Genome Editing of HBG1/2 Promoter Leads to Robust HbF Induction *In Vivo* While Editing of BCL11A Erythroid Enhancer Shows Erythroid Defect


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Overview

Etiology of Sickle Cell Disease

In Vivo Study Design to Evaluate Two Approaches to Increase Fetal Hemoglobin (HbF) Expression

Effect of Downregulating BCL11A Expression by Targeting its Erythroid Enhancer

Editing Cis-regulatory Elements in β-Globin Locus

Conclusion
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.
Harnessing Natural Anti-sickling Hemoglobin to Treat Sickle Cell Disease

β-globin locus

Embryo

Fetus

Adult

Insulator (5'HS)
Enhancer (HS1-4)
LCR

HBE

HBG2
HBG1

HBD
HBB

Insulator (3'HS1)

Globin switch

Onset of SCD Symptoms

% Globin Synthesis

0 25 50 75 100

Months Post-conception

0 3 6 9 12 15 18

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Adapted from Canver and Orkin, Blood, 2016
Genome Editing to Reverse Hemoglobin Switching for Treating Sickle Cell Disease

CRISPR-based disruption of HbF-repressing machinery

Patient CD34+ cells*

Engineered patient CD34+ cells* with switching reversed

HbS: ααββ
HbF: ααγγ
HbS: ααββ

*CD34+ hematopoietic stem and progenitor cells (HSPCs)
Preclinical Target Criteria

- Successful editing of long-term HSCs
- Maintenance of normal HSPC functions
- Robust long-term induction of HbF
Study Design for Assessment of Multilineage Engraftment Potential of Edited HSPCs

Edited of mock transfected CD34+ cells

Intravenous (IV) infusion

Bone marrow (BM) collection

NBSGW Mice

8-16 weeks

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

1 Lineage reconstitution by flow cytometry

<table>
<thead>
<tr>
<th>Human Chimerism</th>
<th>Erythroid</th>
<th>B Cell</th>
<th>Monocyte</th>
<th>Neutrophils</th>
<th>HSPCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCD45/WBC</td>
<td>CD235a/total</td>
<td>hCD19/hCD45</td>
<td>hCD14/hCD45</td>
<td>hCD15/hCD45</td>
<td>hCD34/hCD45</td>
</tr>
</tbody>
</table>

2 Editing analysis by Next-Gen Sequencing (NGS)

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

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

4 Apoptosis assessment of cultured erythroid cells by flow cytometry

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

chr2

BCL11A

Intron2

GATAA

Erythroid-specific enhancer

BCL11A

chr11

β-globin locus

Insulator (5'HS) Enhancer (HS1-4) LCR

HBE

HBG2 HBG1

HBD HBB

Insulator (3'HS1)

HbF

HbS

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

**Population (%)**

- hCD45 Leukocyte
- CD235a Erythroid
- CD14 Monocyte
- CD15 Neutrophil
- CD19 B-cell
- CD34 HSPC

**Control vs BCL11A**

- *** p<0.001
Reduced \textit{BCL11A} Erythroid Output Coincided with Increased Non-productive Indels and Increased Apoptosis

\begin{align*}
\text{BM} & \quad \text{Sorted erythroid cells} & \quad \text{Cultured erythroids} \\
\text{Reduced erythroid output} & \quad \text{Less editing} & \quad \text{Enrichment Of non-productive edits} & \quad \text{Increased apoptosis} \\
\% \text{Control (Erythroid)} & \quad \text{Normalized Indels (\%)} & \quad \text{Normalized non-productive Indels (\%)} & \quad \% \text{Caspase+ Cells} \\
\text{Control} & \quad \text{BM} & \quad \text{BM} & \quad \text{Control} \\
\text{BCL11A} & \quad \text{Erythroid} & \quad \text{Erythroid} & \quad \text{BCL11A} \\
\end{align*}

$p<0.001$

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BCL11A Erythroid Enhancer-editing Failed to Meet Preclinical Target Criteria

- Successful editing of long-term HSCs
- Maintenance of normal HSPC functions
- Robust long-term induction of HbF
Approach 2: Editing Cis-regulatory Elements in 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
Robust HbF Induction Achieved with Editing of HBG1/2 Promoters Ex Vivo

Multiple enzymes including SpCas9, SaCas9, Cpf1 (Cas12a) and gRNA combinations were tested.
**HBG1/2 Promoter-editing Displayed Normal Erythroid Output in BM of NBSGW Mice**

- **hCD45**
  - Leukocyte
- **CD235a**
  - Erythroid
- **CD14**
  - Monocyte
- **CD15**
  - Neutrophil
- **CD19**
  - B-cell
- **CD34**
  - HSPC

The graph shows the population (%) of each cell type for control and HBG1/2 conditions.
$HBG1/2$ Promoter Editing Demonstrated
No Erythroid Defect

Similar levels of erythroid output

Similar levels of indels in erythroid compared to BM

Similar levels of apoptosis

% Control (Erythroid)

Normalized Indels (%)

Normalized non-productive Indels (%)

% Caspase+ Cells

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**HBG1/2 Promoter Editing Demonstrated**

**Long-term HbF Induction**

![Graph showing HbF (γ/β-like) (%) for Control and HBG1/2 in CD235a Erythroid and Cultured erythroid from BM.](image-url)

- P-value: p<0.001

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Editing *HBG1/2* Promoters Met Critical Preclinical Target Criteria

- Successful editing of long-term HSCs
- Maintenance of normal HSPC functions
- Robust long-term induction of HbF
Conclusions

Long-term engraftment 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

Robust induction of HbF in HBG1/2 promoter-edited erythroid cells from long-term in vivo study