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Multiplex Genome Engineering Strategies to Enable Next-Generation Shielded HSPC Transplants for Treatment of Acute Myeloid Leukemia

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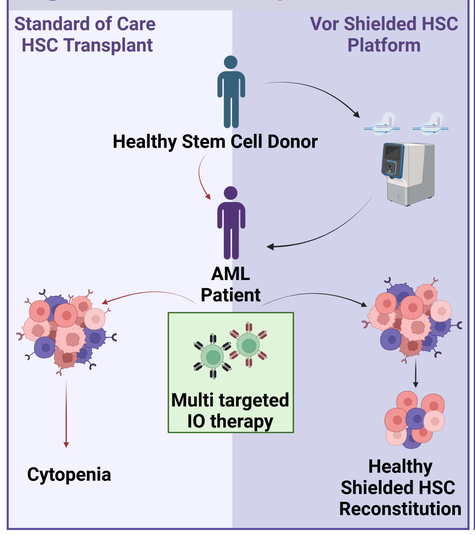
INTRODUCTION

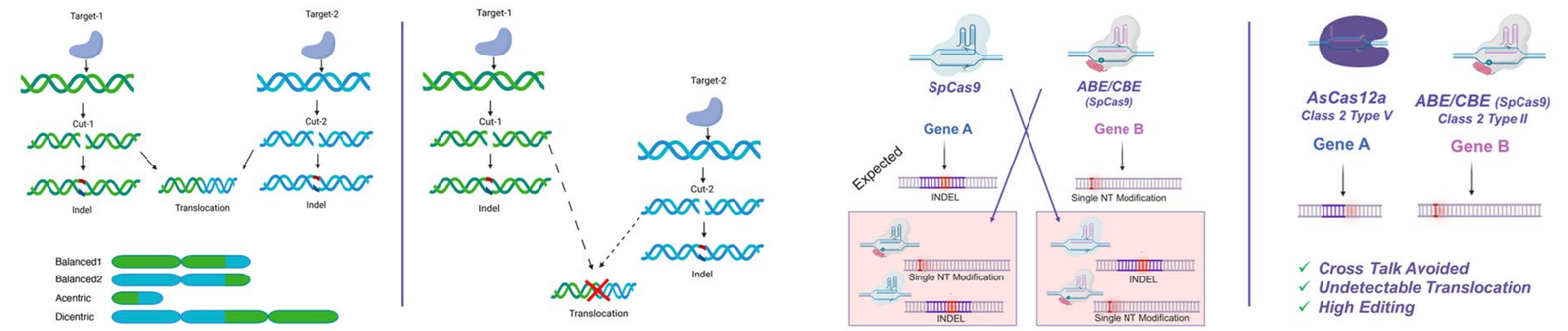
- Targeted immunotherapy of Acute Myeloid Leukemia (AML) has been limited due to lack of tumor-specific antigens resulting in "on-target, off-tumor" effects that can lead to severe cytopenia.
- To unlock the full potential of targeted treatments, we created treatment-resistant HSPCs by genetically ablating target antigens from healthy, HLA-matched donor-derived HSPCs for hematopoietic cell transplant (HCT)
- This allows compatible immuno-therapy to specifically kill leukemic cells bearing the AML target-antigen while protecting the target antigen-null allogeneic graft.
- Targeting multiple antigens simultaneously increases the potential to avoid antigen escape and addresses the issue of antigen heterogeneity of tumor cells.
- We examined unique combinations of CRISPR editing tools, including Cas9, Cas12a and Adenine Base Editors (ABE) to evaluate therapeutic potential.

Fig. 1. Shielded Multiplex Platform



GENOME ENGINEERING METHODS

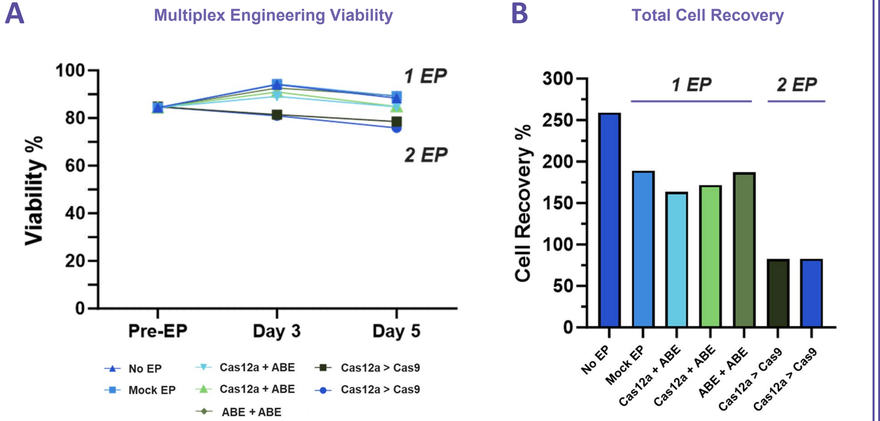
Fig. 2. Multiplex Delivery Strategies and Precise Engineering Approach



- Multiplex editing of HSPCs with CRISPR nucleases enables next generation cell transplant but poses a potential for genomic translocations. Through optimized engineering approaches, that potential can be reduced.
- (A) Si delivery of independent CRISPR nucleases leads to two double stranded DNA breaks (DSB) occurring in parallel that can result in translocations between the targeted cut sites. To reduce the translocation potential, a Se delivery of two RNPs staggers the two DSBs to allow the primary RNP to cut and begin repair before delivering and editing with the second RNP.
- (B) To enable a single delivery and preserve precise editing, either the desired edits must be produced with the same editing tool or a unique combination of orthogonal editors to avoid 'cross-talk' editing at gene specific target sites. Pairing Cas12a and ABE only creates one DSB during engineer to reduce translocations to an undetectable level.

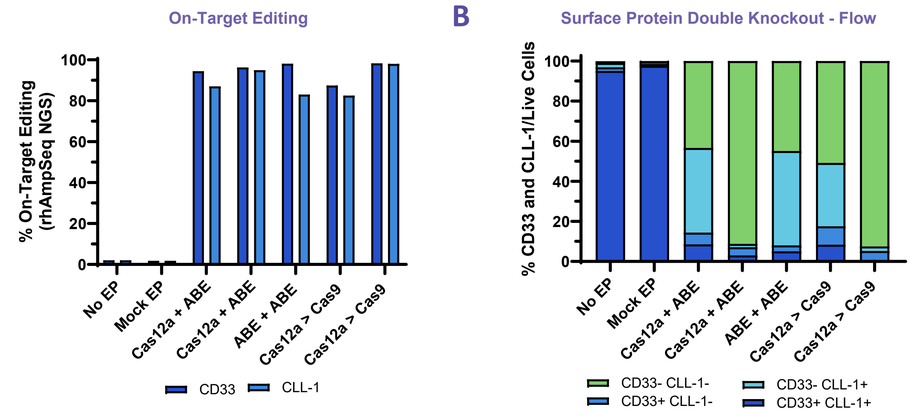
RESULTS

Fig. 3. Single Electroporation Delivery Preserves Overall Cell Health



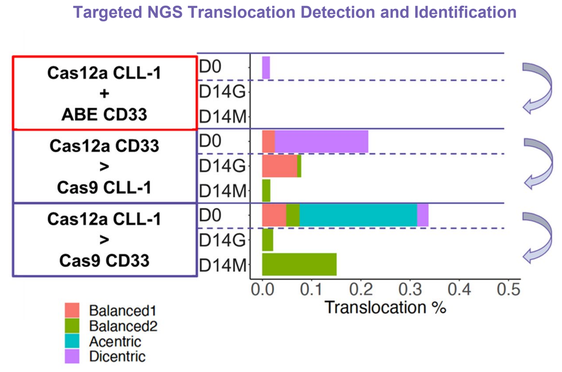
- (A) High viability is maintained with a single electroporation (EP) multiplex HSC samples as compared to No EP or Mock EP conditions.
- (B) CD34+ total cell recovery is dependent on the sample processing during engineering *in vitro*. Si delivery increases overall cell recovery as compared to 2 EP delivery.

Fig. 4. High On-Target Editing Leads To Protein Loss In Myeloid IVD



- (A) On-target editing by NGS in Monocytes (CD14+, CD11b+) on day 14 Myeloid in vitro differentiation (IVD); Granulocyte (CD15+, CD11b+) lineage editing is comparable (not shown).
- (B) Protein expression of CD33 and CLL-1 on Day 14 of Myeloid IVD. Unedited samples displayed high levels of both CD33 and CLL-1 expression. High levels of CD33 KO was observed across all combinations. CLL-1 expression after targeting with Cas12a in combination with ABE CD33 or Cas9 CD33 produced 90%+ dKO.

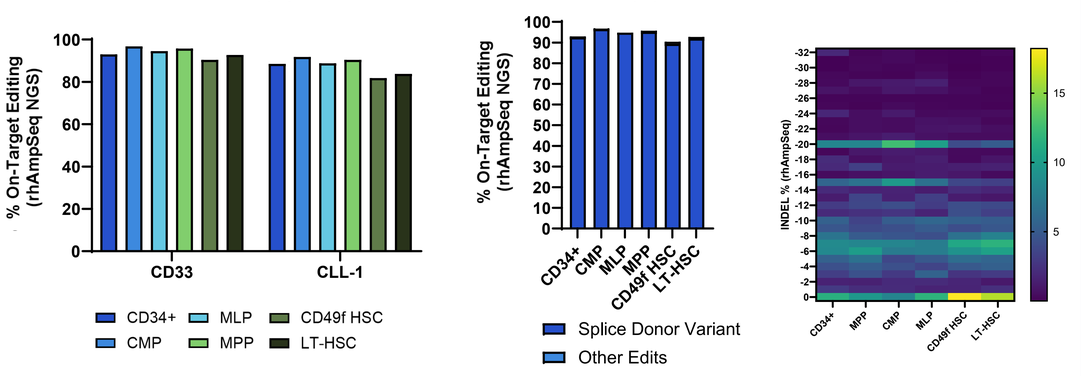
Fig. 5. Demonstration of Translocation Reduction



- Targeted NGS assays designed to identify and quantify on-target/on-target translocation species: acentric, dicentric and two balanced translocation species.
- Samples day 0 (D0) of Myeloid *in vitro* differentiation (IVD), 2 days post EP, and at Day 14 (D14) for both Monocytic (M) and Granulocytic (G) lineages.
- Cas12a CLL-1 + ABE CD33 contained undetectable translocations for both Myeloid lineages (G – Granulocytes and M – Monocytes) post 14-day in vitro differentiation.

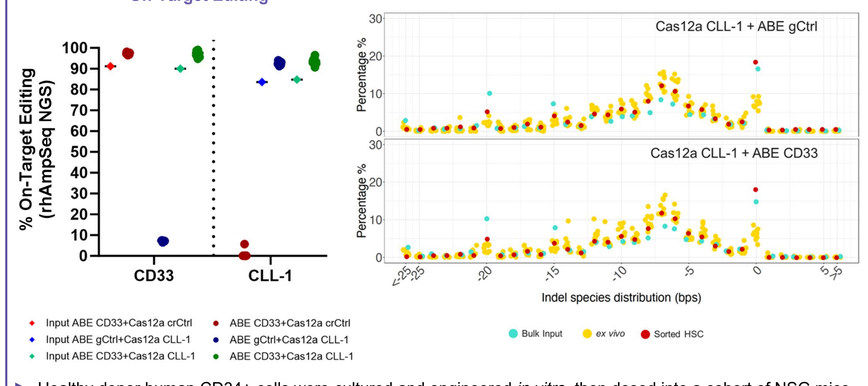
RESULTS

Fig. 6. All CD34+ Subpopulations Are Edited Equally with Cas12a/ABE Multiplex



- Multiplex edited HSPCs were sorted into subpopulations with comparable frequency between Mock EP and multi-edited cells.
- (A) High on-target editing within each subpopulation: CD34+, CMP, MLP, MPP, and HSC CD49f+ HSC[2], and Lt-HSC (EPCR+ and ITGA3+)[3].
- (B) Variant effect predictor (VEP) analysis as a result of ABE CD33 editing was consistent Splice Donor site disruption.
- (C) Cas12a CLL-1 indel species are represented in each subpopulation as in the bulk CD34+ population with similar distribution.

Fig. 7. LT-HSC Editing Is Representative Of *in vivo* Persistence



- Healthy donor human CD34+ cells were cultured and engineered *in vitro*, then dosed into a cohort of NSG mice. After 16-weeks bone marrow was harvested and gDNA was isolated. (A) Molecular analysis (NGS) was completed on *in vivo* input material and *ex vivo* bone marrow (n=10) comparing total on-target editing.
- (B) Cas12a Indel spectra was compared across bulk input CD34+ samples, *ex vivo* bone marrow (n=10) and Sorted LT-HSC (from input material.

Fig. 8. Multiplex Platform Considerations

CONCLUSION

- Combination of Cas12a and ABE in a single delivery to HSPCs showed high cell recovery and maintained cell viability similar to control samples, in addition to high editing at both loci.
- HSPC CD34+ subpopulation frequency as identified by flow cytometry was maintained post editing, with a distribution comparable to the control samples.
- Multiplex edited HSPCs were *in vitro* differentiated into myeloid lineages and analyzed through flow cytometry revealing a productive double knockout for both target Ags in >90% of the cell population with no detectable on-target translocations as measured by multiplex NGS.
- LT-HSC Editing profile is a predictive measure of *ex vivo* bone marrow analysis productive edits persisted *in vivo* both on the molecular and cellular level.
- These findings support the promising utility of Cas12a editing, enhanced by our improved cell engineering process, to generate next-generation HCTs enabling administration of multi-specific targeted therapies with reduced on-target, off-tumor toxicity in AML patients.

References

1. Clement K, Rees H, Canver MC, et al. Nature Biotechnology (2019)
2. Notta et al. Science (2011)
3. Tomellini et al. Cell Rep (2019)

Disclosures

All authors listed above are current or former employees of Vor Bio

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