Increased Engraftment of Gene Modified HSPCs Overexpressing ALDH1 in vivo
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INTRODUCTION
- Hematopoietic stem-cell transplantation (HSCT) has been studied extensively for a variety of diseases including HIV.
- Two persons living with HIV (PLWH), who also developed hematologic cancers, have been cured by allogeneic HSCT from donors with a genetic mutation that prevents expression of a key co-receptor for HIV: C–C chemokine receptor type 5 (CCR5).
- However, allogeneic HSCT carries a high rate of morbidity and mortality and is not feasible for widespread applications.
- Aldehyde dehydrogenase-1 (ALDH1), a naturally occurring enzyme in human stem/progenitor cells (HSPCs), is known to confer enhanced cellular resistance to cytotoxic agents, including cyclophosphamide (CY).
- We hypothesized that low-dose CY could potentially increase engraftment of HSPCs genetically modified to overexpress ALDH1.

MECHANISM OF ACTION
- Overexpression of the naturally occurring enzyme ALDH1 confers increased resistance to CY.
- Pre-conditioning with fludarabine opens space in the bone marrow and reduces rejection of transplanted cells.
- In syngeneic mice transplanted with cells modified to overexpress ALDH1, low dose CY treatment could promote chemoselection and potentially result in increased engraftment (Figure 1).

METHODS
- Syngeneic mouse (C57/Black6) stem/progenitor cells were transduced with either Enochian Lentivirus 800 (LV-800), overexpressing ALDH1 under EF1a promoter or Enochian control Lentivirus vector 802 (LV-802) expressing knockdown of CCR5 (shRNA-CCR5), as well as, C44, a C-peptide inhibiting HIV fusion (Figure 2).

RESULTS
- Percentage of peripheral blood granulocytes overexpressing ALDH1 increased from week 7 through 12 for all doses but was highest at 16mg/kg (95.2%) and 19mg/kg (93.5%) (Figure 5A).
- ALDH1 expression increased in absolute number of granulocytes compared to control (blue bars) at all dose levels (1252-4976 cells; orange bars) but was highest at 16 mg/kg (Figure 5B).
- At the end of study, average VCN in bone marrow cells was highest (0.13) at 16mg/kg CY (group 4A; Figure 6A).
- VCN increased 1.47 fold (147%) with 13 mg/kg CY and 1.64 fold (164%) with 16 mg/kg CY in bone marrow cells modified to overexpress ALDH1 (Figure 6B).

DISCUSSION
- ALDH1 overexpression promotes increased engraftment of HSPCs in vivo when combined with low dose cyclophosphamide chemoselection.
- This effect is dose dependent, as exhibited by 95% of gene-modified peripheral granulocytes overexpressing ALDH1 and a 164% increase in relative bone marrow VCN with 16mg/kg of CY.
- Combining ALDH1 overexpression with other genetic modifications to protect cells from HIV infection could be an important strategy to treat or cure HIV.
- Additional in vivo efficacy and safety studies are currently in progress.

CONCLUSIONS
- We demonstrate an 164% increase in engraftment of HSPCs overexpressing ALDH1 in vivo with low dose cyclophosphamide chemoselection.
- This could be used as a strategy to improve engraftment of gene-modified stem cells in non-myeloablative autologous bone marrow transplants for a wide variety of diseases, including potentially, as an approach to cure HIV.