Targeted Lipid Nanoparticle Delivery in Non-Human Primates Enables *In Vivo* *HBG1/2* Promoter Editing for β-hemoglobinopathies

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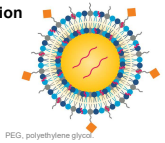
INTRODUCTION

- Genome editing of regulatory elements to reactivate γ-globin gene (*HBG1/2*) expression, resulting in fetal hemoglobin (HbF) induction, has been clinically validated as a therapeutic strategy for sickle cell disease (SCD) and transfusion-dependent β-thalassemia (TDT).
- This validation is based on clinical trial data for renizgamglogene autogedtemcel (reni-cel), an *ex vivo* autologous gene editing therapy. In these trials, patients who received reni-cel exhibited rapid and sustained HbF induction and normalization of total hemoglobin. Of 28 treated patients with SCD, 27 were vaso-occlusive event–free and all 8 treated patients with TDT with >1 month of follow-up achieved transfusion independent, as of the data cutoff date of October 29, 2024 and November 12, 2024, respectively.
- While reni-cel has shown promising clinical results, *ex vivo*-based autologous genome editing therapies present significant challenges across the patient journey and manufacturing process. Thus, an *in vivo*-based genome editing approach targeting the *HBG1/2* promoters offers an attractive alternative with the potential to significantly reduce patient burden and facilitate access to treatment globally.
- Lipid nanoparticle (LNP)–based delivery systems have been successfully utilized to deliver nucleic acid therapeutics, including gene editing cargo. However, the currently available LNPs have been designed to efficiently deliver to the liver. Through iterative optimization design cycles, we have developed a proprietary LNP containing a targeting moiety that enables delivery of editing cargo to the hematopoietic stem cells (HSCs).
- Here, we report results from a non-human primate (NHP) study showing editing in HSCs in the bone marrow following intravenous (IV) administration of a targeted LNP (tLNP) containing *HBG1/2* editing cargo.

LNP composition



PEG, polyethylene glycol

- Lipid-anchored PEG
- Helper lipid
- Ionizable lipid
- Cholesterol
- Targeting moiety
- Cargo

AIM

- The primary goal of the study is to evaluate the *in vivo* editing efficiency of a tLNP containing *HBG1/2* promoter-specific gene editing cargo in the HSCs of NHPs.
- The study also evaluates the delivery of a tLNP to the HSCs and biodistribution in the representative non-target tissues using a green fluorescent protein (GFP) mRNA surrogate cargo.

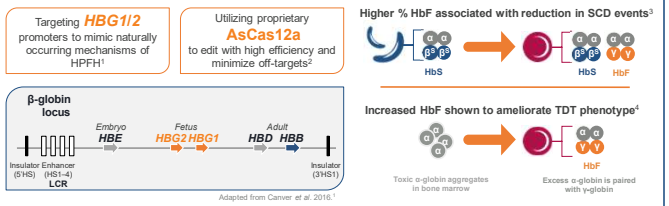
METHODS

- Through an iterative process, tLNPs were optimized for *in vivo* delivery of the *HBG1/2* gene editing cargo to the hematopoietic stem and progenitor cells (HSPCs) and HSCs in the bone marrow using NBSGW mice engrafted with human cluster of differentiation 34$^+$ (CD34$^+$) cells. This evaluation in mice informed the selection of a tLNP for NHP testing.
- For NHP testing, an optimized tLNP contained either *HBG1/2* editing cargo (tLNP-*HBG1/2*) for assessment of editing efficiency or GFP mRNA cargo (tLNP-GFP) to assess biodistribution. Female NHPs (three per group) were dosed with either tLNP-*HBG1/2* (Group 1) or tLNP-GFP (Group 2) via a single IV injection. Editing in Group 1 animals was assessed in HSCs obtained from the bone marrow aspirate at various time points. Group 2 animals were sacrificed 24 hours post-dose, and GFP delivery was assessed in the HSCs. **Plasma pharmacokinetics (PK) was assessed in both groups by quantifying mRNA using reverse transcription-droplet digital PCR.** In addition, representative non-target tissues were collected for biodistribution assessment using an in situ hybridization (ISH) assay for detecting GFP mRNA, and an immunohistochemistry (IHC) assay for detecting the corresponding GFP protein.

DESIGN PROCESS AND RESULTS

Figure 1. Editas' *in vivo* HSC program uses a clinically validated target and enzyme

Editing the HBG1/2 *promoters with AsCas12a leads to HbF induction and correction of anemia*

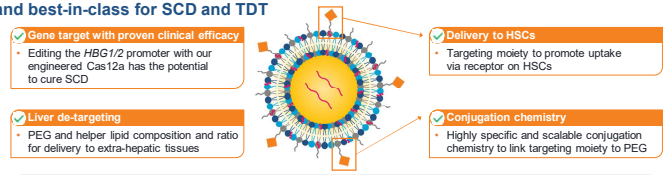


Adapted from Canver *et al.* 2016.[1]

Naturally occurring HbF-inducing mutations in HPFH predict the clinical relevance and safety of editing at the *HBG1/2* promoters

AsCas12a, *Acidaminococcus* sp. CRISPR-associated protein 12a; CRISPR, clustered regularly interspaced short palindromic repeats; *HBB*, β-globin gene; *HBD*, δ-globin gene; *HBE*, embryonic hemoglobin gene; HbF, fetal hemoglobin; *HBG*, γ-globin gene; HbS, sickle hemoglobin; HPFH, hereditary persistence of fetal hemoglobin; HS, hypersensitive site; HSC, hemopoietic stem cell; LCR, locus control region; SCD, sickle cell disease; TDT, transfusion-dependent β-thalassemia.

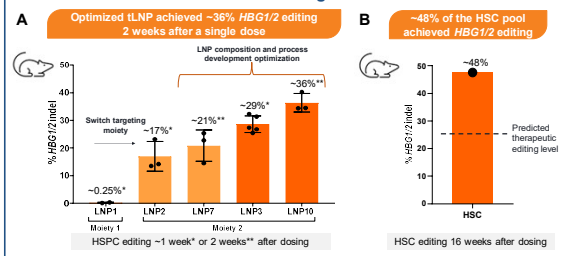
Figure 2. Editas' *in vivo* HSC program is positioned to potentially be first-in-class and best-in-class for SCD and TDT



Integration of multiple unique components has enabled Editas to achieve high efficiency HSC editing *in vivo*

Cas12a, CRISPR-associated protein 12a; CRISPR, clustered regularly interspaced short palindromic repeats; *HBG*, γ-globin gene; HSC, hemopoietic stem cell; PEG, polyethylene glycol; SCD, sickle cell disease; TDT, transfusion-dependent β-thalassemia.

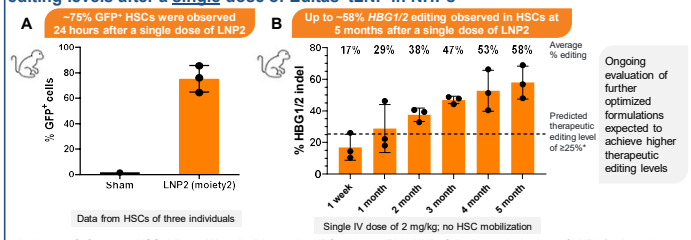
Figure 3. Optimization of Editas' tLNP platform provides capability to achieve efficient and sustainable *HBG1/2* editing in humanized mouse model



In vivo proof-of-concept editing at the *HBG1/2* locus in HSPCs **(A)** and HSCs **(B)** in a humanized mouse model following a single dose of tLNP. Editing in HSCs exceeded the predicted clinical threshold needed for therapeutically relevant editing.

In vivo model: NBSGW mouse strain (NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ [NSG] crossed with C57BL/6J-*Kit*$^{W-41J}$ [C57BL/6.KitW41J]) engrafted, without irradiation, with human CD34$^+$ cells from peripheral blood after plerixafor mobilization of cells from bone marrow. HSC defined as Lin$^-$CD34$^+$CD38$^-$CD90$^+$CD45RA$^-$ cells. HSPC defined as Lin$^-$CD34$^+$CD38$^-$ cells. CD, cluster of differentiation; *HBG*, γ-globin gene; HSC, hemopoietic stem cell; HSPC, hematopoietic stem and progenitor cell; LNP, lipid nanoparticle, tLNP, targeted LNP.

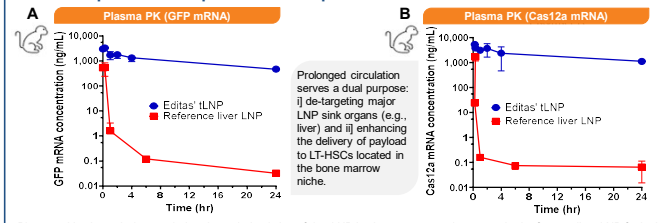
Figure 4. High efficiency HSC delivery achieved therapeutically relevant *HBG1/2* editing levels after a single dose of Editas' tLNP in NHPs



In vivo proof-of-concept HSC delivery **(A)** and editing at the *HBG1/2* locus **(B)** in NHPs following a single dose of LNP2 (moiety *2*). Editing in HSCs exceeded the predicted clinical threshold set for therapeutically relevant editing.

HSCs defined as CD34$^+$CD90$^+$CD45RA$^-$ cells based on Radtke S *et al.* 2017.[2] Clonal profile is consistent across all timepoints. *Therapeutically relevant editing threshold of ≥25% determined on the basis of editing dynamics and allogeneic HSC transplantation data from Fitzhugh CD *et al.* 2017.[4] CD, cluster of differentiation; GFP, green fluorescent protein; *HBG*, γ-globin gene; HSC, hematopoietic stem cell; IV, intravenous; LNP, lipid nanoparticle; NHP, non-human primate; tLNP, targeted LNP.

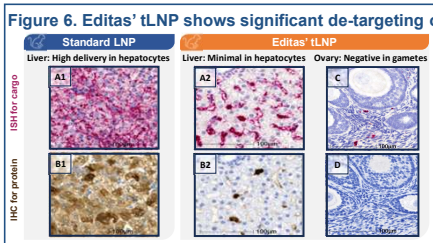
Figure 5. Pharmacokinetic analysis shows that the Editas' tLNP exhibits a longer circulation profile in the plasma as compared with a standard reference liver LNP



Pharmacokinetic analysis revealed prolonged circulation of the tLNP in plasma compared to a standard reference liver LNP for both tLNP-GFP **(A)** and tLNP-*HBG1/2* **(B)**.

Cas12a, CRISPR-associated protein 12a; CRISPR, clustered regularly interspaced short palindromic repeats; GFP, green fluorescent protein; LNP, lipid nanoparticle; LT-HSC, long-term hematopoietic stem cell; ND, not determined; PK, pharmacokinetic; tLNP, targeted LNP.

Figure 6. Editas' tLNP shows significant de-targeting of the liver in NHPs



NHPs dosed with GFP mRNA encapsulated in tLNP show favorable biodistribution results 24 hours post-end of infusion. ISH (red puncta) was used to measure GFP mRNA (top row), and IHC (brown signal) was used to measure corresponding protein (bottom row). Representative images are shown. Compared with a standard formulation (**A1**, **B1**) at the same timepoint, the tLNP shows distribution limited to the sinusoidal spaces and sinusoidal cells (**A2**, **B2**), suggesting a liver de-targeted profile. Importantly, there was no detectable signal in the developing oocytes (**C**, **D**) of the ovaries. Bone marrow samples were also analyzed for Cas12a protein. No detectable Cas12a protein was observed in the available Day 30 samples, consistent with the expectation of lack of nuclease persistence (data not shown).

Cas12a, CRISPR-associated protein 12a; CRISPR, clustered regularly interspaced short palindromic repeats; GFP, green fluorescent protein; IHC, immunohistochemistry; ISH, in situ hybridization; LNP, lipid nanoparticle; NHP, non-human primate; tLNP, targeted lipid nanoparticle.

CONCLUSIONS

- In NHPs, IV administration of tLNP-*HBG1/2* resulted in on-target editing levels in the *HBG1/2* promoter region of up to an average of ~58% in HSCs (CD34+CD90+CD45RA−) without requiring mobilization or conditioning.
- A single dose of the optimized tLNP-GFP resulted in a positive GFP signal distribution in ~75% of HSCs. Additionally, this tLNP demonstrated a favorable biodistribution profile based on GFP ISH and IHC signals in the non-target tissues.
- These data demonstrate high efficiency delivery, therapeutically relevant editing levels, and a favorable biodistribution profile, thereby warranting further development of Editas' proprietary HSC-tLNP for editing of the *HBG1/2* promoters for the treatment of β-hemoglobinopathies.

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