EDIT-301: AN AUTOLOGOUS CELL THERAPY TO PROMOTE FETAL HEMOGLOBIN EXPRESSION FOR THE POTENTIAL TREATMENT OF SICKLE CELL DISEASE

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Jack Heath is a full-time employee and shareholder in Editas Medicine
Overview

Inducing Anti-Sickling Fetal Hemoglobin to Treat Sickle Cell Disease

Desirable Editing with Cas12a at the \textit{HBG1/2} Promoter in a Region Where Fetal Hemoglobin Inducing Mutations Naturally Occur

Highly Efficient, Productive and Durable Editing and an Improved Phenotype in Edited Sickle Patient Red Blood Cells
Harnessing Natural Anti-Sickling Hemoglobin to Treat Sickle Cell Disease

β-globin locus

Insulator (5’HS) Enhancer (HS1-4) LCR

Embryo
HBE

Fetus
HBG2 HBG1

Adult
HBD HBB

Insulator (3’HS1)

Globin switch

% Globin Synthesis

Onset of SCD Symptoms

Adapted from Canver and Orkin, Blood, 2016.
Naturally Occurring Mutations Support Clinical Relevance and Safety of Editing at the \textit{HBG1/2} Promoter Region

\begin{itemize}
  \item \textbf{β-globin locus}
  \begin{itemize}
    \item \textbf{Embryo}
      \begin{itemize}
        \item HBE
      \end{itemize}
    \item \textbf{Fetus}
      \begin{itemize}
        \item HBG2
        \item HBG1
      \end{itemize}
    \item \textbf{Adult}
      \begin{itemize}
        \item HBD
        \item HBB
      \end{itemize}
  \end{itemize}

\end{itemize}

Multiple Naturally Occurring HbF Inducing Mutations located in \textit{HBG} Distal CCAAT Box

*CD34+ hematopoietic stem and progenitor cells (HSPCs)
Large NHEJ Deletions at *HBG* Distal CCAAT Box Region Are Durable and Induce High Levels of HbF

Long-Term HSCs Rely Heavily on NHEJ-mediated Editing

- > 3bp NHEJ deletions induce long-term, robust HbF expression

* Only showing levels of \( \gamma \) chain which represents the gene product of *HBG2*
Cas12a Demonstrates a Superior Editing Profile to SpCas9 at HBG Distal CCAAT Box Region for Persistent and High HbF Expression

Enzyme Cleavage Sites

Enzyme Cleavage Sites

TSS:-130
CCAGCCTTGCATTGACCAATAGCCTTGACAAGGCAAAC
GGTCGGAACGGAACGTTCTCGGAACTGGTTCTCGTTTG

Cas12a  SpCas9

TSS:-92

NHEJ Indels

SpCas9

Indel Size

Cas12a

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Cas12a Editing is Efficient and Results in Long-Term Robust and Pancellular HbF Expression *In Vivo*

**Editing**

- Preinfusion: n = 1
- 16 Weeks: n = 5

**In vivo HbF Expression**

- HbF (γβ-like %)
- Mock: n = 5
- Edited: n = 5

**In vivo HbF-positive RBCs (F-cells)**

- F + RBC (%)
- Mock: n = 5
- Edited: n = 5

*Data from one representative experiment*
Cas12a Edited CD34+ Hematopoietic Stem Cells Provide Stable, Polyclonal Engraftment with No Lineage Skewing

Engraftment

<table>
<thead>
<tr>
<th>Population (%)</th>
<th>hCD45</th>
<th>hCD19</th>
<th>hCD15</th>
<th>CD235a</th>
<th>hCD34</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leukocyte</td>
<td>B-cell</td>
<td>Neutrophil</td>
<td>Erythroid</td>
<td>HSPC</td>
</tr>
</tbody>
</table>

Mock
Edited

16 weeks post infusion. n = 5 mice / group

Blood

Bone Marrow

Indels (%)

Weeks

(0: Pre-infusion)

Representative data from one mouse

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Comparable Editing and Robust HbF Induction Achieved in Normal and Sickle Donor CD34+ Cells (EDIT-301)

Efficient editing and robust HbF induction observed in normal donors translate to sickle patient derived CD34+ cells and their progeny

Untreated cells did not undergo electroporation.
EDIT-301 RBCs from Sickle Patient Donors Have Significantly Reduced Sickling, Lower Point of Sickling and Improved Deformability

- **Reduced Sickling**
  - HbF 19.9% HbF 53.8%
  - When exposed to sodium metabisulfite

- **Lowered Point of Sickling**
  - HbF 19.9% HbF 53.8%
  - Measured with Lorrca Oxygenscan

- **Improved Deformability at Low O₂**
  - HbF 19.9% HbF 53.8%
Conclusions

Cas12a demonstrates a superior editing profile to SpCas9 at HBG distal CCAAT box region for persistent and high HbF expression.

Potentially therapeutically-relevant levels of HbF were expressed long-term with pancellular distribution in vivo.

High levels of editing were achieved with EDIT-301, leading to a significant reduction in sickling, lowered point of sickling and improved deformability of sickle patient RBCs.

Plan to file IND for EDIT-301 by end of 2020.
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