P #2939

Multiplex Engineering of Human CD34+ HSPCs Enables Dual Gene Knockout While Maintaining High Engraftment Potential and Safety

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INTRODUCTION

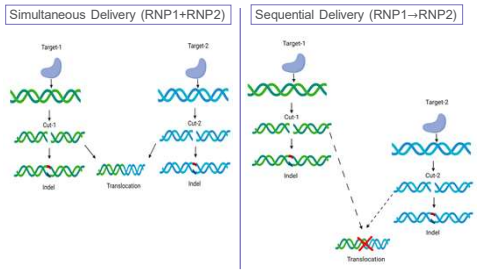
- Targeted therapies for acute myeloid leukemia (AML) exhibit off-tumor toxicity due to their inability to differentiate between leukemic cells from healthy blood cells that express the same cell surface antigen.
- Removal of surface antigens from allogeneic hematopoietic stem cell transplants (HSCTs), thereby allows the therapies to specifically target leukemic cells by sparing the gene-edited, antigen null grafts, is a novel approach to enable post-transplant targeted therapies for AML. This strategy has the potential to enable the next generation of HSCTs.[1-3]
- AML may still pose challenges due to target antigen heterogeneity as well as the phenomenon of antigen escape. Use of multi-specific immuno-therapies, simultaneously targeting multiple cell surface antigens, may provide greater efficacy.
- Our approach to create multi-knockout HSCTs will allow the multi-therapies to specifically target leukemic cells. However, multiplex editing with CRISPR/Cas9 poses translocation risk.
- Here, we propose an optimized editing process which generates efficient knockout of two targets with drastically reduced translocation as evidenced by a long-term engraftment study in a xeno-transplant mouse model.

OBJECTIVE

- Determine long-term reconstitution potential of multiedited HSPCs.
- Determine persistence of on-target editing and translocations through the long-term reconstitution process.

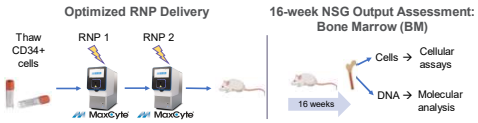
METHODS

Fig. 1. Multiplex Editing Schematic



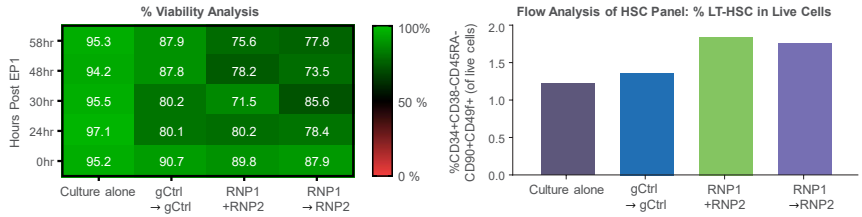
- Sequential ribonucleoprotein complex (RNP) delivery to reduce translocation risk.

Fig. 2. Experimental Overview



RESULTS

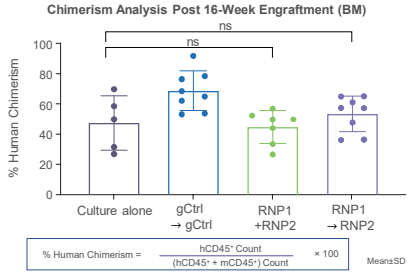
Fig. 3. Dual-Edited HSPCs Maintain High Viability and %LT-HSCs



*gCtrl → gCtrl = Sequential delivery of non-targeting guide, RNP1+RNP2 = Simultaneous delivery group, RNP1 → RNP2 = Sequential delivery group

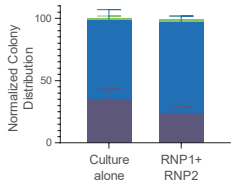
- AO/PI staining was used to assess viability of all samples, and the overall viability of dual-edited input HSPCs was >70%.
- The proportion of CD34+CD38-CD45RA-CD90+CD49f+ putative long-term hematopoietic stem cells (LT-HSCs) in input cells were similar (1-2%) between unedited and dual-edited cells.[4]

Fig. 4. Engraftment and Differentiation Potential of Dual-Edited HSPCs Are Comparable to Culture-Alone HSPCs



- *In vivo* engraftment potential of dual-edited cells was maintained long term, with average chimerism of hCD45+ cells in mice transplanted with dual knock-out (KO) HSPCs similar to those transplanted with unedited HSPCs (53.36±11.80% vs 47.45±18.03%, respectively). Dots represent individual animals. (Statistical test, 1-way analysis of variance)

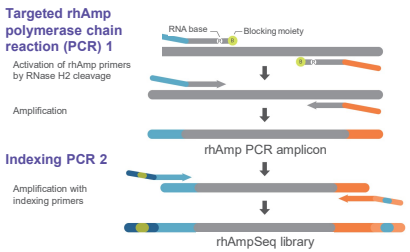
***In Vitro* Colony-Forming Unit (CFU) Assay Post 16-Week Engraftment**



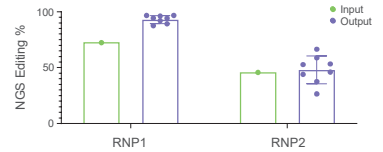
- Dual-edited cells also maintained normal differentiation potential as judged by multilineage reconstitution by *in vitro* CFU assay.

Fig. 5. On-Target Editing Persist Both Targets Post 16-Week Engraftment

- IDT rhAMP-Seq was used for on-target editing assessment.[5]



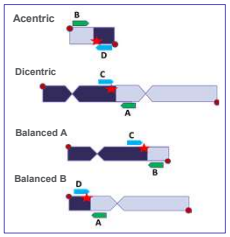
On-Target Editing of RNP1 → RNP2 Input and BM Output Material Using rhAmp-Seq Assay



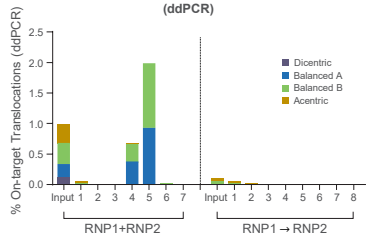
- RNP1 → RNP2 Sequential delivery group.
- Quantification of on-target editing by multiplexed next-generation sequencing (NGS) revealed no reduction in total editing between dual-edited input and BM cells, indicating the gene modifications in dual engineered cells persist after engraftment.

Fig. 6. Sequential RNP Delivery Reduces On-Target Translocations

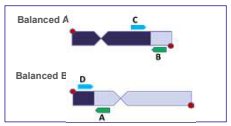
Anticipated On-Target Translocation Species



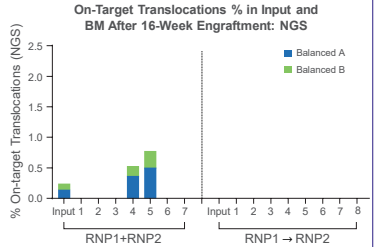
On-Target Translocations % in Input and BM After 16-Week Engraftment: Droplet Digital PCR (ddPCR)



- Translocation assessment using ddPCR: Compared to simultaneous delivery (RNP1 + RNP2), Sequential RNP delivery (RNP1 → RNP2) reduces persistence of translocation to near-background levels of detection (0.02%).
- Number on the X-axis represent individual animal.



On-Target Translocations % in Input and BM After 16-Week Engraftment: NGS



- Multiplexed NGS showed similar trend of reduction as ddPCR for on-target translocations.
- Number on the X axis represent individual animal.

CONCLUSION

- These findings support the promising utility of multiplex editing, enhanced by our improved cell engineering process, to generate multiplex-engineered next-generation HSCTs—enabling the administration of multi-targeted therapies with reduced on-target, off-tumor toxicity in AML.

References

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Illustrations were created with BioRender.com

Acknowledgments

Robert Ang, Amy Mendel, Christopher Slapak, Hilary Eaton, John King, Nathan Jorgensen, Robert Pietrusko, Sarah Spencer, Tobias Brambrink

Disclosures

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

Presented at

63rd ASH Annual Meeting & Exposition, December 11–14, 2021