P # 3002

Multiplex Editing of Hematopoietic Stem and Progenitor Cells (HSPCs) with CRISPR-Cas Nucleases Achieves High On-Target Editing with Undetectable Translocations

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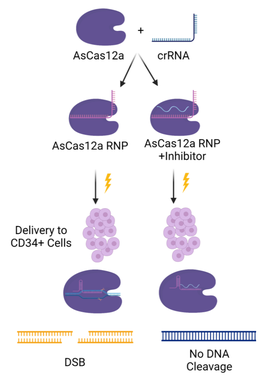
INTRODUCTION

- 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).
- Tumor antigen heterogeneity is one of the hurdles in treating AML, but use of multi-specific therapies targeting multiple antigens may provide greater efficacy in AML treatment and help avoid potential antigen escape.
- However, multiplex editing with CRISPR/Cas9 poses translocation risk.
- We describe a novel multiplex editing approach that utilizes sequential delivery of orthogonal CRISPR-Cas nucleases and incorporation of AsCas12a inhibitors to reduce or eliminate the potential risk of translocation events.

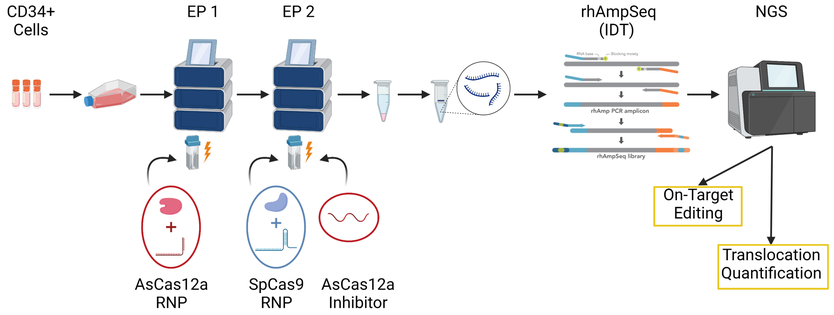
Fig. 1. AsCas12a Inhibition



- Phosphorothioate-modified (ps) DNA oligonucleotides are designed to inhibit AsCas12a-mediated genome editing[1]

METHODS

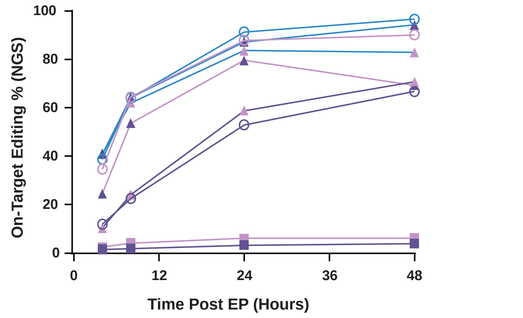
Fig. 2. Multiplex Experimental Schema



- CD34+ cells are thawed and allowed to recover in culture. AsCas12a RNP is delivered to cells through electroporation (EP) and recovered in culture. AsCas12a/SpCas9 sequential multiplexing allows for the addition of inhibitor in second EP during delivery of editing technology to block editing that occurs during first EP, while inducing the second targeted gene editing events. After further culture, cell are harvested and genomic DNA is extracted. Samples are prepped using Integrated DNA Technologies, Inc (IDT) rhAmpSeq and then deep sequenced using next generation sequencing (NGS). Data collected was used to evaluate on-target editing and translocations.

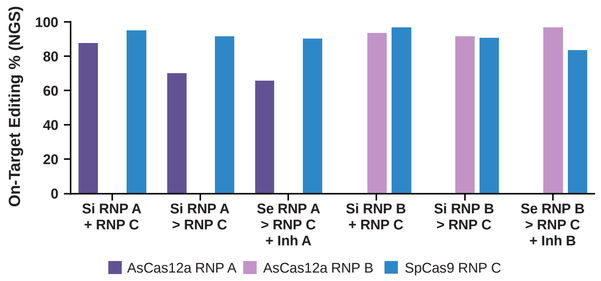
RESULTS

Fig. 3. AsCas12a Inhibitors are Target Sequence Specific



- Single target NGS analysis[2] pipeline of samples with and without inhibitors
- Inhibition of AsCas12a activity is time dependent, dose dependent and target sequence specific

Fig. 4. High On-Target Multiplex Editing Maintained with Inhibitor



- High on-target editing was achieved with both simultaneous (Si) and sequential (Se) delivery with or without AsCas12a inhibitor
- On-target editing utilize a pipeline/workflow[2] to measure on-target editing of multi-edited CD34+ cells through IDT rhAmpSeq when combining AsCas12a RNP A with SpCas9 RNP C or AsCas12a RNP B with SpCas9 RNP C

Fig. 5. Sequential Multiplex with Inhibitors Produce No Detectable Translocation

- Translocation quantification of multi-edited CD34+ cells through IDT rhAmpSeq.
- AsCas12a RNP A is combined with SpCas9 RNP C or AsCas12a RNP B combined with SpCas9 RNP C and elivered either simultaneously (Si) or sequentially (Se)
- Translocation quantification utilize an optimized in-house NGS analysis workflow/pipeline

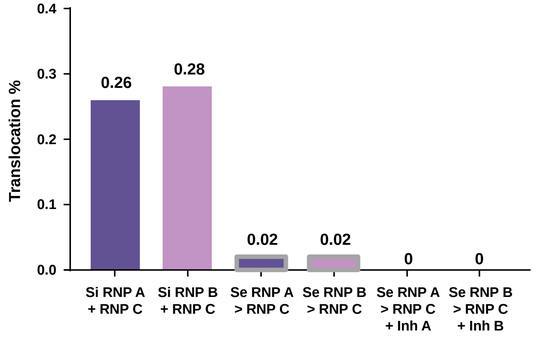
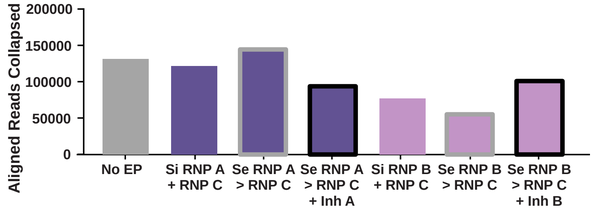


Fig. 6. High Sequencing Depth Across All NGS Samples

- High sequencing depth for all samples
- Total raw sequencing reads were subjected to QC
- Quantification of translocation is based on total number of sequencing reads aligned



CONCLUSION

- AsCpf1/SpCas9 combinatorial multiplexing in a sequential electroporation (EP) delivery allows for the addition of an inhibitor in the second EP to block editing that occurs after first EP while inducing the second targeted gene editing.
- With this approach, high viability (>80%) and high on-target editing (>65%) were achieved at both targets in HPSCs while reducing translocation to an undetectable level as assayed by next-generation sequencing.
- These findings support the promising utility of multiplex editing, enhanced by our improved cell engineering process, to generate next-generation HSCTs enabling administration of multi-specific targeted therapies with reduced on-target, off-tumor toxicity in AML patients.

References

1. Li et al., 2018, Cell Reports 25, 3262–3272 December 18, 2018
2. Clement K, Rees H, Canver MC, et al. CRISPResso2 provides accurate and rapid genome editing sequence analysis. Nature Biotechnology. 2019 Mar;37(3):224-226.

Disclosures

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

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