CCR5^+CD4^+$ T cells and would require higher fractions of gene-edited cells (~97%) than those that maintained immunologic control (~60%). Our results provide a framework to predict the likelihood of post-rebound control *in vivo,* based on the percentage of CCR5-edited cells in peripheral blood and the loss of HIV-specific immunity following autologous HSPC.

41 **(Main text: 3789/4000 words, including headings)**

42 **INTRODUCTION**

43 The major obstacle to HIV-1 eradication is the latent reservoir of long-lived infected
44 cells[1-3]. Cure strategies aim to eliminate all infected cells or prevent sustained viral reactivation
45 from latency. The only known case of HIV cure[4,5] and an additional, recently-reported case of
46 prolonged remission[6], resulted from allogeneic hematopoietic stem cell transplant with
47 homozygous CCR5Δ32 donor cells[4-6]. The success of this procedure is likely multifactorial—in
48 part attributable to HIV resistance of the transplant product, the conditioning regimen that
49 facilitates engraftment, some graft-versus-host effect, and immunosuppressive therapies for
50 graft-versus-host disease[7-11].

51 A current research focus is to recapitulate this method of cure with minimal toxicity. One
52 method is to perform autologous transplantation following ex vivo editing of the CCR5 gene
53 with a zinc finger nuclease, eliminating the need for allogeneic CCR5-negative donors[12,13].
54 While this procedure is safe and feasible in pigtail macaques infected with simian-human
55 immunodeficiency virus (SHIV)[13-16], only a fraction of HSPCs can be genetically modified *ex*
56 *vivo* to be HIV-resistant.

57 Due to this challenge, we developed a mathematical model that can be used to predict the
58 minimum threshold of persisting, gene-modified cells necessary for functional cure. First, we
59 modeled the kinetics of $CD4^+CCR5^+$, $CD4^+$ $CCR5^-$, and $CD8^+$ T cell reconstitution after
60 autologous transplantation. We then modeled SHIV rebound kinetics following analytical
61 treatment interruption (ATI) and identified the degree of loss of anti-HIV cytolytic immunity
62 following transplantation. Finally, we projected the proportion of gene-modified cells and the
63 levels of SHIV-specific immunity required to eliminate viral replication following ATI.

64 # METHODS

65 **Experimental Data**

66 Eight juvenile pigtail macaques were intravenously challenged with 9500 TCID50 SHIV-
67 1157ipd3N4 (SHIV-C)[14,17]. After 6 months, the macaques received combination antiretroviral
68 therapy (cART: tenofovir [PMPA], emtricitabine [FTC], and raltegravir [RAL]). After ~25
69 weeks on cART, four animals received total body irradiation (TBI) followed by transplantation
70 of autologous HSPCs. After an additional 25 weeks following transplant, when viral load was
71 fully suppressed, animals underwent analytical treatment interruption (ATI)[14]. A control group of
72 four animals did not receive TBI or HSPCs transplantation and underwent ATI after ~50 weeks
73 of treatment (**Fig. 1A**). Plasma viral loads and absolute quantified $CD4^+CCR5^-$, $CD4^+CCR5^+$ and
74 $CD8^+$ total and subsets (naïve, central memory [T_{CM}], and effector memory [T_{EM}]) T cell counts
75 from peripheral blood were measured as described previously[14,17]. We analyzed peripheral T cell
76 counts and plasma viral load from transplant until 43 weeks (~25 weeks pre-ATI and ~18 weeks
77 post-ATI).

78

79 **Mathematical modeling**

80 We employed several series of ordinary differential equation models of cellular and viral
81 dynamics after transplantation. First, we modeled T cell dynamics and reconstitution following
82 transplant and before ATI, assuming that low viral levels do not affect cell dynamics (**Fig. 1B)**.
83 After curation of that model, we introduced viral dynamics and fit those to the T cell and viral
84 rebound dynamics from the animals (**Fig. 1C)**. Lastly, we used our complete model in a
85 transplant scenario with gene editing of CCR5 to predict the minimal threshold of editing for
86 functional HIV cure.

87

88 *T cell reconstitution after transplantation:* We modeled the kinetics of $CD4^+$ and $CD8^+$ T cell
89 subsets in blood, transplanted cells that home to the BM, and progenitor cells in the BM/thymus
90 as shown in **Fig. 1B**. We included $CD8^+$ T cells in the model because $CD8^+$ and $CD4^+$ T cells
91 may arise from new naïve cells from the thymus and compete for resources that impact clonal
92 expansion and cell survival[18-20]. At the moment of HSPC infusion, transplanted animals are
93 lymphopenic due to TBI. The control group did not have a transplanted-cell compartment, and
94 all other compartments remained in steady state. We assumed that $CD4^+$ and $CD8^+$ T cell
95 expansion may have two possible drivers: (1) lymphopenia-induced proliferation of mature cells
96 that persist through myeloablative TBI[18,21-25], and (2) differentiation from naïve cells from
97 progenitors in the thymus (from transplanted $CD34^+$ HSPCs[26,27] or $CD34^+$ HSPCs that survive
98 TBI) and further differentiation to an activated effector state[24,25,28-32]. We assumed that in a
99 lymphopenic environment, factors that drive T cell proliferation are more accessible (i.e., self-
100 MHC molecules on antigen-presenting cells[28,29,33,34] and γ-chain cytokines such as IL-7 and IL-
101 15[21-23,35-37]). However, as they grow, cells compete for access to these resources, limiting clonal
102 expansion[18] such that logistic growth models are appropriate[19]. We assume that new peripheral
103 $CD4^+$ and $CD8^+$ T naïve cells come from a progenitor compartment in the BM/Thymus[38,39]. For
104 $CD4^+$ T cells the models assume that naïve cells do not express CCR5[40-42], and subsequently up-
105 and/or down-regulate expression of the CCR5 receptor[30]. For $CD8^+$ T cells we included a single
106 $CD8^+$ memory precursor compartment of T_N and T_{CM} cells that differentiate linearly into T_{EM}
107 during lymphopenia[43-45]. The details of the model are presented in the **Supp. Material** and in
108 **Fig. 1B,** with the symbols described in **Table 1**. A parsimonious model based on the one

109 described above was selected from a series of models with varying statistical complexity as
110 presented in the **Supp. Materials.**
111
112 *T cell and viral dynamics:* We adapted the previous model combining several adaptations of the
113 canonical model of viral dynamics[43-53] as shown in **Fig. 1C**. The model assumes that SHIV only
114 infects $CD4^+CCR5^+$ T cells[17], and that only a small fraction (~ 5%) of those infected cells are
115 able to produce infectious virus[51,54,55]. We modeled cART by reducing infection rate to zero, and
116 modeled ATI by assuming infection is greater than zero after some time Δ_t after interruption.
117 This model assumes also that productively infected cells arise also from activation of a steady set
118 of latently infected cells. The presence of both unproductively and productively infected cells
119 leads to the expansion of $CD8^+$ $T_{naïve}$ and T_{CM} cells, from which the majority of dividing cells
120 differentiate into SHIV-specific effector cells[30,46,47,52,53]. The details of the model are presented in
121 the **Supp. Material** and in **Fig. 1C,** with the symbols described in **Table 1**. A parsimonious
122 model based on the one described above was selected from a series of models with varying
123 statistical complexity as presented in the **Supp. Materials.**
124
125 *Viral and T cell dynamics in the setting of ΔCCR5 HSPC transplantation:* We next adapted our
126 model to simulate scenarios in which autologous transplantation includes cells that are CCR5-
127 edited. We added variables representing CCR5-edited HSPCs, T cell progenitors in BM/thymus,
128 and $CD4^+CCR5^-$ T cells in blood. These compartments have the same structure as CCR5-non-
129 edited cells but with two differences. First, the value of HSPCs at transplantation is a fraction f_p
130 of the total number of infused cells, i.e. representing the proportion of infused HSPCs that were

131 CCR5-edited. Second, mature $CD4^+CCR5^-$ cells with CCR5 edition do not upregulate CCR5 (see
132 full model in **Supp. Materials**).
133

134 **Fitting procedure and model selection**

135 We used a nonlinear, mixed-effects modeling approach[56] described in detail in **Supp.**
136 **materials**. Briefly, parameters for individual animals were sampled from a probabilistic model
137 that describes the population with a fixed effect or population median, and a random effect with
138 a standard deviation that describes the parameter variability in the population. We first fit
139 instances of the model in **Fig. 1B** to blood T cell counts during transplant and before ATI (**Table**
140 **S1** includes all 19 competing models). Then, we fit several instances of the model **Fig. 1C** to
141 blood T cell counts and plasma viral load during the period after transplant including ATI, using
142 the best competing model for the model in **Fig. 1B** (**Table S2** includes all 15 competing models).
143 For each step we fit each competing model to all data points from all animals simultaneously
144 using a maximum likelihood approach. We estimated the standard deviation of the measurement
145 error for the observations, and each parameter fixed effects and standard deviation of the random
146 effects using the Stochastic Approximation of the Expectation Maximization (SAEM) algorithm
147 embedded in the Monolix software (www.lixoft.eu)[56]. For best fit of the different possible
148 versions of the models we computed the log-likelihood (log L) and the Akaike Information
149 Criteria (AIC=-2log L+2m, where m is the number of parameters estimated)[57] to obtain the most
150 parsimonious model. We assumed a model has similar support from the data if the difference
151 between its AIC and the best model (lowest) AIC is less than two[57] (see **Supp. materials** for
152 details).
153

154 **RESULTS**

155 **$CD4^+CCR5^+$ and $CD8^+$ T cells recover more rapidly than $CD4^+CCR5^-$ T cells after HSPC**
156 **transplantation.** We analyzed the kinetics of peripheral blood $CD4^+CCR5^+$ and $CD4^+CCR5^-$ T-
157 cells, and total, $T_{naïve}$, T_{CM}, and T_{EM} $CD8^+$ T-cells in macaques after HSPC transplantation (**Fig.**
158 **1A**). In controls, levels of $CD4^+$ and $CD8^+$ T cells oscillated around a persistent set point (blue
159 lines in **Fig. 2A-C** and **Fig. S1A-B**). In the control group, $CD4^+$ $CCR5^+$ T cell levels were ~100
160 cells/µl and were uniformly lower than the $CD4^+CCR5^-$ T cell counts (~1200 cells/µl) (p=0.01,
161 Paired t-test of the averaged measures post-transplant. See **Fig. 2D**). Total $CD8^+$ T cell levels in
162 the control group were ~1400 cells/µl with a greater contribution from T_{EM} (73%) than T_N+T_{CM}
163 (27%) (Based on median values. See **Fig. 2D).** In the transplant group immediately post-TBI, the
164 levels of $CD4^+CCR5^+$ T cells started at 1-10 cells/µl and reconstituted to levels similar to the
165 control group over 5-10 weeks (**Fig. 2A**). After TBI, $CD4^+CCR5^-$ T cells remained at higher
166 levels (~100 cells/µl) than $CD4^+CCR5^+$ T cells but expanded more slowly and did not reach the
167 values of the control group after 25 weeks (**Fig. 2B**). The $CD4^+CCR5^+$ T cell compartment
168 expanded 8-fold more rapidly than the $CD4^+CCR5^-$ compartment (p=0.01, Paired t-test. See **Fig.**
169 **2E**). Following TBI in the transplanted group, $CD8^+$ T cells decreased to levels between 10 and
170 100 cells/µl after TBI but recovered to levels below the control group in 5 weeks (**Fig. 2C**); these
171 cells recovered as fast as the $CD4^+CCR5^+$ population (**Fig. 2E**). $CD8^+$ T cell levels were highly
172 correlated with the $CD8^+$ T_{EM} subtype but not to the naïve or T_{CM} subtypes (**Fig. 2F** and **Figures**
173 **S1C-D**). Overall, these results show that during post-transplantation there is a faster
174 reconstitution of $CD4^+CCR5^+$ and $CD8^+$ T cells compared to $CD4^+CCR5^-$ cells, and that $CD8^+$ T
175 cells are composed mostly of T_{EM}.
176

177 **Lymphopenia-induced proliferation favors $CD4^+CCR5^+$ and $CD8^+$ T cell reconstitution**
178 **after HPSC transplantation.** To understand what are the main drivers of T cell reconstitution
179 we developed a mathematical model that included the plausible mechanisms underlying differing
180 T cell subsets reconstitution following autologous transplantation (**Fig. 1B**). We built 19 versions
181 of that model by assuming that one or multiple mechanisms are absent, or by assuming certain
182 mechanisms have equal kinetics (**Table S1**). Using model selection theory based on AIC, we
183 identified the model in **Fig. 3A** which most parsimoniously reproduces the data (**Table S1**). The
184 main features of this model are: (1) $CD4^+CCR5^+$ T cell reconstitution after transplant is driven
185 by proliferation and upregulation of CCR5; (2) $CD4^+CCR5^-$ T cell expansion is driven only by
186 new naïve cells from the thymus; and (3), thymic export rates are equal between $CD4^+$ and $CD8^+$
187 naïve T cells.
188 The best fits of this model are presented in **Figures S2-S3** and **Fig. 3B-E** with the
189 respective parameter estimates in **Tables S3-S4**. The best fitted model predicts that $CD4^+CCR5^-$
190 T cells have a delayed reconstitution that occurs only when cells from the thymus (estimated
191 with rate ~0.01/day) outnumber their loss during death, trafficking to tissues or upregulation of
192 CCR5. Furthermore, the estimated $CD4^+CCR5^+$ T cell proliferation rate (~0.1/day) far exceed
193 the estimated CCR5 upregulation (~0.004/day) and thymic export rates. Therefore, total cell
194 proliferation in this cell compartment is predicted by the model to be up to 40-fold higher than
195 the concentration of cells that up-regulate CCR5 **(Fig. 3F** and **Fig. S4)**.
196 Our model also predicts that $CD8^+$ T cells follow a similar pattern to $CD4^+CCR5^+$ T cells
197 (**Fig. 3D-E**), with $CD8^+$ T_{EM} proliferation rate up to 10-fold higher than the $CD8^+$ T cell
198 differentiation rate (**Fig. S4**). Overall, these results suggest that following autologous HSPC
199 transplant: (1) slow thymic export is the main driver of $CD4^+CCR5^-$ T cell growth, and (2) rapid

200 lymphopenia-induced proliferation of remaining cells (rather than transplanted cells) after TBI is
201 the main driver for $CD4^+CCR5^+$ and $CD8^+$ T cell expansion.
202
203 **Reduction of blood $CD4^+CCR5^+$ T cell counts correlates with plasma viral rebound after**
204 **ATI in animals that underwent HSPC transplantation.** We next aimed to compare plasma
205 viral load rebound kinetics to $CD4^+CCR5^+$ and $CCR5^-$ T cell subset dynamics after ATI, in the
206 context of previous plasma viral load analyses that we have performed from these animals[14,51].
207 **Fig. 4A-C** presents the plasma viral loads and the blood $CD4^+CCR5^+$ and $CD4^+CCR5^-$ T cell
208 counts before and after ATI. The median peak viral load after treatment interruption was 10-fold
209 higher for the transplant group (p=0.06, Mann-Whitney test. See **Fig. 4D**). Similarly, the median
210 of the final viral load measurements at necropsy was 2-$\log_{10}$ higher in the transplant group
211 (p=0.06, Mann-Whitney test. See **Fig.4E**). $CD4^+CCR5^+$ T-cell counts decreased after ATI in the
212 transplant group: nadir was significantly lower (~8-fold) than in the control animals (p=0.01,
213 Mann-Whitney test. **Fig. 4F**). The maximum reduction of $CD4^+CCR5^+$ T cells occurred in the
214 same two animals with the highest viral set points. There was no difference between control and
215 transplant groups in the $CD4^+CCR5^-$ T cell compartment: both groups had an average of ~200
216 cells/μL (**Fig.4G**). None of the individual plasma viral load observations in the control group
217 post-ATI did not correlate with the corresponding $CD4^+CCR5^+$ T-cell counts post-ATI.
218 However, in three animals in the transplant group, viral load observations post-ATI correlated
219 negatively with their corresponding $CD4^+CCR5^+$ T cell counts (**Fig. 4H**). On the other hand,
220 plasma viral loads were negatively correlated with the $CD4^+CCR5^-$ T cell count in two animals
221 in both the control and transplant groups (**Fig. S5**). These results suggest that autologous HSPC

222 transplantation (without gene editing) affects SHIV-infected macaques so that the presence of the
223 virus leads to more depletion of $CD4^+CCR5^+$ T cells.
224
225 **Higher viral set points and $CD4^+CCR5^+$ T-cell depletion following transplantation and ATI**
226 **are due to a reduction in SHIV-specific immunity.** To understand why transplantation may
227 have an effect in virus and $CD4^+CCR5^+$ T cell kinetics during ATI, we modified our
228 mathematical model to include SHIV infection as described in **Fig. 1C** (**Methods**). Using model
229 selection theory based on AIC, we found that the most parsimonious model to explain the data
230 was the one shown in **Fig. 5A** (**Table S2**). In the best fit model SHIV-specific $CD8^+$ effector
231 cells reduce virus production in a non-cytolytic manner[58-60] (e.g. possibly by secretion of HIV-
232 antiviral factors[61-64]—not included in the model). Finally, the model assumes that infection leads
233 to enhanced activation of $CD4^+CCR5^-$ T cells leading to replenishment of $CD4^+CCR5^+$ T cells,
234 explaining the concentration reduction of the $CD4^+CCR5^-$ compartment after ATI[65-68].
235 The model simultaneously recapitulates plasma viral rebound, and the kinetics of $CD4^+$
236 $CCR5^+$ and $CCR5^-$ T cells in each animal as shown in **Fig. 5B** and **Fig. S6-S7** with
237 corresponding estimated parameters in **Table 1** and **Table S5-S6**. From the estimated
238 parameters, only SHIV-based $CD8^+$ proliferation rate, ω_8, correlated with post-ATI viral load set
239 point and $CD4^+CCR5^+$ T-cell nadir (**Fig. 5C-D**). We also found that the estimated SHIV-based
240 $CD8^+$ proliferation rate (ω_8) was significantly lower in the transplant group, and the estimated
241 time to viral rebound (Δ_t) was significantly higher in the transplant group (**Fig. 5E-F**). The
242 projected fraction of SHIV-specific $CD8^+$ T cells in the transplant group approached zero (**Fig.**
243 **S8**). Overall, these results suggest that HSPC transplantation may lead to varying degrees of loss

244 of the immune response to SHIV infected cells, in turn leading to higher viral loads and loss of
245 $CD4^+CCR5^+$ T cells.
246
247 **Greater loss of immunologic control during TBI/transplant requires higher numbers of**
248 **CCR5-edited HSPCs to control viral rebound after ATI.** To calculate the minimum threshold
249 of CCR5-edited cells necessary to induce cART-independent virus suppression, we next added a
250 population of transplanted, gene-edited CCR5 HSPCs to the model in equation 2. We assumed
251 that in the infused product there is a fraction f_p of HSPCs that have a biallelically modified CCR5
252 gene. Hence, $CD4^+$ T-cells derived from these HSPCs should not express CCR5. In the model
253 we added state variables for protected progenitors and $CD4^+CCR5^-$ T cells that cannot become
254 $CD4^+CCR5^+$ T cells (**Fig. 6A**, full model in **Supp. Materials**). **Fig. 6B** depicts projections of the
255 model using the best estimates from the fits of the model to transplanted animal Z09144**,** using
256 six values of f_p. For this animal, an initial fraction of protected cells smaller or equal than 40%
257 will not lead to post-rebound viral control after ATI, even after a year. However, it is possible to
258 have a spontaneous post-rebound viral control at ~40 weeks and 10 weeks after ATI when f_p is
259 60% or greater than 80%, We then simulated the model using parameter values obtained from
260 the best fit in the previous section for each animal in the transplant group using 100 values of f_p
261 from zero to one (0-100% CCR5-edited HSPCs). The heatmaps in **Fig. 6C-F** show plasma viral
262 load projections after 2 years after the start of ATI for different values of f_p. The model predicts
263 that the minimum f_p to maintain post-rebound control for 2 years after ATI is higher for animals
264 with lower estimated SHIV-specific immune response rates. Using parameters estimates from the
265 two animals in the transplant group with lower viral setpoints, the minimum f_p for viral control
266 was 35% and 19% (**Fig. 6E-F**). In contrast, for the other two animals the minimum f_p for viral

267 control was 56% and 97% (**Fig. 6C-D**). These model projections suggest that a larger loss of
268 immunologic control during TBI/HSPC transplant requires a higher fraction of CCR5 gene-
269 edited cells to control viral rebound after ATI.

270 The model also predicts that for some values of f_p it is possible to have two viral set
271 points: an initial high viral set point in the first weeks after ATI and then a delayed ART-free
272 viral remission (e.g. when f_p=60% in **Fig. 6B** and between 60% and 70% in **Fig. 6C**). Therefore,
273 in some cases the viral load set point during the initial weeks after ATI might not be a sufficient
274 surrogate to predict viral control further in the future. However, we found that for the simulations
275 predicting a delayed viral remission, the maximum decrease of $CD4^+CCR5^+$ T cells during the
276 first 10 weeks after ATI has a linear relationship with the minimum initial fraction of protected
277 cells required to obtain post-rebound control after 2 years **(Fig. 6G)**. We repeated these
278 projections by adding the estimated measurement error to the simulations and obtained similar
279 results (**Fig. 6H**). Therefore, the maximum initial change in $CD4^+CCR5^+$ T cells 10 weeks after
280 ATI might predict late viral control.

281 **Discussion**

282 Here we introduce a data-validated mathematical that to our knowledge is the first to
283 simultaneously recapitulate SHIV viral loads, as well as $CD4^+$ and $CD8^+$ T cell count subsets.
284 The model predicts that post-rebound viral control might be possible during autologous gene-
285 edited HSPC transplantation if therapy achieves (1) a sufficient fraction of gene-protected,
286 autologous HSPCs, and (2) maintenance or enhancement of SHIV-specific immune responses
287 following transplantation. Specifically, the model predicts that increasing amounts of
288 conditioning regimen-dependent depletion of the SHIV-specific immune response leads to a
289 higher threshold of CCR5-gene-edited cells in the transplanted HSPC product required to obtain

290 stable, ART-free viral control. These results are consistent with the cure achieved by the Berlin
291 patient who received transplant with 100% HIV-resistant cells after intense conditioning[4,5]. In
292 the autologous setting where 100% CCR5 editing may not be feasible, adjunctive measures that
293 augment virus-specific immunity, such as therapeutic vaccination, infusion of HIV-specific CAR
294 T cells or use of neutralizing antibodies, may synergize with HSPC transplantation to achieve
295 post-treatment control[11,69].

296 We systematically selected from a series of mathematical models to arrive at a set of
297 equations that most parsimoniously explains the available data. We recapitulated (1) peripheral
298 $CD4^+$ and $CD8^+$ T-cell subset reconstitution dynamics following transplant, and (2) T-cell
299 dynamics and SHIV viral rebound following ATI. Before ATI, all animals suppressed plasma
300 viral load below the limit of detection, allowing analysis of T cell reconstitution dynamics
301 independent of virus-mediated pressure. At each step, we applied model selection theory to
302 select the simplest set of mechanisms capable of explaining the observed data[57].

303 The best model predicts that the lack of complete elimination of lymphocytes by TBI
304 prevents $CD4^+CCR5^-$ cells from predominating post-transplant: the rapid expansion of
305 $CD4^+CCR5^+$ and $CD8^+$ T cells during the first few weeks after HSPC transplantation is most
306 likely due to lymphopenia-induced proliferation of remaining cells after TBI via a thymus-
307 independent pathway; the slower expansion of $CD4^+CCR5^-$ T cells is more likely due to thymic
308 export of both transplanted and remaining cells. An important future research question will be to
309 identify anatomic sites and mechanisms allowing activated $CD4^+CCR5^+$ to survive conditioning.

310 A challenge is that more intense conditioning may decrease remaining $CD4^+CCR5^+$ cells
311 but will also lower SHIV specific immunity. We previously demonstrated the link between
312 disruption of the immune response during transplant and increased magnitude of viral rebound

313 during treatment interruption[14,51]. Here we predict that the magnitude of the SHIV-specific
314 immune response is correlated not only with viral load set point, but also with the reduction of
315 $CD4^+CCR5^+$ T cells after ATI. $CD4^+CCR5^+$ T cell depletion might be predictive of the loss of
316 depletion of virus-specific immunity following conditioning.

317 A final important observation from the model is that viral control may be delayed beyond
318 the first ten weeks after ATI, rather occurring after many months of treatment interruption. Thus,
319 viral load levels during the initial weeks after ATI may not completely define success (stable
320 ART-free remission), whereas $CD4^+CCR5^+$ T-cell nadir should more strongly correlate with the
321 degree of depletion of virus-specific immunity. In this sense, minimal $CD4^+CCR5^+$ T-cell nadir
322 may predict post-rebound viral control, if the starting fraction of protected cells is known.

323 Our results are limited by a small sample size of eight animals. For that reason, several
324 model parameters were assumed to be the same among the population (i.e., without random
325 effects). However, the number of observations for each animal was large enough to discriminate
326 among different plausible model candidates. Therefore, we performed projections using only the
327 individual estimated parameters. Reassuringly, our results align with prior mechanistic studies of
328 cellular reconstitution after stem cell transplantation[18,26,38,70,71]. Our analysis also suggests that
329 the majority of reconstituting $CD4^+CCR5^-$ T cells do not proliferate and have a slow expansion
330 that concurs with estimates of thymic export from previous studies[26,70,71].

331 While our work highlights the complexity of the interplay between reconstituting HIV
332 susceptible $CD4^+$ T cells, HIV-resistant $CD4^+$ T cells, infected cells, virus-specific immune cells,
333 and replicating virus following autologous, CCR5-edited HPSC transplantation, our results
334 illustrate the capabilities of modeling to glean insight from such a complex system. A

335 comprehensive understanding of these and similar systems will be required to optimize strategies
336 for pre-clinical HIV cure studies, both in the macaque model, as well as in HIV^+ individuals.

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346 Disclosure of conflicts of interest

347 The authors declare no competing financial interests.

348 Animal welfare.

349 The data used in this work were collected in strict accordance with the recommendations in the
350 *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health. The
351 study protocol was approved by the Institutional Animal Care and Use Committees (3235-03) of
352 the Fred Hutchinson Cancer Research Center and the University of Washington.

353 # Tables

354 **Table 1. Parameters of the model.** Values are from steady state equations, using population
355 estimates from best model fits or assumed from the references as described. When assumed from
356 steady state equations, population estimates were used. See **Supp. Materials** for more details.
357

Parameter	Units	Description	Value		Source
			Control	Transplant	
$\boldsymbol{T(t_0)}$	cells	Number of cells in the transplant product.	0	$4*10^7$	Fixed, assumed from reference [14].
$\boldsymbol{P(t_0)}$	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.
$\boldsymbol{N(t_0)}$	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.
$\boldsymbol{S(t_0)}$	cells/μL	Blood $CD4^+CCR5^+$ T cell	115	2	Control: Computed from

		concentration at the moment of transplant.			the median of steady state equations. Transplant: Fitted.
$\boldsymbol{M(t_0)}$	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.
$\boldsymbol{E(t_0)}$	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.
$\boldsymbol{E_h(t_0)}$	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.
$\boldsymbol{I_p(t_0)}$	cells/μL	Productively infected blood $CD4^+CCR5^+$ T cell concentration at the	$2*10^{-6}$	$2*10^{-6}$	Computed from steady state equations.

		moment of transplant.			
$\boldsymbol{I_u(t_0)}$	cells/μL	Unproductively infected blood $CD4^+CCR5^+$ T cell concentration at the moment of transplant.	0	0	Computed from steady state equations.
$\boldsymbol{V(t_0)}$	RNA copies/mL	Plasma viral load at the moment of transplant.	0.5	0.5	Computed from steady state equations.
$\boldsymbol{k_e}$	1/day	Homing rate of transplanted cells into the bone marrow.	1		Fixed, assumed from references [72,73].
$\boldsymbol{\hat{r}_p = r_p - \lambda_p - d_p}$	1/day	Renewal rate of stem and progenitor cells in the bone marrow/thymus.	0.04		Fitted.
$\boldsymbol{\hat{r}_s = r_s - d_s}$	1/day	Proliferation rate of blood $CD4^+CCR5^+$ T cells.	0.14		Fitted.
$\boldsymbol{\hat{r}_m = r_m - \lambda_m - d_m}$	1/day	Proliferation rate of blood $CD8^+$ T_N + T_{CM} cells.	0.003		Fitted.

$\hat{r}_e = r_e - d_e$	1/day	Proliferation rate of $CD8^+$ T_{EM} cells.	0.09	Fitted.
$\hat{d}_n = \lambda_n + d_n$	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.
λ_n	1/day	CCR5 upregulation rate in $CD4^+$ T cells.	0.004	Fitted.
λ_m	1/day	Differentiation rate of $CD8^+$ $T_N + T_{CM}$ cells to $CD8^+$ T_{EM} cells.	0.09	Fitted.
$K_p = K\frac{\hat{r}_p}{r_p}$	cells/μL	Effective carrying capacity of progenitor cells.	1664	Fitted.
$K_s = K\frac{\hat{r}_s}{r_s}$	cells/μL	Effective carrying capacity of $CD4^+CCR5^+$ T cells.	1328	Fitted.
$K_m = K\frac{\hat{r}_m}{r_m}$	cells/μL	Effective carrying capacity of $CD8^+$ $T_N + T_{CM}$ cells.	49	Fitted.

$K_e = K\frac{\hat{r}_e}{r_e}$	cells/μL	Effective carrying capacity of $CD8^+$ T_{EM} cells.	1257	Fitted.
β	μL/ copies/day	Infectivity rate.	0.0003	Fitted.
Δ_t	days	Time to rebound after ATI.	7.5	Fitted.
δ_I	1/day	Death rate of infected $CD4^+CCR5^+$ T cells.	1	Fixed, assumed using estimates from references [74,75].
τ	-	Fraction of infected cell that produce infectious virus.	0.05	Fixed, assumed from reference [54].
$\xi\bar{L}$	cells/μL/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.
π	1/day	Viral production rate.	$5*10^4$	Fixed, assumed using estimates from reference [76].
γ	1/day	Virus clearance rate.	23	Fixed, assumed using estimates from reference [77].
ω_4	μL/cells/day	SHIV-dependent replenishment of	0.19	Fitted.

		$CD4^+CCR5^+$ T cells.		
$\boldsymbol{\omega_8}$	μL/cells/day	SHIV-dependent proliferation rate of $CD8^+$ T cells.	0.002	Fitted.
$\boldsymbol{I_{50}}$	cells/μL	50% maximum value of adaptive infected cells, allows bounded growth.	0.20	Fitted.
$\boldsymbol{f}$	-	Fraction of SHIV-$CD8^+$-responding cells that become SHIV-specific effectors.	0.9	Fixed, assumed from reference [46].
$\boldsymbol{d_h}$	1/day	Death rate of SHIV-specific effector $CD8^+$ T cells.	0.05	Fitted.
$\boldsymbol{\frac{1}{\theta}}$	μL/cells	50% maximum value of SHIV-specific immune cells to block virus production.	1	Fixed.

358

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552

553 ## Figure Legends

554 **Figure 1. Study design and mathematical modeling. A.** Four animals were infected with
555 SHIV, suppressed with cART and underwent TBI/HSPC transplantation without editing of
556 CCR5 (Transplant group). A control group of four animals did not receive TBI or HSPC
557 transplantation. Both groups underwent ATI approximately one year after cART initiation. **B.**
558 Mathematical model for T cell reconstitution. Each circle represents a cell compartment: T
559 represents the HSPCs from the transplant; P, the progenitor cells in bone marrow (BM) and
560 Thymus; S and N, $CD4^+CCR5^+$ and $CD4^+CCR5^-$ T cells, respectively; M and E, the $CD8^+$ T cells
561 with naïve and central memory phenotypes, and effector memory phenotypes, respectively. N
562 cells come from the thymus at a rate λ_f, grow with maximum rate r_n, upregulate CCR5 at rate λ_n,
563 and are cleared at rate d_n. S cells grow with maximum division rate r_s, downregulate CCR5 at a
564 rate λ_s, and are cleared at rate d_s. M cells have thymic input of λ_e, grow with maximum division
565 rate r_m, differentiate to effector memory at rate λ_m, and are cleared at rate d_m. The E compartment
566 grows with maximum division rate r_e and is cleared at rate d_e. All $CD4^+$ and $CD8^+$ T cell subsets
567 compete to grow logistically with same carrying capacity K. **C.** Mathematical model for virus
568 dynamics. We adapted the previous model by including the following assumptions. Susceptible
569 cells, S, are infected by the virus, V, at rate β. A fraction τ of the infected cells produce virus, I_p,
570 and the other fraction become unproductively infected, I_u. All infected cells die at rate δ_I. I_P cells
571 arise from activation of latently infected cells at rate ξL, produce virus at a rate π, that is cleared
572 at rate γ. $CD8^+$ M cells proliferate in the presence of infection with rate ω_8 from which a fraction
573 f become SHIV-specific $CD8^+$ effector T cells, E_h, that are removed at a rate d_h. These effector
574 cells reduce virus production or infectivity by $1/(1+\theta E_h)$, or $1/(1+\varphi E_h)$, respectively. Non-

575 susceptible $CD4^+$ T cells upregulate CCR5 in the presence of infection and replenish the
576 susceptible pool with rate ω_4.
577
578 **Figure 2. $CD4^+$ and $CD8^+$ T cell dynamics post-transplantation, pre-ATI. A.** $CD4^+CCR5^+$,
579 **B.** $CD4^+CCR5^-$, and **C.** total $CD8^+$ T cells from animals in the control (blue) and transplant
580 groups (red). **D.** Range of blood $CD4^+$ and $CD8^+$ T cell counts using all data points for the period
581 before ATI in control animals (p-value calculated with a paired t-test for averaged measurements
582 post-transplant). **E.** Distribution of the growth rate estimates of $CD4^+CCR5^+$, $CD4^+CCR5^-$, and
583 $CD8^+$ T cells using all data points from time of transplant until their levels reached set point in
584 transplanted animals (p-value calculated using a paired t-test). We assumed set point as the data
585 point after which the sum of consecutive changes from the moment of transplant in T cell counts
586 was smaller or equal to zero. **F.** Correlation between total $CD8^+$ T cell and $CD8^+$ T_{EM} cell counts
587 for all data points post-transplant in transplanted animals (p-value computed using repeated
588 measures correlation test).
589
590 **Figure 3. Mathematical modeling of T cell reconstitution dynamics. A.** Mathematical model
591 that most parsimoniously explains the T cell reconstitution data. Each circle represents a cell
592 compartment: *T* represents the HSPCs from the transplant; *P*, the progenitor cells in bone
593 marrow (BM) and Thymus; *S* and *N*, $CD4^+CCR5^+$ and $CD4^+CCR5^-$ T cells, respectively; *M* and
594 *E*, the $CD8^+$ T cells with naïve and central memory phenotypes, and effector memory
595 phenotypes, respectively. **B-E:** Model predictions (black solid lines) vs. empirical data (red
596 diamonds) for peripheral subset counts from animal A11200 in the transplant group. **B.**
597 $CD4^+CCR5^+$, **C.** $CD4^+CCR5^-$, **D.** $CD8^+$ Total, **E.** $CD8^+$ T_{EM} and T_{naive}+T_{CM}. F. Model prediction

598 of the proliferation of $CD4^+CCR5^+$ T cells (solid line) and upregulation of CCR5 (dashed line)
599 over time for animal A11200.
600
601 **Figure 4. Plasma viral load and $CD4^+$ T cell kinetics after ATI. A.** Plasma viral loads, **B.**
602 peripheral blood $CD4^+CCR5^+$ T-cell counts, and **C.** $CD4^+CCR5^-$ T-cell counts from animals in
603 the control (blue) and transplant (red) groups, respectively. **D-G**: Distributions of **D.** peak viral
604 load post-ATI, **E.** viral load at endpoint necropsy, **F.** $CD4^+CCR5^+$ T-cell nadir post-ATI, **G.**
605 $CD4^+CCR5^-$ T-cell nadir post-ATI. P-values were calculated using Mann-Whitney test. **H.**
606 Correlations between plasma viral load and peripheral blood $CD4^+CCR5^+$ T-cells in each animal
607 post-ATI. Each panel shows the timepoints post-ATI for each animal. P-values in each panel
608 were calculated using Spearman's rank test for all time points post-ATI for the corresponding
609 animal.
610
611 **Figure 5. Mathematical modeling of virus and T cell kinetics during HSPC transplantation.**
612 **A.** Mathematical model that most parsimoniously describes the relationship between plasma viral
613 load and peripheral T-cell counts. $CD4^+CCR5^+$ T cells, S, are susceptible to the virus, V. Infected
614 cells are divided into those that are able to produce virus, I_p, or not, I_u. Precursor $CD8^+$ cells, M,
615 divide and differentiate in the presence of infected cells becoming SHIV-specific effector cells,
616 E_h. E_h cells reduce virus production by a factor of $1/(1+\theta E_h)$. The model assumes a constant
617 activation of latent cells, $\bar{L}$. **B.** Best fits of the model (black lines) to SHIV RNA, and blood
618 $CD4^+CCR5^+$ and $CD4^+CCR5^-$ T cell counts. Scatterplots of the SHIV-dependent CD8
619 proliferation rate (ω_8) vs. **C.** $CD4^+CCR5^+$ nadir, and **D.** final observed viral load from all
620 animals; (p-values calculated using Spearman's rank test). **E-F:** Individual parameter estimates

621 of **E.** the SHIV-dependent CD8 proliferation rate (ω_8) and **F.** the time of rebound after ATI (see
622 text). Blue: control, and red: transplant groups (p-values calculated by Mann-Whitney test).
623
624 **Figure 6. Model predictions for post-rebound viral control after CCR5 gene-edited HSPC**
625 **transplant. A.** Schematic of the extended mathematical model that now includes CCR5-edited,
626 protected cells. Now, protected cells from transplant: T_p, protected progenitor cells in bone
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633 cells required to obtain post-rebound control after 2 years using parameter estimates for each
634 animal with (**G.**) and without (**H.**) measurement error.
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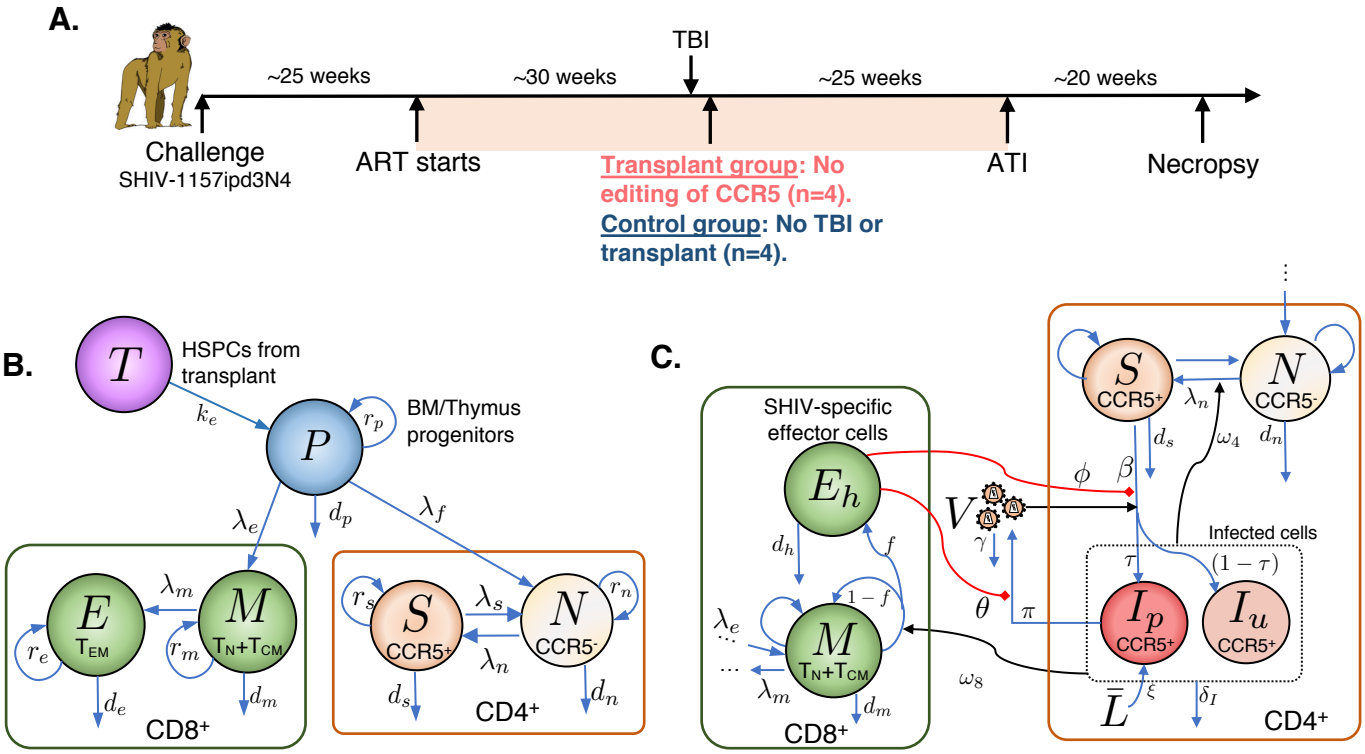

Figure 1. Study design and mathematical modeling. A. Four animals were infected with SHIV, suppressed with cART and underwent TBI/HSPC transplantation without editing of CCR5 (Transplant group). A control group of four animals did not receive TBI or HSPC transplantation. Both groups underwent ATI approximately one year after cART initiation. **B.** Mathematical 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. *N* cells come from the thymus at a rate λ_f, grow with maximum rate r_n, upregulate CCR5 at rate λ_n, and are cleared at rate d_n. *S* cells grow with maximum division rate r_s, downregulate CCR5 at a rate λ_s, and are cleared at rate d_s. *M* cells have thymic input of λ_e, grow with maximum division rate r_m, differentiate to effector memory at rate λ_m, and are cleared at rate d_m. The *E* compartment grows with maximum division rate r_e and is cleared at rate d_e. All CD4$^+$ and CD8$^+$ T cell subsets compete to grow logistically with same carrying capacity *K*. **C.** Mathematical model for virus dynamics. We adapted the previous model by including the following assumptions. Susceptible cells, *S*, are infected by the virus, *V*, at rate β. A fraction τ of the infected cells produce virus, I_p, and the other fraction become unproductively infected, I_u. All infected cells die at rate δ_I. I_P cells arise from activation of latently infected cells at rate ξL, produce virus at a rate π, that is cleared at rate γ. CD8$^+$ *M* cells proliferate in the presence of infection with rate ω_8 from which a fraction f become SHIV-specific CD8$^+$ effector T cells, E_h, that are removed at a rate d_h. These effector cells reduce virus production or infectivity by $1/(1+\theta E_h)$, or $1/(1+\phi E_h)$, respectively. Non-susceptible CD4$^+$ T cells upregulate CCR5 in the presence of infection and replenish the susceptible pool with rate ω_4.

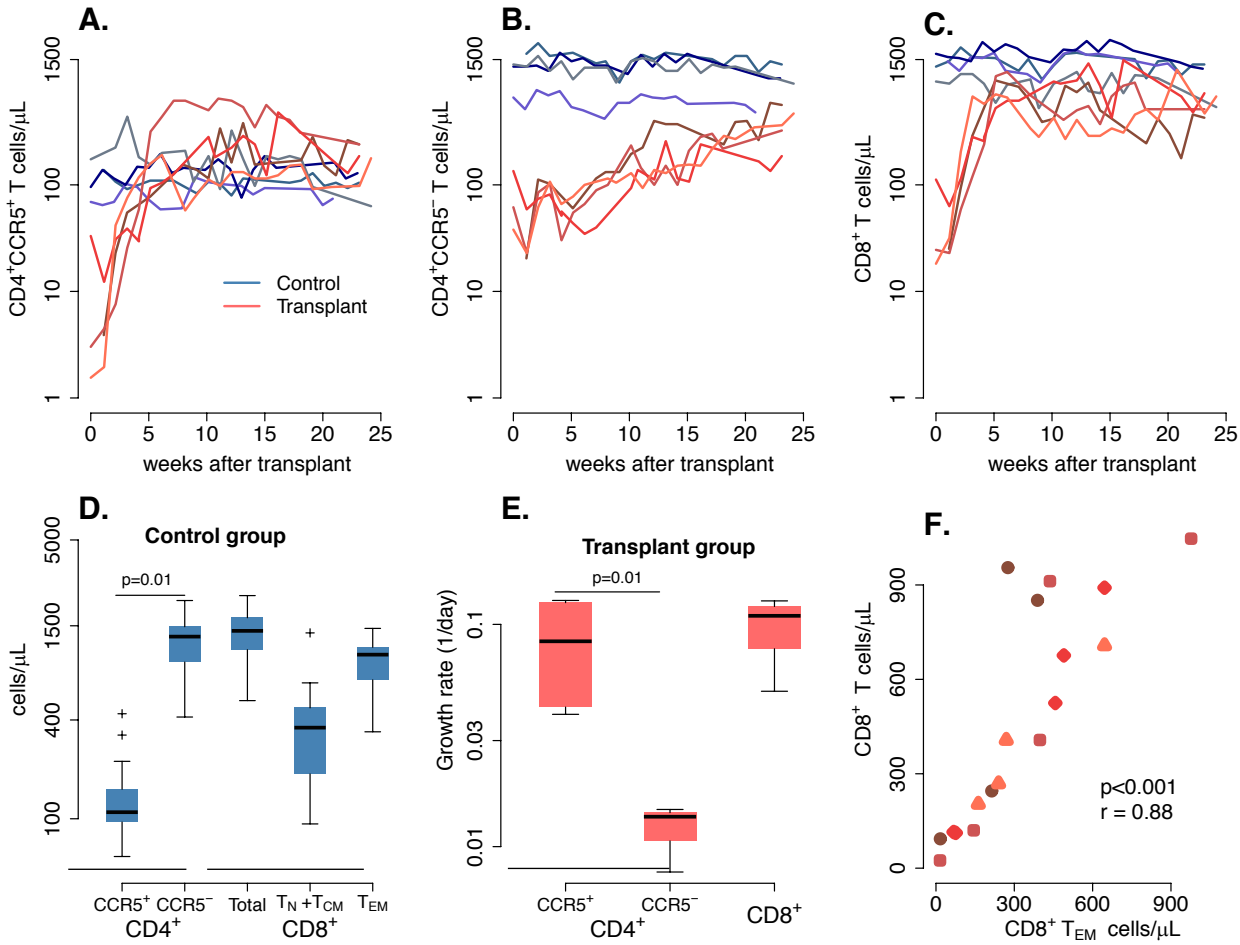

Figure 2. CD4+ and CD8+ T cell dynamics post-transplantation, pre-ATI. A. CD4+CCR5+, **B.** CD4+CCR5-, and **C.** total CD8+ T cells from animals in the control (blue) and transplant groups (red). **D.** Range of blood CD4+ and CD8+ T cell counts using all data points for the period before ATI in control animals (p-value calculated with a paired t-test for averaged measurements post-transplant). **E.** 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. **F.** Correlation between total CD8+ T cell and CD8+ T_{EM} cell counts for all data points post-transplant in transplanted animals (p-value computed using repeated measures correlation test).

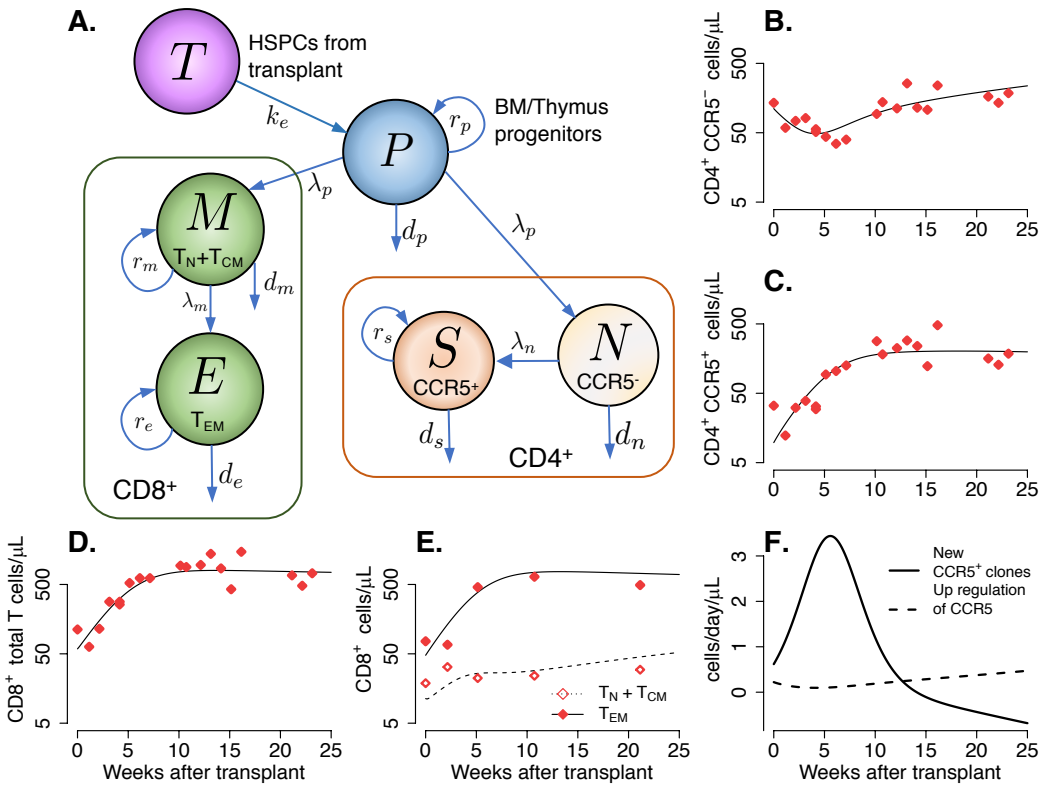

Figure 3. Mathematical modeling of T cell reconstitution dynamics. A. Mathematical model that most parsimoniously explains the T cell reconstitution data. Each circle represents a cell compartment: *T* represents the HSPCs from the transplant; *P*, the progenitor cells in bone marrow (BM) and Thymus; *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. **B-E**: Model predictions (black solid lines) vs. empirical data (red diamonds) for peripheral subset counts from animal A11200 in the transplant group. **B.** CD4+CCR5+, **C.** CD4+CCR5-, **D.** CD8+ Total, **E.** CD8+ T_{EM} and $T_{naive}+T_{CM}$. **F.** Model prediction of the proliferation of CD4+CCR5+ T cells (solid line) and upregulation of CCR5 (dashed line) over time for animal A11200.

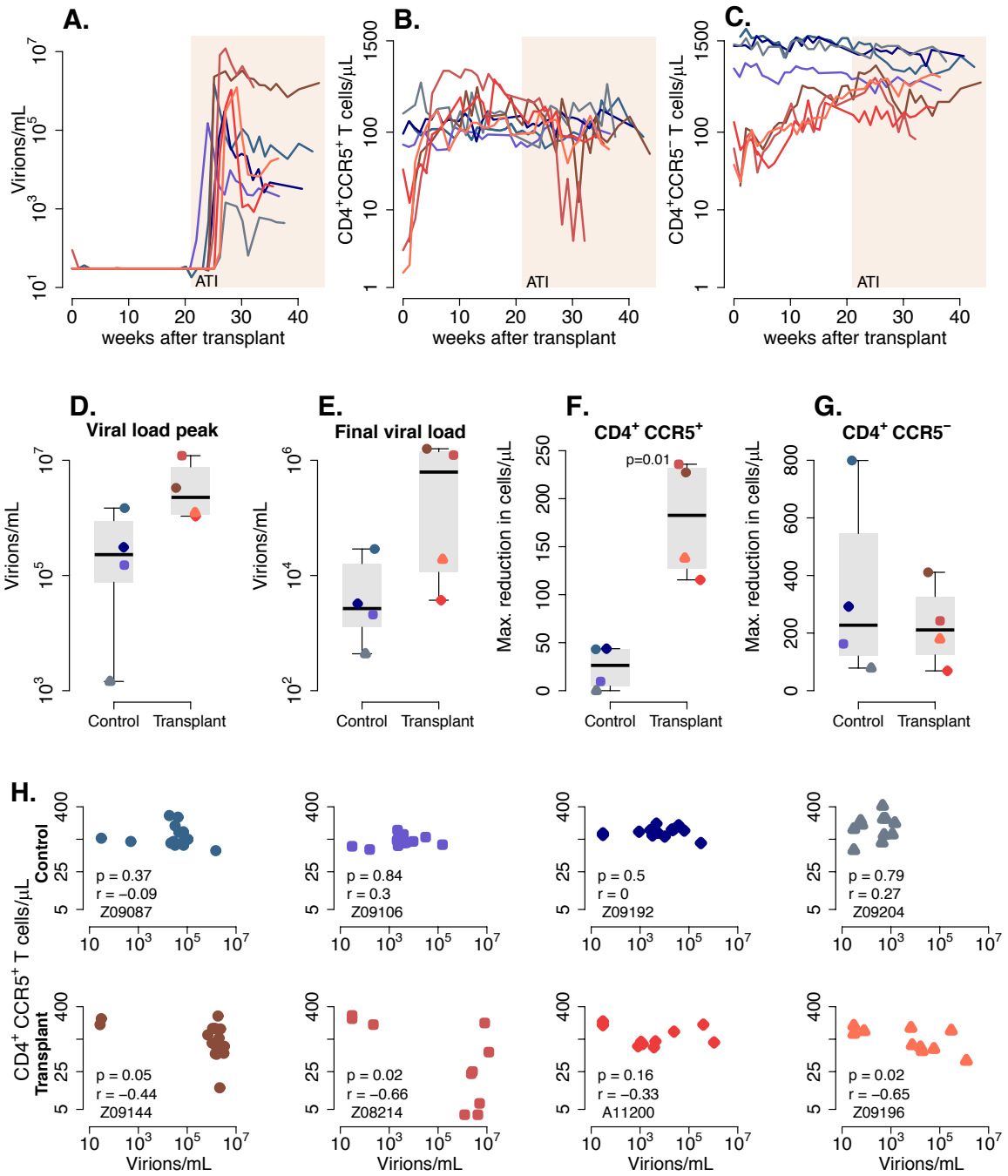

Figure 4. Plasma viral load and CD4+ T cell kinetics after ATI. A. Plasma viral loads, **B.** peripheral blood CD4+CCR5+ T-cell counts, and **C.** CD4+CCR5- T-cell counts from animals in the control (blue) and transplant (red) groups, respectively. **D-G**: Distributions of **D.** peak viral load post-ATI, **E.** viral load at endpoint necropsy, **F.** CD4+CCR5+ T-cell nadir post-ATI, **G.** CD4+CCR5- T-cell nadir post-ATI. P-values were calculated using Mann-Whitney test. **H.** Correlations between plasma viral load and peripheral blood CD4+CCR5+ T-cells in each animal post-ATI. Each panel shows the timepoints post-ATI for each animal. P-values in each panel were calculated using Spearman's rank test for all time points post-ATI for the corresponding animal.

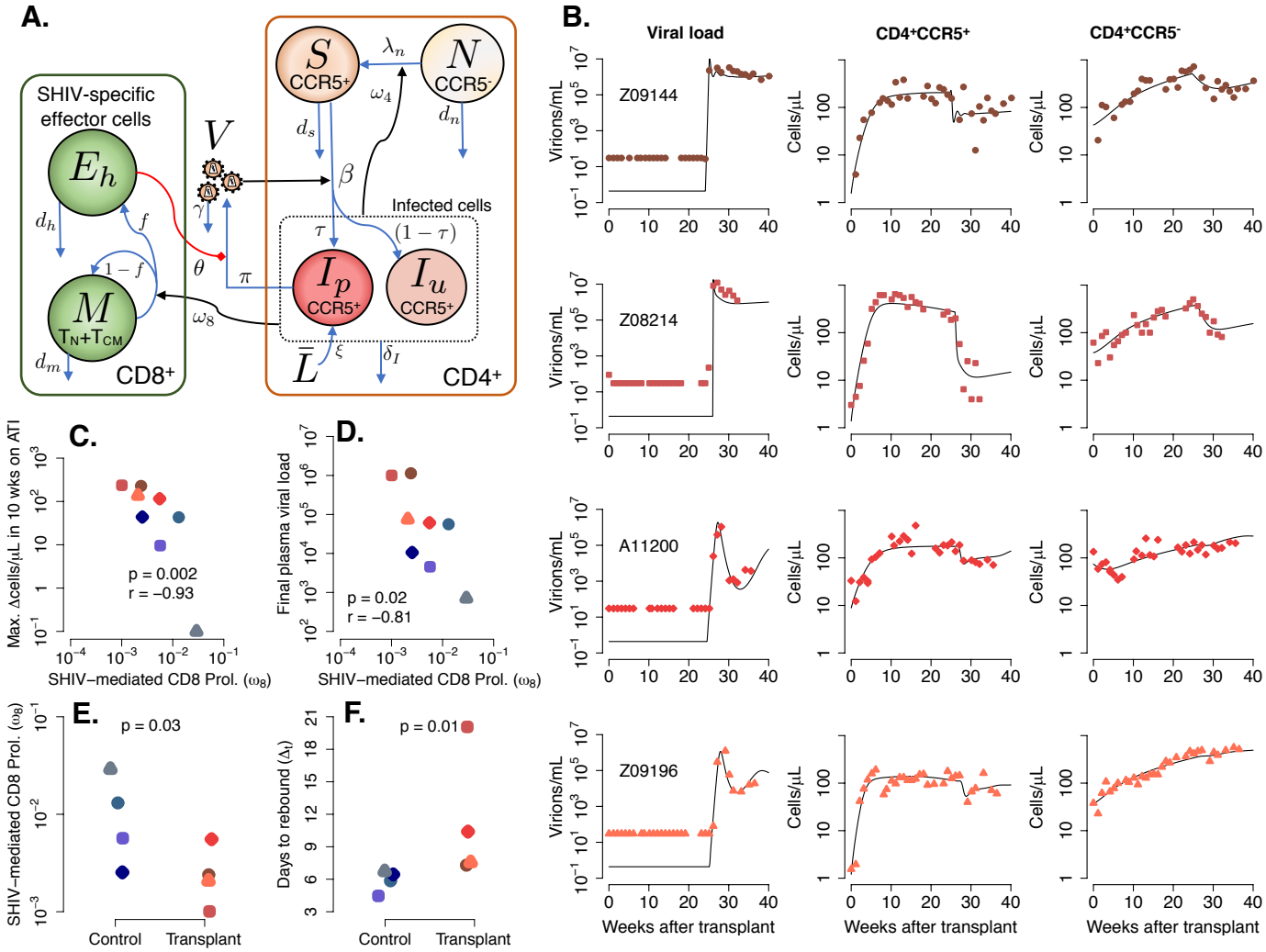

Figure 5. Mathematical modeling of virus and T cell kinetics during HSPC transplantation. A. Mathematical model that most parsimoniously describes the relationship between plasma viral load and peripheral T-cell counts. CD4+CCR5+ T cells, *S*, are susceptible to the virus, *V*. Infected cells are divided into those that are able to produce virus, I_p, or not, I_u. Precursor CD8+ cells, *M*, divide and differentiate in the presence of infected cells becoming SHIV-specific effector cells, E_h. E_h cells reduce virus production by a factor of $1/(1+\theta E_h)$. The model assumes a constant activation of latent cells, $\bar{L}$. **B.** Best fits of the model (black lines) to SHIV RNA, and blood CD4+CCR5+ and CD4+CCR5- T cell counts. Scatterplots of the SHIV-dependent CD8 proliferation rate (ω_8) vs. **C.** CD4+CCR5+ nadir, and **D.** final observed viral load from all animals; (p-values calculated using Spearman's rank test). **E-F**: Individual parameter estimates of **E.** the SHIV-dependent CD8 proliferation rate (ω_8) and **F.** the time of rebound after ATI (see text). Blue: control, and red: transplant groups (p-values calculated by Mann-Whitney test).

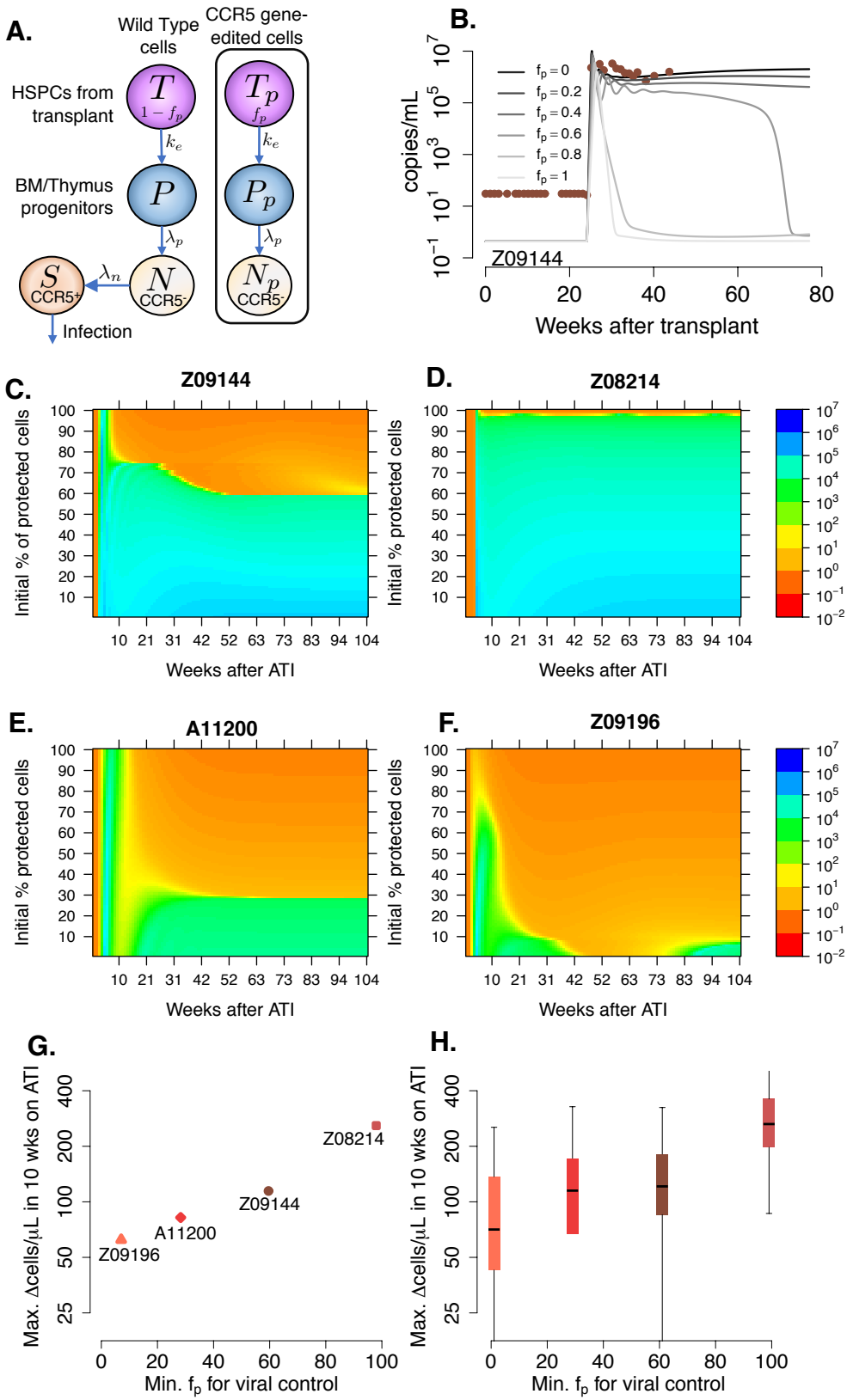
A.
Wild Type cells
CCR5 gene-edited cells
HSPCs from transplant
BM/Thymus progenitors
Infection
CCR5+
CCR5-
B.
copies/mL
Z09144
Weeks after transplant
C.
Z09144
D.
Z08214
E.
A11200
F.
Z09196
Initial % of protected cells
Weeks after ATI
G.
Max. Δcells/μL in 10 wks on ATI
Min. fp for viral control
H.

Figure 6. Model predictions for post-rebound viral control after CCR5 gene-edited HSPC transplant. A. Schematic of the extended mathematical model that now includes CCR5-edited, protected cells. Now, protected cells from transplant: T_p, protected progenitor cells in bone marrow/thymus: P_p, and protected $CD4^+CCR5^-$ T cells: N_p are included. The initial fraction of protected cells is represented by the parameter f_p. **B.** Predictions for plasma viral load up to one year post-ATI using the adapted model for varying values of f_p (using parameter estimates from animal Z09144). **C-F.** 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). **G-H.** 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 with (**G.**) and without (**H.**) measurement error.