Poster # 1233

NON-VIRAL DNA DELIVERY ASSOCIATED TO TALEN® GENE EDITING LEADS TO HIGHLY EFFICIENT CORRECTION OF SICKLE CELL MUTATION IN LONG-TERM REPOPULATING HEMATOPOIETIC STEM CELLS

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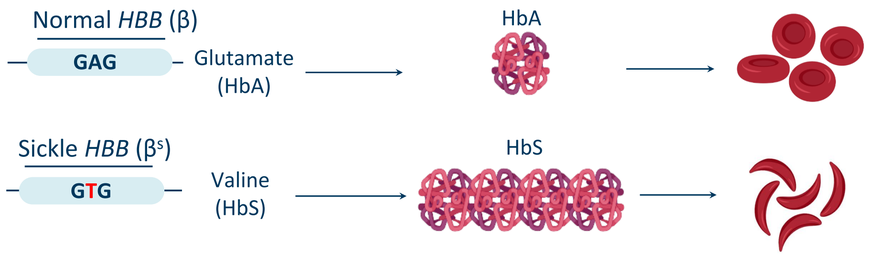
Background

Sickle Cell Disease (SCD)

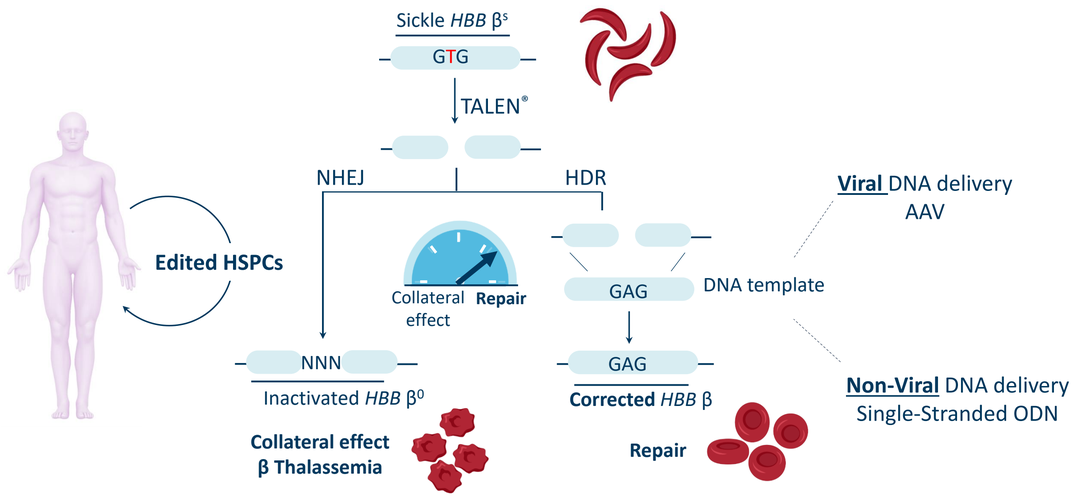
- SCD is an inherited blood disorder that stems from a single point mutation (A>T) in exon 1 of the *HBB* gene. *HBB* encodes the hemoglobin beta subunit (Hb) that associates with hemoglobin alpha subunit to generate the tetrameric protein complex called adult hemoglobin (HbA).[1]
- Mutated HbA polymerization results in sickle shaped red blood cells (RBCs) that cause reduced oxygen transfer to tissues throughout the body and alter normal blood flow.[1,2]



- People with SCD often suffer from anemia, painful vaso-occlusive crises, frequent infections, stroke and many other symptoms that can, ultimately, reduce quality of life and expected lifespan[2].
- SCD can be cured with allogenic hematopoietic stem cell transplant. However, this procedure is only available to patients with severe disease, it requires an HLA-matched donor and is associated to a substantial rate of morbidity and mortality[2]. Thus, alternative treatments are highly regarded
- This work proposes an autologous gene therapy approach based on *ex vivo HBB* gene correction in HSPCs to treat SCD.

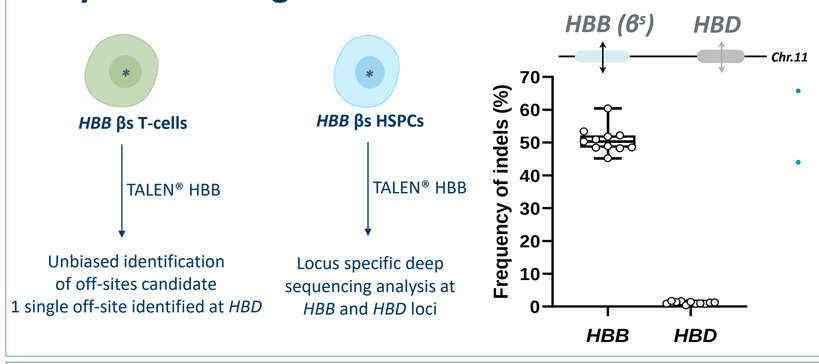
References: 1. Renaudier P, Transfus Clin Biol. 2014; 2. Piel FB et al, Lancet 2013;Eapen et al. Lancet Hematol. 2019

TALEN®-mediated *HBB* gene correction strategy



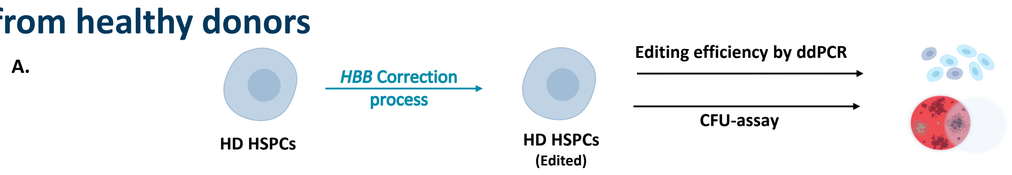
- We developed an autologous gene therapy strategy to treat SCD with a TALEN® targeting the mutated *HBB* (TALEN®-HBB) gene followed by the delivery of a DNA template to repair the HBB mutation via homologous directed repair (HDR).
- Here, we report that our protocol 1), efficiently corrects *HBB* in long-term repopulating hematopoietic stem cells (LT-HSCs) and repairs the sickle cell phenotype in differentiated RBCs 2), provides a direct comparison of viral and non-viral strategies for DNA delivery and 3), mitigates collateral effect derived from bi-allelic inactivation of *HBB*.

TALEN®-HBB displays editing activity at *HBB* locus and high specificity with only one off-target site detected

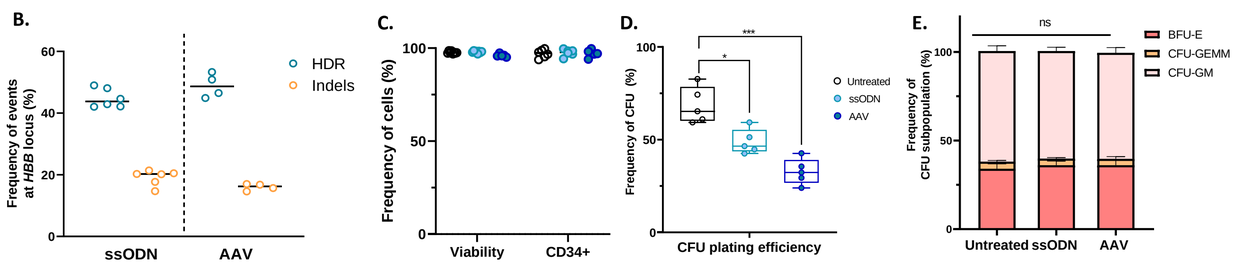


- High activity of TALEN®-HBB detected at *HBB* onsite in *HBB* βs HSPCs
- High specificity of TALEN®-HBB assessed by unbiased Oligo Capture Assay and locus specific deep sequencing. Only one off-site was confirmed at the *HBD* gene. TALEN® cleavage off-site activity was found to be very low compared to the on-site cleavage activity (1.2% +/- 0.4 at the off-site versus 50.7% Indels +/- 3.9 at the on-site).

Highly efficient TALEN® based gene editing at *HBB* locus in mobilized HSPCs from healthy donors

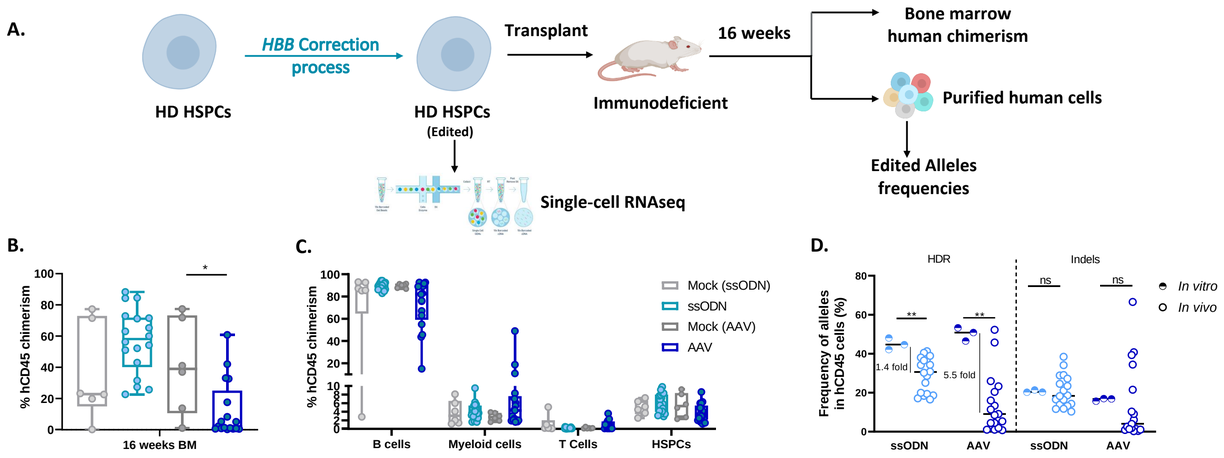


- Mobilized HSPCs from healthy donors were expanded, edited with TALEN® coupled to either AAV or ssODN delivering the correct *HBB* sequence. Edited cells were analyzed 48h after editing to assess editing efficiency, viability, purity and clonogenic potential by CFU-assay (A).

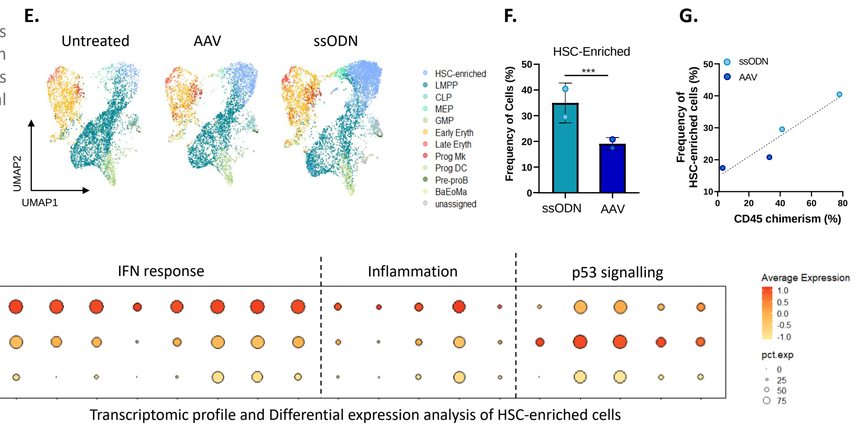


- Our process led to high HDR frequencies (>50%), while maintaining low levels of indels (<20%) either using AAV or ssODN (B). It was reproducible in six different donors and did not affect HSPCs viability (>95% mean viable cells), or purity (> 95% mean CD34+ cells assessed by flow cytometry) (C). We observed a decreased HSPCs clonogenic potential after editing which was further impacted by AAV transduction (D), without lineage skewing between edited and untreated HSPCs (E).

Non-viral gene editing mitigates p53 response activation, maintains LT-HSC pool and fitness and enables high level gene correction *in vivo*

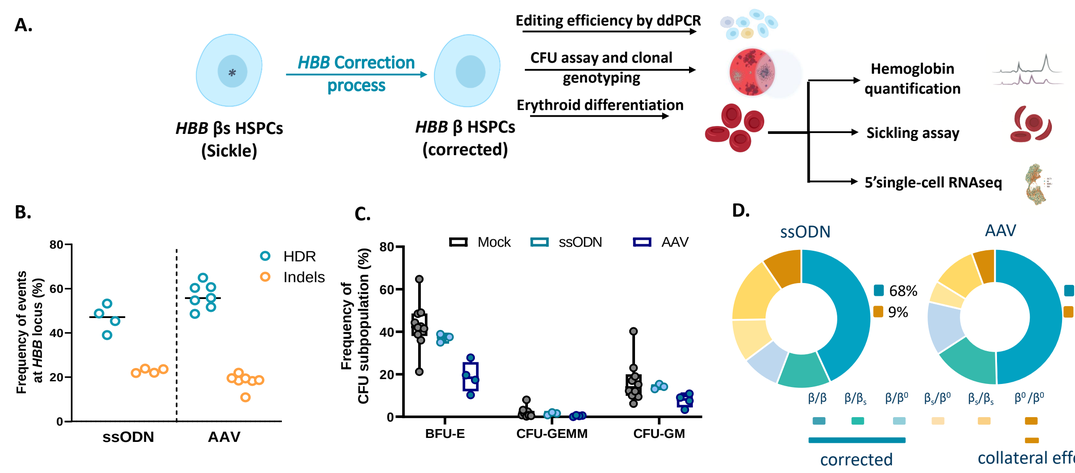


- To assess the ability of TALEN® editing to achieve high HDR efficiencies in LT-HSCs, edited plerixafor-mobilised HSPCs from three healthy donors were injected in immunodeficient mice and analyzed 16 weeks post-engraftment to assess bone marrow (BM) human chimerism, multilineage engraftment and editing efficiencies (A).
- Non-viral DNA delivery performed better than viral-mediated editing *in vivo*, reaching high level BM engraftment, comparable to non-edited HSPCs (B), multilineage reconstitution (C) and long-term maintenance of HDR in engrafted LT-HSCs at levels comparable to the input (average 30% HDR at 16 weeks in BM) (D).
- Single-cell RNAseq (scRNAseq) was performed in HSPCs before *in vivo* injection to better characterize the discrepancies observed between viral and non-viral editing of LT-HSCs (A).

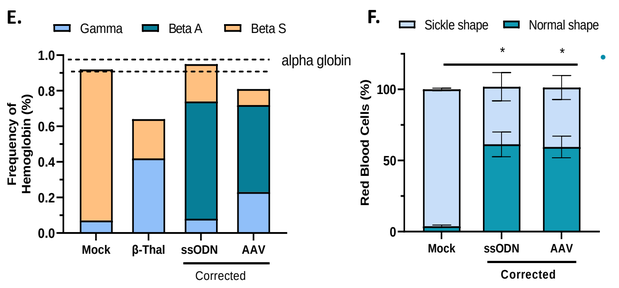


- A higher proportion of primitive HSC-enriched cells was found in ssODN- vs AAV-edited sample (E-F) correlating with engraftment data (G). By looking at transcription profile of HSC-enriched cells, we observed that edited HSCs upregulated several pathways including interferon, inflammation and p53 signaling, compared to untreated sample (H-I). Interestingly, a mitigated p53 signaling activation was observed in ssODN edited samples suggesting that non-viral gene editing better preserves HSC fitness than viral gene editing (I).

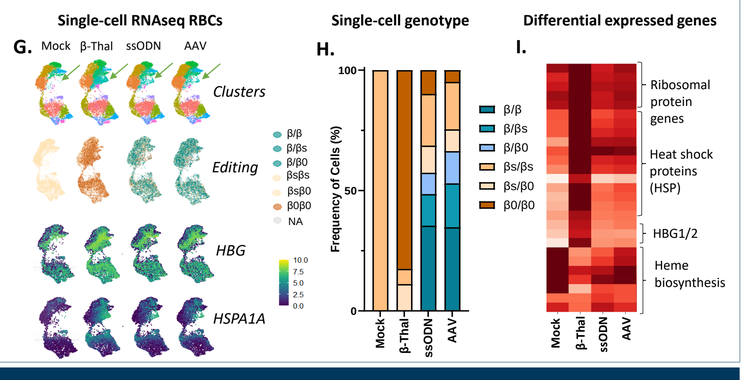
TALEN®-mediated gene editing reaches high level of gene correction in SCD patient's derived HSPCs with minimal collateral effect



- To assess whether our gene editing protocol would efficiently correct sickle-cell HSPCs, we purified HSPCs from multiple non-mobilized homozygous sickle patients (nmHbSS) and assessed gene editing efficiencies in CD34+ bulk and BFU-E single clones and clonogenic potential. We also differentiated edited HSPCs into RBCs to evaluated hemoglobin expression, sickling properties and the potential beta-thalassemic phenotype through scRNAseq (A)
- Our editing strategy achieved high gene correction frequencies (>50%), while maintaining low levels indels (<20%)(B). It was reproducible in multiple different donors and did not impact HSPCs clonogenic potential (C). Notably we reached an average of 68% and 79% of corrected BFU-E clones while generating less than 10% bi-allelic indels clones.



- Our protocol allowed high expression of adult hemoglobin (HbA) (>50%), while maintaining a correct level of total hemoglobin as assessed by alpha to non-alpha globin ratio (alpha globin level set to 1) (E). Moreover, it mitigated collateral effect derived from *HBB* inactivation observed in a β-thalassemic-like control (β-Thal) that was generated by inducing up to 70% indels. Finally, TALEN® gene editing enables rescuing the sickling phenotype (up to 70% of normal cells obtained) in hypoxic conditions (F).
- scRNAseq analysis enabling to simultaneously characterize the genotype and transcription profiles of edited RBCs, highlighted a cell cluster in the β-Thal, which was absent in the mock (G). Interestingly, this cluster was mainly composed of RBCs harboring bi-allelic indels and was reduced in the edited samples (<10% cells) (G-H).
- A β-thalassemic signature characterized by upregulation of heat shock protein genes (HSP), ribosomal proteins genes and genes encoding for fetal Hb was observed in the β-Thal control, while edited RBCs had a transcription profile similar to the mock control (I). These data confirm the reduced risk of TALEN®-gene correction to generate β-Thal phenotype which is restricted only to the cluster of cells harboring bi-allelic indels as shown by the co-localization of *HSPA1A* expression and β0β0 population (G).



Conclusions

-TALEN®-mediated engineering efficiently corrects the mutated *HBB* gene in clinically relevant HSPCs (68-79% corrected progenitors) and this translates into phenotype correction in fully mature RBCs (Up to 50% HbA expression)

-Our optimized TALEN® gene editing process mitigates potential safety challenges by reducing the frequency of *HBB* gene inactivation (<10% β-thal cells)

-Non-viral DNA template mediated repair mitigates p53 DNA damage response activation and preserve LT-HSCs fitness

-Non-viral DNA delivery template coupled to TALEN® editing enables higher engraftment and maintenance of gene correction in LT-HSCs (Up to 40% corrected LT-HSCs)

Image Credit: Images of cells were adapted from "Cell Biology", by BioRender.com (2021); Retrieved from https://app.biorender.com/biorender-templates