

## **Genetically modulated Substrate Reduction Therapy for Mucopolysaccharidoses – in vitro studies**

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Mucopolysaccharidoses (MPSs) are caused by dysfunction in enzymes responsible for the intralysosomal degradation of glycosaminoglycans (GAGs). We have designed an RNA-based strategy based upon the selective downregulation of genes involved in the biosynthesis of GAGs, which is currently under evaluation. Our goal is to promote an effective reduction of the accumulating substrate, ultimately decreasing or delaying MPSs' symptoms.

Taking advantage of the RNA interference (RNAi) technology potential, we have designed and assayed specific siRNAs targeting genes on those biosynthetic cascades to decrease the levels of production of each one of the four substrates: dermatan sulphate (DS), heparan sulphate (HS), keratan sulphate (KS), and chondroitin sulphate (CS). MPSs were divided into two major sub-groups: (1) those that accumulate DS/CS and (2) those that accumulate HS. 'Group 1' included MPS types I, II, VI and VII, while 'group 2' includes the Sanfilippo syndrome, or MPS III, which subdivides into four different diseases: IIIA, IIIB, IIIC and IIID. Proof of principle on the effect of siRNAs targeting CHSY1 and 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 each siRNA as low as 20nM. Subsequent analysis on the effect of those same siRNAs on patients' cell lines resulted in significant CHSY1 expression decrease in MPS I/MPS VI cell lines ('group 1'), as well as that of XYLT1 in MPS IIIA and IIID fibroblasts ('group 2'). Initial studies evaluated mRNA levels after 24-48h incubation with each siRNA. Even though relevant decreases were observed for all tested cell lines, it became evident that the treatment efficacy may depend on the features of each specific MPS cell line, with some lines requiring higher siRNA concentrations to promote similar inhibition levels.

In order to assess the effect of that treatment on substrate reduction, we have used both the routine Alcian blue and a modified, more sensitive 1,9-dimethylmethylene blue assay on the culture media collected after seeding and incubation, at different time points. Nevertheless, the low confluency levels required for siRNA transfection did not allow detection of GAGs excreted to the culture media. Similar problems have been noted by other authors, particularly in small samples, like the ones we used. Thus, we are currently quantifying GAGs' storage by direct measurement of tissue samples after papain extraction. By doing so, we can access the intralysosomal levels of GAGs instead of their excretion.

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.