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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Sickle cell disease stems from a single point mutation in the *HBB* gene which results in sickle hemoglobin. For patients who are not eligible for an allogeneic stem cell transplantation, nuclease-based gene therapy approaches provide a relevant therapeutic alternative to restore functional hemoglobin production.

Here, we leveraged TALEN® technology to develop a gene editing process leading to highly efficient *HBB* gene correction via homology directed repair, while mitigating potential risks associated to *HBB* gene knock-out. Furthermore, we compared viral (TALEN-V) and non-viral (TALEN-NV) DNA template delivery strategies in mobilized healthy donor (HD) or non-mobilized homozygous sickle patient (HbSS) hematopoietic stem and progenitor cells (HSPCs).

Both strategies led to high and comparable efficiencies of *HBB* gene correction *in vitro* in HD and HbSS, without affecting viability, purity or clonogenic potential of corrected HSPCs. Moreover, they both elicited high and similar expression of normal adult hemoglobin in red blood cells differentiated from edited HbSS HSPCs.

Interestingly, when evaluated *in vivo* using an immunodeficient mouse model, transplanted TALEN-NV edited HSPCs showed higher levels of engraftment and gene correction compared to TALEN-V edited HSPCs. Further characterization of edited HSPCs by single-cell RNAseq enabled us to identify distinct transcriptomic signatures associated to each strategy, allowing to better understand the molecular and cellular basis of this discrepancy.

Overall, these results show that non-viral DNA delivery associated to TALEN gene editing reduces the toxicity usually observed with viral DNA delivery and allows high levels of *HBB* gene correction in long-term repopulating hematopoietic stem cells.