

Introduction

- Engraftment remains a critical challenge for hematopoietic stem cell (HSC) transplant.
- Genetic modification of HSC to improve engraftment with non-myeloablative cytotoxic treatment regimens could substantially improve engraftment.
- Aldehyde dehydrogenase-1 (ALDH1) is known to confer enhanced cellular resistance to cytotoxic agents, including cyclophosphamide (CY).
- Certain diseases, e.g. HIV, have known genetic modifications that can be curative.
- Therefore, infusion of HSC with genetic modifications known to be curative and to increase expression of ALDH1 combined with low-dose CY could significantly increase engraftment and treatment success.

Mechanism of Action

- Without the ALDH1 enzyme, cells exposed to mafosfamide (MFA), a metabolite of CY that does not require liver enzymes, undergo apoptosis.
- In cells with basal levels of ALDH1 expression, a dose dependent number of cells will survive MFA treatment.
- In cells transduced to overexpress ALDH1, more cells will survive MFA treatment at any dose when compared with wild type cells.

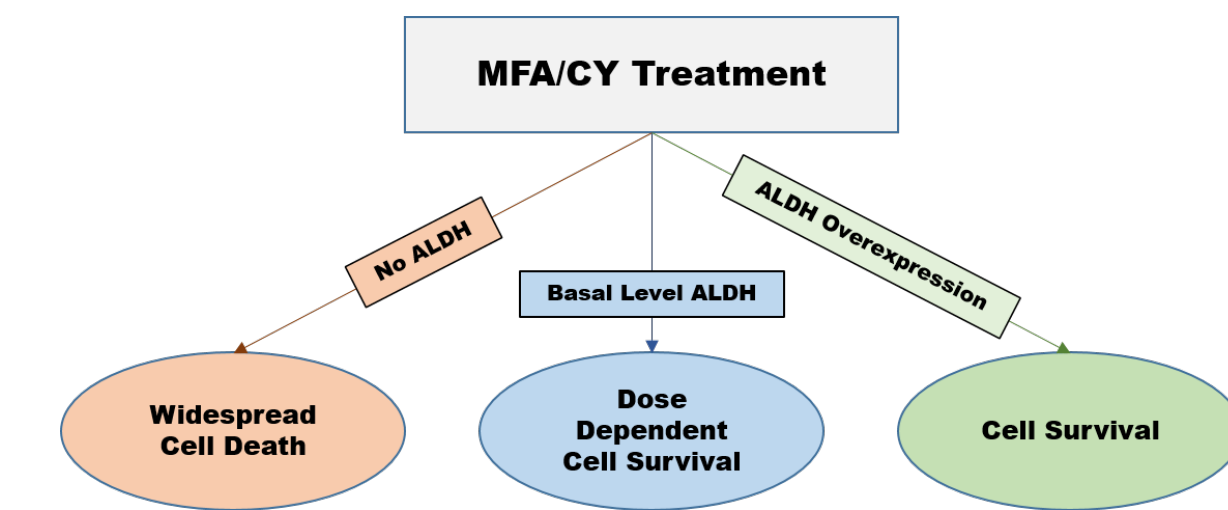


Figure 1. Mechanism of Action

Methods and materials

- CD34+TF1a cell line (ATCC, CRL-2451) and primary huCD34+ cells were transduced with lentiviral vectors overexpressing ALDH1 (LV800), or ALDH1/shRNA-CCR5/C44 (LV801) (Figure 2). shRNA-CCR5 causes a knock-down of CCR5, a key HIV co-receptor, and C44 expresses an HIV fusion inhibitor C-peptide.
- Transduction was evaluated using qPCR and vector copy number (VCN) analysis. AldeFluor kits (StemCell) were used to assess ALDH1 enzymatic activity. Transduced cells were treated with 0.0 - 50 μM of (MFA) and cultured for 48, 72, 96, and 168 hours.
- Results were evaluated for each condition using the ChemoMetec NC-200 cell counter, or CountBright Absolute Counting Beads added before flow cytometry evaluation. Cells were stained using CD34 Ab, and propidium iodide or Annexin-V with 7-AAD and analyzed for viability using the MACSQuant-10 flow cytometer.
- For flow cytometry evaluation we employed hierarchical gating for all lymphocytes, then all singlets; of all singlets we recorded data for number of viable cells. Absolute cell counts were calculated using the formula:

$$\text{Absolute Cell count} = \left(\frac{\# \text{ cells}}{\# \text{ beads}} \times \frac{\# \text{ beads in sample}}{\text{sample volume}} \right) \times (\text{well volume})$$

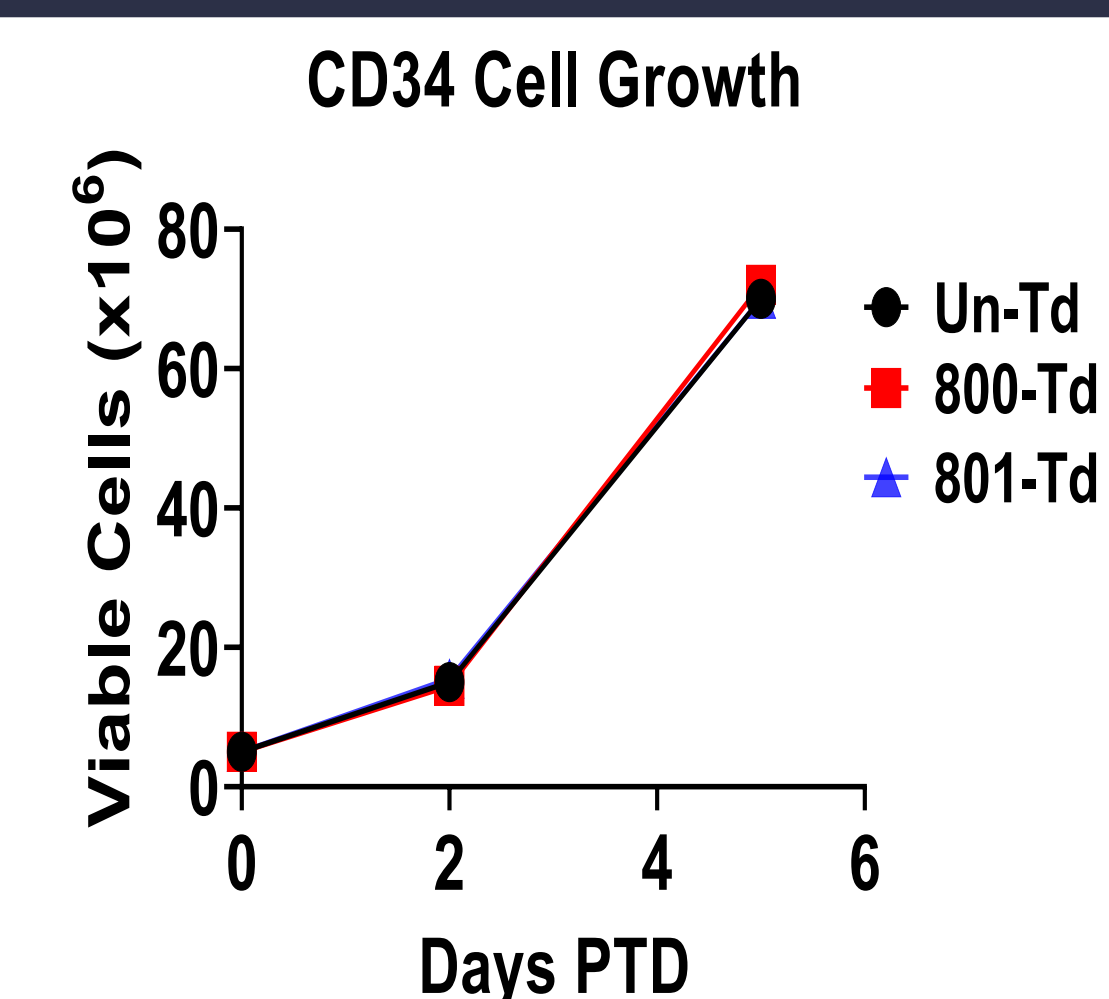
Figure 2: Lentiviral Vectors



Results

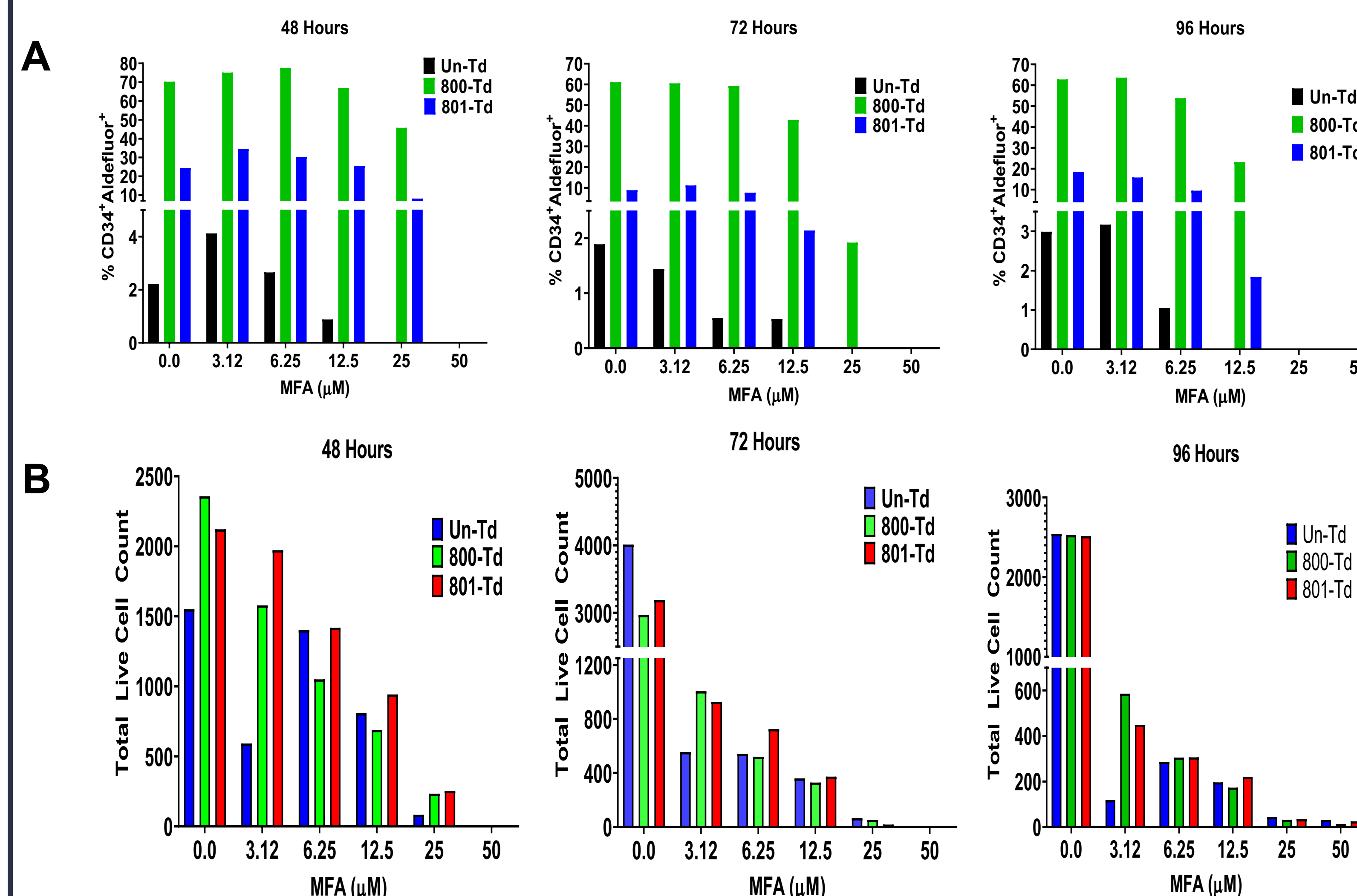
- Primary huCD34+ cells displayed extensively similar growth curves in culture when untransduced, and when transduced with LV800, or LV801 (Figure 3).
- TF-1a cells transduced with LV800 or LV801 demonstrated an increase in cells with high levels of ALDH1 activity of 25-35 fold, and 5-10 fold, respectively, as compared with untransduced cells (Figure 4, A).
- Transduced TF-1a cells treated with 3.12 μM of MFA showed higher total live cell counts of 2-3 fold at 48 hours, 1.5 fold at 72 hours, and 5 fold at 96 hours (Figure 4, B).
- Primary huCD34+ cells transduced with LV800 or LV801 and treated with 1.25 μM MFA showed higher absolute cell counts by 50,000 cells and 20,000 cells, respectively, 7 days after treatment.
- High dose MFA (10 or 20 μM) overcame chemoprotective effect of the overexpression of ALDH1 (Figure 5).

Figure 3: Primary huCD34+ cells transduced with LV800 or LV801 do not show cytotoxic effect



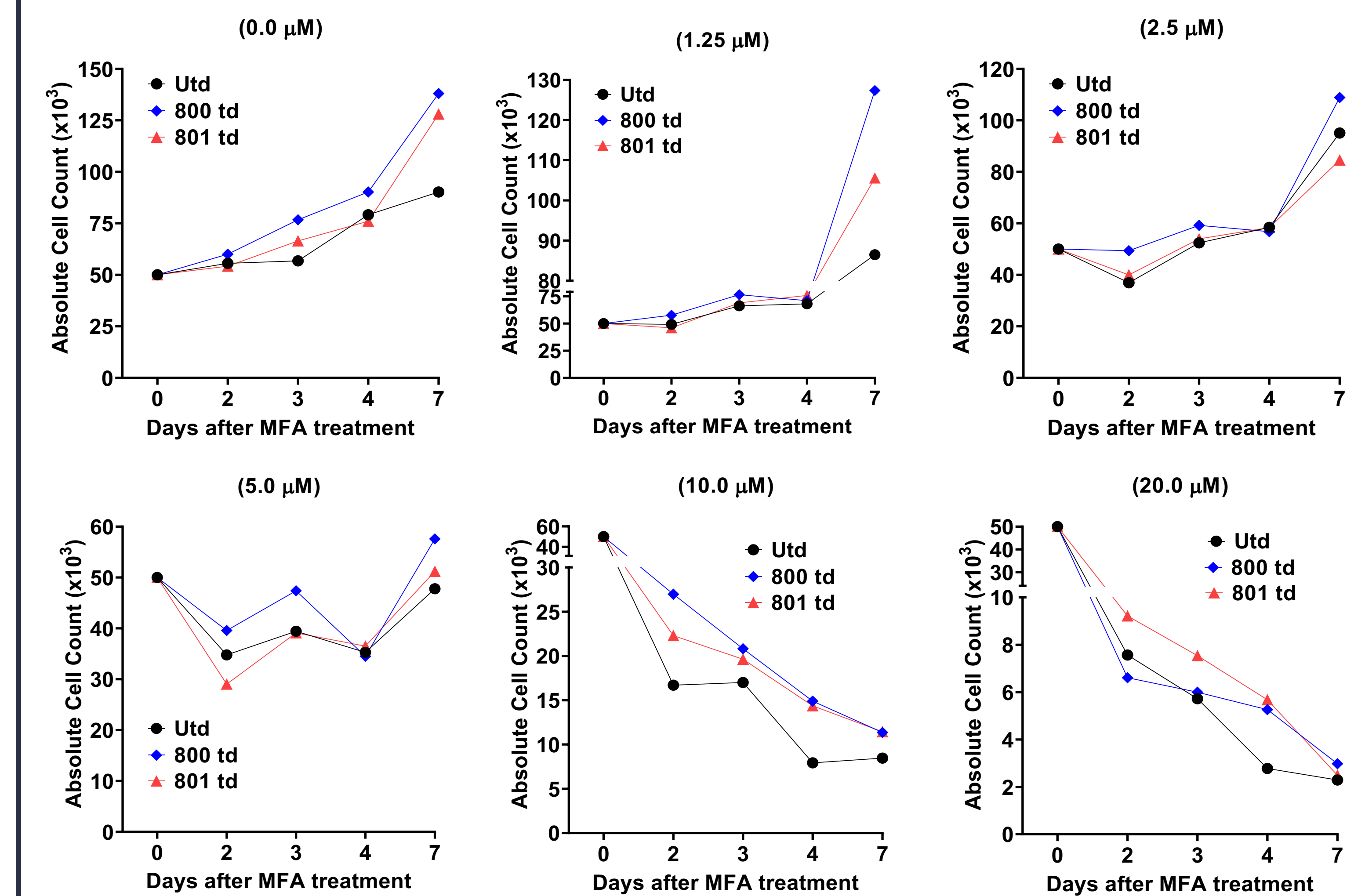
Legend: Primary huCD34+ cells were transduced with LV800, or LV801, and cultured for five days. Cells in culture were counted on days 0, 2, and 5. This data shows that there is no reduced cell number in the LV800, or LV801 transduced cell cultures, as compared to the untransduced cell culture.

Figure 4: ALDH1 activity and total live cell count increase in transduced CD34+TF-1a cells after MFA treatment



Legend: Chemoprotection in LV800 and LV801 transduced CD34+TF-1a cells against Mafosfamide *in vitro*. AldeFluor expression (A) and total live cell count (B) were shown as the bar graphs from the summarize of the flow data in untransduced, LV800, and LV801 transduced CD34+TF-1a cells.

Figure 5: Primary huCD34+ cells are protected from MFA cytotoxicity when transduced with vectors over expressing ALDH1



Legend: Chemoprotection in LV800 and LV801 transduced human CD34+ cells against Mafosfamide *in vitro*. Absolute cell count was shown as the line graphs from the summarize of the flow data in untransduced, LV800, and LV801 transduced cells.

Discussion

- Transduction with lentiviral vectors that induce ALDH1 overexpression successfully protects both TF-1a and primary huCD34+ cells from cytotoxicity with low dose MFA.
- Therefore, over-expression of ALDH1 may increase the engraftment of gene modified HSCs in a non-myeloablative HSCs transplant.
- The effects of MFA were observed with vectors (ENOB-HV-1 800 and 801) containing cassettes both to increase ALDH1 expression and to protect them from HIV expression. Therefore, the approach could be a potential approach to treat or cure HIV.
- The results should be verified with *in vivo* systems, with the goal of increasing engraftment levels for infused modified CD34+ cells by using administration of cyclophosphamide in humanized mice.

Conclusion

- Overexpression of ALDH1 provided chemoprotection to CD34+ cell line and human stem/progenitor cells when exposed to low dose mafosfamide, a metabolite of cyclophosphamide.
- Genetically modifying autologous human stem/progenitor cells to overexpress ALDH1 along with other genetic modifications known to treat or cure diseases, for example HIV, could lead to increased engraftment with the use of low dose cyclophosphamide.