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Multiplex deletion of myeloid antigens by base editing in human hematopoietic stem and progenitor cells (HSPCs) enables potential for next generation transplant for acute myeloid leukemia (AML) treatment

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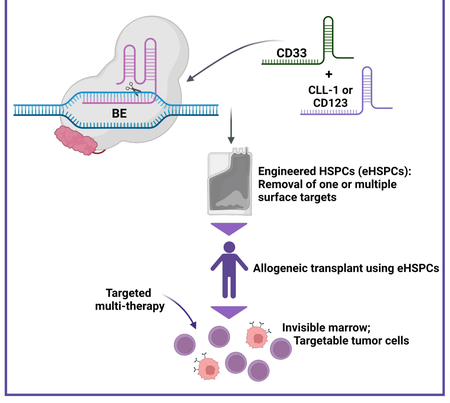
INTRODUCTION

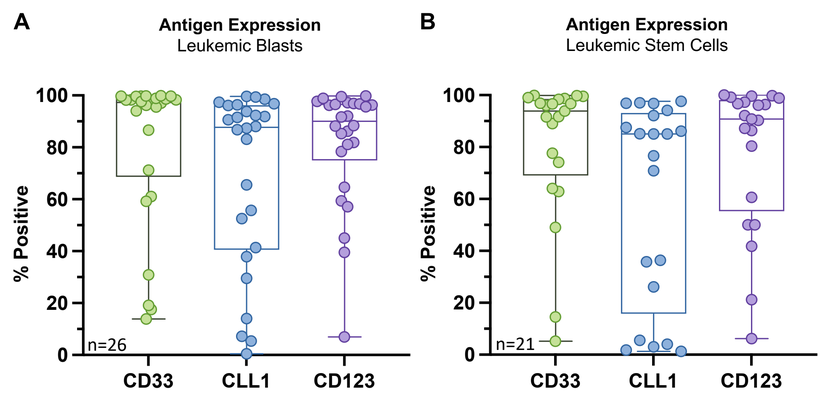
- Targeted immunotherapy of AML exhibit off-tumor toxicity due to their inability to differentiate between tumor blasts and healthy blood cells that express the same surface antigens.
- To unlock the full potential of targeted treatments, we engineer treatment-resistant HSPCs by genetically ablating target antigens from healthy donor-derived HSPCs for hematopoietic stem cell transplant.
- This approach allows specific immunotherapy targeting of leukemic cells while protecting the target antigen null allogenic graft.
- Targeting multiple antigens simultaneously avoids potential antigen escape and addresses the issue of antigen heterogeneity of tumor cells
- Here we present multiplex base editing approaches using cytosine base editors (CBE)[1,2] or adenine base editors (ABE)[3] to simultaneously induce gene knock-out (KO) of clinically relevant AML surface antigens in CD34+ HSPCs from healthy donors.
- This approach may enable administration of combinatorial targeted therapeutics with reduced on-target, off-tumor toxicity for AML patients.

Fig. 1. Therapeutic Schema



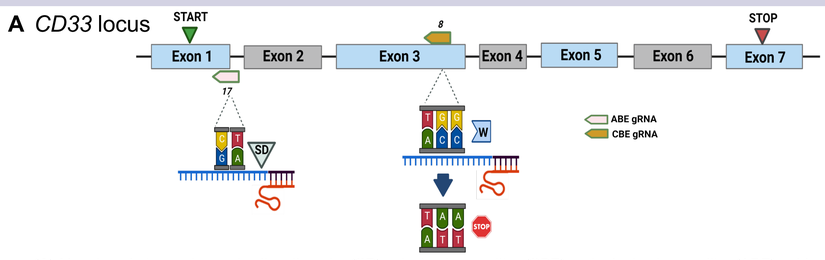
RESULTS

Fig. 2. CD33, CLL-1, and CD123 are highly expressed on the surface of leukemic blasts and stem cells



- Percentage of CD33, CLL-1, and CD123 positive cells by flow cytometry in AML blasts (left) and leukemic stem cells (right) obtained from bone marrow aspirates of patients with AML.

Fig. 3. BE KO Strategies to delete CD33 in combination with CLL-1 or CD123

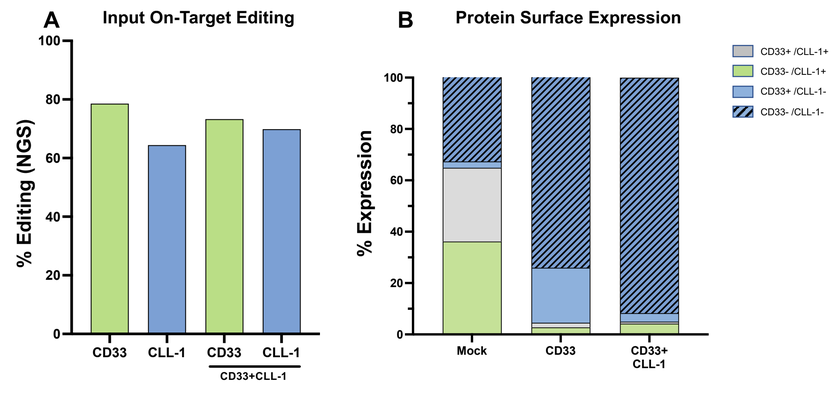


(A) Mapping of stop codon and splice disrupter (SD) cytosine base editor (CBE) and adenine base editor (ABE) guide-RNA (gRNA), respectively, on the *CD33* locus with schema indicating BE impact. CD33 g8 multiplexed with CLL-1 g3; CD33 g17 multiplexed with CD123 g18.

(B) Mapping of stop codon CBE gRNA, g3, on the *CLL-1* locus. Multiplexed with CD33 CBE gRNA 8.

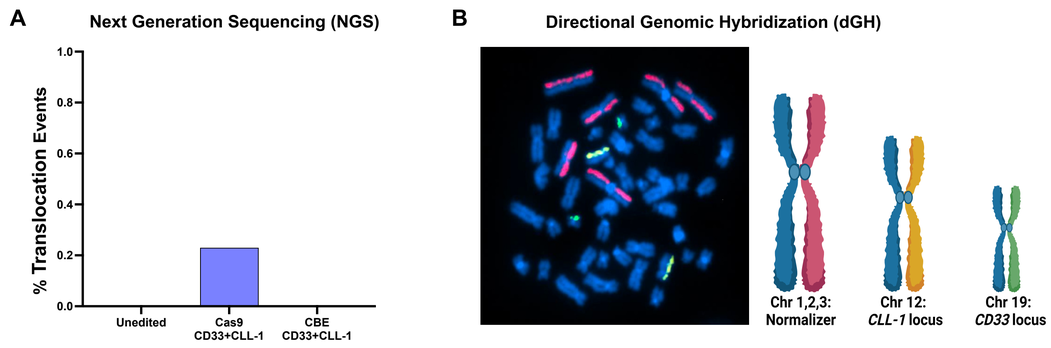
(C) Mapping of splice disrupter ABE gRNA on the *CD123* locus. Multiplexed with CD33 ABE gRNA 17.

Fig. 4. Multiplex CBE of CD33 and CLL-1 loci shows efficient on-target editing and dual protein expression knockdown



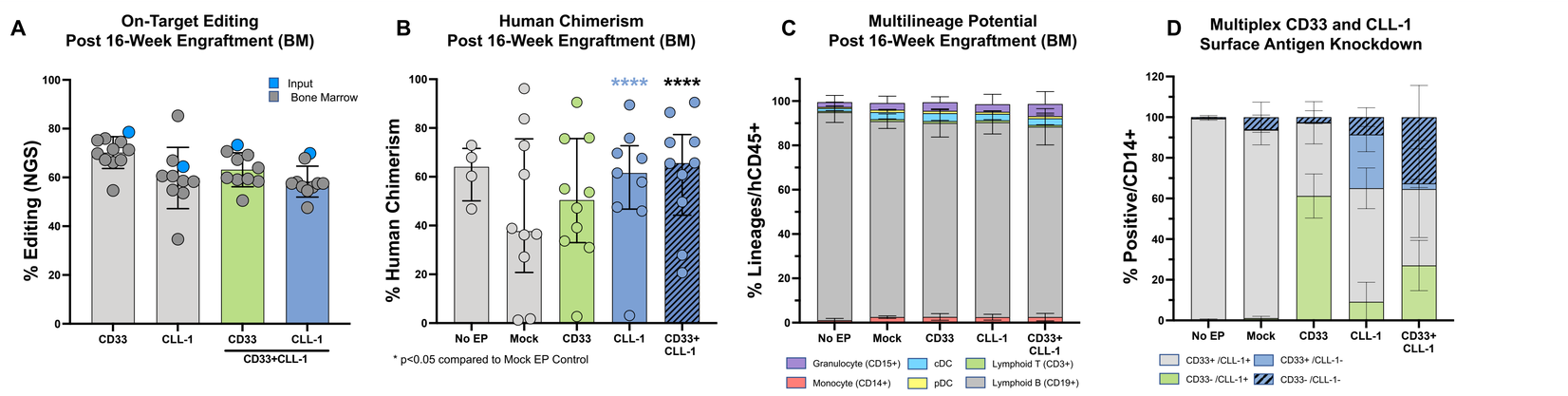
- On-target editing efficiency 2 days post-electroporation (EP) of single and multiplex base edited cells.
- CD33 and CLL-1 surface expression in unedited (Mock EP) and edited CD34+ HSPCs using flow cytometry, 6 days post-electroporation.

Fig. 5. Translocations were not detected in CD33+CLL-1 Multiplex Base Edited samples



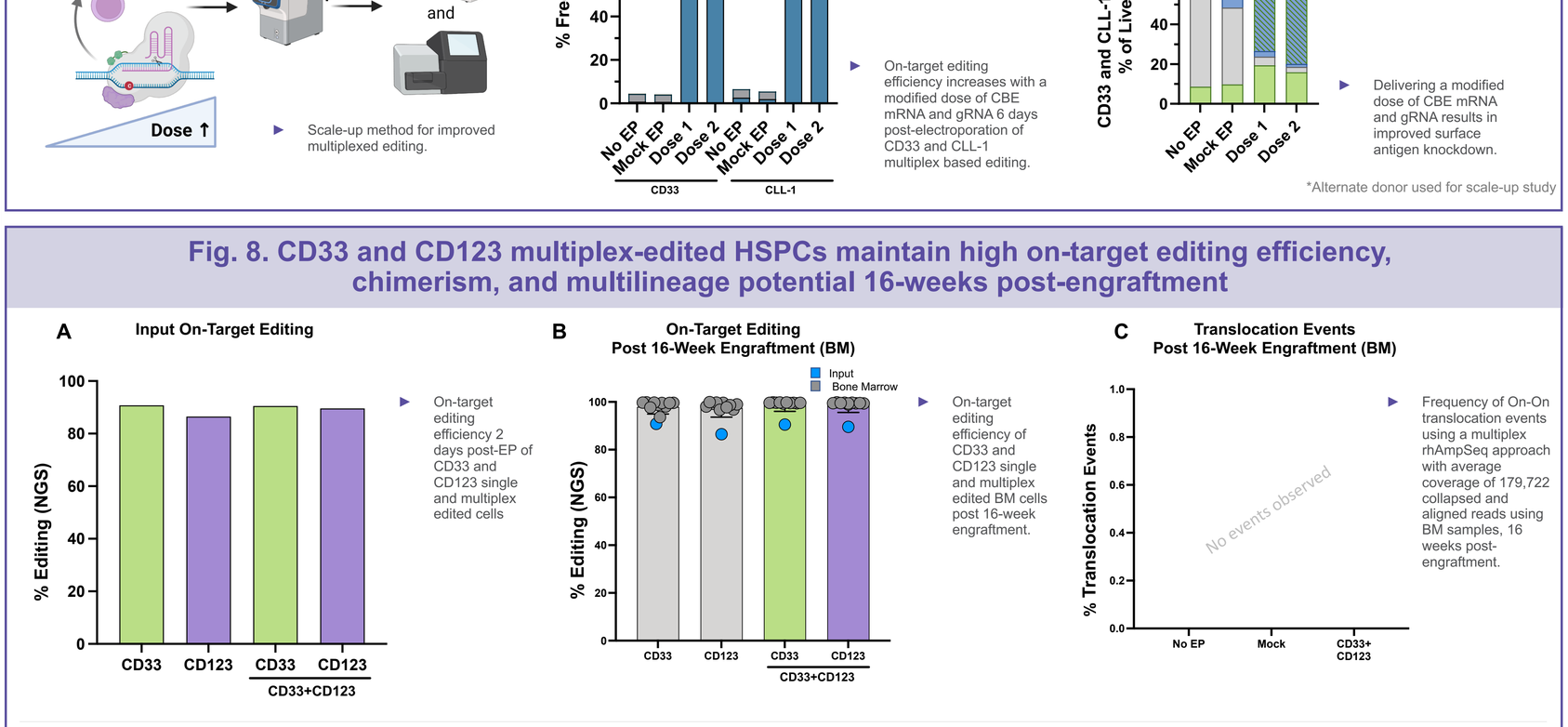
- **(A)** Frequency of On-On translocation events using a multiplex rhAmpSeq approach with coverage of 217,442 collapsed and aligned reads to a 223 bp junction of the expected translocation between the two different loci. Cas9 CD33-CLL-1 are a control RNP in which the CD33 + CLL-1 loci are simultaneously edited using CRISPR/Cas9.
- **(B)** Representative metaphase spread using a directional genomic hybridization (dGH) assay in edited and unedited samples showing chromosomal paints in pink (chromosomes 1, 2, 3) used as normalizers to account for donor variability (dosimetry); yellow (chromosome 12, CLL-1 locus) and green (chromosome 19, CD33 locus).

Fig. 6. CD33 and CLL-1 multiplex-edited HSPCs maintain editing efficiency, chimerism, and multilineage potential 16-weeks post-engraftment



- On-target editing efficiency of CD33 and CLL-1 single and multiplex base edited bone marrow (BM) cells post 16-week engraftment.
- Human chimerism of CD33 and CLL-1 single and multiplex base edited BM cells from 16-week post-engraftment mice.
- Multilineage potential of CD33 and CLL-1 single and multiplex base edited BM cells from 16-week post-engraftment mice.
- Monocyte (CD14+) surface expression of CD33 and CLL-1 from single and multiplex CBE-edited HSPCs 16 weeks post-engraftment.

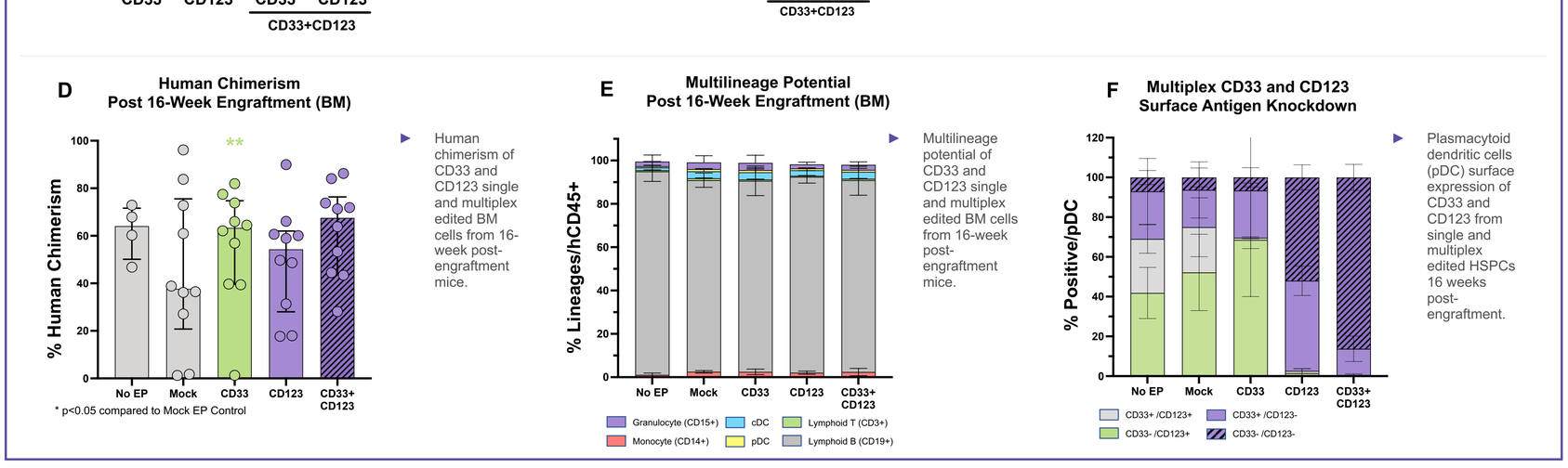
Fig. 7. Scale-Up Optimization of CBE CD33+CLL-1 Multiplex Editing



- Scale-up method for improved multiplexed editing.
- On-target editing efficiency increases with a modified dose of CBE mRNA and gRNA 6 days post-electroporation of CD33 and CLL-1 multiplex based editing.
- Delivering a modified dose of CBE mRNA and gRNA results in improved surface antigen knockdown.

*Alternate donor used for scale-up study

Fig. 8. CD33 and CD123 multiplex-edited HSPCs maintain high on-target editing efficiency, chimerism, and multilineage potential 16-weeks post-engraftment



- On-target editing efficiency 2 days post-EP of CD33 and CD123 single and multiplex edited cells
- On-target editing efficiency of CD33 and CD123 single and multiplex edited BM cells post 16-week engraftment.
- Frequency of On-On translocation events using a multiplex rhAmpSeq approach with average coverage of 179,722 collapsed and aligned reads using BM samples, 16 weeks post-engraftment.
- Human chimerism of CD33 and CD123 single and multiplex edited BM cells from 16-week post-engraftment mice.
- Multilineage potential of CD33 and CD123 single and multiplex edited BM cells from 16-week post-engraftment mice.
- Plasmacytoid dendritic cells (pDC) surface expression of CD33 and CD123 from single and multiplex edited HSPCs 16 weeks post-engraftment.

CONCLUSION

- Our data shows high base editing efficiency, robust surface protein KO, and no detection of translocation of multiplex edited cells in *ex vivo* edited cells.
- Pharmacology studies using NOD/SCID-gamma mice showed unaltered long-term engraftment and multilineage differentiation in the multiplex-edited cohorts.
- NGS analysis revealed no change in total editing between dual knockout input and bone marrow cells post-engraftment, indicating the edits in dual-engineered cells persisted long-term and loss of multiple antigens was well-tolerated.
- Multiplex Base Editing in CD34+ HSPCs of one or multiple surface targets provides an efficient strategy for multi-gene disruptions in HSPCs and can enable next-generation AML treatments.

References

1. Komor A. *Nature* **533,** 420–424 (2016)
2. Yu et al. *Nat Commun.* **11,** 2052 (2020)
3. Gaudelli et al. *Nature* **551,** 464–471 (2017)
4. Billon et al. *Mol Cell*. **67**(6):1068-1079.e4 (2017)

Disclosures

All authors listed above are current or former employees at Vor Biopharma

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