Assessing the potential of RNA-based therapeutics for a group of Lysosomal Storage Diseases with neurological involvement

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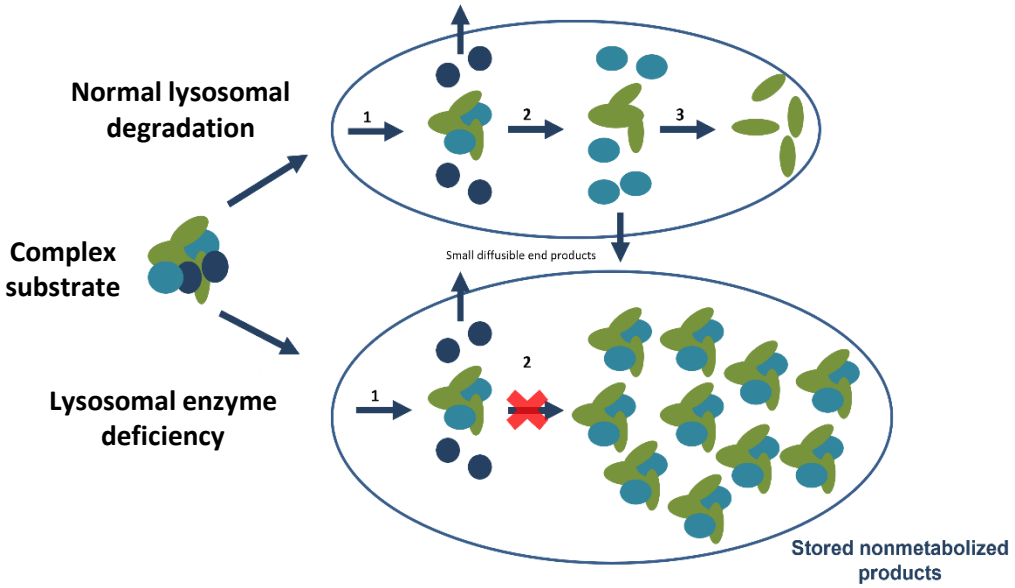
Mucopolysaccharidosis (MPSs) - overview



- Group of **Lysosomal Storage Disorders** (LSD) caused by defects in the degradation of **glycosaminoglycans** (GAGs)
- **11 different disorders**, each one associated with a defect in one of the enzymes responsible for the degradation of GAGs
- In general, the **clinical features** of different MPSs may be classified as **somatic** or **neuronopathic**

MPS III, or Sanfilippo syndrome, has a predominance of CNS disease ⟹ Treatments are only **symptomatic**

That is why ⇓

Further investment is necessary to create a therapy that targets the **neurological manifestations!**

RNA-based therapies

- Novel class of **highly specific and promising drugs** –already reached the market
- Constitute a potential **alternative** or an **adjuvant** therapeutic strategy for MPS III, **<u>acting at</u>**:

1) Pre - mRNA level → **Splicing modulation/ correction** → *Design of mutation-specific approaches to **correct** **abnormal splicing** process in MPS IIIC-related gene, using **U1snRNAs***

2) mRNA level → **Gene expression modulation** → *Selective downregulation of **one gene** involved in the very early stages of the GAGs biosynthetic cascade to promote **substrate reduction** in MPS III, using **siRNAs***

U1snRNA to correct abnormal splicing process in MPS IIIC caused by the c.234+1G>A mutation

U1snRNAs Mutation-specific approach to correct abnormal splicing process in MPS IIIC caused by the c.234+1G>A mutation

We demonstrated in patient fibroblast cells that a modified U1snRNA vector (comprising exon 1 to exon 3) designed to improve the definition of exon 2 5' ss of the *HGSNAT* can restore the splicing defect caused by the mutation c.234+1G>A (Matos *et al.*, 2014).

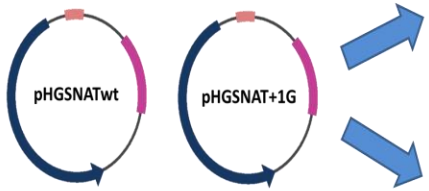
GOAL: Evaluate *in vivo* the therapeutic potential of the modified U1snRNA by testing it in mice expressing the human splicing defect

Generation of full-length (*HGSNAT* cDNA sequence plus part of introns 1 and 2) *HGSNAT* constructs of **wild-type (wt) (pHGSNATwt)** and **c.234+1G>A (pHGSNAT+1G)** by cloning the **wt** or the **mutated *HGSNAT* splicing-competent cassettes** into the **pcDNA 3.1 backbone**

In vitro

Transfection of 3µg of each construct using Lipofectamine® 2000

HEK 293T cells



In vivo

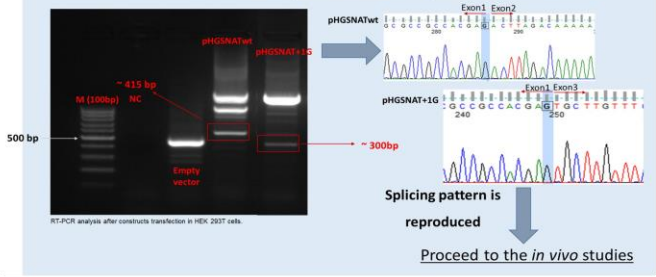
Administration in mice by hydrodynamic injection (using the tail vein)

C57BL/6 mouse strain

RESULTS

In vitro

Transfection of HEK 293T with **pHGSNATwt** and **pHGSNAT+1G** constructs:

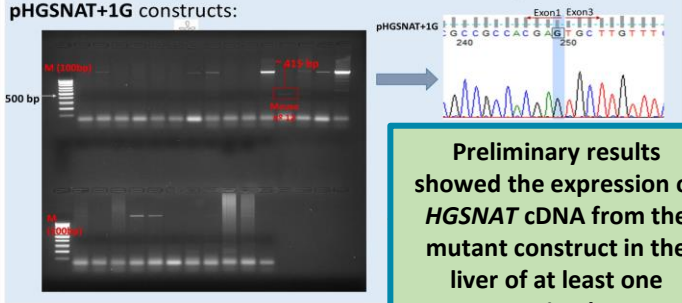


Splicing pattern is reproduced

Proceed to the *in vivo* studies

In vivo

Hydrodynamic injection of C57BL/6 mice with **pHGSNATwt** and **pHGSNAT+1G** constructs:



Preliminary results showed the expression of *HGSNAT* cDNA from the mutant construct in the liver of at least one animal

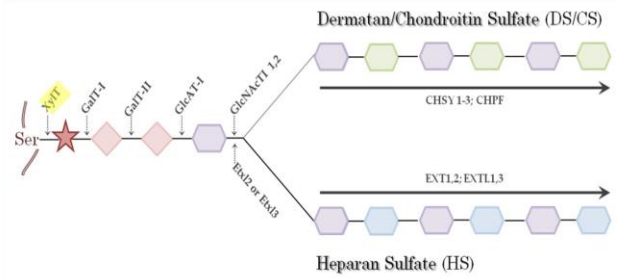
- The lack of expression in the majority of animals was probably due to the **suboptimal hydrodynamic injection conditions**
- When we tried to administrate a volume of **10%** of mice body weight **animals died**
  ↳ we lowered to **7%**
- The mouse strain used is **aggressive**, has a **hyperactive behaviour** and a **black color**, which difficults the visualization of the tail vein

REPEAT

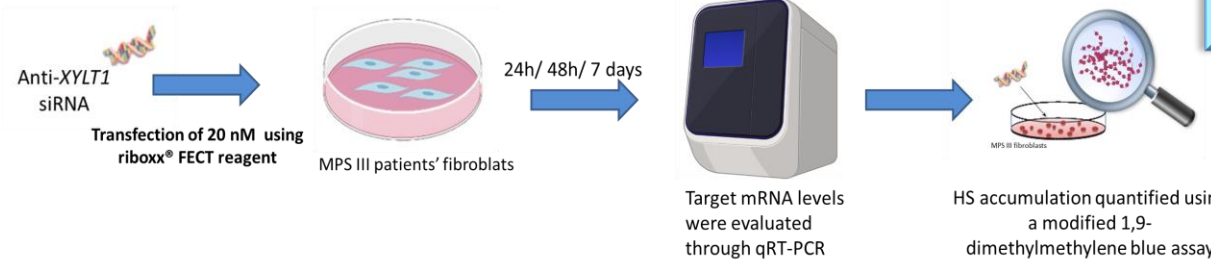
- Use an albine strain, more easier to handle and has a docile behaviour **(ICR mice strain)**
- Increase the volume of injection for **8-9%** of the mice body weight

siRNAs to promote substrate reduction

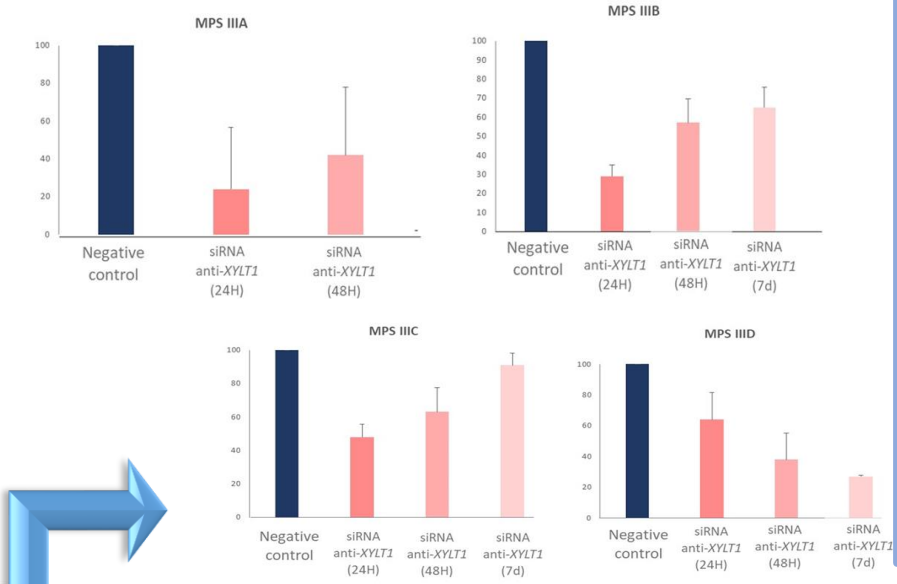
siRNAs **Selective downregulation** of one gene involved in the very early stages of the glycosaminoglycans' (GAG) biosynthethic cascade to promote substrate reduction in Sanfilippo disease



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: xylosyl-transferase 1.



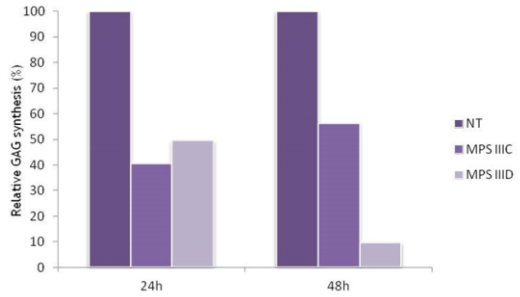
RESULTS



We observed **significant decreases** of the **target gene** expression at the different time points analyzed

We noticed some differences in the expression of the target gene between **different cell lines** treated with the same **anti-*XYLT1* siRNA pool**, at the same concentration (also observed by other authors)

These results reinforce the idea that there is a **variability** between the different subtypes of MPS III



A reduction in **GAGs levels** was observed in this first assessment:

MPS IIIC – 50% at both incubation periods and MPS IIID – 50% at 24h and 80% at 48h

We are now evaluating its effect 7 days post-transfection and for other MPS III subtypes, also with promising results.

Further validation:

- qGAG by MS/MS
- Immunocytochemistry (anti-HS antibody)

Aknowledgements

Research and Development Group on LSD

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