Increased Engraftment of Gene Modified HSPCs Overexpressing ALDH1 *in vivo*

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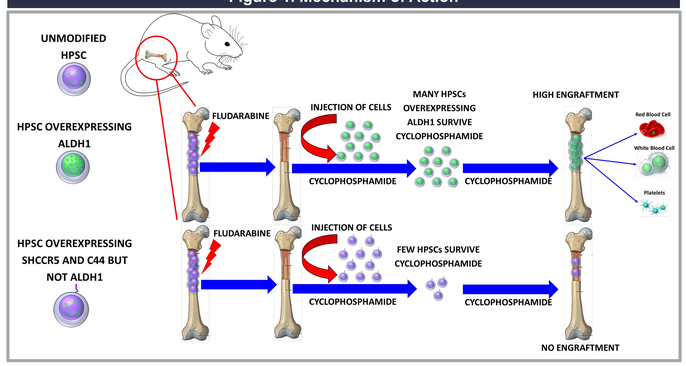
INTRODUCTION

- 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)**.

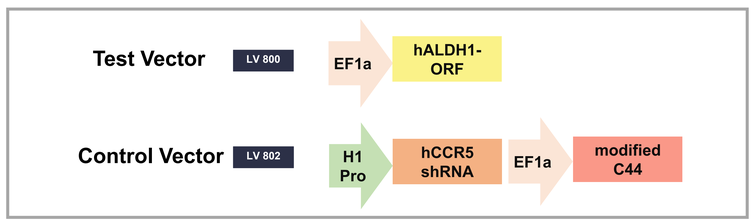
Figure 1. Mechanism of Action



METHODS

- Syngeneic mouse (C57/Black6) stem/progenitor cells were transduced with either Enochian Lentivirus 800 (LV-800), overexpressing ALDH1 under EF1a promotor 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).**

Figure 2. Lentiviral Vectors

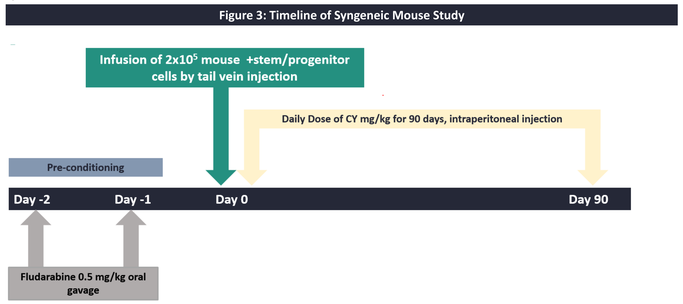


- Mice received fludarabine (0.5 mg/kg oral gavage) preconditioning for two days (day -2 and day -1) **(Figure 3)**.

METHODS

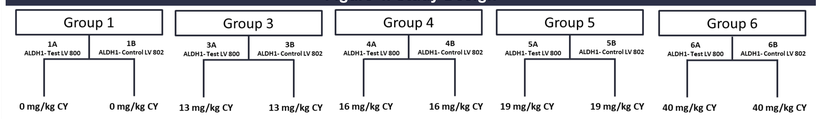
- On day 0, mice were Infused with of 200,000 mouse +stem/progenitor cells by tail vein **(Figure 3)**.

Figure 3: Timeline of Syngeneic Mouse Study



- Mice were divided into groups and treated with once-daily intraperitoneal CY at doses of 0,13, 16,19 or 40mg/kg **(Figure 4)**.

Figure 4. Study Design



- From Week 7 through Week 12 blood samples were collected from each mouse by retro-orbital bleeding, followed by lysis with ammonium chloride and staining with ALDEFLOUR™ (StemCell) for flow cytometry to assess ALDH1 activity. Hierarchical gating was used for the granulocyte population, then all singlets, then all viable cells, then ALDH1^{hi} cells.
- At week 12, the mice were sacrificed by cardiac puncture, and the blood was lyzed with ammonium chloride followed by assessment of: (1) transduction efficiency by qPCR for vector copy number (VCN)[for VCN analysis gDNA was extracted then for analysis using the Lenti-X™ Provirus Quantitation kit (Takara) per manufacturer's and Enochian-developed protocols],(2) the presence of the granulocyte marker Gr-1 using an APC-conjugate anti-Gr-1 antibody by flow cytometry, and (3) ALDH1 activity by ALDEFLOUR flow cytometry staining. For flow cytometry at end of study we employed hierarchical gating for the granulocyte population, then all singlets, then all viable cells (7-AAD negative cells), then Gr-1 positive granulocytes, then ALDEFLOUR positive cells.
- To calculate the change in counts in Gr-1 positive-ALDELOUR positive cells, we used the N,N-diethylaminobenzaldehyde (DEAB) (which inhibits ALDH1 activity) as an internal control in both Test and Control groups. To obtain the average change in the counts across the CY treatments in the Test samples the following formula was used: **[Test non-DEAB] – [Test DEAB] = Δ in counts**. We then conducted a similar analysis on the Control samples across CY treatments.

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)**.

Figure 5. Increased Percent of Granulocytes Overexpressing ALDH1 at Varying Doses of CY

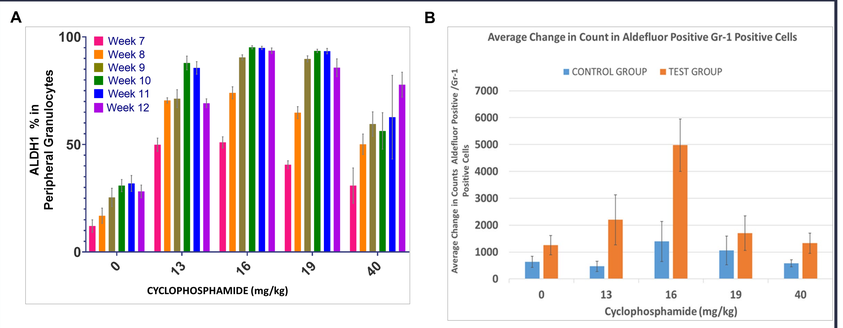


Figure 5: ALDH1 Percent Increase (ALDEFLOUR) in Mouse Peripheral Blood Granulocytes at Various Doses of Cyclophosphamide
(A) Evaluation of ALDH1 percentage (as measured by ALDEFLOUR flow cytometry) in granulocytes from the peripheral blood of mice transplanted with HSPCs transduced with VL800 to overexpress at varying doses of cyclophosphamide from week 7 through week 12. Error bars indicate standard error (n= 6 or 12 mice depending on the groups). **(B)** Evaluation of Change in the Counts in ALDEFLOUR Positive/Gr-1 Positive Granulocytes from peripheral blood at end of study in control mice (blue bar) and test mice (orange bar).

Figure 6. Increased Engraftment of Bone Marrow Cells Genetically Modified to Overexpress ALDH1 in Response to Cyclophosphamide Treatment in a Does-dependent Manner

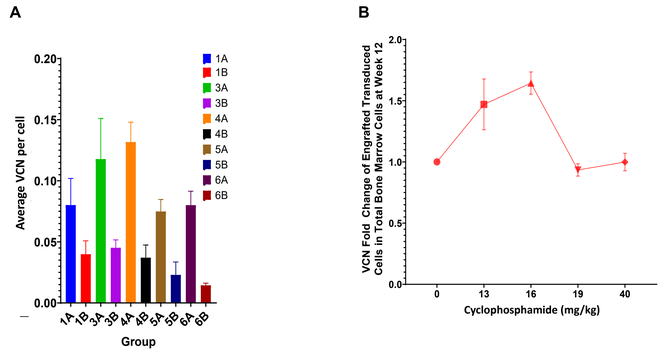


Figure 6. VCN Analysis of Mouse Bone Marrow at Week 12 End of Study.
(A) End of study VCN of BM of Mice Transplanted with HSPCs Transduced with LV800 to overexpress ALDH1 and LV802 (no ALDH1) for each treatment group. Error bars indicate standard error (n= 6 or 12 mice depending on the groups). **(B)** The fold change VCN for each treatment group. Error bars indicate standard error (6 or 12 mice, depending on group).

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/d 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 HPSCs 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.