P # 3001

Multiplex Base Editing in Human Hematopoietic Stem and Progenitor Cells (HSPCs) Enables Efficient Removal of Multiple Surface Antigens in Acute Myeloid Leukemia (AML) Immunotherapy

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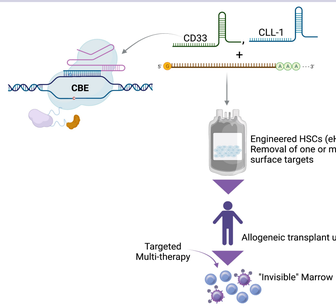
INTRODUCTION AND RATIONALE

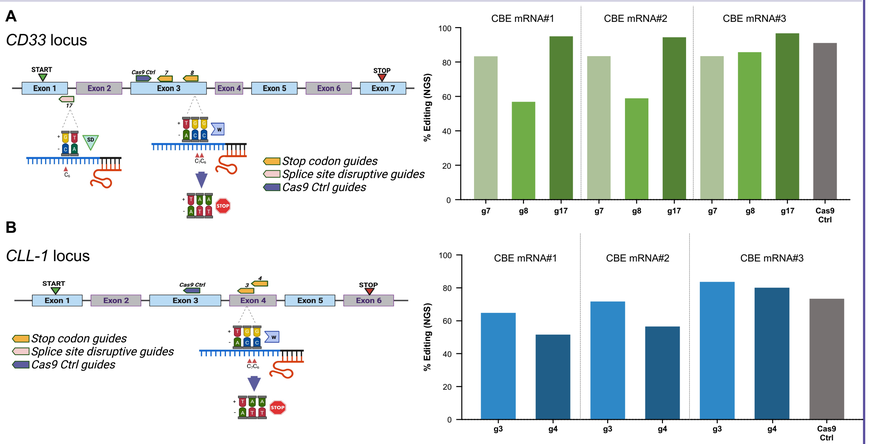
- Removal of surface antigens by editing the genome of hematopoietic stem and progenitor cells (HSPCs) in allogeneic transplants is a novel approach to enable post-transplant targeted therapies in diseases, such as acute myeloid leukemia (AML).
- This approach allows for compatible therapeutic modalities to specifically target leukemic cells while protecting the target antigen null allogenic draft.
- One of the hurdles in treating AML is tumor antigen heterogeneity and removing one surface target might not be sufficient.
- Combinatorial therapies targeting multiple antigens may provide greater efficacy in AML treatment and help avoid potential antigen escape.
- Here we present a multiplex base editing approach using cytosine base editors (CBE) 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. Multiplex Base Editing using CBEs in CD34+ HSPCs



RESULTS

Fig. 2. CBE4 Base Editing guide screen of CD33 and CLL-1 generated high efficient editing in CD34+ HSPCs

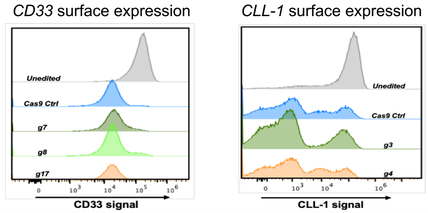


- *Left panel:* Mapping of stop codon and splice disrupter base editing guides on the *CD33* locus and Cas9 control binding sites.
- *Left panel:* Mapping of stop codon and splice disrupter base editing guides on the *CLL-1* locus and Cas9 control binding sites.

Right panel: On-target base editing efficiency of top 3 CBE gene KO inducing single guides using 3 different CBE4 mRNA encoding constructs. Gray bar: Cas9-induced indel frequency on the CD33 locus.

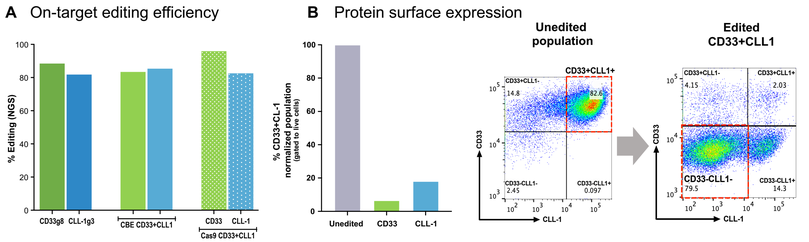
Right panel: On-target base editing efficiency of top 3 CBE gene KO inducing single guides using 3 different CBE4 mRNA encoding constructs. Gray bar: Cas9-induced indel frequency on the CLL-1 locus.

Fig. 3. Efficient Base Editing of CD33 and CLL-1 abrogate protein surface expression



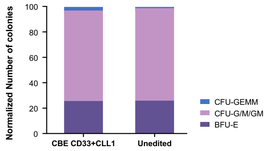
- CD33 and CLL-1 surface protein expression quantified in edited CD34+ HSPCs by flow cytometry 9 days post-electroporation (EP).
- Histograms are color coded per guide and show percentage of positive population for CD33 or CLL-1 edited samples compared to control untreated cells.

Fig. 4. Multiplex Base Editing of CD33 and CLL-1 loci shows efficient on-target editing and dual protein surface expression knock-out



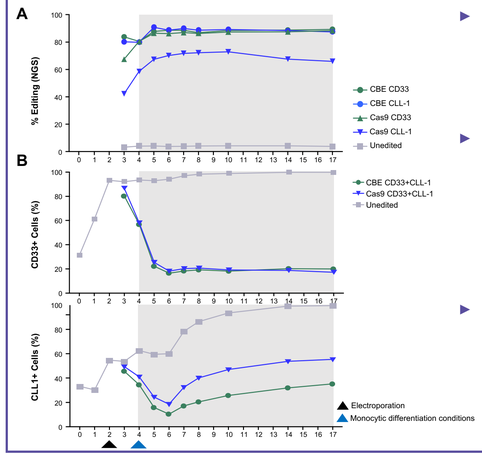
- On-target editing efficiency of single and multiplex base edited cells for CD33 and CLL-1 compared to multiplex Cas9 control edited cells.
- Normalized CD33 and CLL-1 surface protein expression in edited and unedited CD34+ HSPCs using flow cytometry 9 days post-EP in myeloid differentiating culture conditions. Panel on the right shows 80% true double KO population.

Fig. 5. Multilineage potential of double edited CD34+ HSPCs is maintained



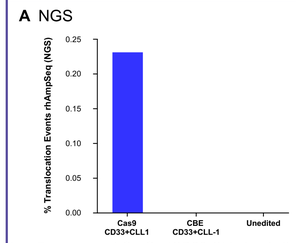
- Edited and untreated control HSPCs were cultured in differentiating culture conditions to support growth of multiple progenitor cell lineages. Colony-forming unit (CFUs) were measured 14 days after plating and Erythroid (BFU-E), Myeloid (CFU-G/M/GM) and Mixed (CFU-GEMM) lineages were quantified.

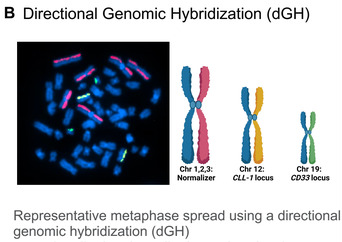
Fig. 6. Myeloid *In vitro* differentiation shows editing persistence in monocytes and protein KO expression of CD33 and CLL-1 multiplexed edited cells



- CD34+ HSPCs were electroporated with CBE4 encoding mRNA or Cas9 ribonucleoprotein complex using CD33,CLL1 synthetic guides and in vitro differentiated into monocytic lineage.
- On-target editing efficiency of CD33 and CLL-1 in base edited and Cas9-edited samples harvested at different time points post-EP (Day 2) and throughout monocytic differentiation. Editing efficiency was calculated and annotated using CRISPResso v2.0.30 and variant effector predictor (VEP).
- CD33 and CLL-1 protein expression in edited and unedited samples measured throughout monocytic differentiation using flow cytometry. Bulk population throughout multiplex base edited cells showed a decrease in CD33 and CLL-1 expression in monocyte differentiated CD34+ HSPCs.

Fig. 7. Translocations were not detected in CD33+CLL1 Multiplex Base Edited samples





- Frequency of On-On (CD33-CLL-1 cut site) translocation events using a multiplex rhAmpSeq approach with coverage of 217442 collapsed and aligned reads to a 223bp junction of the expected translocation between the two different loci.
- 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).

CONCLUSION

- Simultaneous delivery of base editing guides preserves health, expansion and stemness of HSPCs which could facilitate the process and manufacturing of this therapeutic.
- Our data shows high base editing efficiency, robust surface protein KO, and no detection of balanced translocation of multiplex edited cells.
- Multiplex Base Editing in CD34+ HSPCs of one, two, or multiple surface targets offers a valuable, safe, and efficacious alternative to engineer the next generation of transplants to treat AML patients.

References

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Disclosures

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

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