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Intronic editing enables lineage-specific expression of therapeutics relevant for HSPC gene therapy

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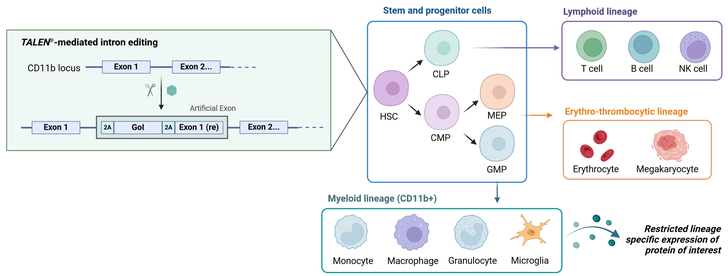
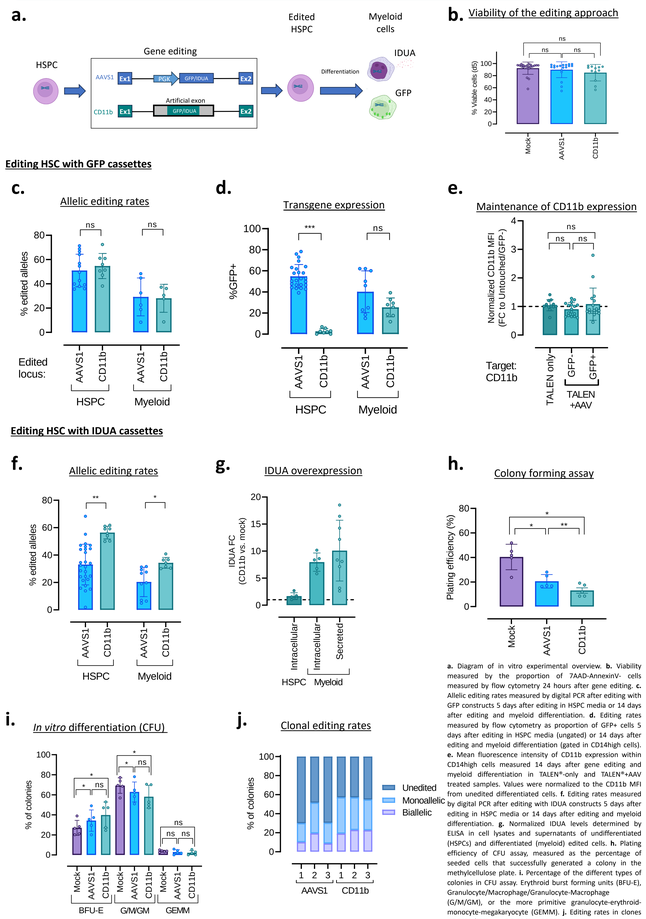
#1 Abstract



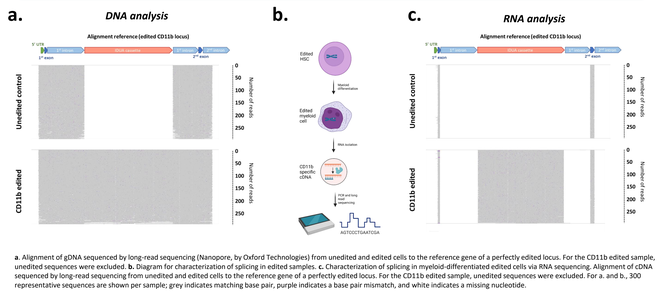
Autologous transplant of gene edited hematopoietic stem and progenitor cells (HSPCs) could become the treatment of choice in the near future for multiple genetic diseases including lysosomal storage diseases (LSDs). Traditional gene therapy approaches for HSPCs are based on the integration of a transgene by a lentiviral vector, and more recently targeted cassette integration usually supported by designer nucleases. Either case, expression of the transgene is generally sustained by an exogenous ubiquitous promoter, which can alter or dysregulate the expression of surrounding proto-oncogenes and/or tumor suppressors. Furthermore, ubiquitous promoters induce expression of the desired transgene at the stem cell level, which could affect its functionality, as it has been suggested for the overexpression of galactocerebrosidase (Krabbe) or glucocerebrosidase (Gaucher). We propose a novel gene editing system for HSPCs based on the integration of a splicing-competent cassette into the intron of a lineage-specific locus. This approach is meant to prevent expression of the transgene at the stem cell level, only triggering transgene expression after cellular differentiation. As a proof of concept, we edited the intron of CD11b in HSPCs and induce myeloid-specific expression of a transgene (GFP or IDUA for the treatment of Mucopolysaccharidosis type I) in the myeloid lineage after *in vitro* differentiation and *in vivo* myeloid engraftment. We demonstrate the transportability of this approach to the CD20 and CD4 genes.

#2 Intron editing of CD11b in HSPCs lead to myeloid-specific expression of a desired transgene



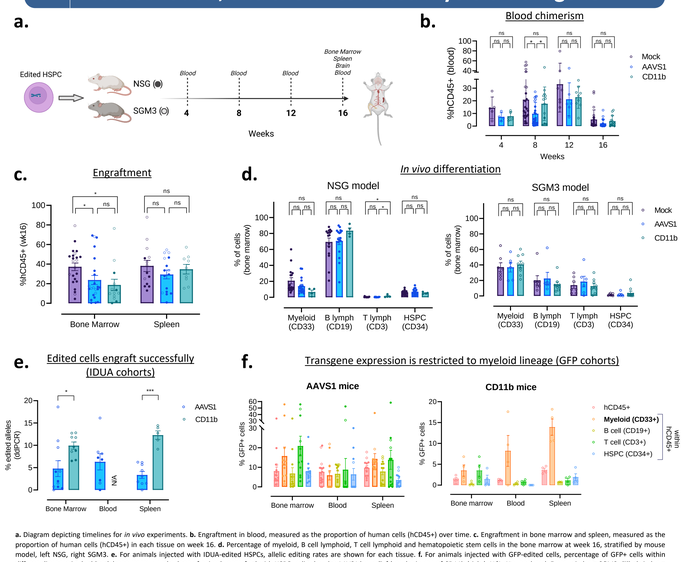
a. Diagram of in vitro experimental overview. **b.** Viability measured by the proportion of 7AAD-AnnexinV- cells measured by flow cytometry 24 hours after gene editing. **c.** Allelic editing rates measured by digital PCR after editing with GFP constructs 5 days after editing in HSPC media or 14 days after editing and myeloid differentiation. **d.** Editing rates measured by flow cytometry as proportion of GFP+ cells 5 days after editing in HSPC media (ungated) or 14 days after editing and myeloid differentiation (gated in CD14high cells). **e.** Mean fluorescence intensity of CD11b expression within CD14high cells measured 14 days after gene editing and myeloid differentiation in TALEN®-only and TALEN®+AAV treated samples. Values were normalized to the CD11b MFI from unedited differentiated cells. **f.** Editing rates measured by digital PCR after editing with IDUA constructs 5 days after editing in HSPC media or 14 days after editing and myeloid differentiation. **g.** Normalized IDUA levels determined by ELISA in cell lysates and supernatants of undifferentiated (HSPCs) and differentiated (myeloid) edited cells. **h.** Plating efficiency of CFU assay, measured as the percentage of seeded cells that successfully generated a colony in the methylcellulose plate. **i.** Percentage of the different types of colonies in CFU assay. Erythroid burst forming units (BFU-E), Granulocyte/Macrophage/Granulocyte-Macrophage (G/M/GM), or the more primitive granulocyte-erythroid-monocyte-megakaryocyte (GEMM). **j.** Editing rates in clones isolated from CFU plates (n=3 donors).

#3 IDUA cassette is inserted at the CD11b locus and spliced correctly



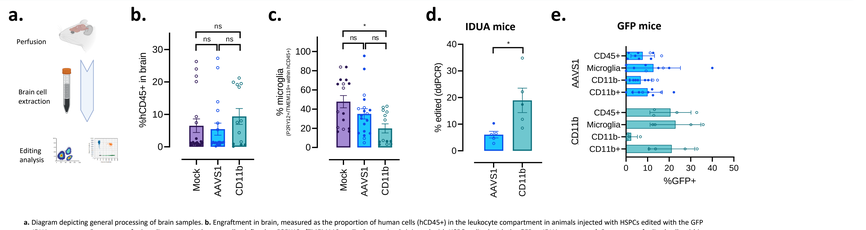
a. Alignment of gDNA sequenced by long-read sequencing (Nanopore, by Oxford Technologies) from unedited and edited cells to the reference gene of a perfectly edited locus. For the CD11b edited sample, unedited sequences were excluded. **b.** Diagram for characterization of splicing in edited samples. **c.** Characterization of splicing in myeloid-differentiated edited cells via RNA sequencing. Alignment of cDNA sequenced by long-read sequencing from unedited and edited cells to the reference gene of a perfectly edited locus. For the CD11b edited sample, unedited sequences were excluded. For a. and b., 300 representative sequences are shown per sample; grey indicates matching base pair, purple indicates a base pair mismatch, and white indicates a missing nucleotide.

#4 Edited HSPCs engraft successfully in two immunodeficient mouse models, and show restricted myeloid transgene



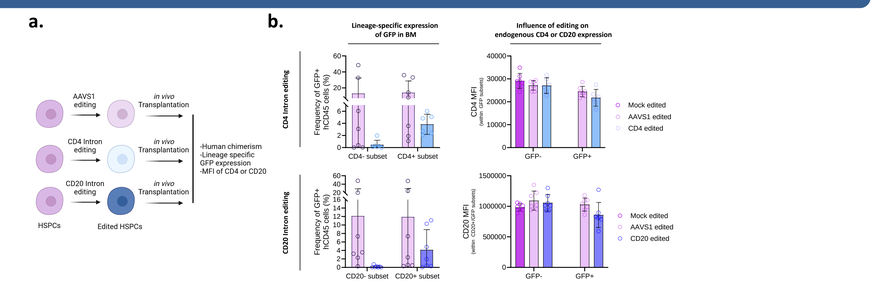
a. Diagram depicting timelines for *in vivo* experiments. **b.** Engraftment in blood, measured as the proportion of human cells (hCD45+) over time. **c.** Engraftment in bone marrow and spleen, measured as the proportion of human cells (hCD45+) in each tissue on week 16. **d.** Percentage of myeloid, B cell lymphoid, T cell lymphoid and hematopoietic stem cells in the bone marrow at week 16, stratified by mouse model, left NSG, right SGM3. **e.** For animals injected with IDUA-edited HSPCs, allelic editing rates are shown for each tissue. **f.** For animals injected with GFP-edited cells, percentage of GFP+ cells within different lineages in the blood, bone marrow and spleen of animals engrafted with HSPCs edited at the AAVS1 locus (left) or the intron of CD11b (right). N/A: Not analyzed. Empty circles = SGM3. Filled circles = NSG.

#5 Edited cells reach the brain compartment efficiently



a. Diagram depicting general processing of brain samples. **b.** Engraftment in brain, measured as the proportion of human cells (hCD45+) in the leukocyte compartment in animals injected with HSPCs edited with the GFP or IDUA cassettes. **c.** Percentage of microglia among the human cells, defined as P2RY12+/TMEM119+ cells, from animals injected with HSPCs edited with the GFP or IDUA cassettes. **d.** Percentage of edited cells within the brain of animals engrafted with HSPCs edited with a IDUA cassette. **e.** Percentage of edited cells within the brain amongst human cells from animals engrafted with HSPCs edited with a GFP cassette. Empty circles = SGM3. Filled circles = NSG.

#6 Transportability of the intron-editing approach



a. Diagram of *in vitro*/*in vivo* experimental plan to study CD4 and CD20 intron editing by GFP reporter system. **b.** left panel, Lineage-specific expression of GFP by Mock-, AAVS1-, CD40- and CD20-edited cells in the BM of transplanted NSG mice. B. right panel, Influence of GFP transgene intron specific insertion on CD4 and CD20 endogenous gene expression

#7 Conclusions

- We developed a TALEN®-based gene editing protocol for HSPCs that restricts transgene expression to the myeloid lineage after inserting a splicing-competent cassette into the intronic region of CD11b.
- *In vitro and in vivo* data shows lack of transgene expression at the stem cell level, as well as specific myeloid overexpression for both GFP and IDUA cassettes.
- Edited HSPCs engrafted in multiple tissues *in vivo*, including the brain compartment, and showed myeloid-specific GFP expression when analyzed.
- Intron editing is transportable to other loci including CD4 and CD20, as non limiting examples.

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Some illustrations were created with BioRender.com