# EFFICIENT DELETION OF CCR5 PROVIDES COMPLETE PROTECTION AGAINST HIV IN XENOGRAFT MICE

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## BACKGROUND

Hematopoietic stem cell transplant (HSCT) with *CCR5*$^{d32/d32}$ defective stem cells has resulted in long-term remission of HIV infection in three patients ("Berlin", "Dusseldorf", and "Oxford") that received allogeneic HSCT for co-occurring malignancies. However, the scarcity of HLA-matched, *CCR5d32* homozygous stem cell donors represents a significant hurdle to more widespread adoption of HSCT for treatment of HIV infection. The ability to effectively delete *CCR5* in autologous, mobilized, CD34$^{+}$ hematopoietic stem progenitor cells (mobHSPCs) would overcome this hurdle and provide a path toward an autologous HSCT cure for HIV infection.

## METHODS

Guides were screened for editing efficiency by CRISPR/Cas9 ribonucleoprotein (RNP) nucleofection of primary human T cells **(Figure 1A,B)**. Edited CD4$^{+}$ T cells were then stimulated and challenged with R5-tropic HIV **(Figure 1C)**. A dual guide approach engendered the highest level of *CCR5* editing and complete protection from high titer HIV challenge *in vitro* and was selected for HSCPC editing and transplant.

**Figure 1A: Guide screen in vitro**

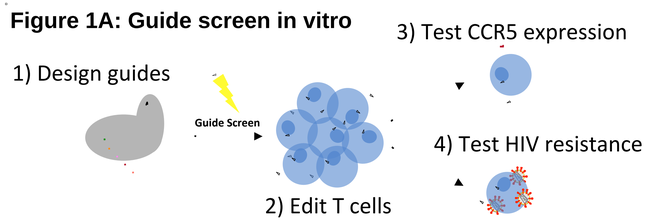


**Figure 1B: Highly efficient ablation of CCR5 in T cells**

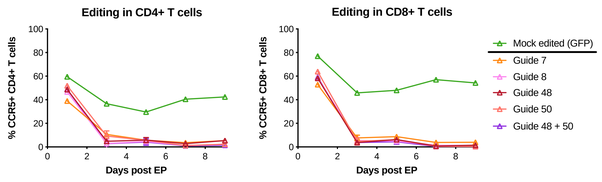


**Figure 1C: CCR5 ablation results in HIV resistance**

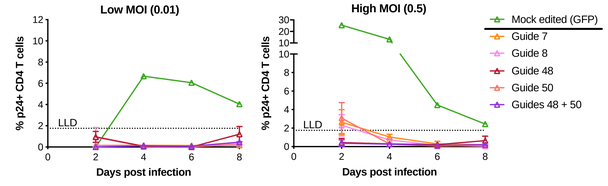


**Figure 2: Experimental Design**

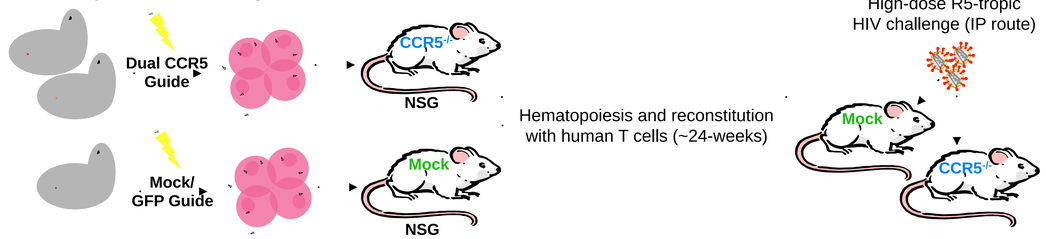


**Figure 2. CRISPR/Cas9-mediated editing of adult mobilized HSCs and engraftment in NSG mice.** Adult mobilized HSCs from a healthy donor were nucleofected with Cas9 complexed to CCR5-specific guides (guide 48 and 50) or Cas9 complexed to an irrelevant guide (mock, guide specific for GFP). HSCs were rested for 2 days after nucleofection and cryopreserved. Female NSG mice were irradiated with 200cGy and given 1 x 10$^{6}$ CCR5-edited or mock-edited HSCs. After 24-weeks of reconstitution, groups of mice were challenged with HIV-JRCSF, a CCR5-tropic HIV isolate.

**Figure 3**

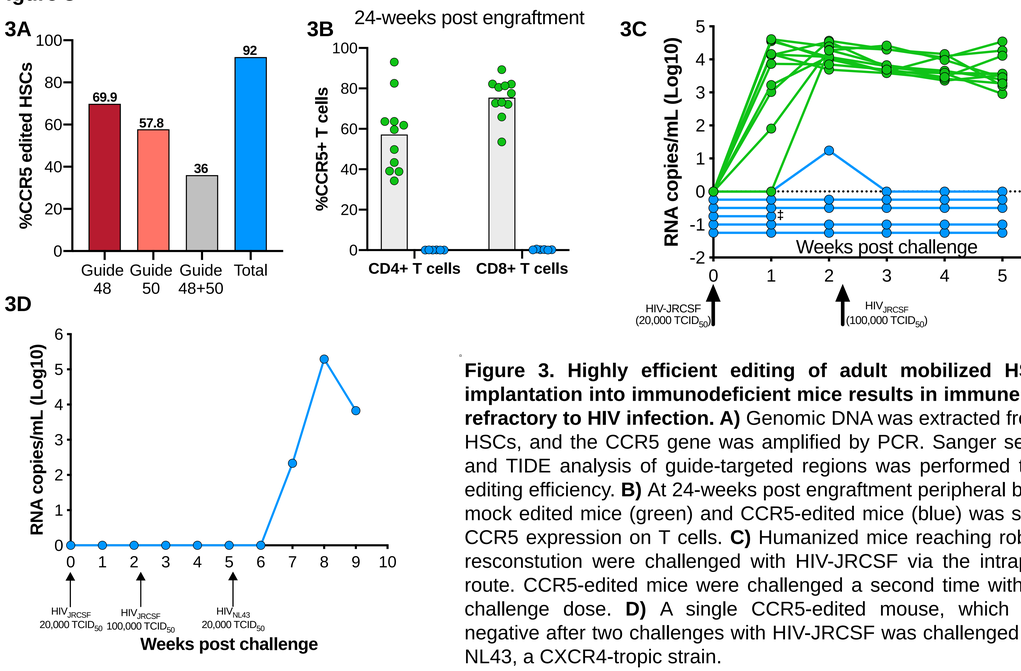


**Figure 3. Highly efficient editing of adult mobilized HSCs and implantation into immunodeficient mice results in immune systems refractory to HIV infection. A)** Genomic DNA was extracted from edited HSCs, and the CCR5 gene was amplified by PCR. Sanger sequencing and TIDE analysis of guide-targeted regions was performed to assess editing efficiency. **B)** At 24-weeks post engraftment peripheral blood from mock edited mice (green) and CCR5-edited mice (blue) was stained for CCR5 expression on T cells. **C)** Humanized mice reaching robust T cel resconstution were challenged with HIV-JRCSF via the intraperitoneal route. CCR5-edited mice were challenged a second time with a higher challenge dose. **D)** A single CCR5-edited mouse, which remained negative after two challenges with HIV-JRCSF was challenged with HIV-NL43, a CXCR4-tropic strain.

## RESULTS

Dual guides achieved a 92% *CCR5* editing frequency in mobHSPCs from an anonymous HIV-negative donor (guide 1: 70%; guide 2: 58%; total: 92%). After transplant into NSG mice, *CCR5*$^{edit}$ HSPCs displayed slightly delayed but otherwise normal hematopoiesis resulting in human immune cell reconstitution with frequencies of human monocytes, B cells, and T cells comparable to the control sham (GFP guide) edited mice. High frequency *CCR5* editing was detected in descendant monocytes, B cells, and T cells (median 89%), and the frequency of circulating T cells expressing CCR5 on the cell surface was <0.25% compared to 57% in the sham edited controls. Importantly, *CCR5*$^{edit}$ mice were completely refractory to challenge with an $ID_{100}$ of a CCR5-tropic HIV (0/5 *CCR5*$^{edit}$ mice infected) that infected 8/8 control mice. *CCR5*$^{edit}$ mice further resisted a challenge dose of 50x $ID_{100}$. In contrast, subsequent intraperitoneal challenge of a *CCR5*$^{edit}$ mouse with a CXCR4-tropic HIV strain resulted in robust infection and plasma viremia confirming CCR5-specific protection.

## RESULTS

**Figure 4**

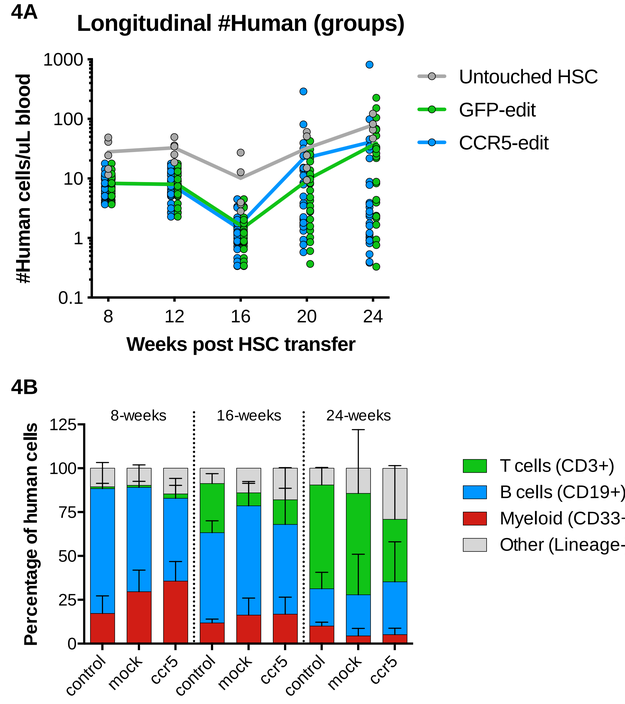


**Figure 4. Efficient engraftment intact hematopoiesis of CCR5-edited HSCs in NSG mice. A)** The number of human cells was quantified in the peripheral blood via flow cytometric analysis in NSG mice reconstituted with untouched HSCs (no culture or nucleofection; gray), mock-edited HSCs (irrelevant GFP guide; green), or CCR5-edited HSCs (dual guide approach; blue). B) Human cell lineages were identified via flow cytometric analysis of peripheral blood and are depicted as the average percentage of T cells (CD3+), B cells (CD19+), or myeloid cells (CD33+) in each of the 3 experimental groups.

## CONCLUSIONS

- A dual guide approach achieves highly efficient editing (>90%) of human T cells and HSCs
- CCR5-edited HSCs engraftment efficiently in NSG mice, displaying intact hematopoiesis
- CCR5-edited HSCs generate immune systems in humanized mice that are completely refractory to HIV infection
- Protection from HIV infection was CCR5 specific, as mice displayed robust infection with a CXCR4-tropic strain