When less is actually more: *in vitro* assessment of the potential of anti-*XYLT1* siRNAs to promote substrate reduction in Mucopolysaccharidosis type III

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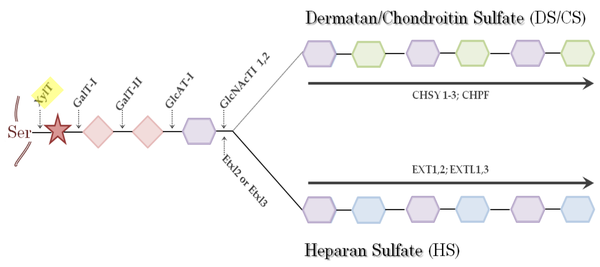
Introduction

Mucopolysaccharidosis type III (MPS III) refers to a group of four autosomal recessive neurodegenerative lysosomal storage disorders (LSD) caused by the incomplete lysosomal degradation of the heparan sulphate (HS) that accumulates in patient cells and triggers disease.

Degeneration of the central nervous system is the major hallmark of these disorders, resulting in mental retardation and hyperactivity. By their mid-teenage years most affected patients are dependent on their caregivers for all needs and death occurs at the end of the second or early in the third decade of life. The classical therapeutic approach for LSDs, enzyme replacement therapy, would hardly rise as a potentially successful tool to reduce the disease burden in MPS III patients due to the inability of the recombinant enzymes to cross the blood-brain barrier (BBB), having no impact in neuropathology. Thus, there is no effective therapy available, with treatment limited to clinical management of neurological symptoms. A tempting alternative, however, would be to block substrate accumulation upstream, by decreasing its synthesis. That concept is known as substrate reduction therapy (SRT).

Methods

In order to decrease HS storage inside the lysosomes, we designed and assayed in MPS III patients' fibroblasts a specific siRNA pool targeting *XYLT1,* a gene that encodes an enzyme involved in an early stage of the HS biosynthetic cascade.

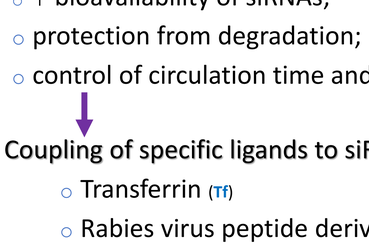


Overall, our rationale is simple: **if we decrease GAG synthesis by downregulating this specific gene we will actively decrease substrate accumulation and, hopefully, slow down pathology.**

Fibroblasts from MPS IIIA, B, C and D patients were transfected with the designed siRNAs pool to inhibit *XYLT1.* Cell pellets were collected 24/48 hours and 7 days post- transfection and total RNA extracted. Target mRNA levels were evaluated through qRT-PCR using the $2^{-\Delta\Delta Cq}$ method. Additionally, the effect on HS accumulation was quantified, until now, for 24 and 48h after transfection using a modified 1,9-dimethylmethylene blue assay.

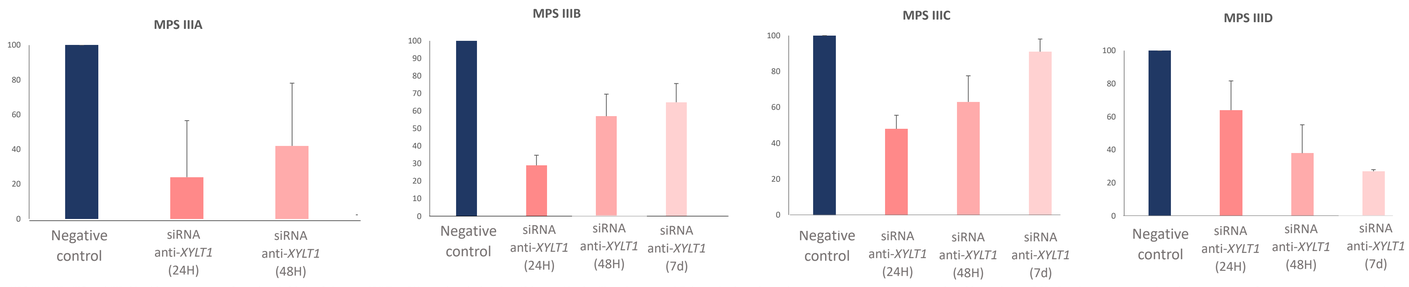
Results

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



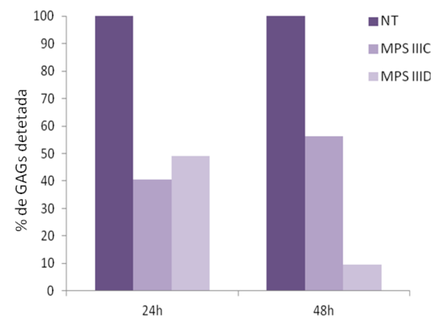
Proof of principle on the *in vitro* effect of anti-*XYLT1* siRNAs was obtained for Sanfillippo syndrome, with significant decreases of the target gene expression at different time points (24h-7 days).

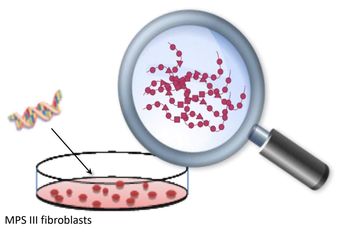
In general, our results also reinforce the idea that there is variability amongst different cell lines in terms of response to treatment with siRNAs. In fact, we observe substantial differences between different cell lines treated with the same anti-*XYLT1* siRNA pool, at the same concentration. In studies 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, IIIB, IIIC and IIID fibroblasts were transfected with 20nM of the anti-*XYLT1* siRNA pool and after 24, 48 hours (H) (for IIIA subtype) and 7 days (d) (for other subtypes), 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.

2. 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 and for other MPS subtypes, also with promising results.

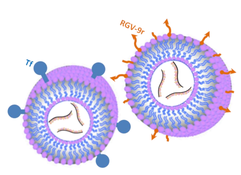
Further validation

- qGAG by MS/MS;
- immunocytochemistry *(anti-HS antibody)*

A look forward

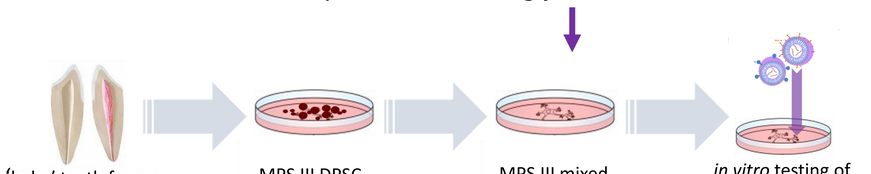
Our goal is to develop **targeted stable nucleic acid lipid particles** (t-SNALPs) coupled with different ligands, to promote cell uptake of the 'anti-GAG' siRNAs in a variety of cells, including **patient-derived neurons**:

- ↑ bioavailability of siRNAs;
- protection from degradation;
- control of circulation time and release rate.



Coupling of specific ligands to siRNA-carrying liposomes → Efficiency assessment + Targeting of brain cells

- Transferrin (Tf)
- Rabies virus peptide derivative (RGV-2r) > both of them have already been shown to enable nanoparticles to **cross the blood brain barrier**.



- non-invasive;
- cost-effective method approach using **dental pulp stem cells** (DPSC)

Conclusion

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 patient-derived **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

Aknowledgements

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2021 RNA Therapeutics: From Concept to Clinic

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