Design and Development of LNP Targeting Ligands for *In Vivo* Hematopoietic Stem Cell Editing

Stephen E. Sherman, Katerina Tsiounis, Li Li, Peisheng Zhang, Ying Shao, Tallie Anne Algiere, Ryan Santosh, Harry Wang, Ramya Viswanathan, Swarali Lele, Jieni Xu, John Zuris, Sheryl Bowley

Editas Medicine, Cambridge, MA, United States

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Introduction

- Lipid nanoparticle (LNP) technology enables the non-viral delivery of nucleic acid cargo to cells. Loading LNPs with gene-editing cargo (Cas12a mRNA and guide RNA) provides an opportunity to perform *in vivo* gene editing. [1-2]
- The lack of LNP selectivity after systemic administration remains a challenge to minimize broad off-target editing to non-target cells after systemic administration of LNPs with gene-editing cargo.
- Here, we describe the development and demonstration of proof-of-concept for a targeted LNP (tLNP) strategy to enable the selective editing of discreet cellular compartments *in vivo*.
- Our proof-of-concept data towards the targeting of hematopoietic stem cells (HSCs) *in vivo* demonstrate both the validity of our targeting strategy and the potential for our *in vivo* gene-editing strategy to provide transformational medicines to patients without the need for complex *ex vivo* therapeutic regimens.

Targeted Lipid Nanoparticle Composition

Targeted LNP Strategy

Targeting ligand helps deliver the gene-editing cargo to a specific cell type, as well as improve uptake of the LNP

Methods

A screening platform was designed to select targeting moieties that maximize the internalization of cell-surface targets while maintaining cellular specificity for HSCs. First, this approach utilized a combination of available omics datasets paired with flow cytometry–based measurements with the target-of-interest. After identifying candidate surface markers based on expression, potential targeting candidates were further assessed for their internalization potential to validate their candidacy as a viable target for LNP delivery. Lastly, we generated a tLNP format against our candidate target(s) using multiple moiety configurations to select an optimal targeting moiety before testing the platform *in vivo* for proof-of-concept.

Results: Screening platform

Development of a targeting moiety screening platform for HSC targeting

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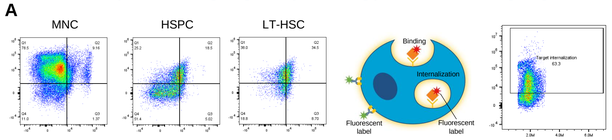

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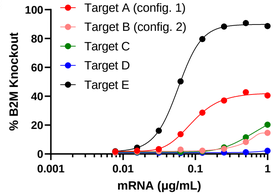

(A) Quantification of cell surface presence of prospective targets and confirmation of internalization was determined by flow cytometry on CD34+ cells.

(B) Functionalized LNPs with multiple targeting moiety configurations to select the optimal binder.

HSC, hematopoietic stem cell, HSPC, hematopoietic stem and progenitor cells; LNP, lipid nanoparticle; LT-HSC, long-term hematopoietic stem cells; MNC, mononuclear cells.

Results: Initial target finding campaign for LT-HSC targets

Assessment of target internalization on LT-HSC

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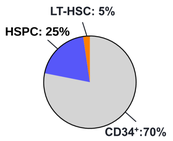

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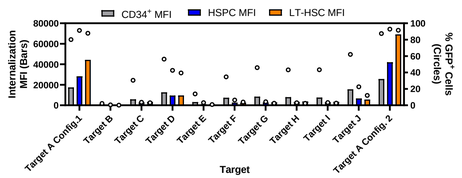

(A) Approximately 1% of the bone marrow MNCs are CD34+. Of the CD34+ cells in the bone marrow, approximately 5% represent the LT-HSC, while 25% represent the HSPC.

(B) Internalization assessment for possible LT-HSC targeting moieties was conducted using bulk CD34+ cells isolated from bone marrow MNCs. Prospective targets with high internalization in LT-HSC were identified.

CD, cluster of differentiation; GFP, green fluorescent protein; HSPC, hematopoietic stem and progenitor cells; LT-HSC, long-term hematopoietic stem cells; MFI, mean fluorescence intensity; MNC, mononuclear cell.

Results: Assessing targeting moiety functions on LNPs

Selecting an optimal targeting moiety configuration and proof-of-concept *in vivo* targeting.

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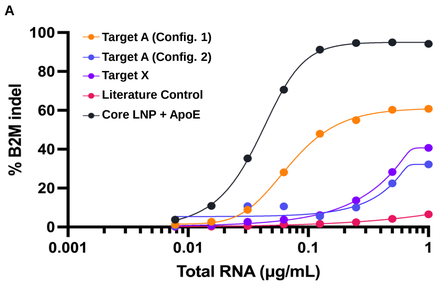

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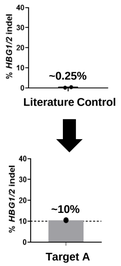

(A) The optimal targeting moiety for use with the LNP was determined by delivering editing cargo to bone marrow CD34+ cells *in vitro*. HSPC treated with LNPs were cultured for 4 days to ensure adequate editing. The cells were subsequently assessed for *B2M* expression using flow cytometry. *B2M* indels must be in both alleles for a negative signal, per cell, by flow cytometry. (B) The most potent targeting moiety configuration was selected and tested *in vivo* for initial proof-of-concept *HBG1/2* editing in human HSC.

ApoE, apolipoprotein E; CD, cluster of differentiation; HSPC, hematopoietic stem and progenitor cells; LNP, lipid nanoparticle.

Results: *In vivo* editing of HSCs at the *HBG* locus

***In vivo* proof-of-concept editing of HSPC/HSCs across species**

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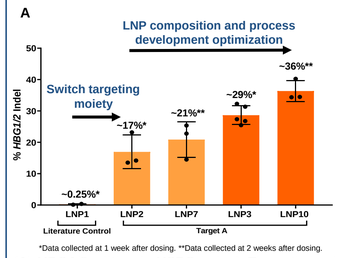

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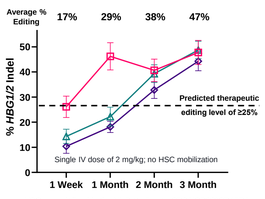


*Data collected at 1 week after dosing. **Data collected at 2 weeks after dosing.

HSC were defined in the NHP studies as CD34+CD90+CD45RA-.

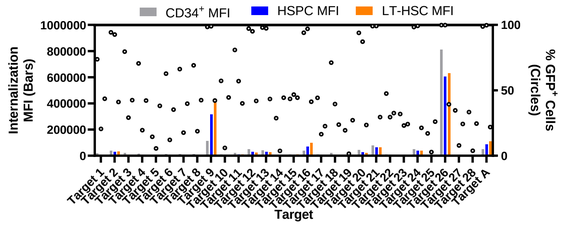
(A) *HBG1/2* editing in HSPC (mice). The optimized tLNP achieved ~40% *HBG1/2* editing after a single dose of the optimized tLNP.

(B) *HBG1/2* editing in HSCs (NHPs). Approximately 47% of the HSC pool achieved *HBG1/2* editing in NHPs, exceeding the predicted clinical threshold set for therapeutically relevant editing.

CD, cluster of differentiation; HSC, hematopoietic stem cell; HSPC, hematopoietic stem and progenitor cells; IV, intravenous; LNP, lipid nanoparticle; NHP, non-human primate; tLNP, targeted LNP.

Results: Additional target-finding campaign for HSCs

Assessment and discovery of additional targets for HSCs



After establishing the discovery platform could identify viable targeting moieties for HSCs, a wider study to discover additional targets for HSCs was conducted. The rates of internalization of 28 additional targets were screened and compared against the Target A targeting moiety. Of the 28, ~6 targets were identified to be comparable or superior to the current lead targeting moiety.

CD, cluster of differentiation; GFP, green fluorescent protein; HSC, hematopoietic stem cell; HSPC, hematopoietic stem and progenitor cells; LT-HSC, long-term hematopoietic stem cells; MFI, mean fluorescence intensity.

Results: Top targeting moieties for HSCs

Assessment and discovery of additional targets for HSCs

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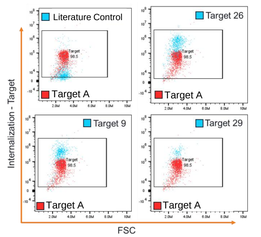

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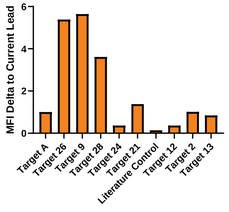

(A) MFI comparison of prospective targets versus Target A. Improvement in the signal intensity was demonstrated by overlaying the mean fluorescence intensity of the new prospective targets against Target A.

(B) Internalization delta compared to Target A. An approximate 6-fold improvement in internalization of other targets compared with Target A.

FSC, forward scatter; HSC, hematopoietic stem cell; MFI, mean fluorescence intensity.

Results: Assessment of targeting moiety configurations

Targeting ligand test on BM CD34+ cells

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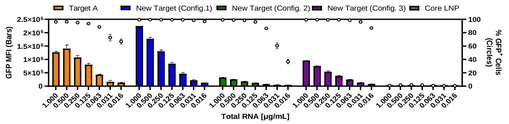

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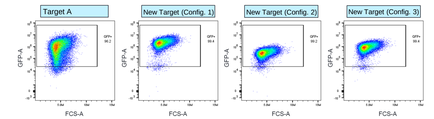

Functional efficacy of our new prospective target. (A) The efficacy of various configurations of the new targeting moiety was assessed by GFP expression on target cells 24 hours after LNP treatment *in vitro*. (B) We observed that our new target demonstrated more consistent GFP expression compared with the Target A targeting moiety in an LNP format.

BM, bone marrow; CD, cluster of differentiation; FCS, forward scatter; GFP, green fluorescent protein; LNP, lipid nanoparticle; MFI, mean fluorescence intensity.

Conclusions

Editas has developed a target-finding platform which maximizes target specificity and LNP efficacy by targeting the LNPs to discreet cell targets in highly heterogeneous environments. Our target discovery campaign enabled the development of a novel LT-HSC targeting molecule that can be conjugated to LNPs to improve specificity and overall delivery of editing machinery to HSC in the bone marrow compartment. Using our clinically validated editing approach for sickle cell disease, we demonstrated meaningful editing of LT-HSC *in vivo* after a single dose of tLNPs to the *HBG1/2* locus. Further targeting moiety discovery has yielded potential targets to further improve the functional outcomes of tLNPs in the context of HSC targeting for *in vivo* editing programs.

References

1. Hou X *et al. Nat Rev Mater* 2021; 6 (12): 1078–1094. 2. Shi D *et al. Nano Lett* 2023; 23 (7): 2938–2944.

NOTE: Figures including 'TARGET' labels denote unique targeting moieties.

Acknowledgments and disclosures

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