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

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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 \( \beta \)-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.

Study Objective

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

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

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

Approach 2: Editing Cis-regulatory Elements in the \( \beta \)-globin Locus

Results: Downregulation of BCL11A Expression

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

Results: Editing Cis-regulatory Elements in the \( \beta \)-globin Locus

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

HBG1/2 Promoter Editing Demonstrated Long-term HbF Induction

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 erythrocytes 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 BCL11A-edited CD34+ cell-derived erythroid cells from long-term (8-16 week) in vivo studies.
- Further optimization of nuclease and gRNA combinations led to HbF expression of ~40%.
- IND-enabling activities have been initiated.

References


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

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