Effective Gene Editing in HSPCs through a Novel Targeted Lipid Nanoparticle

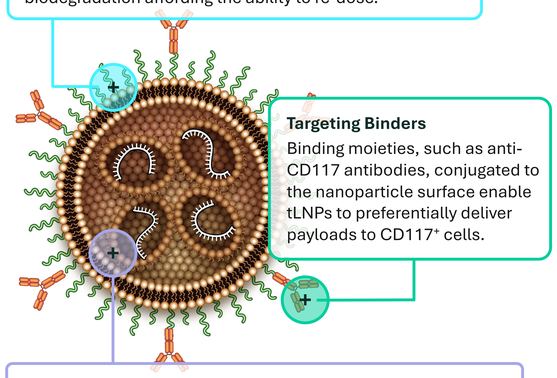
Esther Yu-Tin Chen, Xianghong Li, Ferran Soldevila-Casals, Theresa L. Hunter, Aaron Beams, Diti Desai, Duy P. Nguyen, Donald Jhung, Stuart A. Sievers, Daiki Matsuda, Yanjie Bao, John J. Li, David Chu, Michelle Nguyen, Michael Pica, Jack Lee, Duncan Ugland, Claudia Fernandez, James Vestal, Jeffrey Chen, Josephine Nguyen, Michael Rosenzweig, Gregor B. Adams, Priya Karmali, Adrian I. Bot, Haig Aghajanian

Background

Ex vivo Hematopoietic stem cell (HSC) gene therapy has achieved substantial progress over the past decade, advancing as a feasible therapeutic strategy for various blood and immune disorders, including sickle cell anemia, beta-thalassemia, and other immunodeficiencies. However, the complexities and challenges of *ex vivo* HSC gene therapy, such as manufacturing, scalability, cost, and accessibility, highlight the need for an *in vivo* engineering solution that could simplify the process and address many of the current limitations. This study describes a novel lipid nanoparticle (LNP) system designed to selectively deliver gene editing (GE) payloads to hematopoietic stem and progenitor cells (HSPCs) *in vivo*. The targeted lipid nanoparticles (tLNPs) is a product of the CellSeeker™ platform technology, which comprise a proprietary LNP formulation and are conjugated to a recombinant antibody that binds to a specific surface marker on HSPCs, enabling efficient delivery of CRISPR-Cas components to genetically edit HSPCs *in vivo*.

LNP Delivery Vehicle

Capstan's proprietary lipid nanoparticle is a non-viral system demonstrated in preclinical models to have rapid biodegradation affording the ability to re-dose.



Targeting Binders

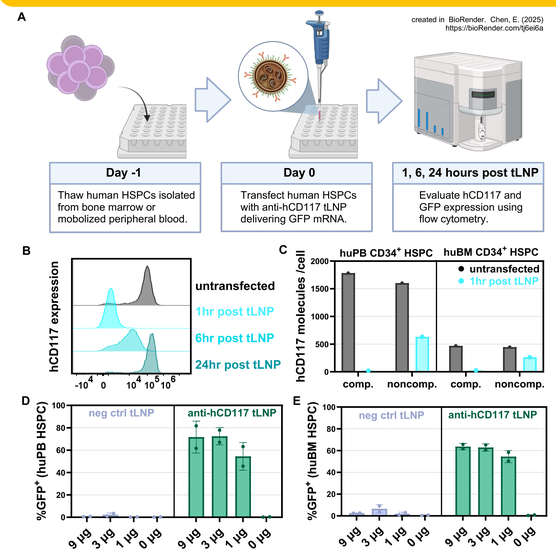
Binding moieties, such as anti-CD117 antibodies, conjugated to the nanoparticle surface enable tLNPs to preferentially deliver payloads to CD117⁺ cells.

Payloads

RNA payloads comprise gene editing components such as single guide RNA (sgRNA) and mRNA-encoded CRISPR/Cas9.

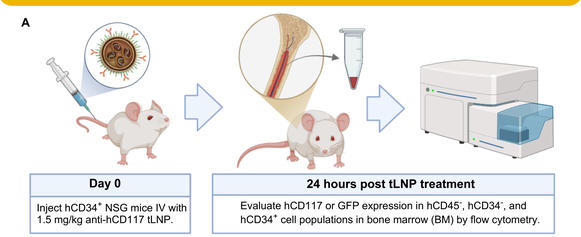
To generate proof of concept, single-payload or combined-payload anti-hCD117 tLNPs were designed to deliver Cas9 mRNA and single guide RNA (sgRNA) targeting beta-2 microglobulin (B2M) to human HSPCs. These tLNPs were formulated with a novel and biodegradable ionizable lipid and an antibody targeting the stem cell factor receptor, CD117, which is expressed on the surface of HSPCs. The dosage listed in this study refers to the total amount of RNA. We evaluated the efficiency of tLNP-mediated B2M knock-out (B2M-KO) in vitro in HSPCs and *in vivo* using a human CD34⁺ hematopoietic stem cell-engrafted mouse model.

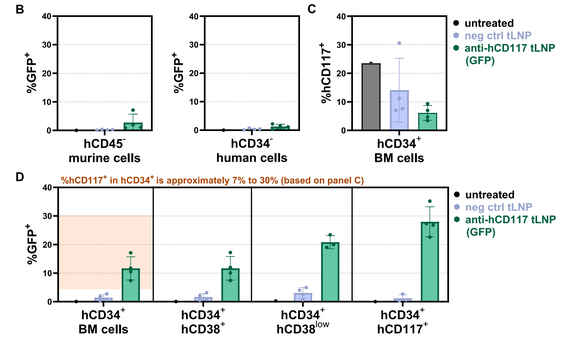
Figure 1. Efficient in vitro tLNP transfection of HSPCs with anti-hCD117 tLNP



A) Schematic of in vitro transfection of HSPCs using anti-hCD117 tLNP delivering GFP-encoding mRNA. **B)** Histogram showing hCD117 levels on human plerixafor-mobilized peripheral blood (huPB) CD34⁺ HSPCs at 1, 6, or 24 hours post anti-hCD117 tLNP transfection. **C)** Quantification of hCD117 expression 1 hour after tLNP transfection using either a competitive (comp.) or a noncompetitive (noncomp.) antibody (relative to the targeting antibody on tLNP). **D)** Percentage of GFP⁺ cells 24 hours after transfection of huPB CD34⁺ HSPCs using a negative control tLNP (anti-HIV) or anti-hCD117 tLNP. **E)** Percentage of GFP⁺ cells 24 hours after transfection of human bone marrow (huBM) CD34⁺ HSPCs using tLNPs. GFP expression was measured by flow cytometry and data are presented as mean ± SD (n=2 donors).

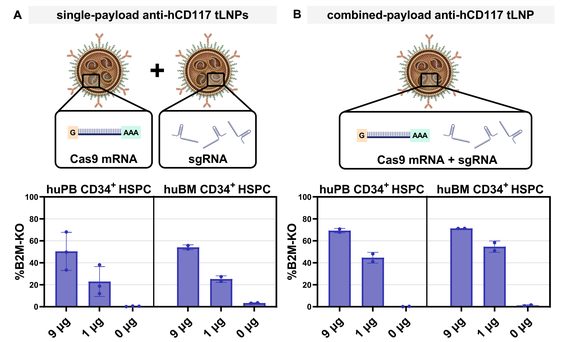
Figure 2. *In vivo* delivery of mRNA to human HSPCs using anti-hCD117 tLNP





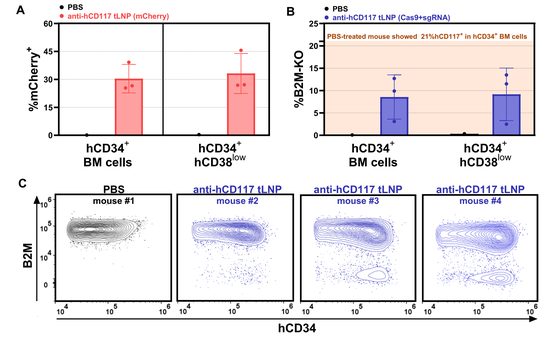
A) Schematic of the *in vivo* study in hCD34⁺ NSG mice. **B)** Minimal off-target delivery, suggested by the percentage of GFP expression in hCD45⁻ or hCD34⁻ cells in bone marrow (BM) 24 hours post negative control tLNP (anti-HIV) or anti-hCD117 tLNP treatment. **C)** Percentage of hCD117 expression in BM hCD34⁺ cells in hCD34⁺ NSG mice 24 hours after treatment. Note that %hCD117⁺ in BM hCD34⁺ cells is approximately 7% to 30% based on untreated group and neg ctrl tLNP group. **D)** Percentage of GFP expression in BM hCD34⁺ cells and hCD34⁺ cell subsets. Expression was measured by flow cytometry. Data are presented as mean ± SD (n=4; n=1 for untreated group).

Figure 3. Robust in vitro gene editing efficacy achieved by single- and combined-payload tLNPs



In vitro gene editing using anti-hCD117 tLNPs delivering Cas9 mRNA and sgRNA with either single-payload tLNPs or a combined-payload tLNP. **A)** Percentage of B2M-KO 5 to 7 days after transfection of huPB or huBM CD34⁺ HSPCs using single-payload anti-hCD117 tLNPs. **B)** Percentage of B2M-KO 5-7 days after transfection of huPB or huBM CD34⁺ HSPCs using combined-payload anti-hCD117 tLNP. B2M expression was measured by flow cytometry. Data are presented as mean ± SD (n=2-3).

Figure 4. *In vivo* gene editing of HSPCs in the bone marrow of humanized mice



A) Confirmatory anti-hCD117 tLNP delivery in hCD34⁺ NCG mice using reporter mCherry mRNA. Percentage of mCherry expression in hCD34⁺ NCG mice 24 hours post 1.5 mg/kg tLNP treatment. **B)** Percentage of B2M-KO in BM hCD34⁺ cells 8 days after treatment with 1.5 mg/kg anti-hCD117 tLNP delivering Cas9 mRNA and B2M-targeting sgRNA. Note that %hCD117⁺ in BM hCD34⁺ cells is approximately 21% in the PBS-treated group. mCherry or B2M expression was measured by flow cytometry. Data are presented as mean ± SD (n=3; n=1 for PBS groups). **C)** Representative contour plots of BM hCD45⁺hCD34⁺ cells on Day 8.

Results and Conclusions

Anti-hCD117 tLNP demonstrated robust transfection of huPB and huBM CD34⁺ HSPCs in vitro (up to 75% GFP⁺ , Figure 1), as well as in BM hCD34⁺ cells in hCD34⁺ NCG mice upon intravenous tLNP dosing. Notably, higher transfection efficiency, reaching 20%, was observed in the hCD34⁺hCD38^{low} cell population (Figure 2). It is important to highlight that CD117 expression in human CD34⁺ cells exceeds 95%, whereas in the BM hCD34⁺ cells of the NSG and NCG mouse models, hCD117 expression is significantly lower, ranging between 7% to 30% (based on untreated and neg ctrl-treated tLNP groups), pointing to the fact that a large fraction of target cells were edited *in vivo*.

For genomic engineering, both single-payload and combined-payload tLNP formats achieved high levels of B2M-KO, up to 70%, in human CD34⁺ HSPCs in vitro (Figure 3). Importantly, the gene-editing capability is translatable *in vivo* in humanized hCD34⁺ NCG mice, as suggested by efficient B2M-KO within the hCD34⁺hCD38^{low} population (Figure 4).

Collectively, the distinctive targeting moiety of these tLNPs enables efficient delivery of both reporter mRNA and gene-editing payloads to human CD34⁺ HSPCs. The demonstrated success of *in vivo* gene editing in the hCD34⁺ NCG mouse model underscores the clinical potential of this delivery system.