Genome Editing of *HBG1/2* Promoter Leads to Robust HbF Induction *In Vivo*, While Editing of *BCL11A* Erythroid Enhancer Results in Erythroid Defects

PS1518

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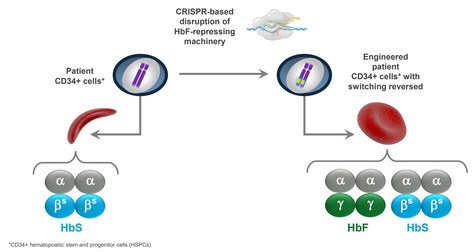
Introduction and Methods

Etiology of Sickle Cell Disease

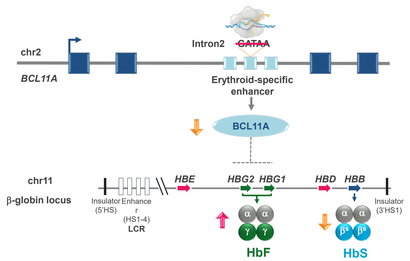
Sickle cell disease (SCD) is caused by a single mutation E6V of the β-globin chain, leading to polymerization of hemoglobin (Hb) and formation of sickle hemoglobin (HbS) fibers when deoxygenated. Symptoms include anemia, acute chest syndrome, pain crises, and an array of other complications. Patients suffer significant morbidity and early mortality.[1]

Study Objective

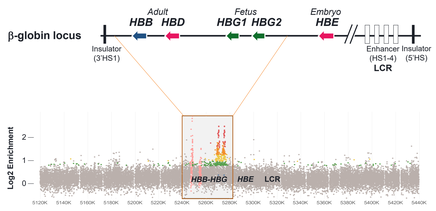
The aim of this study was to assess two different genome editing strategies targeting the *BCL11A* and β-globin loci to reverse hemoglobin switching for the treatment of sickle cell disease using CRISPR/Cas.



Approach 1: Downregulation of BCL11A Expression by Targeting its Erythroid Enhancer



Approach 2: Editing Cis-regulatory Elements in the β-Globin Locus



- ~26,000 gRNAs were tested covering 320kb genomic region.
- ~300 HbF-inducing gRNA were identified.
- Most were mapped to β-globin locus including *HBG*, *HBD*, and *HBB* genes.

Methods used in this study tested for specific preclinical target criteria:

1. Successful editing of long-term HSCs
2. Maintenance of normal HSPC function
3. Robust, long-term induction of HbF

1 Lineage reconstitution by flow cytometry

Human Chimerism	Erythroid	B Cell	Monocyte	Neutrophils	HSPCs
hCD45/WBC	CD235a/total	hCD19/hCD45	hCD14/hCD45	hCD15/hCD45	hCD34/hCD45

2 Editing analysis by Next-Gen Sequencing (NGS)

- Unfractionated BM
- Flow sorted erythroids, B cells, neutrophils, and Lin-HSPCs

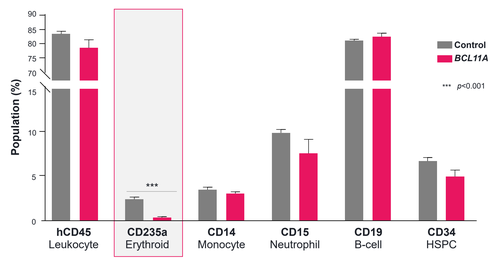
3 Analysis of HbF (γ/β-like) expression by reverse phase UPLC

- Flow sorted CD235a+ erythroid cells
- *Ex vivo* cultured erythroid cells from chimeric BM

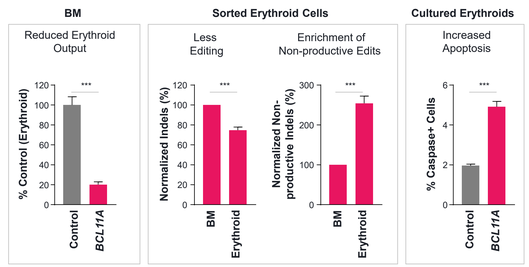
4 Apoptosis assessment of cultured erythroid cells by flow cytometry

Results: Downregulation of BCL11A Expression

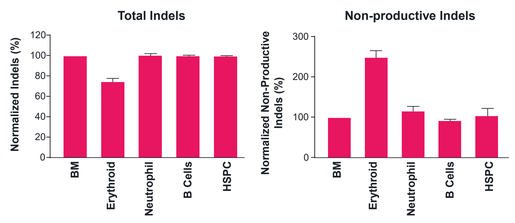
BCL11A Erythroid Enhancer-editing Displayed Reduced Erythroid Output in BM of NBSGW Mice



Reduced *BCL11A* Erythroid Output Coincided with Increased Non-productive Indels and Increased Apoptosis



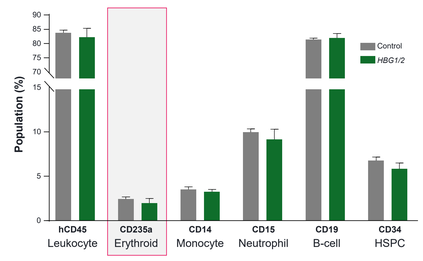
Only Erythroid Progeny Displayed Reduced Total Indels or Enriched Non-Productive Indels Following Transplantation of *BCL11A*-edited CD34+ HSPCs



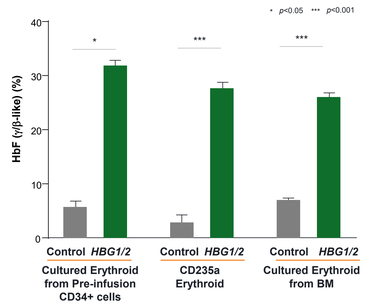
Although editing of long-term HSCs was observed, normal HSPC function was not maintained. Therefore, BCL11A erythroid enhancer-editing did not meet the preclinical target criteria.

Results: Editing Cis-regulatory Elements in the β-globin Locus

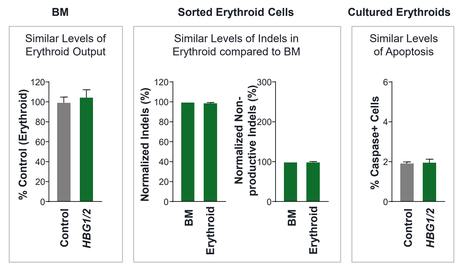
HBG1/2 Promoter-editing Displayed Normal Erythroid Output in BM of NBSGW Mice



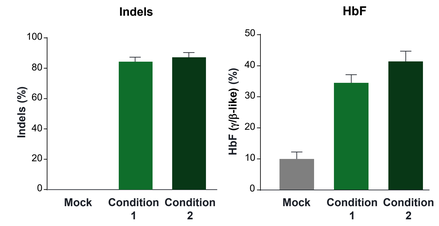
HBG1/2 Promoter Editing Demonstrated Long-term HbF Induction



HBG1/2 Promoter Editing Demonstrated No Erythroid Defect



Optimization of Nuclease and gRNA Pairs Increased HbF Induction to ~40% While Maintaining Editing Efficiency



Conclusions

- Long-term engraftment was observed in immunocompromised NBSGW mice with both *BCL11A* erythroid enhancer-edited and *HBG1/2* promoter-edited CD34+ HSPCs.
- In this study, *BCL11A*-edited CD34+ HSPCs had an erythroid differentiation defect in the NBSGW mouse model that was not observed in *HBG1/2*-edited CD34+ HSPCs.
- *In vivo*-derived erythroid cells from BCL11A-edited CD34+ HSPCs had reduced total indels and increased non-productive indels compared to other tested lineages, a phenomenon not observed with *HBG1/2* promoter editing.
- There was robust induction of HbF in *HBG1/2* promoter-edited erythroid cells from long-term (8-16 week) *in vivo* studies.[2]
- Further optimization of nuclease and gRNA combinations led to HbF expression of ~40%.
- IND-enabling activities have been initiated.

References

[1]Bender MA. In: Gene Reviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018.

[2]Chang et al. Presented at American Society of Hematology (ASH 2018), San Diego, CA

Acknowledgements

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