

# Genetically Modulated Substrate Reduction Therapy for Sanfilippo syndrome – proof of principle

Juliana Inês Santos<sup>1,2\*</sup>, Maria Francisca Coutinho<sup>1\*</sup>, Paulo Gaspar<sup>3</sup> and Sandra Alves<sup>1</sup>

<sup>1</sup>Research and Development Unit, Department of Human Genetics, INSA, Porto, Portugal

<sup>2</sup>Faculty of Sciences, University of Porto, Portugal

<sup>3</sup>Newborn Screening, Metabolism and Genetics Unit, Department of Human Genetics, INSA, Porto, Portugal



\* These authors contributed equally to this work

## INTRODUCTION

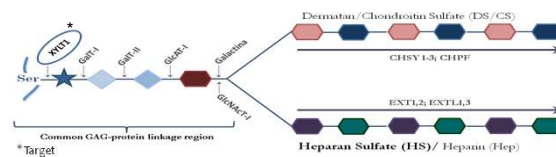
**Sanfilippo syndrome**, or Mucopolysaccharidosis type III (MPS III) refers to a group of five autosomal recessive neurodegenerative lysosomal storage disorders caused by the incomplete lysosomal degradation of the glycosaminoglycan (GAG) heparan sulphate (HS) that accumulates in patient cells and triggers disease.

The main characteristic of MPS III is the degeneration of the central nervous system, resulting in mental retardation and hyperactivity, with a typical early onset.

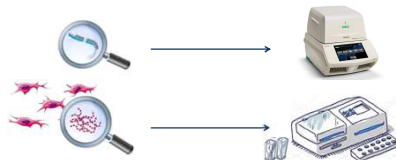
**No effective therapy available, with treatment limited to clinical management of neurological symptoms.**

## AIMS AND METHODS

To decrease production levels of heparan sulfate (HS) we took advantage of the RNA interference (RNAi) technology potential. We have designed and assayed a specific siRNA pool targeting the expression of *XYLT1*, an enzyme that participates in an early stage of the HS biosynthetic cascade.



**Our goal is to promote an effective reduction of the accumulating substrate, ultimately decreasing or delaying MPS III symptoms.**



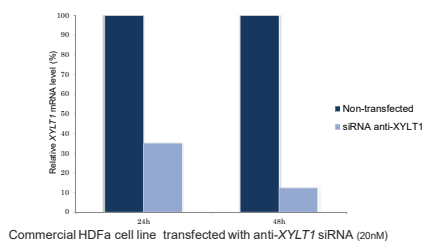
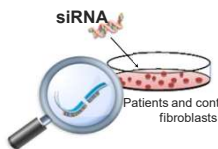
The relative *XYLT1* mRNA levels were evaluated through **quantitative real-time PCR**, and normalized to *GAPDH* endogenous control.

The GAGs accumulation was quantified over time using a **modified 1,9-dimethylmethylene blue assay**.

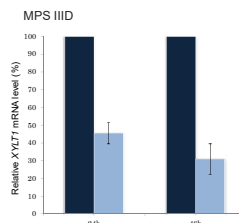
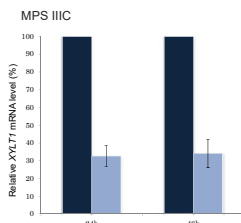
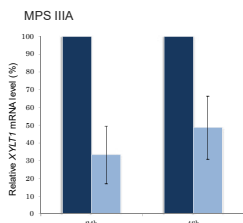
## RESULTS AND DISCUSSION

### (I) Proof of principle on the effect a siRNA pool targeting *XYLT1*

We started to test the siRNA pool in a commercial control cell line, in order to evaluate its effect at the *XYLT1* mRNA levels.

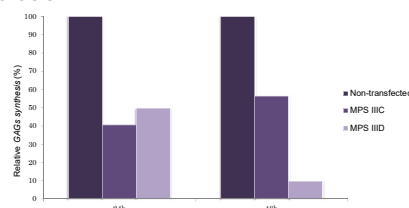
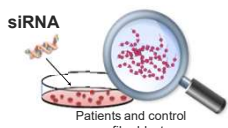


We observed significant reduction in *XYLT1* mRNA levels compared to non-transfected cells, and therefore we proceeded with the experiments in the MPS III cell lines, using the same conditions (20 nM of siRNA).



**We observed a significant lower *XYLT1* mRNA levels (20-50%) after 24-48h incubation in MPS IIIA, IIIC and IIID fibroblasts.**

### (II) Effect of si $XYLT1$ in GAGs accumulation



**A significant reduction on the total GAGs levels was observed: MPS IIIC – 50% at both times and MPS IIID – 50% at 24h and 70% at 48h.**

## CONCLUSIONS AND FUTURE STUDIES

- Proof of principle on the effect of siRNA targeting *XYLT1* was achieved for MPS types IIIA, IIIB and IIID, resulting in significant lower levels of *XYLT1* mRNA. Studies on MPS IIIB are ongoing.
- A significant reduction on GAGs accumulation was observed for MPS IIIC and IIID, and we are currently addressing this storage in the remaining MPS III cell lines.
- An immunocytochemistry assay is being developed to evaluate the accumulation of HS in all cell lines.

## FUNDING

FUNDAÇÃO  
**Millennium**  
bcp

bcp/LIM/DGH/Dz2015

**FCT**

Fundação para a Ciência e a Tecnologia

MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR  
SFRH/BPD/101965/2014; SFRH/BD/124372/2016