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TALEN®-mediated engineering of HSPC enables systemic delivery of IDUA

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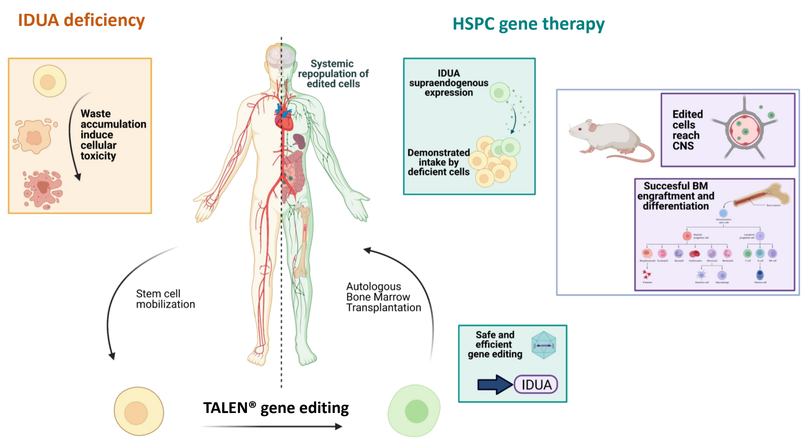
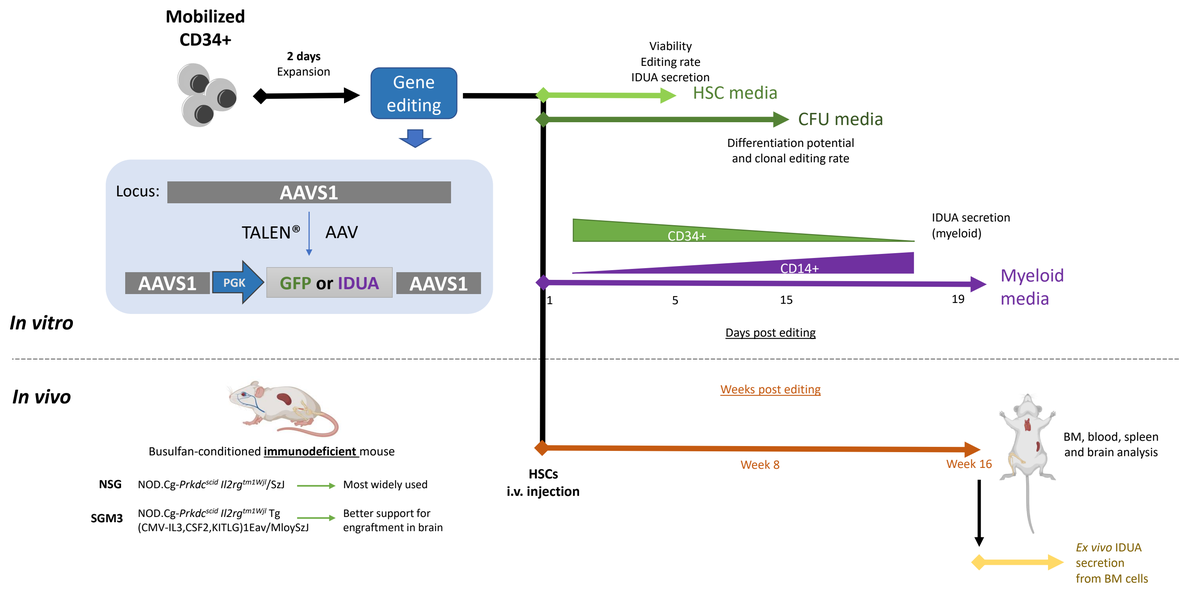
Introduction

- Mucopolysaccharidosis type I (MPS-I) is caused by gene defects in the alpha-L-iduronidase (IDUA) gene.
- Current treatments are limited to enzyme replacement therapy usually preceded by allogenic bone marrow transplantation. Disadvantages include the need of lifelong enzyme infusions that do not address neurological symptoms due to the lack crossover of IDUA through the brain blood barrier.
- Gene editing of hematopoietic stem and progenitor cells (HSPCs) followed by autologous transplantation offers unique therapeutic advantages including systemic and local delivery of IDUA into the brain and could be a therapeutic strategy for MPS-I and other lysosomal storage diseases.

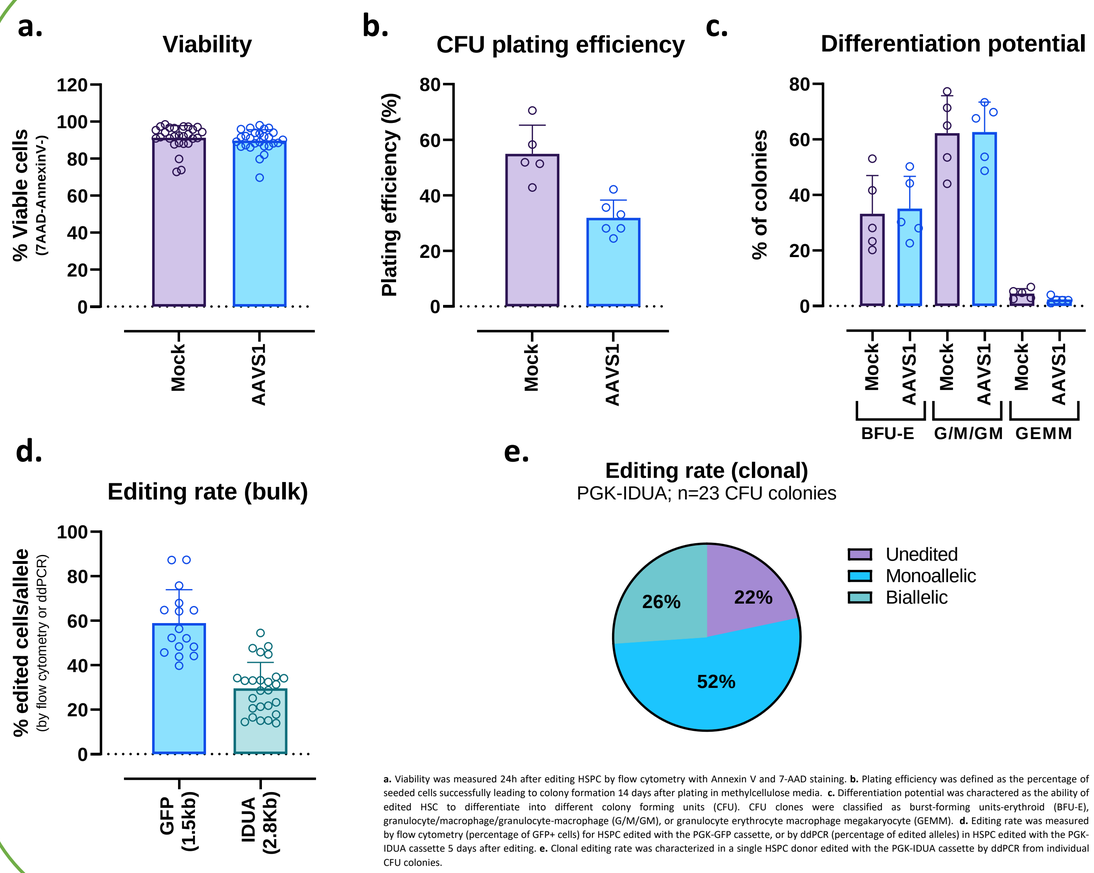


Experimental overview



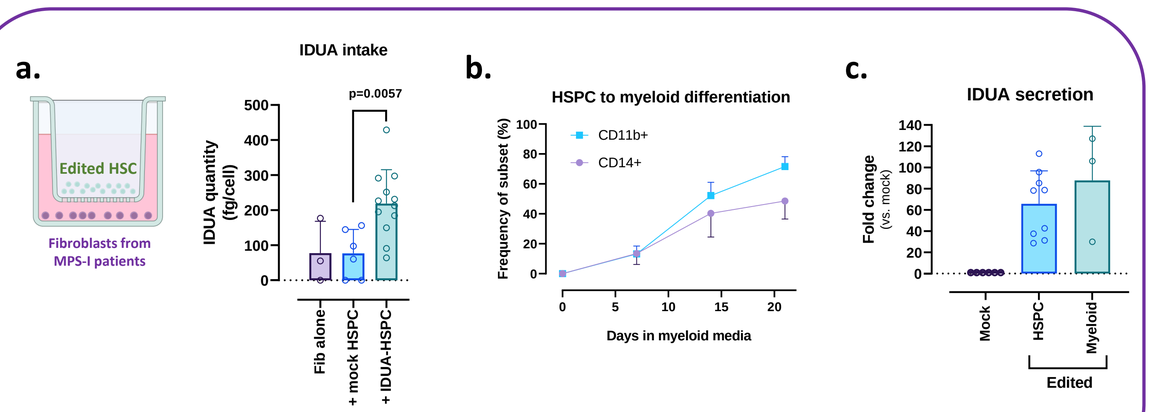
Results

1. HSPC editing protocols are efficient and safe.



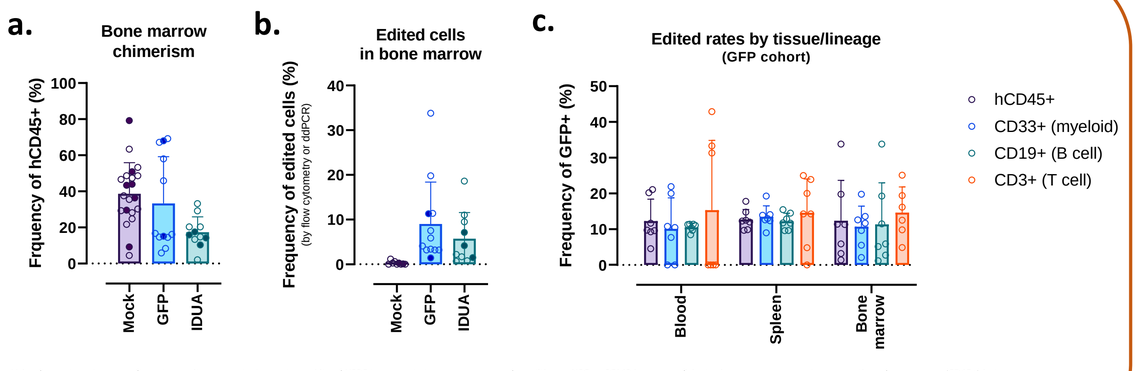
a. Viability was measured 24h after editing HSPC by flow cytometry with Annexin V and 7-AAD staining. **b.** Plating efficiency was defined as the percentage of seeded cells successfully leading to colony formation 14 days after plating in methylcellulose media. **c.** Differentiation potential was charactered as the ability of edited HSC to differentiate into different colony forming units (CFU). CFU clones were classified as burst-forming units-erythroid (BFU-E), granulocyte/macrophage/granulocyte-macrophage (G/M/GM), or granulocyte erythrocyte macrophage megakaryocyte (GEMM). **d.** Editing rate was measured by flow cytometry (percentage of GFP+ cells) for HSPC edited with the PGK-GFP cassette, or by ddPCR (percentage of edited alleles) in HSPC edited with the PGK-IDUA cassette 5 days after editing. **e.** Clonal editing rate was characterized in a single HSPC donor edited with the PGK-IDUA cassette by ddPCR from individual CFU colonies.

2. Potent IDUA secretion from edited HSPC and its progeny.



a. A transwell assay was performed to confirm IDUA intake of IDUA-deficient fibroblasts from MPS-I patients. Briefly, undifferentiated IDUA-edited HSPC were cocultured with MPS-I fibroblasts in different chambers of a permeable transwell plate for 3 days. Then, IDUA from fibroblast cell lysates was quantified by ELISA. Statistical comparison was done by unpaired T test. **b.** Edited HSC were plated in media for myeloid differentiation and myeloid markers were tracked over time. **c.** Edited HSPC (d3) or myeloid-like cells differentiated from edited HSPC (d14) were seeded IDUA secretion. IDUA was measured by ELISA in the supernatant of culture media 3 days after plating. Values were normalized to levels from unedited mock cells.

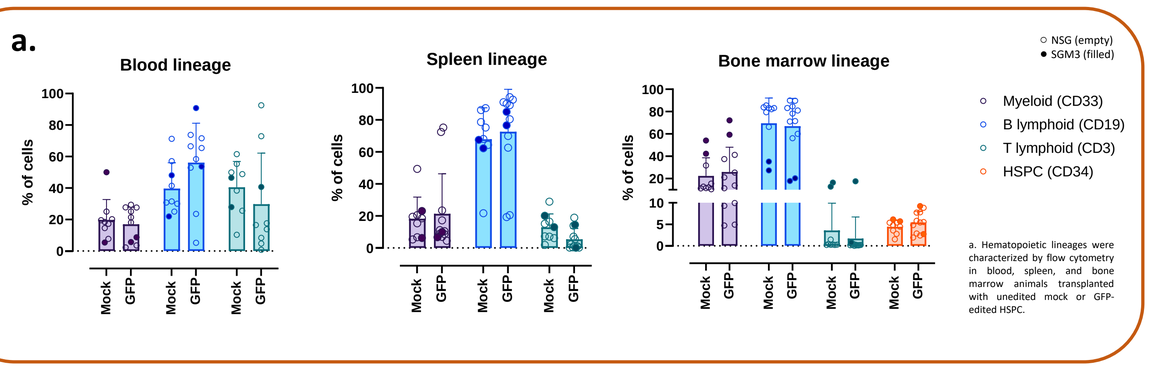
3. Edited HSPC retain engraftment capabilities in multiple tissues and lineages.



a. Using flow cytometry, engraftment capacity was evaluated by the ability of HSPC to populate the bone marrow of conditioned NSG or SGM3 immunodeficient mice, as measured by the proportion of human cells (CD45+) in the bone marrow 16 weeks after intravenous injection with unedited (mock) or edited HSPC. **b.** *In vivo* editing rates were measured by flow cytometry in animals injected with HSPC edited with the PGK-GFP cassette, or by ddPCR from DNA extracted from cells recovered from mice injected with HSPC edited with the PGK-IDUA cassette. **c.** To confirm broad editing in multiple tissues and lineages, the percentage of GFP-edited cells in blood, spleen, and bone marrow was characterized in animals injected with HSPC edited with the PGK-GFP cassette within different hematopoietic lineages, including myeloid (CD33+), B cells (CD19+) and T cells (CD3+).

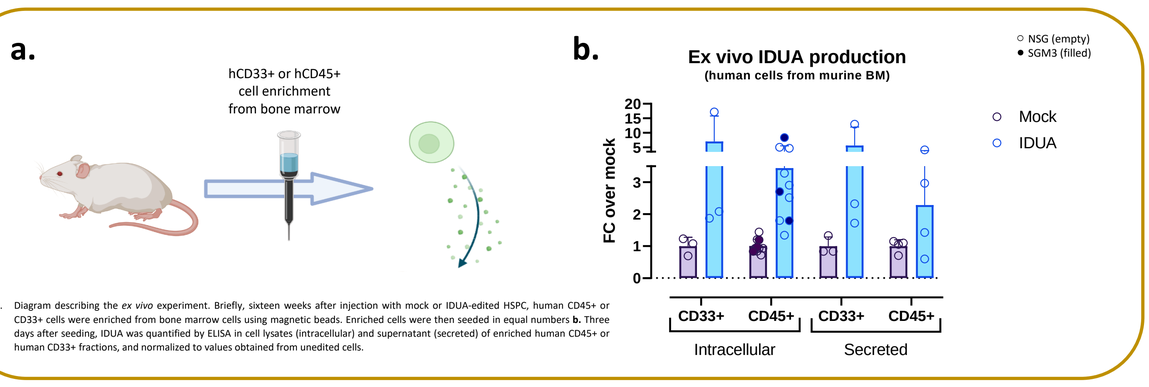
○ NSG (empty)
● SGM3 (filled)

4. Edited HSPC retain differentiation capabilities in multiple tissues and lineages.



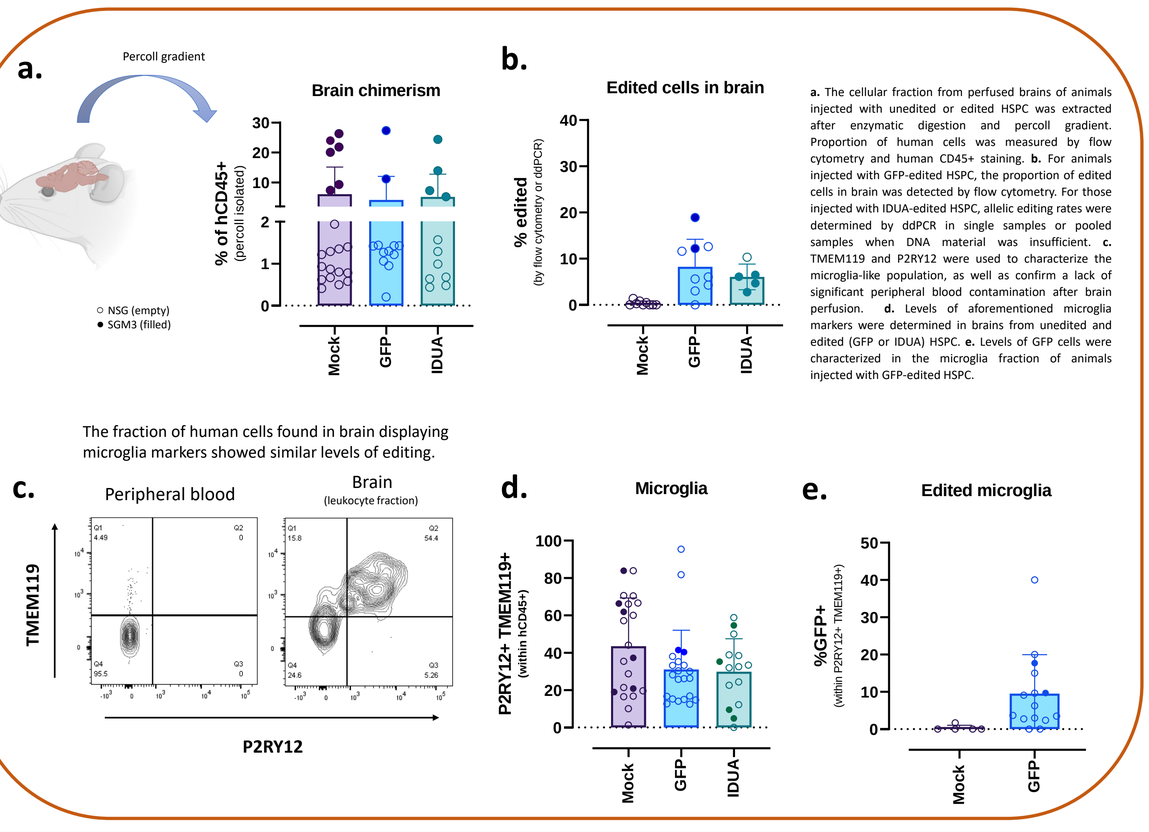
a. Hematopoietic lineages were characterized by flow cytometry in blood, spleen, and bone marrow animals transplanted with unedited mock or GFP-edited HSPC.

5. IDUA secretion is maintained in edited HSPC progeny 16 weeks after transplant.



a. Diagram describing the *ex vivo* experiment. Briefly, sixteen weeks after injection with mock or IDUA-edited HSPC, human CD45+ or CD33+ cells were enriched from bone marrow cells using magnetic beads. Enriched cells were then seeded in equal numbers **b.** Three days after seeding, IDUA was quantified by ELISA in cell lysates (intracellular) and supernatant (secreted) of enriched human CD45+ or human CD33+ fractions, and normalized to values obtained from unedited cells.

6. Edited cells successfully engraft the brain compartment.



a. The cellular fraction from perfused brains of animals injected with unedited or edited HSPC was extracted after enzymatic digestion and percoll gradient. Proportion of human cells was measured by flow cytometry and human CD45+ staining. **b.** For animals injected with GFP-edited HSPC, the proportion of edited cells in brain was detected by flow cytometry. For those injected with IDUA-edited HSPC, allelic editing rates were determined by ddPCR in single samples or pooled samples when DNA material was insufficient. **c.** TMEM119 and P2RY12 were used to characterize the microglia-like population, as well as confirm a lack of significant peripheral blood contamination after brain perfusion. **d.** Levels of aforementioned microglia markers were determined in brains from unedited and edited (GFP or IDUA) HSPC. **e.** Levels of GFP cells were characterized in the microglia fraction of animals injected with GFP-edited HSPC.

Conclusions

- We established a TALEN®-based ex vivo gene editing protocol to safely and efficiently insert an IDUA-expression cassette into HSPCs.
- High levels of editing supported IDUA secretion *in vitro* and *ex vivo*.
- Edited HSPC engrafted in multiple tissues *in vivo*, including the brain compartment.
- These results pave the way towards targeted gene therapy-mediated treatment of MPS-I. The modular nature of this HSPC gene editing platform enable swapping the therapeutic DNA cassette to target other lysosomal storage diseases.
- Effectively reaching the brain tissue with edited HSPC and/or its progeny could help ameliorate neurological symptoms present associated with lysosomal storage diseases, which is not possible with current therapies.
- This platform has the potential to be leveraged for the treatment of other neurological diseases.

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