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# From bench to bioterium and back again: DEVELOPMENT OF A U1snRNA-BASED THERAPEUTIC STRATEGY FOR MUCOPOLYSACCHARIDOSIS IIIC

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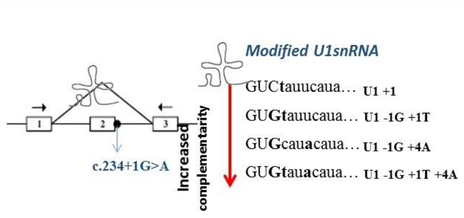
## INTRODUCTION

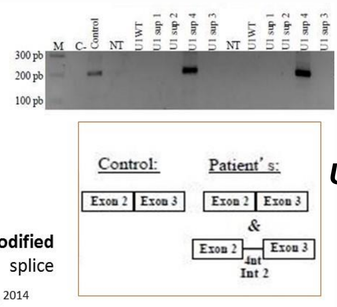
Splicing is an essential cellular process to generate mature transcripts from pre-mRNA. One of the most important factors for mRNA transcription is the U1snRNA, a spliceosomal component that recognizes 5' splicing donor sites (SDS) at specific regions in pre-mRNA. Splicing mutations represent one of the most frequent (~20%) genetic defects in Mucopolysaccharidosis IIIC (MPS IIIC), a Lysosomal Storage Disorder (LSD) caused by mutations in the *HGSNAT* gene, encoding an enzyme involved in heparan sulphate degradation.

Exon-skipping has been demonstrated as, probably, the most frequent aberrant splicing defect, and occurs due to mutations in the 5' SDS. Application of modified U1snRNAs to improve recognition of mutated 5' SDS represent a potential therapeutic strategy to recover the normal splicing process.

The c.234+1G>A is a frequent mutation among patients of countries around the Mediterranean basin (Portugal, Spain, Morocco and Tunisia). It's located in the + 1 position of intron 2 of *HGSNAT* gene and leads to the skipping of exon 2.

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




***A partial recovery was achieved with a modified U1snRNA that completely matches the SDS.***

**Our goal is to evaluate *in vivo* the therapeutic potential of the modified U1snRNA by testing it in mice expressing the human splicing defect**

## METHODS

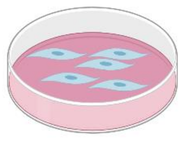
### *In vitro*

1) Generation of **full-length** (containing the full *HGSNAT* cDNA sequence plus part of introns 1 and 2) **splicing competent constructs** of:

- **wild-type (wt)**
- **c.234+1G>A *HGSNAT***

**HOW?** **By cloning the wt or the mutated *HGSNAT* splicing-competent cassettes into the pcDNA 3.1 backbone**

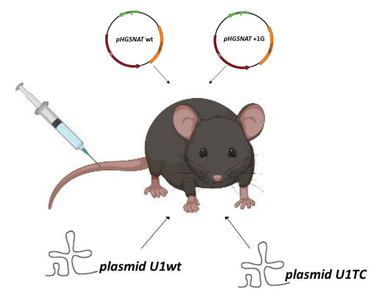
2) **Transfection of those constructs into COS-7 cells** to check if the splicing pattern is reproduced



### *In vivo*

1) Use the constructed plasmid vectors to promote **transient expression** of the human ***HGSNAT* wt** or **mutant** alleles in c57bl/6 mice

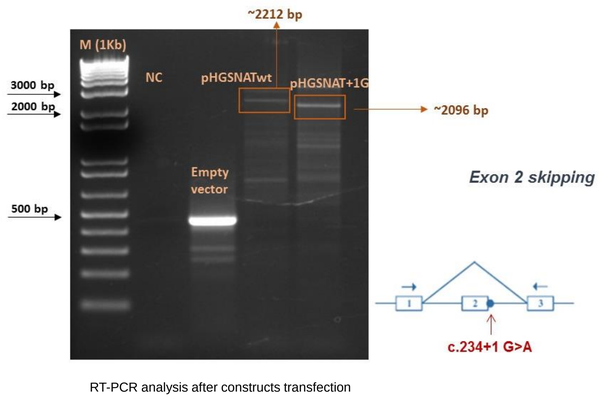
2) **Co-injection** of the **wt** or **mutant** vectors plus **modified U1snRNA**




## RESULTS

### *In vitro*

Transfection of COS-7 with **pHGSNATwt** and **pHGSNAT+1G** constructs:



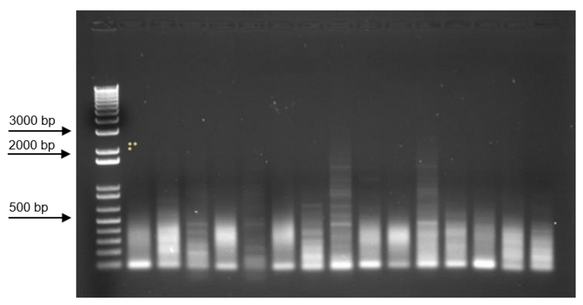

RT-PCR analysis after constructs transfection

**Splicing pattern is reproduced** ✓

proceed to the *in vivo* studies

### *In vivo*

**Hydrodynamic injection** of c57bl/6 mice with **pHGSNATwt** and **pHGSNAT+1G** constructs:




RT-PCR analysis of liver cDNA samples from injected mice to check for pHGSNAT expression

** expected amplicon length for the pHGSNATwt
* expected amplicon length for the pHGSNAT+1G

1) animal sacrifice after 48h ✓
2) organ collection (liver) ✓
3) molecular biology analysis ✓
4) **pHGSNAT cDNA expression** ✗ (i.e., no evidence of effective gene expression in vivo) **REPEAT!**

## CONCLUSION/ FUTURE PERSPECTIVES

We have double checked every step of the overall protocol to assess pHGSNAT expression through RT-PCR and everything was OK!
So, the **lack of pHGSNAT cDNA expression** was probably due to **sub-optimal hydrodynamic injection conditions**. In fact, when we tried to administrate a volume of 10% of the mice body weight, animals died. So, we **lowered the volume to 7%**.
This mice strain is **aggressive**, has a **hyperactive behaviour** and has **black color**, which difficult the visualization of the tail vein.

**In order to overcome these issues we will now repeat the protocol with ICR mice (albine strain, more easier to handle and known for its docile behaviour) with some alterations:**
- Increase the volume of our injection for **8-9%** of the mice body weight
- Dilute our construct in a **commercial delivery solution** to enhance the transfection efficiency

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