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Genetically Modulated Substrate Reduction Therapy for Sanfilippo syndrome - proof of principle

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Introduction: Sanfilippo syndrome, or Mucopolysaccharidosis (MPS) type III refers to a group of five autosomal recessive neurodegenerative lysosomal storage disorders caused by the incomplete lysosomal degradation of the glycosaminoglycan (GAG) heparan sulphate (HS) that accumulates in patient cells and triggers disease. The main characteristic of MPS III is the degeneration of the central nervous system, resulting in mental retardation and hyperactivity, with a typical early onset. Currently, there is no effective therapy available, with treatment limited to clinical management of neurological symptoms. In order to address this issue, we have designed an RNA-based therapeutic strategy based upon the selective downregulation of genes involved in HS biosynthesis.

Methods: Taking advantage of the RNA interference (RNAi) technology potential, we have designed and assayed a specific siRNA targeting an early stage of the HS biosynthetic cascade (XYLT1). Our goal is to promote an effective reduction of the accumulating substrate, ultimately decreasing or delaying the symptoms. Fibroblasts from MPS III patients' were transfected with the designed siRNA. Total RNA was extracted and target mRNA levels evaluated through real-time PCR. In order to evaluate the effect of this approach, the GAGs accumulation was quantified over time using a modified 1,9-dimethylmethylene blue assay.

Results and Discussion: Proof of principle on the effect of an siRNA targeting XYLT1 was achieved for two independent control cell lines, with 8-12 fold decreases on the target mRNA levels, after 24h of incubation with concentrations of 20nM of each siRNA. Subsequent analysis on the effect of the same siRNA on patients' cell lines resulted in significant lower expression of XYLT1 in MPS IIIA, IIIC and IIID fibroblasts. Initial studies evaluated mRNA levels after 24-48h incubation. Studies on MPS IIIB are also ongoing. For MPS IIIC, we have already assessed the treatment effect on storage and observed a significant reduction (50%) on the total GAGs levels. We are currently addressing GAGs' storage in the remaining MPS III cell lines. Here we present an overview of the preliminary results of this project and unveil its next steps towards a full characterization/evaluation of its potential therapeutic effect.

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