CCR5 Gene-Edited Cells Undergo Positive Selection in SHIV-Infected Nonhuman Primates

Christopher W. Peterson[1], Jianbin Wang[2], Patricia Polacino[3], Audrey Baldessari[4], Robert D. Murnane[4], Shiu-Lok Hu[3], Michael C. Holmes[2], and Hans-Peter Kiem[1,3]

[1]Fred Hutchinson Cancer Research Center, Seattle, WA, United States [2]Sangamo Biosciences, Richmond, CA, United States, [3]University of Washington, Seattle, WA, United States, [4]Washington National Primate Research Center, Seattle, WA, United States

Abstract

BACKGROUND: Nuclease-mediated gene editing holds great promise in the cure of HIV infection, but the efficacy of this approach in patients is unclear. We have targeted the CCR5 locus in hematopoietic stem cells (HSCs) and assessed engraftment following autologous transplant in the pigtailed macaque. CCR5 disruption in this model should directly protect against infection with simian/human immunodeficiency virus (SHIV). We are evaluating the extent to which CCR5-disrupted cell progeny impact SHIV infection in naïve animals, and viral reservoirs in previously infected, combination antiretroviral therapy (cART)-suppressed animals.

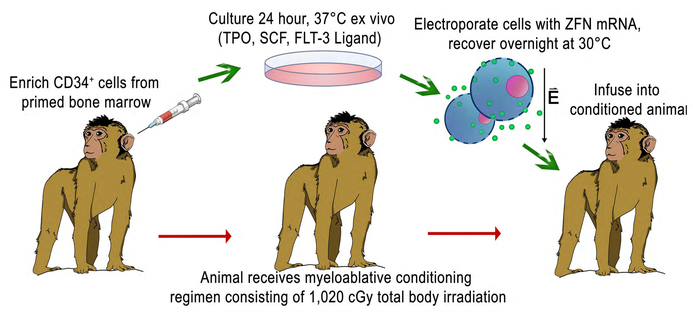
METHODOLOGY: Animals are challenged with CCR5-tropic, HIV enveloped SHIV, and suppressed by three-drug cART following viral set point. Zinc Finger Nucleases (ZFNs) are used to disrupt CCR5 in autologous HSCs; these stem cells and their progeny are subsequently measured ex vivo and in vivo. In previously infected and suppressed animals, assays are conducted to measure the size of the latent SHIV reservoir before and after transplant.

RESULTS: In SHIV-naïve animals, we observe approximately 5% steady state bulk disruption of CCR5 in vivo, which persists following SHIV challenge. This approach is equally feasible in SHIV-infected, cART-suppressed animals. Virus-dependent selection for CCR5-disrupted HSC progeny is observed, namely in memory T-cell subsets. Viral reservoir assays demonstrate that sites of viral persistence are present in cART-suppressed animals, and are impacted by the transplant procedure.

CONCLUSIONS: We described successful long-term, multilineage engraftment of CCR5 gene-edited HSCs in macaques. In addition, we show virus-dependent positive selection of CCR5-edited repopulating cells.

Experimental Design

CCR5 Deletion Gene Therapy in *M. nemestrina*



Zinc Finger Nucleases Efficiently Target the CCR5 Genomic Locus in Macaque CD34+ HSPCs *ex vivo*

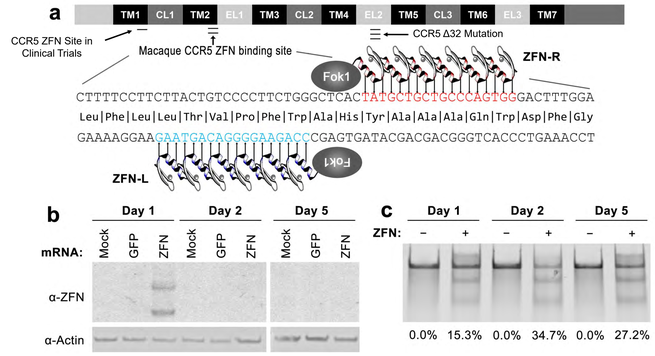


Figure 1: (A) Schematic of the CCR5 locus showing transmembrane (TM), cytoplasmic loop (CL), and extracellular loop (EL) domains, and the ZFN-targeted region. **(B)** At the indicated time points, CCR5 ZFN-electroporated macaque HSPCs were collected and western blots were performed for ZFN and actin protein. **(C)** Representative Cel I nuclease assay demonstrating robust targeting of mCCR5.

CCR5 Edited Macaque HSCs Persist and Undergo SHIV-Dependent Positive Selection *in vivo*

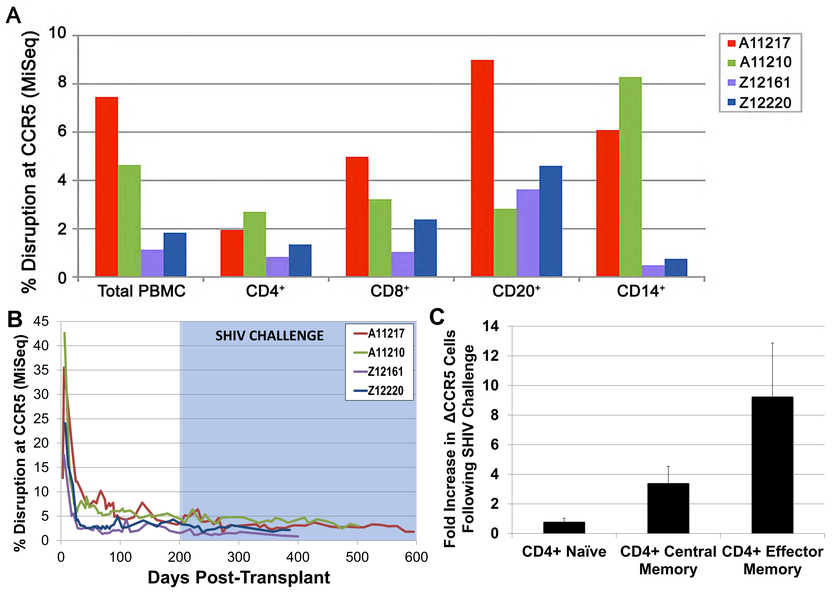


Figure 2: (A) Bead-based positive selection was used to isolate CD4+, CD8+, CD20+, and CD14+ subsets from each animal prior to SHIV challenge. **(B)** Peripheral blood was collected from each animal at the indicated time points post-transplant, and gDNA was isolated from total leukocytes. Gray: post-SHIV challenge. **(C)** Fold increase in CCR5 deleted cells in the indicated subsets following SHIV challenge, relative to pre-SHIV measurements. CCR5 disruption was measured in each sample by Illumina MiSeq.

CCR5 Mutant HSPC "Clones" Can be Tracked Over Time *in vivo*

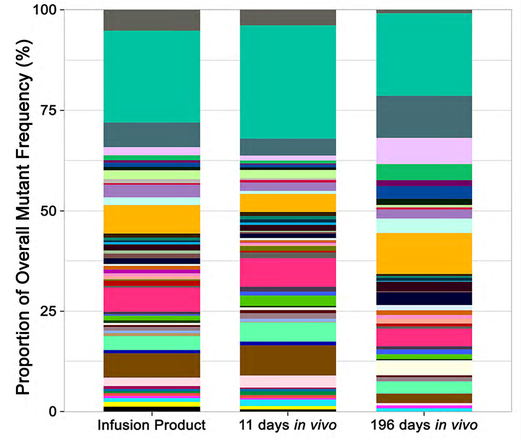


Figure 3: Total genomic DNA was collected from transplanted HSPC products, or from total leukocytes collected 6–12 or 189–198 days following transplantation. Using Illumina MiSeq, CCR5 disruption was measured, and the presence of the indicated CCR5 mutations was tracked over time. The 50 most frequent mutants that were detected in more than one time point are shown in colored bars; frequency is displayed as a proportion of the total pool of mutants that were detected. Top bars (dark gray) represent the sum of mutants that were detected in only one time point ("non-recurrent mutants"). Second bars (teal) represent the sum of recurrent mutants that were not among the 50 most frequent. Data is representative of one of four animals from Figure 2.

Targeted Integration at CCR5 in Macaque HSPCs

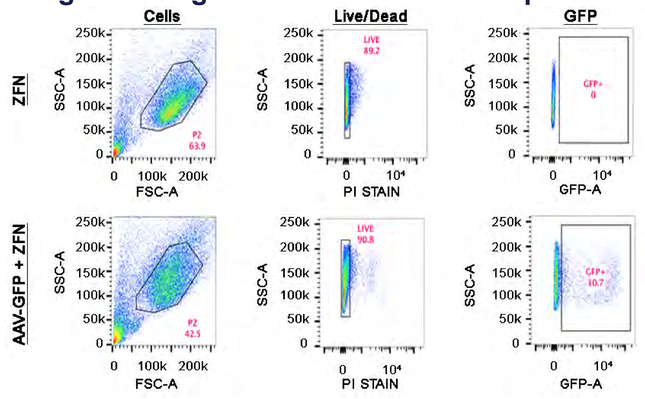


Figure 4: Percentage GFP expression in macaque HSPCs following CCR5 ZFN mRNA electroporation in the presence or absence of a homologous recombination donor template expressing SFFV promoter-driven GFP. Donor template was delivered by adeno-associated virus (AAV-GFP).

Conclusions and Future Plans

- Multilineage engraftment of ΔCCR5 stem cells and SHIV-dependent selection for ΔCCR5 memory T-cell progeny
  - -Immunological correlates of ΔCCR5 engraftment?
- A polyclonal repertoire of of ΔCCR5 is established *in vivo*
  - -Clonal diversity at SHIV reservoir sites, e.g. lymph nodes?
- Macaque HSPCs can be targeted for homologous recombination at the disrupted CCR5 locus
  - -Virus-independent selection for protected cells *in vivo*?