Robust Pre-Clinical Results and Large-Scale Manufacturing Process for EDIT-301: An Autologous Cell Therapy for the Potential Treatment of SCD

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Disclosures


• Nothing to disclose: S.H., D.K.W.
is an autologous cell therapy comprising CD34+ cells from patients with SCD (sickle cell disease) that are edited with CRISPR-Cas12a at the HBG1 and HBG2 promoters to induce the expression of anti-sickling fetal hemoglobin.

Objectives:

To demonstrate the **function and phenotype** of edited red blood cells (RBCs) derived from EDIT-301 *in vitro*

To evaluate the edited CD34+ cell **large-scale manufacturing process**
CRISPR-Cas12a editing at the HBG1 and HBG2 promoter regions induces anti-sickling fetal hemoglobin (HbF) to treat SCD

β-globin locus

Embryo

Fetus

Adult

Unedited CD34+ cells from patients with SCD

EDIT-301: Edited CD34+ cells from patients with SCD

CRISPR-Cas12a editing at the HBG1 and HBG2 promoter regions induces HbF expression

HbF production reduces RBC sickling by inhibiting formation of HbS fibers at low oxygen levels

Naturally occurring HbF-inducing mutations support clinical relevance and safety of editing

α α βs βs Sickle hemoglobin (HbS)

α α α α βs βs Y Y HbF

HbS

HbF

α CD34+ hematopoietic stem and progenitor cell
HS: hypersensitive site; LCR: locus control region; TSS: transcriptional start site
Adapted from Higgs, Engel and Stamatoyannopoulos. Lancet 2012
Comparable editing and robust HbF induction in edited CD34+ cells from normal donors and patients with SCD

Efficient editing

Robust ex vivo HbF expression

Unedited cells did not undergo electroporation
EDIT-301-derived RBCs have reduced sickling and improved rheological properties versus unedited SCD-derived RBCs.

**Reduced sickling**

Mean HbF (%): 19.9 for unedited and 53.8 for EDIT-301-derived RBCs.

*When exposed to sodium metabisulfite*

- Normal donor-derived RBCs
- Unedited SCD-derived RBCs
- EDIT-301 (edited SCD)-derived RBCs

**Improved rheological behavior**

*When placed in microfluidic channels, mimicking blood flow in microvasculature, at a range of oxygen levels*

**HbF levels correlated with velocity**

$R^2 = 0.9321$

Oxygen (%) 1% = 7.6mmHg
Successful development of edited CD34+ cell large-scale manufacturing process

- **Normal human donor**
- **Stem cell mobilization**
- **CD34+ cells**
- **CRISPR-Cas12a editing**
- **Edited CD34+ cells**
- **Edited CD34+ cell product**
- **Infusion**
- **NSG mice**

**In vitro assays**
- Recovery and viability of edited CD34+ cells
- Editing efficiency and purity of the cell product

**In vivo assays**
- Editing efficiency
- **Safety**: Lineage and clonality

**NSG**: NOD scid gamma
Consistent and robust large-scale manufacturing of edited CD34+ cells from normal donors

Robust large-scale manufacturing

Efficient editing maintained in vivo

Bone Marrow
20 weeks post-infusion

n=32 mice/donor
Infusion of edited CD34+ cells manufactured on a large scale to NSG mice leads to polyclonal engraftment with no lineage skewing.

No lineage skewing after engraftment

Stable polyclonal engraftment

Female NSG mice bone marrow 20 weeks post-infusion

n=46–48 mice/treatment

Blood draws over 20 weeks

20 weeks post-infusion

Representative data from one NSG mouse. Each color or color shade represents an individual indel signature.
Conclusions

High levels of editing were achieved in CD34\(^+\) cells, leading to potentially therapeutically relevant levels of HbF expression.

Significant reduction in sickling and improved rheological properties of EDIT-301 (edited SCD)-derived RBCs.

Consistent large-scale process suitable for use in clinical manufacturing showing multilineage, polyclonal engraftment, and persistence of high levels of editing in vivo.

Plan to file Investigational New Drug application for EDIT-301 by end of 2020.
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