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Genetic Substrate Reduction Therapy for Mucopolysaccharidoses type III: toward a siRNA-containing nanoparticle targeted to brain cells

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INTRODUCTION

The classical therapeutic approach for LSD, enzyme replacement therapy, would hardly rise as a potentially successful tool to reduce the disease burden in MPS III patients, as it is long known to have no impact on neuropathology.
A tempting alternative, however, would be to block substrate accumulation upstream, by decreasing its synthesis. That concept is known as **substrate reduction therapy** (SRT).

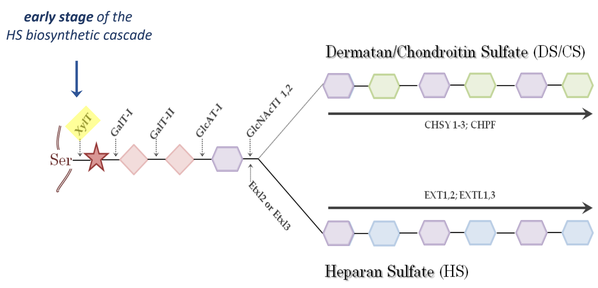
Having this in mind, we designed an **RNA-based strategy** based upon the **selective downregulation** of **one gene** involved in the very early stages of the **glycosaminoglycans' (GAG) biosynthethic cascade**. Our goal is to promote an effective reduction of the accumulating substrate, ultimately decreasing or delaying MPS' symptoms. As tools to achieve substrate reduction, we are evaluating a specific type of antisense oligonucleotides, able to trigger a naturally-occurring post-transcriptional gene silencing process called RNA interference: the small interfering RNAs (**siRNAs**).

PROJECT OVERVIEW

RESULTS AND DISCUSSION

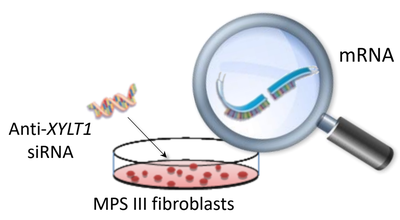
1. Choice of the molecular target

From the biochemical cascades that give origin to heparin sulfate (the GAG that accumulates in MPS III), we chose one major target: *XYLT1*, which codes for an enzyme involved in the early stages of GAG biosynthesis (xylosyltransferase1). By targeting an *'early stage'* gene, we may develop a drug that holds the potential to benefit virtually all MPS.



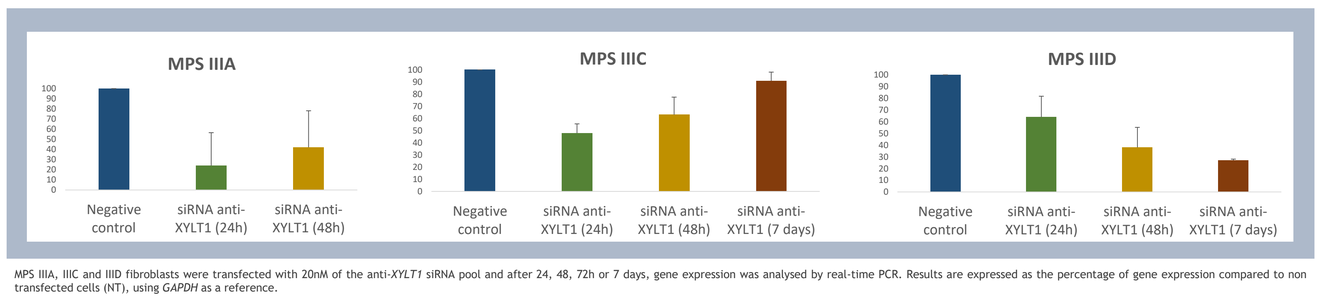
Overall, our **rationale** is simple: if we decrease GAG synthesis by downregulating this specific gene we will actively decrease substrate accumulation and, hopefully, slow down pathology.

2. *In vitro* studies – proof of principle on the treatment's effect on target mRNA levels



Currently, proof of principle on the *in vitro* effect of anti-*XYLT1* siRNAs was already obtained for 3 of the 4 Sanfillippo syndromes (MPS IIIA, IIIC and IIID), with significant decreases of the target gene expression at different time points (24h-7 days).

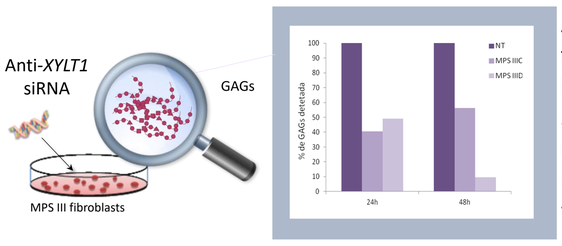
In general, our results also reinforce the idea that there is variability amongst different cell lines in terms of response to treatment with siRNAs. In fact, we observe substantial differences between different cell lines treated with the same anti-XYLT1 siRNA pool, at the same concentration. In studies by other authors, this sort of variability was observed even among cell lines from patients suffering from the same disease and with the same underlying genotype.



MPS IIIA, IIIC and IIID fibroblasts were transfected with 20nM of the anti-*XYLT1* siRNA pool and after 24, 48, 72h or 7 days, gene expression was analysed by real-time PCR. Results are expressed as the percentage of gene expression compared to non transfected cells (NT), using *GAPDH* as a reference.

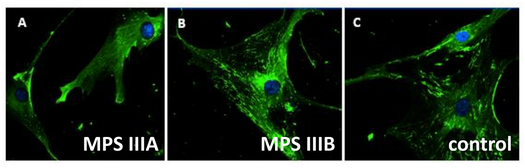
ONGOING STUDIES

3. Evaluation of the treatment's effect over GAG levels



After this first assessment on the effect of anti-*XYLT1* siRNA incubation at 24 and 48h over sulphated GAG levels, we are now evaluating its effect 7 days post-transfection, also with promising results.

Additional data on the effect of the designed siRNAs on substrate accumulation will be collected over the next months and other methods will be used to further address this issue. We are currently optimizing an anti-HS immunofluorescence method to further assess the effect of treatment upon GAG storage, particularly HS, which is the GAG accumulating in MPS III, and expect to have its results by the end of the year.



HS accumulation assessment through immunoflourescence.
HS staining in control (A) and MPS III B (B) and III D (C) fibroblasts (untreated).

FUTURE PERSPECTIVES

4. Development of an appropriate delivery method

4.1. Vector design & siRNA encapsulation into stable nucleic acid lipid particles
- ↑ bioavailability of siRNAs;
- protection from degradation
- control of circulation time and release rate

4.2. Coupling of specific ligands to siRNA-carrying liposomes
- Transferrin (Tf)
- Rabies virus peptide derivative (RGV-2r)

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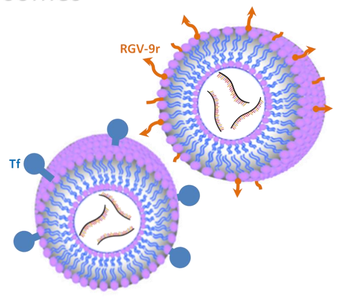
both of them have already been shown to enable nanoparticles to **cross the blood brain barrier**.



4.3. Efficiency assessment + Targeting of brain cells

TAKE-HOME MESSAGE

By the end of this project…

an appropriate **delivery method** for **'anti-GAG siRNAs'** targeted to **neuronal receptors** will be developed and subsequently tested for efficacy in **neuronal cells**, as they represent the **ultimate therapeutic target** for **MPS III.**

Ultimately, the same principle may be extended to other MPS, or even for LSD in general. Amongst other advantages, one such approach would virtually allow for the creation of multifunctional complexed siRNA mixtures, as different diseases share the same accumulating substrates, reducing therapy costs and increasing the number of patients with available therapeutic options

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