EDIT-301: An Experimental Autologous Cell Therapy Comprising Cas12a-RNP Modified mPB-CD34+ Cells for the Potential Treatment of SCD

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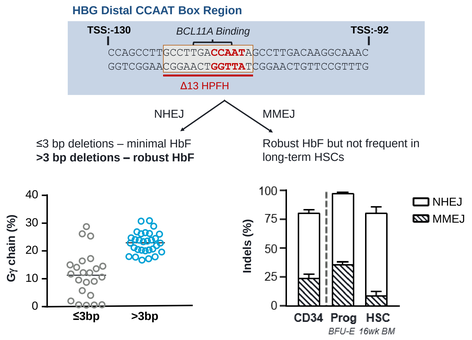
Introduction

Sickle cell disease (SCD) is caused by a single mutation E6V of the β-globin chain, leading to polymerization of sickle hemoglobin (HbS) and formation of HbS fibers. Fetal hemoglobin (HbF) protects against SCD by inhibiting HbS polymerization.[1] Individuals with compound heterozygosity for HbS and deletional hereditary persistence of fetal hemoglobin (HPFH) express approximately 30% HbF with pancellular distribution and exhibit no features of SCD.[2]

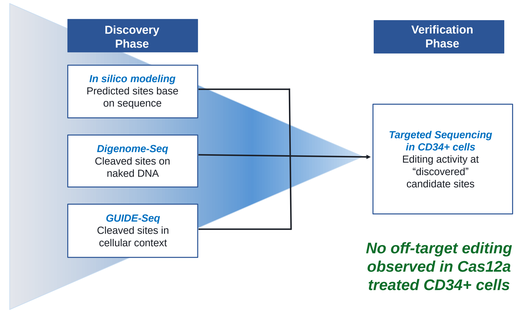
The HBG distal CCAAT box region was identified in our previous screen to be one of the most robust HbF-inducing sites across the beta-globin locus. Several naturally occurring HPFH mutations are also clustered at this region, partially overlapping a BCL11A binding site.[3]

Cas12a, a highly specific gene editing enzyme,[4] was evaluated against spCas9 for its capacity to promote durable and high-HbF inducing indels at the distal CCAAT box. Those studies led us to optimize Cas12a editing for EDIT-301, a potentially best-in-class experimental medicine for SCD.

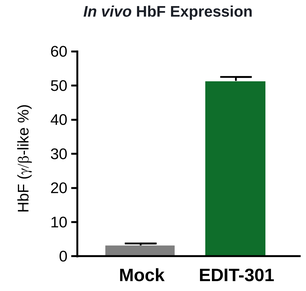
Large NHEJ Deletions Are Durable and Induce High Levels of HbF



EDIT-301 – No Detectable Off-targets



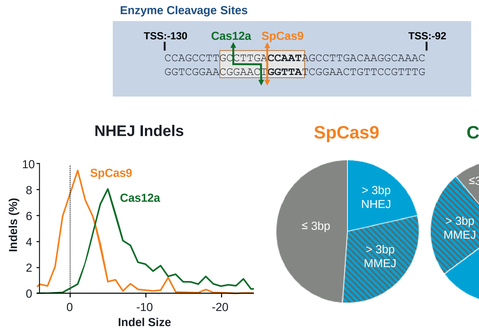
EDIT-301 – 50% HbF *In Vivo*



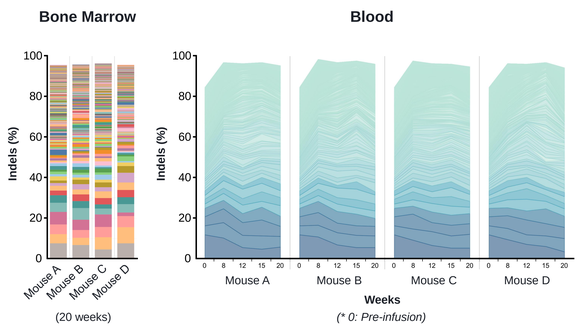
Methods

- Edited cells were generated by transfection of healthy donor mobilized CD34+ cells with spCas9 or Cas12a ribonucleoproteins (RNPs)
- Targeted Next-Gen Seq (NGS) was conducted for on and off-target editing levels and indel profile analysis. Identification of candidate off-target sites was performed using *in silico* prediction, Digenome and Guide-seq.
- HbF-inducing indels were identified in clonal erythroid cultures, derived from RNP-electroporated CD34+ cells
- *In vivo* studies were performed in NBSGW mice or NSG mice infused with mock or edited CD34+ cells.
- Lineage reconstitution (flow cytometry), editing (NGS), and HbF expression (HPLC and flow cytometry) analyses were conducted 16-20 weeks post engraftment.

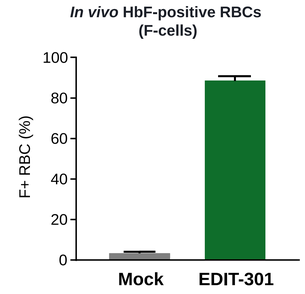
Cas12a Editing Demonstrates a Better Editing Profile for Persistent and High HbF Expression



EDIT-301 – High Polyclonality *In Vivo*



EDIT-301 – Pancellular HbF *In Vivo*



References

[1]Akinsheye I., et al. (2011). Blood, 118, 19-27.

[2]Ngo DA., et al. (2012). Br J Haematol, 156, 259-264.

[3]Liu N., et al. (2018). Cell, 173, 430-442.

[4]Strohkendl I. et al. (2018). Molecular Cell, 71, 816–824.

Acknowledgements

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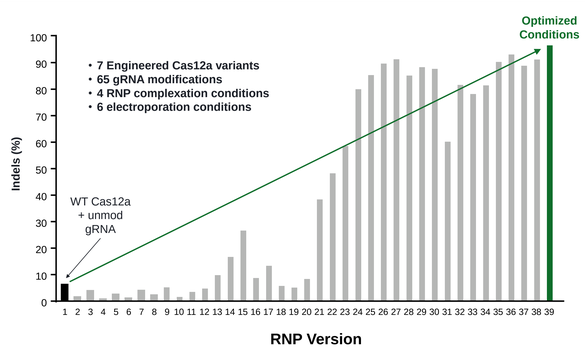
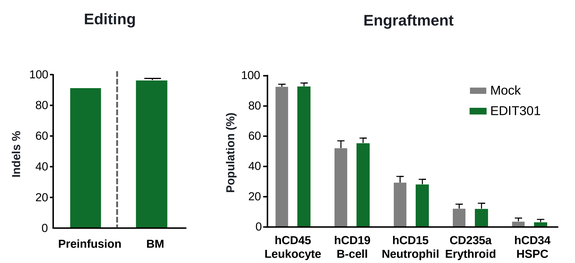
Disclosures

All authors were Editas employees and shareholders when the research was conducted.

Optimization Enabled Editing Levels >90%



EDIT-301 – Efficient Editing without Lineage Skewing *In Vivo*



Conclusions

- Cas12a produced more long-term HbF-inducing indels than SpCas9 at the HBG distal CCAAT box region.
- Greater than 90% indels were achieved after optimization of electroporation conditions and selection of best performing Cas12a variant and gRNA modifications
- EDIT-301 had no detectable off-target editing and contained highly-edited long-term HSCs (>90% indels) that engrafted mice with high polyclonality and no lineage skewing.
- 50% HbF levels were observed *in vivo* with pancellular distribution.

IND-enabling activities are ongoing for EDIT-301: an experimental autologous cell therapy comprising Cas12a-RNP modified mPB-CD34+ cells for the potential treatment of SCD.

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