In vivo delivery of *HBG1/2* promoter editing cargo to HSCs of humanized mouse and non-human primate with lipid nanoparticles

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Li Li, Peisheng Zhang, Stephen Sherman, Ying Shao, Emily Kaye, Katerina Tsiounis, Jieni Xu, He Wang, Tallie Anne Algiere, Ramya Viswanathan, Swarali Lele, Jimit Raghav, Morgan P. Thompson, Ruhong Dong, Meng Wu, Eugenio Marco, Stephen Pietrasiewicz, Mark Jones, Linnea Jansson-Fritzberg, Bryant Chica, Georgia Giannoukos, Steven Wolk, Kai-Hsin Chang, Anshul Gupta, Linda Burkly, John Zuris, Sheryl R. Bowley

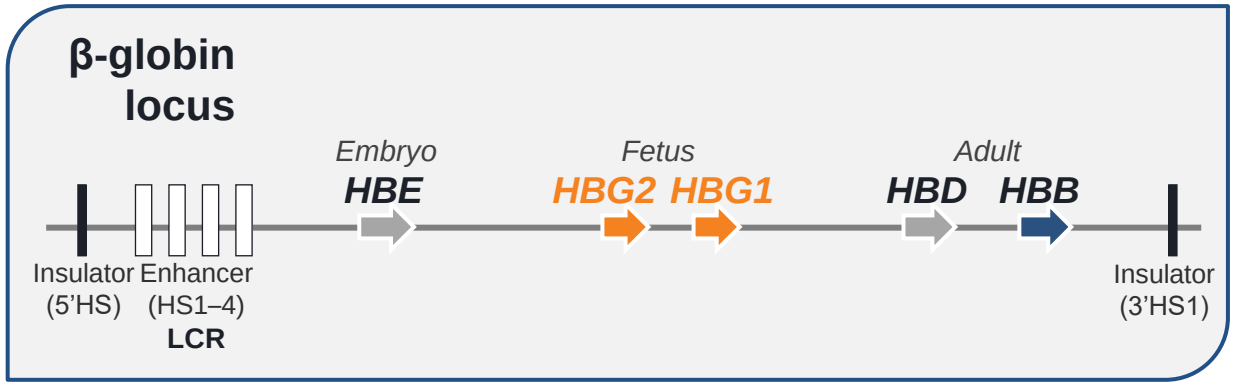
Editas Medicine, Inc., Cambridge, MA, United States

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

Editing *HBG1* and *HBG2* promoters with AsCas12a leads to HbF induction and correction of anemia

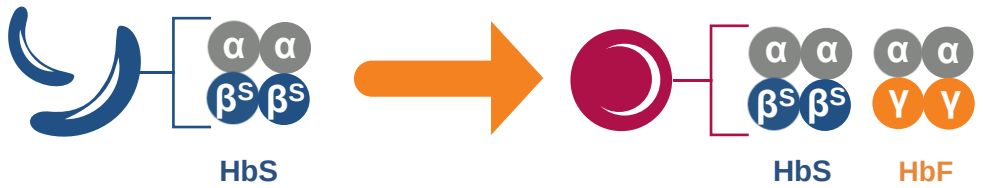
Targeting
HBG1/HBG2 promoters
to mimic naturally occurring mechanisms of HPFH[1]

Utilizing proprietary
AsCas12a
to edit with high efficiency and minimize off-targets[2]

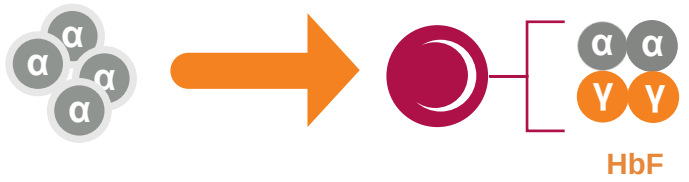


Adapted from Canver *et al*. 2016.

Higher % HbF associated with reduction in SCD events[3]



Increased HbF shown to ameliorate TDT phenotype[4]



Toxic α-globin aggregates in bone marrow

Excess α-globin is paired with γ-globin

Naturally-occurring HbF-inducing mutations in HPFH predict the clinical relevance and safety of editing at the *HBG1/HBG2* 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; LCR, locus control region; SCD, sickle cell disease; TDT, transfusion-dependent beta-thalassemia. 1. Canver MC *et al. Blood* 2016; 127 (21): 2536–2545. 2. Zhang L *et al. Nat Commun* 2021; 12 (1): 4500. 3. Powars DR *et al. Blood* 1984; 63 (4): 921–926. 4. Musallam KM *et al*. *Blood* 2012; 119 (2): 364–367.

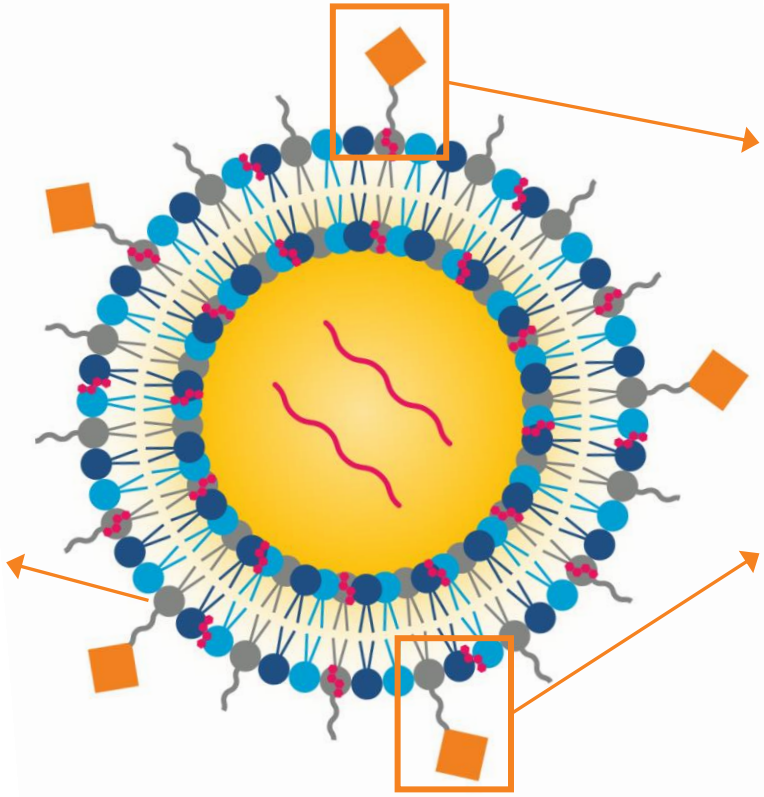
Editas' *in vivo* HSC program is positioned to be potentially first-in-class and best-in-class for sickle cell disease and transfusion-dependent beta-thalassemia

Gene target with proven clinical efficacy

- Editing the *HBG1/2* promoter with our engineered Cas12a has the potential to cure SCD

Liver de-targeting

- PEG and helper lipid composition and ratio for delivery to extra-hepatic tissues



Delivery to HSC cells

- Targeting moiety to promote uptake via receptor on HSC

Conjugation chemistry

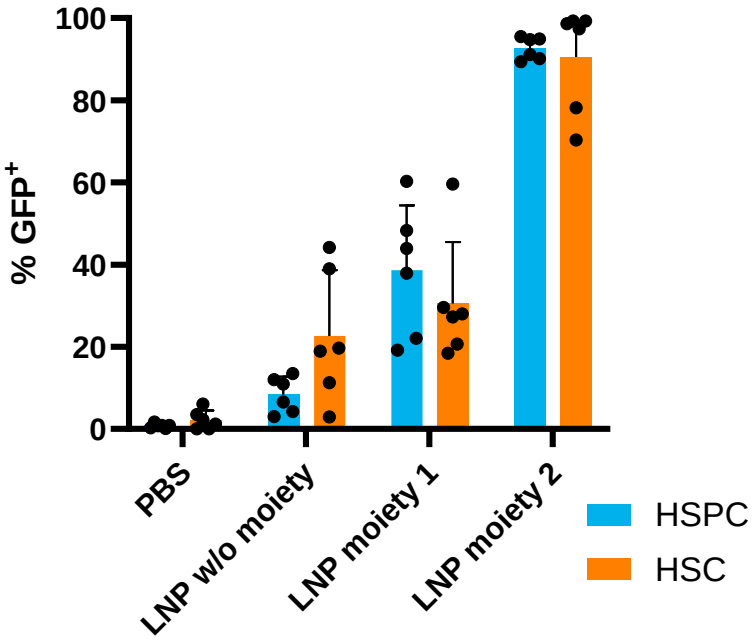
- Highly specific and scalable conjugation chemistry to link targeting moiety to PEG

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.

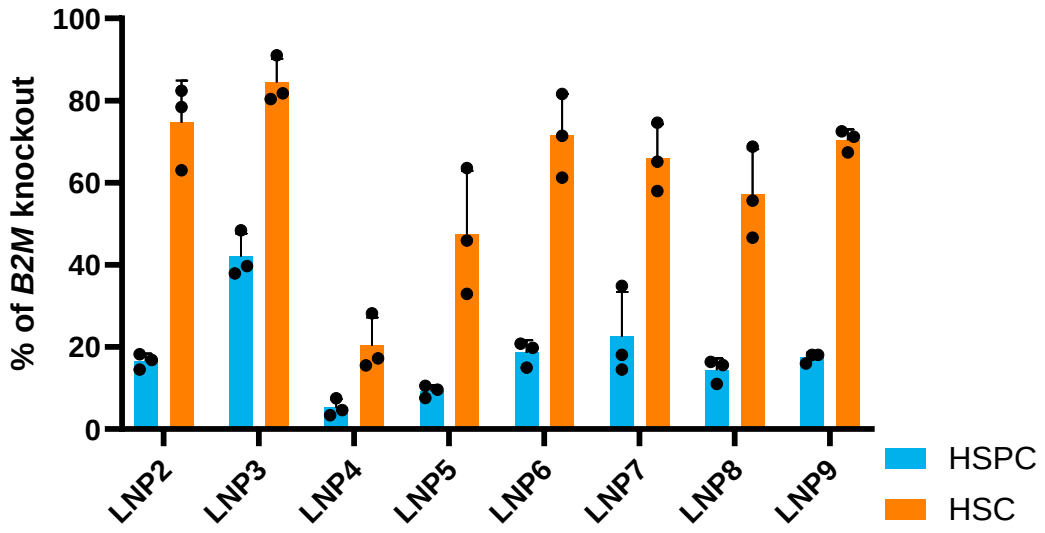
Administration of targeted LNPs achieves efficient delivery of either GFP or *B2M* editing cargo in mice engrafted with human CD34+ cells

Flow cytometry analyses of GFP expression in bone marrow



GFP expression, 24 hours after dosing

Flow cytometry analyses of B2M expression in bone marrow

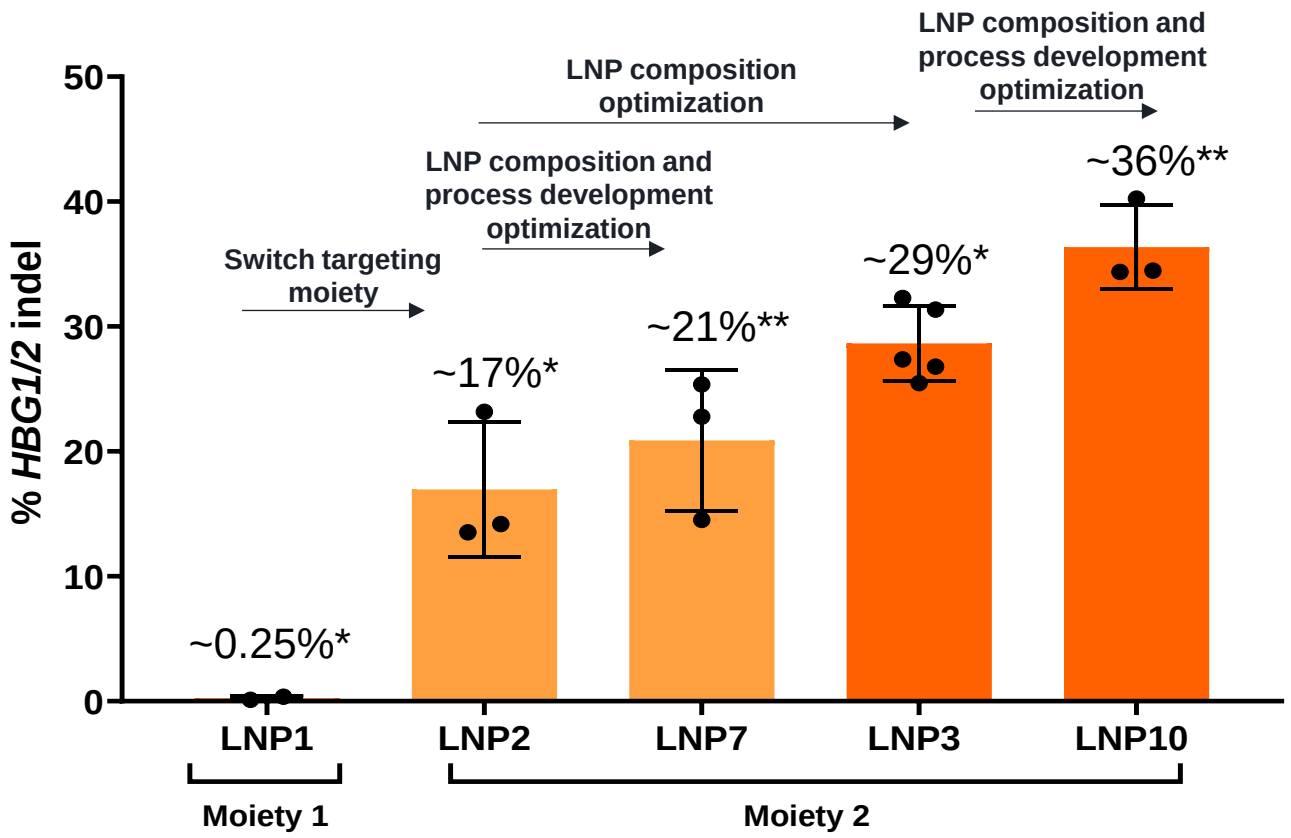


B2M knockout, 7 days after dosing
All LNPs carry targeting moiety 2

In vivo model: NBSGW mouse strain (NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ [NSG] crossed with C57BL/6J-*Kit*$^{W-41J}$/J [C57BL/6.*Kit*^{W41}]) engrafted, without irradiation, with human CD34^{+} cells from peripheral blood after plerixafor mobilization of cells from bone marrow.
B2M, beta-2-microglobulin; CD, cluster of differentiation; GFP, green fluorescent protein; HSC, hemopoietic stem cell defined as CD34^{+}LIN^{-}CD38^{-}CD45RA^{-}CD90^{+}; HSPC, hemopoietic stem and progenitor cell defined as CD34^{+}LIN^{-}CD38^{-}; LNP, lipid nanoparticle; PBS, phosphate-buffered saline; w/o, without.

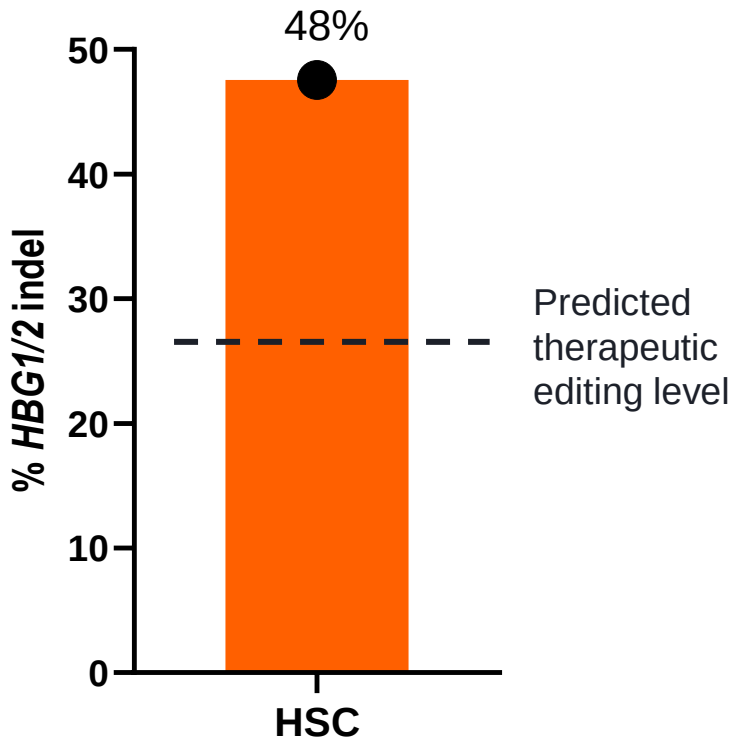
Optimization of tLNP platform provides capability to achieve efficient and sustainable *HBG1/2* editing in humanized mouse model

HBG1/2 editing in HSPC



HSPC editing ~1 week* or 2 weeks** after dosing

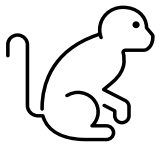
HBG1/2 editing in HSC



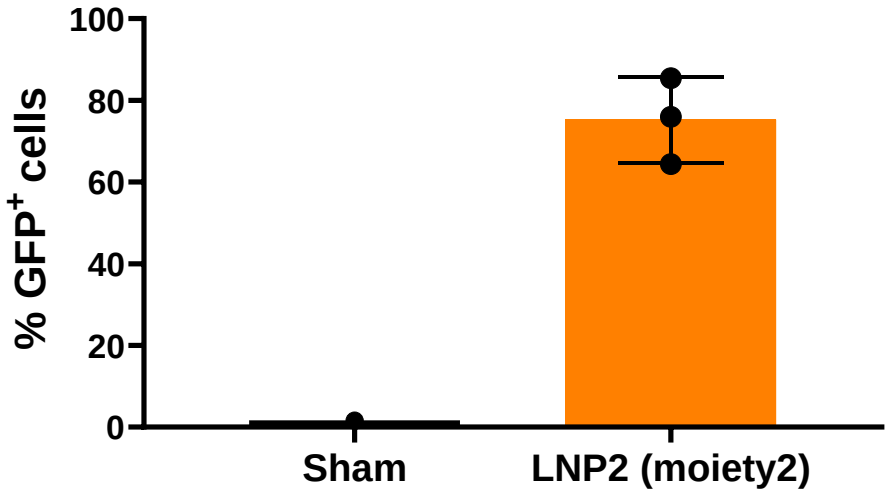
HSC editing 16 weeks after dosing

In vivo model: NBSGW mouse strain (NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ [NSG] crossed with C57BL/6J-*Kit*$^{W-41J}$/J [C57BL/6.*Kit*^{W41}]) engrafted, without irradiation, with human CD34^{+} cells from peripheral blood after plerixafor mobilization of cells from bone marrow.
HBG, γ-globin gene; HSC, hemopoietic stem cell defined as Lin^{-}CD34^{+}CD38^{-}CD90^{+}CD45RA^{-} cells; HSPC, hemopoietic stem and progenitor cell defined as Lin^{-}CD34^{+}CD38^{-} cells; LNP, lipid nanoparticle; tLNP, targeted LNP.

High efficiency HSC delivery achieved therapeutically relevant *HBG1/2* editing levels after a single dose of Editas' tLNP in non-human primates

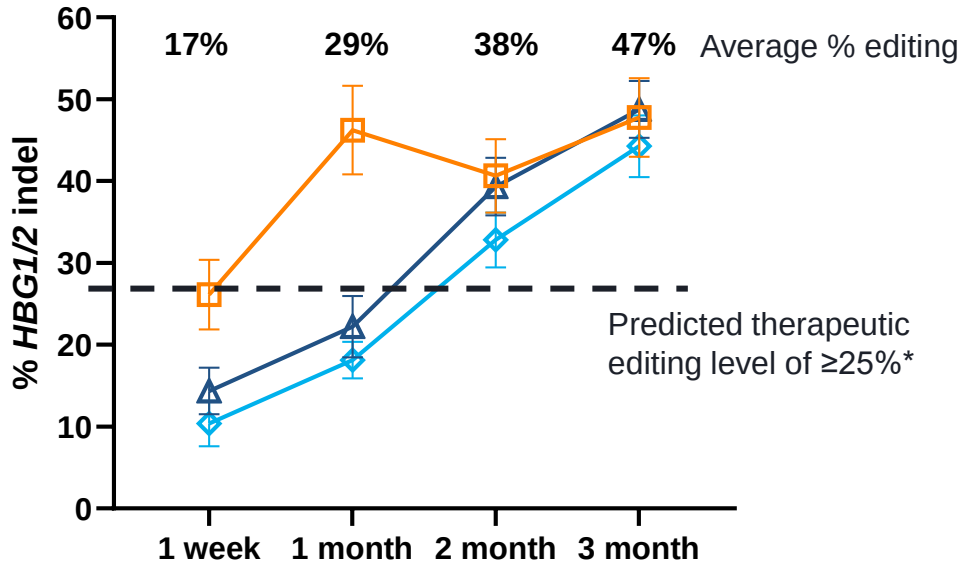


~75% GFP⁺ HSCs observed 24 hours after a single dose of LNP2



Data from HSC of three individuals

Up to ~47% *HBG1/2* editing observed in HSCs at 3 months after a single dose of LNP2 (moiety 2)

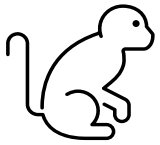


Single IV dose of 2 mg/kg; no HSC mobilization

Ongoing evaluation of further optimized formulations expected to achieve higher editing levels

HSCs defined as $CD34^+CD90^+CD45RA^-$ cells based on Radtke S *et al*. 2017.[1] 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.[2]
GFP, green fluorescent protein; *HBG*, γ-globin gene; HSC, hematopoietic stem cell; IV, intravenous; LNP, lipid nanoparticle; tLNP, targeted LNP.
1. Radtke S *et al*. *Sci Transl Med* 2017; 9 (414): eaan1145. 2. Fitzhugh CD *et al*. *Blood* 2017; 130 (17):1946–1948.

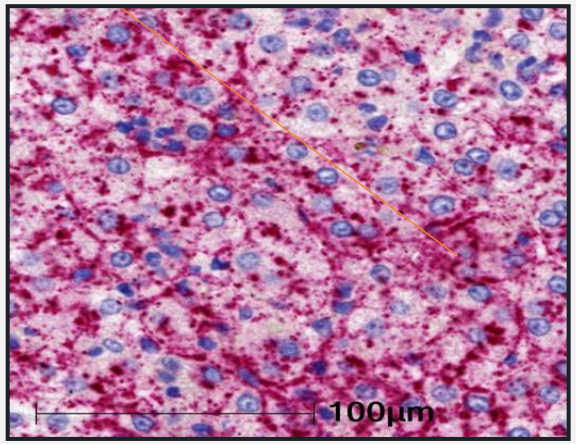
Editas' tLNP shows significant de-targeting of the liver in non-human primates



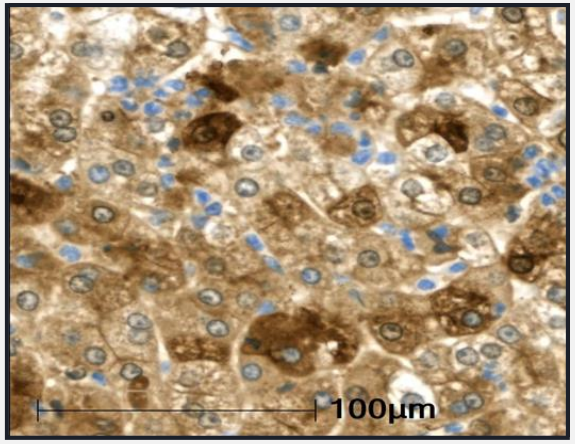
Standard LNP (comparator)

Liver: High delivery in hepatocytes

ISH for cargo

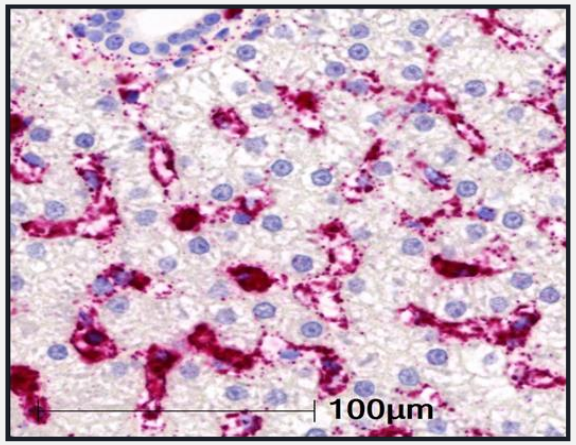


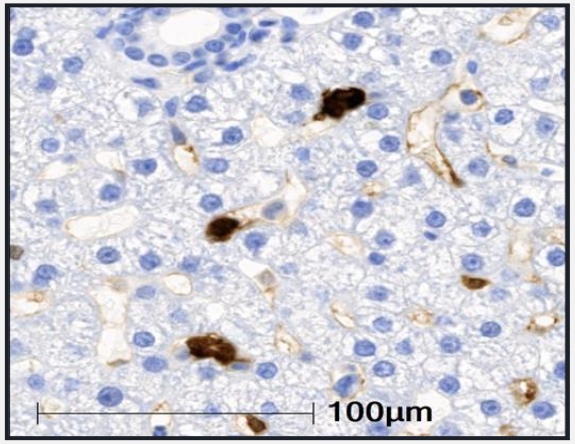
IHC for protein



Editas' tLNP

Liver: Minimal in hepatocytes





Editas' tLNP formulation containing surrogate GFP mRNA cargo was dosed to NHPs as a single IV infusion. Animals were taken down at 24 hours after dose and various non-target tissues were collected. GFP mRNA was detected in FFPE fixed tissue sections via ISH while the corresponding GFP protein was detected via IHC.

FFPE, formalin-fixed, paraffin-embedded; GFP, green fluorescent protein; *HBG*, γ-globin gene; HSC, hematopoietic stem cell; ISH, in situ hybridization; IHC, immunohistochemistry; IV, intravenous; LNP, lipid nanoparticle; NHP, non-human primate; tLNP, targeted LNP.

Summary

- Editas' proprietary LNP formulation with a targeting moiety efficiently delivers either reporter or editing cargo (GFP mRNA or Cas12a mRNA and guide RNA) to HSCs in both a humanized mouse model and NHPs
- Administration of a single dose of proprietary tLNP achieved 48% editing of *HBG1/2* in long-term HSCs in humanized mice, exceeding the threshold required for therapeutic benefit
- An ongoing NHP study demonstrated HSC delivery and achieved therapeutically relevant *HBG1/2* editing levels following administration of a single dose of tLNP
- Preliminary biodistribution data in NHPs with Editas' tLNP shows significant de-targeting of the liver in contrast to standard LNPs

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B2M, beta-2-microglobulin; Cas12a, CRISPR-associated protein 12a; CRISPR, clustered regularly interspaced short palindromic repeats; GFP, green fluorescent protein; *HBG*, γ-globin gene; HSC, hematopoietic stem cell; LNP, lipid nanoparticle; NHP, non-human primate; tLNP, targeted LNP.