P646

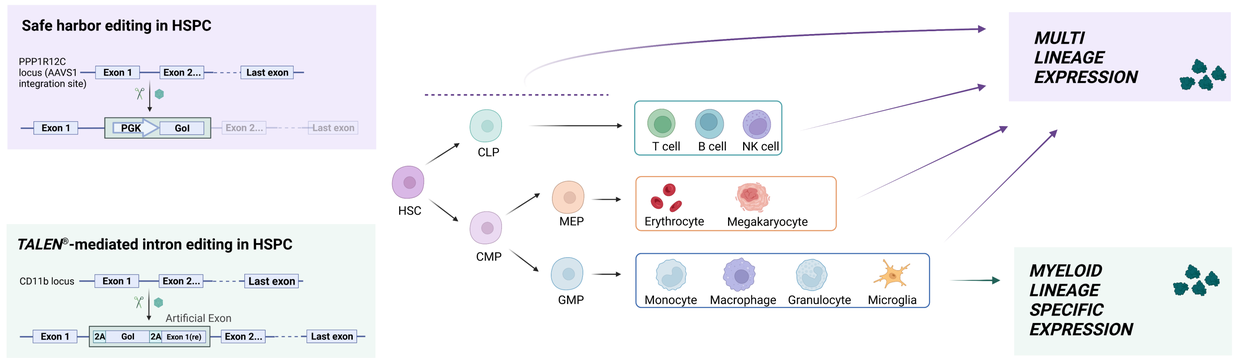
Intronic editing enables lineage-specific expression of therapeutics relevant for HSPC gene therapy

Eduardo Seclen[1], Aminah O Lawal[1], Sylvain Pulicani[2], Alexandre Juillerat[1], Philippe Duchateau[2], Julien Valton[2]
1. Cellectis, New York, USA; 2. Cellectis, Paris, France.
Contact: eduardo.seclen@cellectis.com

Introduction

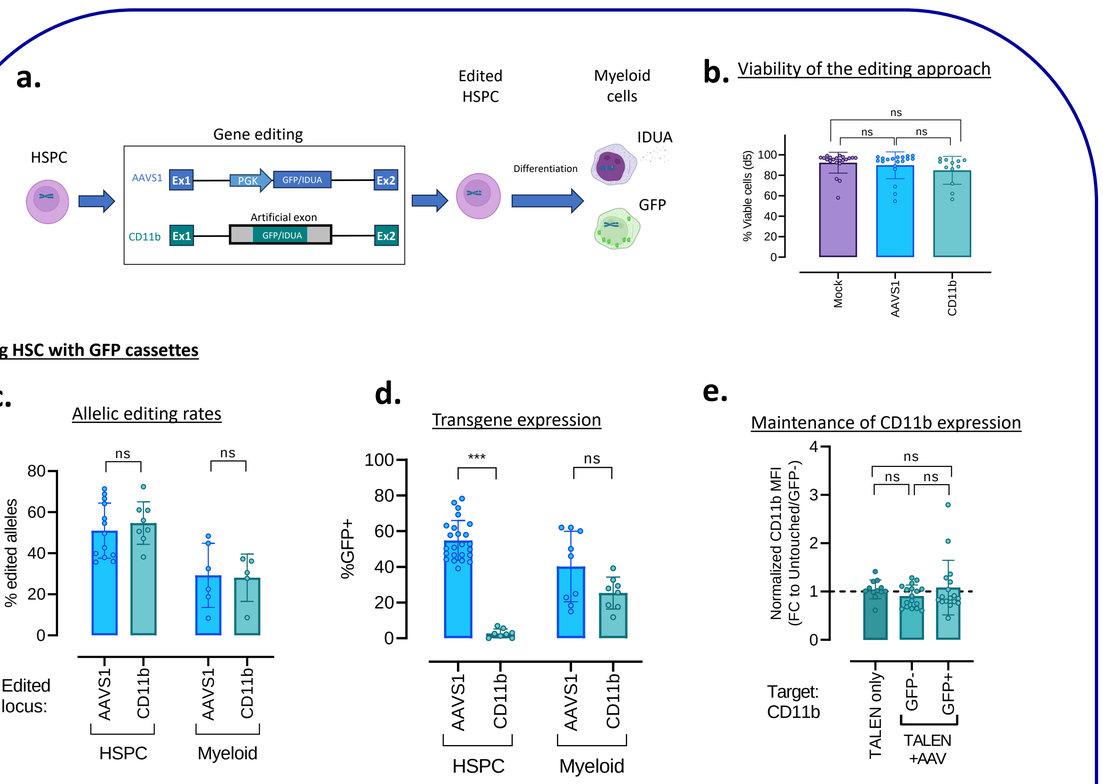
- Autologous transplant of gene edited hematopoietic stem and progenitor cells (HSPC) could become the treatment of choice in the near future for multiple genetic diseases including lysosomal storage diseases (LSDs).
- Traditional gene therapy approaches for HSPC are based on the integration of a transgene 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[1] (Krabbe) or glucocerebrosidase[2] (Gaucher).
- We propose a novel gene editing system for HSPC 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 HSPC 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.

Graphical abstract



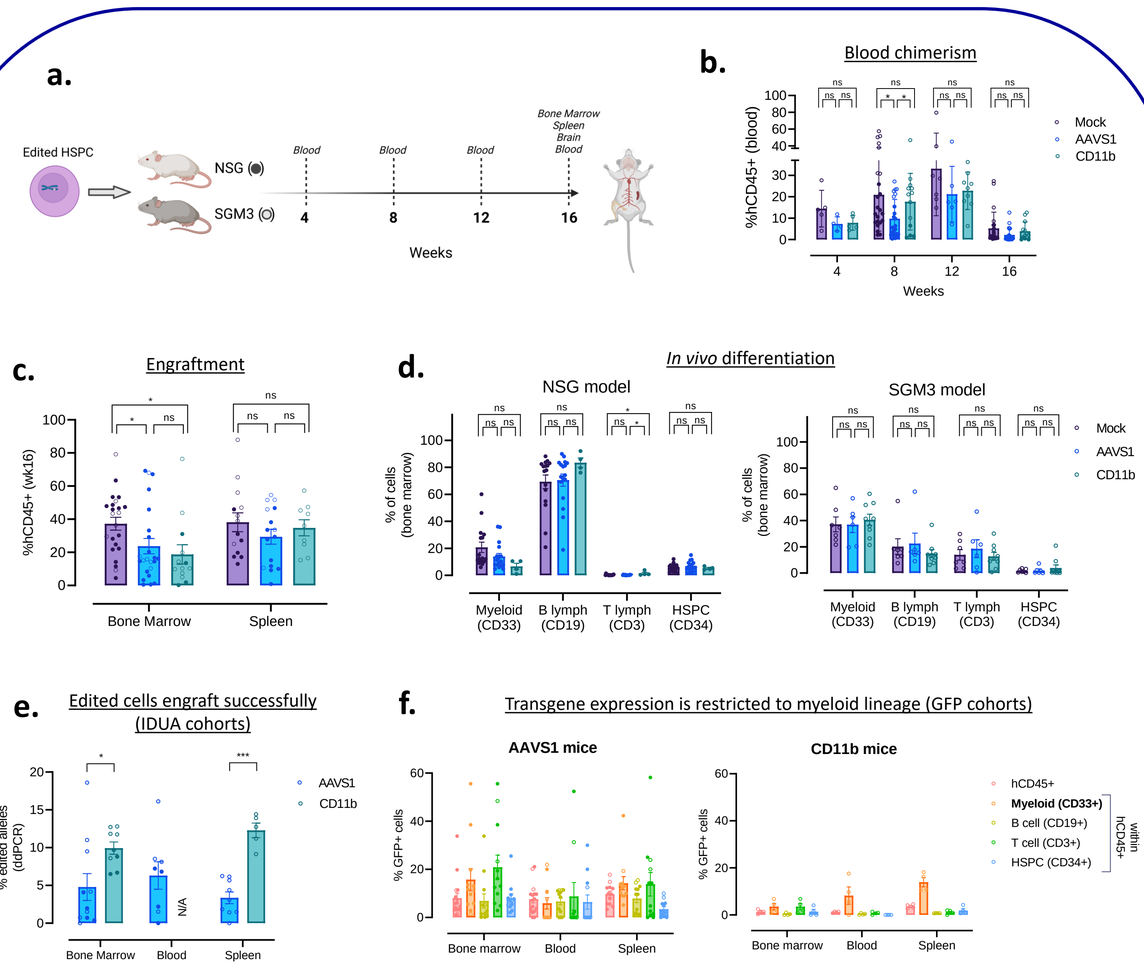
Results

1. Intron editing of CD11b in HSPC lead to myeloid-specific expression of a desired transgene



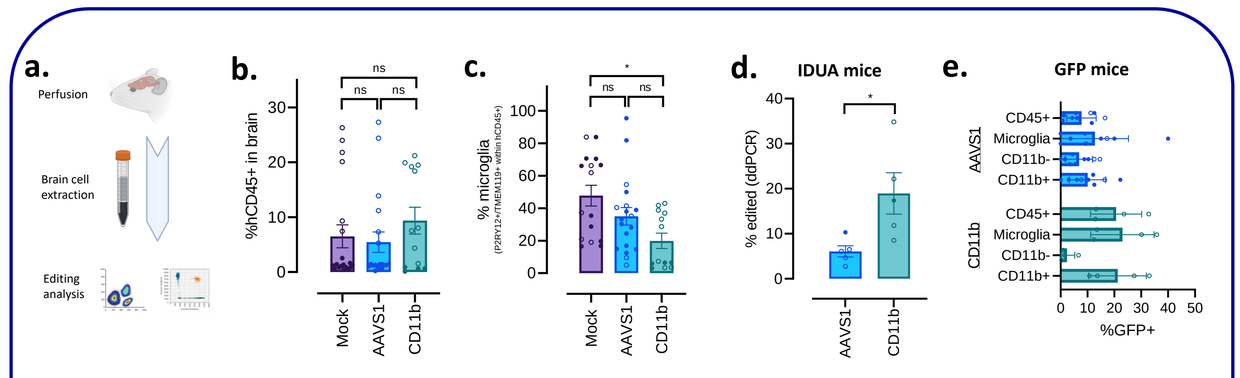
a. Diagram of in vitro experimental overview. **b.** Viability measured by the proportion of 7AAD-AnnexinV- cells measured by flow cytometry 24 hours after gene editing. **c.** Allelic editing rates measured by digital PCR after editing with GFP constructs 5 days after editing in HSPC media or 14 days 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 (HSPC) 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. Edited HSPC engraft successfully in two immunodeficient mouse models, and show restricted myeloid transgene expression



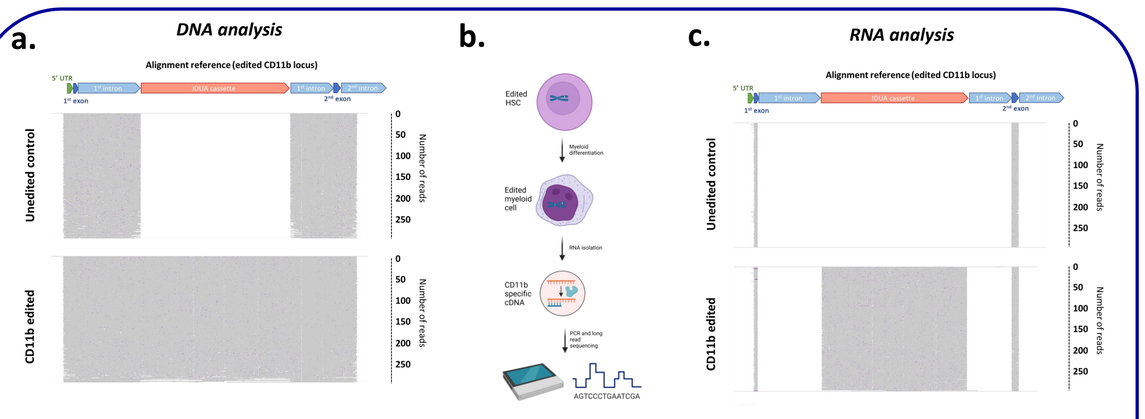
a. Diagram depicting timelines for *in vivo* experiments. **b.** Engraftment in blood, measured as the proportion of human cells (hCD45+) over time. **c.** Engraftment in bone marrow and spleen, measured as the proportion of human cells (hCD45+) in each tissue on week 16. **d.** Percentage of myeloid, 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 HSPC, 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.

4. 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 HSPC edited with the GFP or IDUA cassettes. **c.** Percentage of microglia among the human cells, defined as P2RY12+/TMEM119+ cells, from animals injected with HSPC edited with the GFP or IDUA cassettes. **d.** Percentage of edited cells within the brain of animals engrafted with HSPC edited with a IDUA cassette. **e.** Percentage of edited cells within the brain amongst human cells from animals engrafted with HSPC edited with a GFP cassette. Empty circles = SGM3. Filled circles = NSG.

2. Inserted cassette into the CD11b intron is inserted 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.

Conclusions

- We developed a TALEN®-based gene editing protocol for HSPC that restricts transgene expression to the myeloid lineage after inserting a splicing-competent cassette into the intronic region of CD11b.
- Edited HSPC maintained good viability, engraftment capabilities and differentiation potential *in vitro* and *in vivo*.
- *In vitro* data shows lack of transgene expression at the stem cell level, as well as specific myeloid overexpression for both GFP and IDUA cassettes.
- Edited HSPC engrafted in multiple tissues *in vivo*, including the brain compartment, and showed myeloid-specific GFP expression when analyzed.
- This platform has the potential to be leveraged for the treatment of other LSDs where transgene expression at the HSPC level could be toxic[1, 2].
- Edited cells can efficiently cross the blood brain barrier and express transgene in the myeloid compartment, including the microglia. This could help ameliorate neurological symptoms present in LSDs, which is not possible with current therapies, and could also be leveraged for the delivery of other therapeutics in brain.

Download a poster copy:

References

1. *Visigalli et al. Blood. 2010*
2. *Scharenberg et al. Nat. Commun. 2020.*