Substantial progress has been made in the treatment of HIV/AIDS, but at present, there is still no effective HIV vaccine, nor is there a therapeutic strategy that achieves eradication of HIV from infected individuals. Novel therapeutic approaches are needed that enable full control of HIV infection and restoration of the HIV-damaged immune system. The first case of HIV cure was achieved by nearly complete hematopoietic reconstitution with CCR5-deficient transplanted donor cells. However, it is unlikely that histocompatible donors with naturally occurring CCR5 mutations can be identified for all HIV patients. As a more generally applicable approach with curative potential, we seek to employ stem cell-based gene therapy, in which anti-HIV genes are introduced into autologous hematopoietic stem/progenitor cells (HSPC), to produce immune cells that are resistant to HIV infection. However, studies to date have met with limited success, largely due to the limited efficiency of current methodologies for gene delivery into HSPC, and reduced engraftment and hematopoietic reconstitution by anti-HIV gene modified cells. Here we propose that improved outcomes with anti-HIV gene therapy strategies can be achieved by the development of procedures for selective expansion of genetically-modified HSPC after transplantation. We have developed a novel strategy to selectively amplify HSPC that have been genetically engineered for dual knockout of CCR5 and the purine salvage enzyme hypoxanthine-phosphoribosyltransferase (HPRT). Our preliminary results show that HPRT-knockout HSPC are resistant to the cytotoxic effects of a commonly used purine analog drug, 6-thioguanine (6TG). In the proposed studies, genome editing strategies will be optimized for dual knockout of CCR5 and HPRT in human HSPC, and the efficiency of 6TG chemoselection to expand genetically modified HSPC will be evaluated both in vitro (Aim 1) and in murine models of humanized hematopoiesis in vivo (Aim 2).

### Background

- Hematopoietic stem/progenitor cell (HSPC)-based gene therapy approaches hold great promise for potential cure of HIV with a single treatment
- Several anti-HIV reagents have been developed by targeting chemokine receptor 5 (CCR5):
  - Ribozymes
  - Trans-dominant mutant forms of CCR5
  - Intrabodies
  - Intracellular chemokines ("intrakines")
  - Short hairpin RNA (shRNA)
  - Zinc finger nucleases (ZFNs)

### Problem:

- Limited efficiency for gene delivery into CD34+ HSPCs
- Reduced engraftment by anti-HIV gene modified CD34+ HSPCs

> Efficiency of engraftment needs to be improved and combined with more effective procedures that allow selective expansion of genetically modified HIV-resistant cells in vivo

### Methods

#### Comparison of two different methods to achieve dual disruption of the CCR5 and HPRT gene loci in HSPCs

##### Delivery of HPRT and CCR5 ZFNs

- Optimization of reagents and techniques to achieve dual disruption of the CCR5 and HPRT gene loci in human CD34+ HSPCs, and evaluation of the phenotypic and functional effects of dual gene disruption on 6TG chemoresistance in vitro

##### Assay for ZFN-driven gene disruption

- Investigation of the efficiency of this approach for expansion of genetically modified cells by in vivo chemoselection of CCR5/HPRT-deficient HSPC after transplantation in the NSG mouse model, and evaluation of safety by monitoring for any potential adverse effects of CCR5/HPRT dual gene disruption on hematopoietic development.

### Results

#### CD34+ HSPC treated with HPRT ZFNs can be efficiently selected with 6TG in vitro

- ZFNs targeting the human HPRT locus for gene disruption. GFP control plasmid alone, or ZFNs were transiently transfected into human CD34+ HSPCs. CD34+ cells were cultured with 6 µM 6TG for 12 days. The percentage of HPRT gene disrupted by ZFNs was measured by performing PCR across the ZFN target site followed by digestion with the Cel I nuclease (Surveyor assay).

#### K562 cells co-transfected with both CCR5 and HPRT ZFNs can be efficiently selected with 6TG in vitro

- ZFN-mediated dual disruption of both HPRT and CCR5 gene loci. GFP control plasmid alone, or a combination of HPRT- and CCR5-targeted ZFNs, were transiently transfected into human K562 cells. The percentage of HPRT and CCR5 gene disruption was evaluated by Surveyor assay and TOPO cloning.