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Dual Guide CRISPR/Cas9 Editing of the CCR5 Gene Provides Complete Protection Against HIV in Humanized Mouse Models

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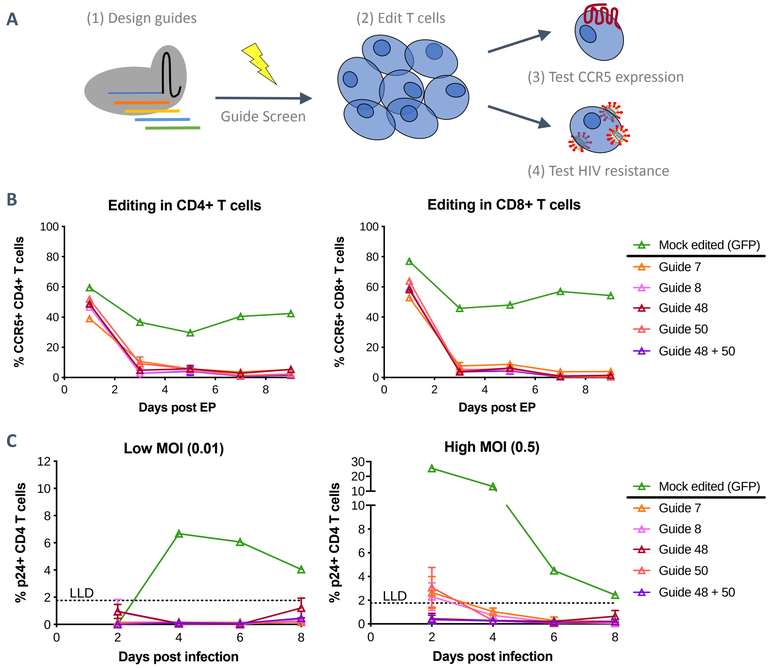
Abstract

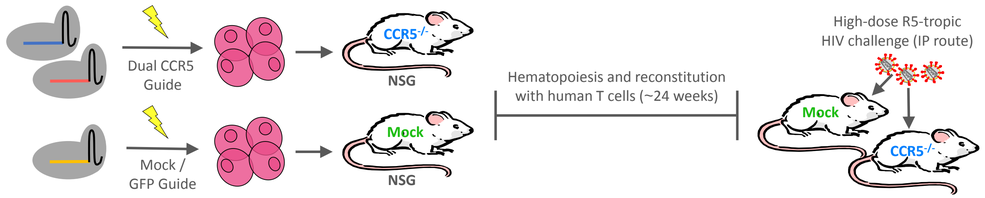
Hematopoietic stem cell transplant (HSCT) with CCR5d32/d32 defective stem cells has resulted in long-term remission of HIV infection in three patients ("Berlin", "Dusseldorf", and "Oxford") that received allogeneic HSCT for co-occurring malignancies. However, the scarcity of HLA-matched, CCR5d32 homozygous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

Figure 1: *In vitro* guide screen identifies guides that protect T cells from R5-tropic HIV



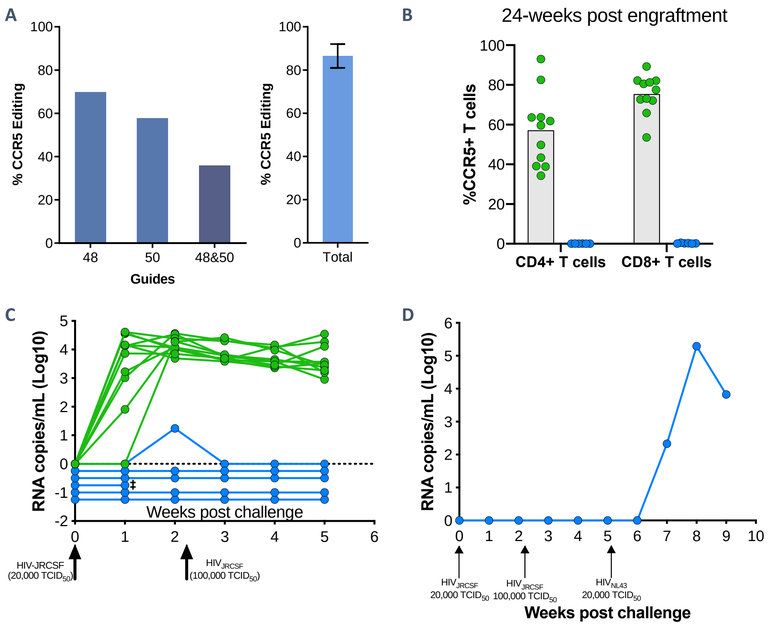
(A) Human T cells were nucleofected with SpCas9 complexed to one of four CCR5-specific guides. Guides 48 and 50 were also nucleofected together with SpCas9 in a dual guide approach. A mock edited control was included using SpCas9 complexed to a non-targeting guide (GFP). **(B)** T cells were stained for CCR5 and analyzed via flow cytometry. CCR5 edited T cells expressed little CCR5 by day 3, while mock edited T cells maintained 40-60% CCR5 expression through 9 days. **(C)** CCR5 edited T cells were then stimulated and challenged with R5-tropic HIV. CCR5 edited T cells were protected from the HIV, while the mock edited T cells became infected. The dual guide approach resulted in the highest level of CCR5 gene editing and complete protection from high titer HIV challenge *in vitro*.

Figure 2: Schematic of CRISPR/Cas9-mediated editing of adult mobilized HSPCs and engraftment in NSG mice



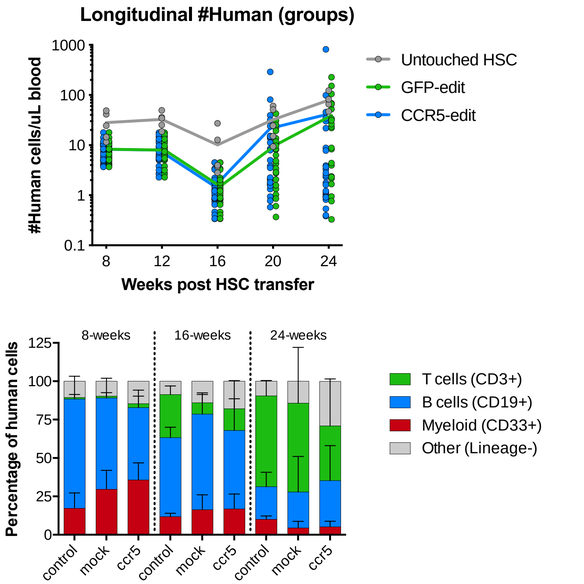
Adult mobilized HSPCs from a healthy donor were nucleofected with SpCas9 complexed to CCR5-specific guides (Guide 48 and 50) or SpCas9 complexed to a non-targeting guide (GFP) for a mock edited control. HSPCs were rested for 2 days after nucleofection and cryopreserved. Female NSG mice were irradiated with 200cGy and given 1x10^6 CCR5-edited or mock edited HSPCs. After 24 weeks of reconstitution, groups of mice were challenged with HIV-JRCSF, a CCR5-tropic HIV isolate.

Figure 3: Highly edited HSPCs engrafted into NSG mice results in immune systems refractory to HIV infection



(A) Genomic DNA from edited HSPCs was analyzed for small insertions and deletions via Sanger Sequencing analysis and for large deletions via ddPCR analysis. The dual guides achieved high CCR5 gene editing in HSPCs from an anonymous HIV-negative donor (Guide 48: 70%; Guide 50: 58%; Deletion: 36%; Total: 81-92%). **(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. The frequency of circulating T cells expressing CCR5 on the cell surface was <0.25% compared to 57% in the mock edited controls. **(C)** Humanized mice reaching robust T cell reconstitution were challenged with HIV-JRCSF via the intraperitoneal route. CCR5 edited mice were challenged a second time with a higher challenge dose. CCR5 edited mice were completely resistant to an infection challenge with an ID100 of a CCR5-tropic HIV (0/5 CCR5 edited mice infected), which was able to infect all 8 mock edited control mice. **(D)** A single CCR5 edited mouse, which remained negative after two challenges with HIV-JRCSF was challenged with HIV-NL43, a CXCR4-tropic strain. This challenge resulted in a robust infection and plasma viremia confirming CCR5-specific protection.

Figure 4: Edited HSPCs reconstitute normal hematopoiesis 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 HSPCs (no culture; gray), mock edited HSPCs (GFP guide; green), or CCR5 edited HSPCs (dual guide; blue). Transplantation of CCR5 edited HSPCs into NSG mice resulted in normal hematopoiesis with efficient human immune cell reconstitution. **(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. The populations of human monocytes, B cells, and T cells in CCR5 edited mice were comparable to the mock edited control (GFP) mice. High frequency CCR5 gene editing was detected in descendant monocytes, B cells, and T cells (median 89%) (data not shown).

Summary & Conclusion

- A dual guide approach achieves highly efficient editing (>81%) of human T cells and HSPCs
- CCR5 edited HSPCs engraft efficiently in NSG mice, displaying an intact hematopoiesis
- CCR5 edited HSPCs 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