Pre-clinical Development of a Highly Efficient TALEN®-based Correction of β-globin Gene in Patient-derived Hematopoietic Stem and Progenitor Cells (HSPCs) to Treat Sickle Cell Disease

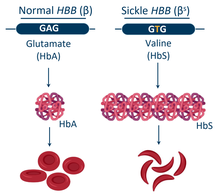
Arianna Moiani[1], Patrick Hong[2], Gil Letort[1], Sabrina Lizot[1], Alexa Chirinos[1], Sophie Leduc[1], Noémie Pinard[1], Chloe Foray[1], Sonal Temburni-Blake[2], Sara Nik[2], Louisa Mayer[2] ,Vladlena Lee[2], Alex Boyne[2], Selena Kazancioglu[2], Giacomo Frati[3], Tristan Felix[3], Anne Chalumeau[3], Annarita Miccio[3], Mathilde Dusseaux[1], Agnès Gouble[1], Mark Frattini[2], Carrie Brownstein[2], Aymeric Duclert[1], Cecile Shiffer-Mannoui[1], Alexandre Juillerat[2], Philippe Duchateau[1], Julien Valton[1]

[1]Cellectis S.A., 8 Rue de la Croix Jarry, 75013 Paris, France; [2]Cellectis, Inc., 430 East 29th Street, New York, NY 10016, USA; [3]Université de Paris, Imagine Institute, Laboratory of Chromatin and Gene Regulation During Development, INSERM UMR 1163, Paris, France

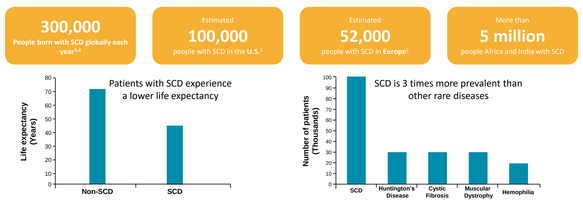
Background

Sickle Cell Disease (SCD)

- SCD is an inherited blood disorder that stems from a single point mutation (A>T) in exon 1 of the hemoglobin subunit beta (*HBB)* gene, which allows hemoglobin to polymerize.[1]



- Polymerization can result in crescent or sickle shaped red blood cells (RBCs) that cause reduced oxygen transfer to tissues throughout the body and slow or stop 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 by up to 30 years.[2]

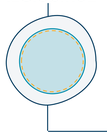


- SCD can be cured with hematopoietic stem cell transplant, but this option is unavailable to most people with SCD.[2] There is an urgent need to provide alternative treatment options for this devastating disease.
- *Ex vivo* gene therapy approaches have shown to be a promising therapeutic option for patients with SCD.

Objectives

- To develop an autologous HSPC-based gene therapy that has the potential to directly repair the mutated *HBB* gene in patients with SCD using highly efficient and precise TALEN®-based gene editing technology.
- To demonstrate TALGlobin01 can be manufactured at large scale with consistent efficiency, precision, and cell viability.

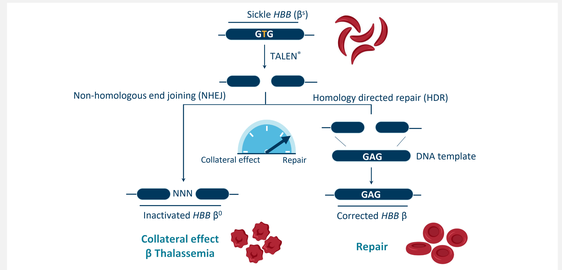
TALGlobin01



- TALGlobin01 is an autologous cell-based gene therapy product designed to repair the mutated b-globin gene (*HBB*), and subsequently restore production of Hemoglobin A in HBSS sickle cell disease.

- The edited HSPCs are assayed for editing precision, efficiency, off-target cleavage, cell viability, differentiation potential, and restoration of normal cell function in differentiated RBCs.
- The potential of TALGlobin01 to maintain edits in long-term engraftment of hematopoietic stem cells was evaluated by transplanting TALGlobin01 cells into immunodeficient NSG mice and quantifying persistence of gene correction for up to 16 weeks.

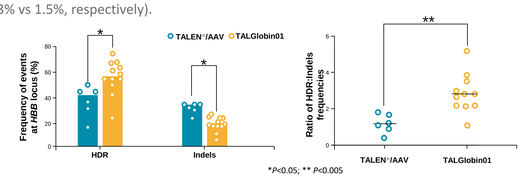
Precise gene editing of HBB mutation with TALEN®



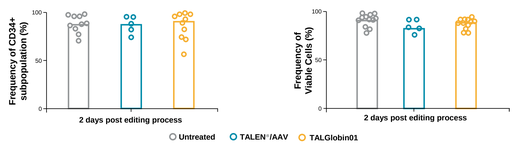
HSPCs from patients with homozygous mutation in *HBB* (HbSS) are engineered with an optimized TALEN® called TALEN-HBB01, and adeno-associated virus (AAV)-based engineering to cleave and correct the sickle *HBB* gene and mitigate edits that result in an inactivated *HBB* gene.

TALGlobin01 reaches high level of gene correction in SCD patient's derived HSPCs with minimal collateral effect

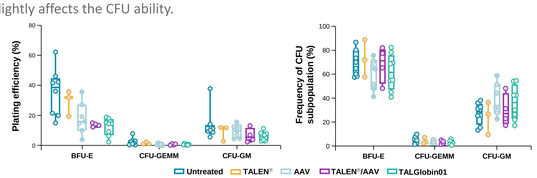
- The TALGlobin01 optimized gene editing process allowed a higher level of HDR mediated correction of the *HBB* gene compared to TALEN®/AAV (57% vs 43%), while decreasing the level of Indels from 37% to 19%, respectively. The ratio HDR:Indels is also higher with TALGlobin01 (3% vs 1.5%, respectively).



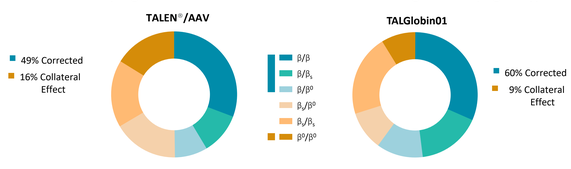
- During the gene editing process, viability and the proportion of CD34+ cells are maintained at levels comparable to the untreated control sample.



- The ability of TALGlobin01 to generate CFU colonies was two-fold lower compared to untreated controls without lineage skewing. This difference was mainly due to AAV treatment as it is comparable to the AAV only sample, while TALEN® electroporation only slightly affects the CFU ability.

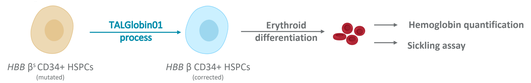


- Clonal analysis showed that the process to make TALGlobin01 corrects SCD mutation in 60% of BFU-E progenitors with less than 10% of clones harboring bi-allelic β^0 genotype.

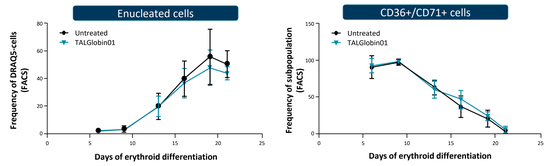


HSPCs | CFU | BFU-E single colonies

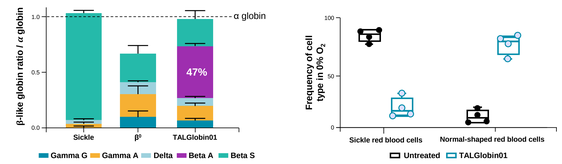
TALGlobin01 restores HbA expression and rescues sickling phenotype in fully differentiated RBCs



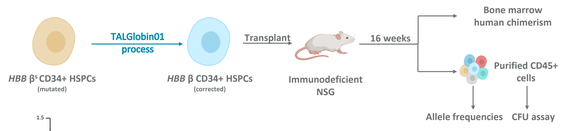
- Erythroid differentiation potential was not affected in TALGlobin01 compared to untreated control as evaluated by FACS.

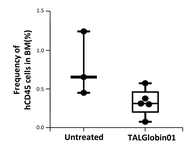


- TALGlobin01 allowed high expression of adult hemoglobin (HbA) while maintaining a correct level of total hemoglobin as assessed by alpha to non-alpha globin ratio (alpha globin level set to 1). TALGlobin01 mitigates the collateral effect derived from *HBB* inactivation observed in β^0 control.
- TALGlobin01 enables rescue of sickling phenotype in cells maintained at 0% O_2.

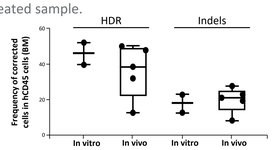


TALGlobin01 retained the capacity of long-term engraftment in immunodeficient NSG mice

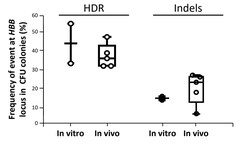




- Non–mobilized HbSS patient derived HSPCs retained the capacity to engraft long-term. The level of engraftment in bone marrow at 16 weeks were two-fold lower in TALGlobin01 compared to untreated sample.



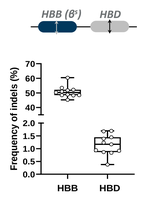
- SCD correction persisted in engrafted progenitors up to 16 weeks with levels comparable to the *in vitro* input (~40%).



- High level of gene correction (~40%) in *ex-vivo* derived CFU colonies confirmed the capacity of TALGlobin01 to edit long-term progenitors. Low level of Indels confirmed the stability of low risk associated to *HBB* inactivation *in vivo*.

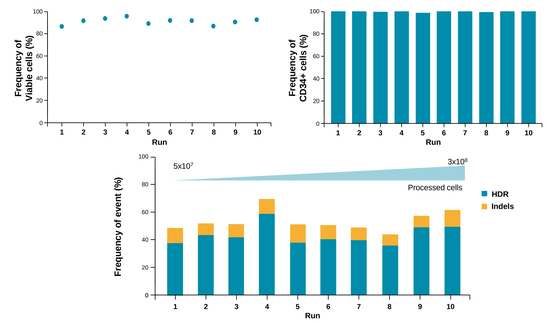
TALGlobin01 has high specificity with only one off-target site detected

- Potential off-target cleavage activity was examined using an unbiased genome wide approach and validated by a target enrichment high-throughput sequencing screening.
- Only one off-site was confirmed by both applications at the level of *HBD* gene.
- TALEN®-HBB01 cleavage activity was assessed at the *HBD* off-target site in HbSS in TALGlobin01 and found to be very low compared to the on-site cleavage activity (50.7% Indels +/- 3.9 at the on-site versus 1.2% +/- 0.4 at the off-site) confirming the high level of specificity of TALGlobin01.



TALGlobin01 can be manufactured at large scale

- Ten large scale processes were performed with increasing number of cells, from 5x10^7 to a maximum of 3x10^8 processed cells.
- HSPCs viability and purity were not impacted by large scale processing.
- Gene correction levels were comparable among large scale processes with an average of 43% HDR and only 10% Indels.
- TALGlobin01 manufacturing at clinical scale (3x10^8 cells) reached up to 49% HDR.



- Large scale manufacturing of TALGlobin01 was performed in mobilized CD34+ cells from healthy donors.

Conclusion

- TALEN®-based engineering could be used to efficiently correct the mutated *HBB* gene with low collateral effects
- TALGlobin01 mitigates potential toxicity or safety issues by reducing the frequency of *HBB* gene inactivation
- TALGlobin01 readily differentiates *in vitro* into normal functioning RBCs and retain the capacity to engraft long-term in immunodeficient mice models
- There was low off-target cleavage generated by the gene correction process
- TALGlobin01 can be manufactured at large scale

Disclosures

Julien Valton, Arianna Moiani, Patrick Hong, Gil Letort, Sabrina Lizot, Sophie Leduc, Noémie Pinard, Alexa Chirinos, Chloe Foray, Sonal Temburni-Blake, Sara Nik, Louisa Mayer, Alex Boyne, Selena Kazancioglu, Vladlena Lee, Mathilde Dusseaux, Agnès Gouble, Mark Frattini, Carrie Brownstein, Aymeric Duclert, Cecile Shiffer-Mannoui, Alexandre Juillerat, and Philippe Duchateau are current employees and current equity holders in a publicly traded company: Cellectis.

Giacomo Frati No conflict of interest

Tristan Felix No conflict of interest

Anne Chalumeau No conflict of interest

Annarita Miccio No conflict of interest

TALEN® is a Cellectis' patented technology.

References: 1. Renaudier P, Transfus Clin Biol. 2014 2. Centers for Disease Control. www.cdc.gov/ncbddd/sicklecell/index.html Last accessed November 4, 2021. 3. Piel FB et al, Lancet 2013. 4. Odame I, Nature 2014.
5. European Medicines Agency. www.ema.europa.eu/en/medicines/human/orphan-designations/eu3182125#key-facts-section Last accessed November 9, 2021. Image Credit: Images of cells were adapted from "Cell Biology", by BioRender.com (2021); Retrieved from https://app.biorender.com/biorender-templates