Preclinical development of EDIT-301, an autologous cell therapy comprising AsCas12a-RNP-modified mobilized peripheral blood CD34⁺ cells for the potential treatment of transfusion-dependent beta thalassemia

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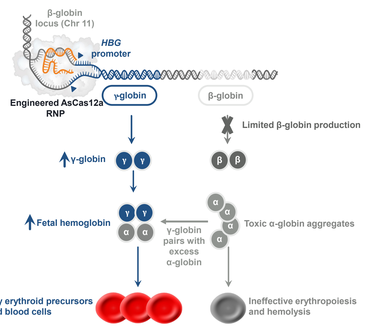
OBJECTIVE

- Based on *ex vivo* assay analysis, confirm that EDIT-301, an autologous cell therapy comprising hematopoietic stem and progenitor cells edited at the *HBG1* and *HBG2* promoters using a highly specific and efficient engineered AsCas12a enzyme, could be an efficacious therapy for patients with transfusion-dependent beta thalassemia

INTRODUCTION

- Beta thalassemia is one of the most common recessive hematologic disorders in the world, characterized by limited or complete absence of β-globin production.[1]
- Insufficient β-globin production leads to the formation of toxic α-globin aggregates, which cause maturation blockage and premature death of erythroid precursors and hemolysis of red blood cells, leading to severe anemia.[1,2]
- Patients with transfusion-dependent beta thalassemia (TDT), the most severe form of beta thalassemia, are burdened with lifelong blood transfusions and iron chelation therapy.[1,2]
- EDIT-301 is a cell therapy product consisting of autologous CD34⁺ hematopoietic stem and progenitor cells edited at the γ-globin gene promoter distal CCAAT box region where multiple naturally occurring fetal hemoglobin-inducing mutations reside, using a highly specific and engineered AsCas12a enzyme.
- Editing at the γ-globin promoter distal CCAAT box region mimics these natural mutations and induces robust γ-globin production.
- EDIT-301 is currently being evaluated in the Phase 1/2 RUBY clinical trial for the treatment of severe sickle cell disease.[3]

EDIT-301 mechanism of action



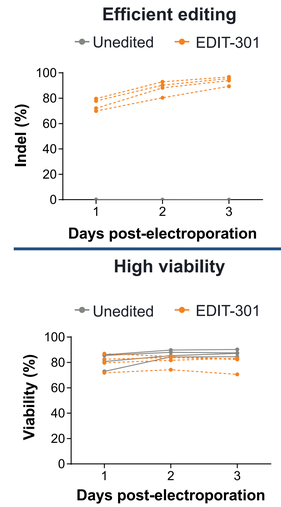
Chr: Chromosome; *HBG*: γ-globin; RNP: Ribonucleoprotein

METHODS

- Three batches of EDIT-301 were generated by electroporating mobilized peripheral blood CD34⁺ cells from three TDT donors with an engineered AsCas12a ribonucleoprotein.
- EDIT-301 was placed in erythroid differentiation conditions and *in vitro* erythropoiesis was monitored using flow cytometry to assess erythroid commitment, maturation, and health.
- Changes in γ-globin and total globin production, both at the mRNA and protein levels, were evaluated using reverse-transcription droplet digital polymerase chain reaction and reverse-phase ultra-performance liquid chromatography.

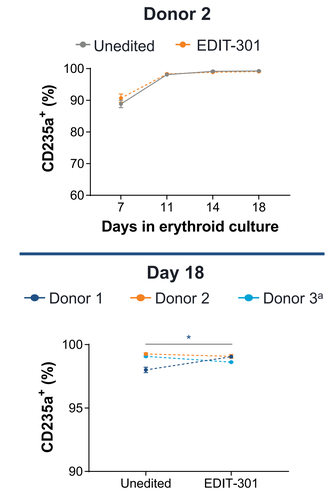
RESULTS

Figure 1. Engineered AsCas12a efficiently edited TDT-derived CD34⁺ cells while maintaining high viability



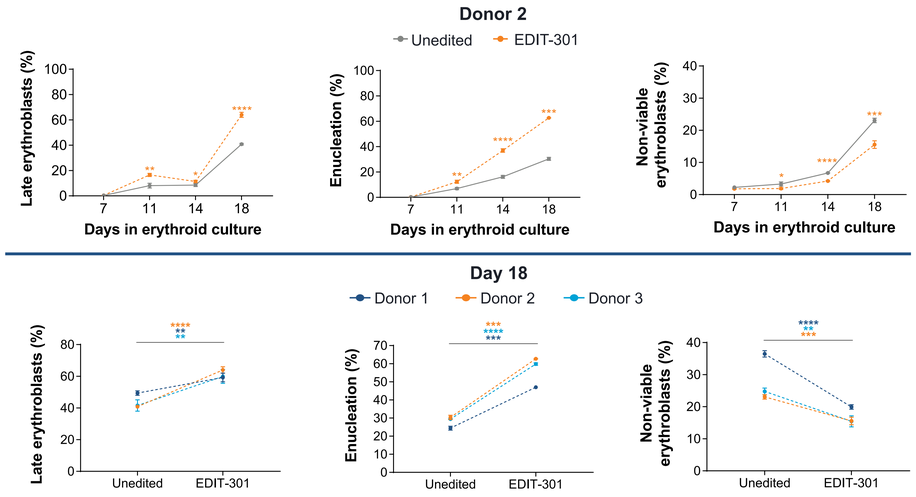
N=3 independent donors

Figure 2. EDIT-301 cells successfully underwent erythroid differentiation at a similar rate to unedited controls



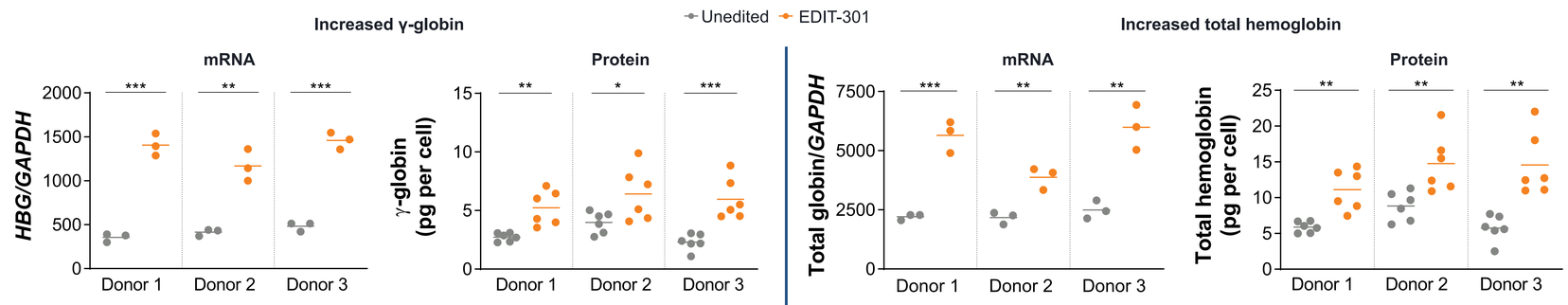
N=3 independent donors with triplicate cultures. *p<0.05. [a]Donor 3 showed a significant decrease (p<0.001) in erythroid commitment (% of CD235a⁺ cells)

Figure 3. EDIT-301 erythroid cells had significantly improved erythroid maturation and decreased erythroid death compared with unedited controls



N=3 independent donors with triplicate cultures. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

Figure 4. EDIT-301 erythroid cells had significantly increased γ-globin and total hemoglobin levels compared with unedited controls at both the mRNA and protein levels



mRNA: N=3 independent donors with three technical replicate cultures. Protein: N=3 independent donors with six technical replicate cultures.*p<0.05; **p<0.01; ***p<0.001
GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; *HBG*: γ-globin

CONCLUSIONS

- EDIT-301 erythroid cells exhibited significantly improved erythroid maturation and decreased erythroid death, therefore reversing the maturation blockage associated with TDT mutations.
- EDIT-301 erythroid cells had significantly increased γ-globin production and total hemoglobin content per cell.
- These preclinical data suggest that EDIT-301, edited at the γ-globin promoters where multiple naturally occurring fetal hemoglobin-inducing mutations reside, may be an efficacious treatment option to correct the ineffective erythropoiesis and severe anemia that characterize TDT, and support the start of clinical investigation.

REFERENCES

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3. Clinicaltrials.gov. NCT04853576

DISCLOSURES

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