TALEN®-mediated engineering of HSPC enables systemic delivery of IDUA

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Mucopolysaccharidosis type I (MPS-I) is caused by deficiencies in the alpha-L-iduronidase (IDUA) gene. Current treatments are limited to enzyme replacement therapy usually preceded by allogenic bone marrow transplantation. Gene editing of hematopoietic stem and progenitor cells (HSPCs) followed by transplantation offers unique therapeutic advantages including local delivery into the brain and could be a therapeutic strategy for MPS-I and other lysosomal storage diseases.

We established a TALEN®-based *ex vivo* gene editing protocol to insert an IDUA-expression cassette into a specific locus of HSPC. *In vitro*, edited HSPC were 90% viable and maintain differentiation potential. Level of gene insertion was up to 60% allelically in bulk edited population, and up to 80% of clones from colony forming unit assay. Edited cells displayed 60- and 80-fold higher IDUA secretion than unmodified cells at the HSPC and myeloid level, respectively.

Edited HSPCs were functionally evaluated in 2 immunodeficient mouse models: NSG and NSG-SGM3. Edited HSPC maintained differentiation potential toward myeloid and lymphoid lineages in vivo, with no differences with unmodified cells. Editing rates *in vivo* were 6-9% sixteen weeks after injection, depending on the tissue analyzed (blood, spleen, bone marrow). Lastly, 8.3% of human cells were edited in the brain compartment.

In conclusion we established a safe TALEN®-based gene editing protocol procuring IDUA-edited HSPCs able to engraft, differentiate into multiple lineages and reach multiple tissues, including the brain. These results pave the way towards targeted gene therapy-mediated treatment of MPS-I.