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Poster Abstract: Therapeutic

Lentiviral Stem Cell Gene Therapy for Pompe Disease

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BACKGROUND

Pompe disease is a rare autosomal recessive metabolic disorder caused by deficiency of lysosomal hydrolase acid α-glucosidase (GAA). GAA degrades glycogen to glucose, and deficiency results in generalized tissue glycogen accumulation leading to cardiorespiratory failure in the early-onset patients within the first year of life. Enzyme replacement therapy (ERT) by administration of recombinant acid α-glucosidase (alglucosidase alfa, Myozyme®) is currently the only effective treatment, requiring high-dose biweekly administration. Although of considerable benefit to many patients, ERT is not curative, requires life-long administration, may result in immune responses to the recombinant enzyme and, partly due to the high doses required for clinical efficacy, the costs are extremely high. Therefore, a corrective intervention with curative intent represents an unmet medical need.

MATERIALS AND METHODS

We have developed a single intervention hematopoietic stem cell (HSC) approach to overexpress GAA in the hematopoietic system. Third-generation self-inactivating lentiviral vectors were constructed for therapeutic expression of GAA. The codon-optimized (GAAco) gene was driven by the spleen focus forming virus promoter. Gaa−/− mice were transplanted with 5×10^5 Lin-cells transduced with therapeutic vector and relevant tissues, particularly skeletal muscles, were evaluated for both GAA activity and glycogen content. In order to further optimize vector design, we have developed an *in vitro* assay using Hepa-1-6 or C2C12 *Gaa* deficient cells through CRISPR/Cas9 genomic engineering. These *Gaa* KO cell lines were applied in a transwell system to test chimeric GAA variants, including fusing GAAco with a portion of insulin-like growth factor II (IGF-II), to create an active, chimeric enzyme with high affinity for the cation-independent mannose 6-phosphate receptor.

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RESULTS

At an average vector copy number of 7, lentiviral vectors expressing GAAco by the strong promoter significantly increased GAA levels compared with the native GAA sequence, resulting in complete correction of the disease phenotype in Pompe mice, including restoration of motor function with immune tolerance induction to the transgene product. In the transwell system, GAAco fused with IGF-II displayed improved excretion and uptake compared with GAAco.

CONCLUSIONS

Lentiviral HSC gene therapy using codon optimized *Gaa* resulted in full correction of the phenotype in Pompe mice. Furthermore, from preliminary data in the developed transwell system, the glycosylation-independent lysosomal targeting-tagged construct resulted in improved excretion and uptake. Currently, this new variant is evaluated *in vivo* for efficacy and safety.

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