

Bolsa SPDM de apoio à investigação
Dr. Aguinaldo Cabral

10th International Symposium
 11th-13th November, 2020

GENETIC SUBSTRATE REDUCTION THERAPY for MUCOPOLYSACCHARIDOSIS: toward a siRNA-containing nanoparticle targeted to brain cells

Maria Francisca Coutinho^{1,2*}, Juliana Inês Santos^{1,3},
 Liliana S. Mendonça^{4,5}, Liliانا Matos^{1,5}, Maria João Prata^{3,6},
 Amália S. Jurado⁷, Maria C. Pedroso de Lima⁴ and Sandra Alves^{1,2}



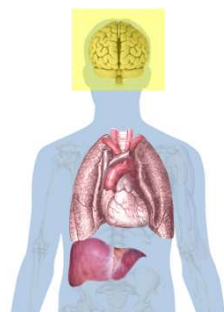
MUCOPOLYSACCHARIDOSIS (MPS) TYPE III

- Autosomal recessive
- Lysosomal Storage Disorders
 - Sub-type of MPSs;
 - Accumulated substrate: heparan sulphate glycosaminoglycans (GAGs)
- 4 different diseases:
 - III A
 - III B
 - III C
 - III D

depending on the defective enzyme

MUCOPOLYSACCHARIDOSES (MPS)

- Chronic
- Progressive
- Large spectrum of severity & symptoms



MPS III
(= Sanfilippo Syndrome)

AVAILABLE THERAPIES

🚫 None!

...only symptomatic!

ameliorate symptoms
support disabled patients

ERT for neurodegenerative MPS would require the introduction of active enzyme into the CNS

↓

extra difficulties!

AVAILABLE THERAPIES

🚫 None!

...only symptomatic!

ameliorate symptoms
support disabled patients

ERT for neurodegenerative MPS would require the introduction of active enzyme into the CNS

↓

Still, it's being attempted with some promising results

AVAILABLE THERAPIES

🚫 None!

...only symptomatic!

**Perfect Target
for
Substrate Reduction
Approaches!**

↓

Still, it's being attempted with some promising results

gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III

genetic substrate reduction

gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III

early stage of the HS biosynthetic cascade

Dermatanan/Chondroitin Sulfate (DS/CS)
 Heparan Sulfate (HS)

gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III

naturally occurring post-transcriptional gene silencing process

Designed to induce RNAi

siRNA
 MPS III fibroblasts

gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III

MPS IIIA
 MPS IIIB

siRNA
 mRNA
 MPS III fibroblasts

gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III

MPS IIIC
 MPS IIID

siRNA
 mRNA
 MPS III fibroblasts

gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III

MPS IIIA
 MPS IIIB
 MPS IIIC

siRNA
 GAGs
 MPS III fibroblasts

gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III

Further validation:

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

The diagram illustrates the experimental workflow. It shows a petri dish containing 'MPS III fibroblasts' being treated with 'siRNA'. A magnifying glass is used to examine 'GAGs' (glycosaminoglycans) from the cells. A callout box indicates that further validation is needed, specifically mentioning that qGAG analysis by MS/MS and immunocytochemistry using anti-HS antibodies are currently not being performed.

A LOOK FORWARD...

- Vector design & siRNA encapsulation into liposomes
 - ↑ bioavailability of siRNAs;
 - protection from degradation
 - control of
 - circulation time
 - release rate
- Coupling of specific ligands to siRNA-carrying liposomes
 - Transferrin (T)
 - Rabies virus peptide derivative (RGV-2)
- Efficiency assessment + Targeting of brain cells

This slide outlines future research directions. It focuses on the design of liposomes to improve siRNA delivery. Key points include increasing bioavailability, protecting siRNAs from degradation, and controlling their circulation time and release rate. Specific ligands like Transferrin (T) and Rabies virus peptide derivative (RGV-2) are mentioned for targeting. The ultimate goal is to assess efficiency and target brain cells.

JUST A LITTLE INSIGHT...

This slide provides a brief overview of the siRNA delivery process. It shows a syringe injecting siRNA into a layer of cells. The siRNA is then internalized by the cells, leading to the formation of endosomes and lysosomes.

This diagram details the cellular uptake of siRNA. It shows the injection of siRNA into a cell layer. The siRNA is internalized via endocytosis, forming endosomes. These endosomes mature and eventually fuse with lysosomes, where the siRNA is released into the cytoplasm.

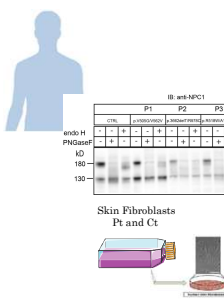
This diagram illustrates the mechanism of action of siRNA. It shows the siRNA being loaded into an Argonaute (AGO) protein. The AGO-siRNA complex then binds to a target mRNA, leading to 'mRNA cleavage'. The process involves the 'passenger strand' of the siRNA and the formation of an 'Activated RISC' complex.

“early stages” GAGs biosynthesis gene

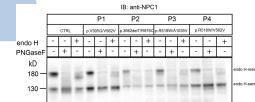
↓ GAG storage

This diagram shows the effect of siRNA on GAG biosynthesis. It highlights the silencing of the “early stages” GAGs biosynthesis gene, which leads to a decrease in GAG storage within the cell. The mechanism involves mRNA cleavage and the formation of an Activated RISC complex.

R&D APPROACH



		IB anti-NPC1				
		P1	P2	P3	P4	
endo H	PNGaseF	+	-	+	-	endo H
+	-	+	-	+	-	+
-	+	-	+	-	+	-
-	-	-	-	-	-	-

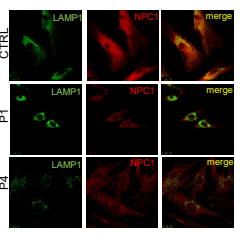


Skin Fibroblasts
Pt and Ct

PROTEIN STUDY

- Western Blot
- Confocal Microscopy

Intracellular Processing and Trafficking of affected protein



R&D APPROACH



Contact us!



sandra.alves@insa.min-saude.pt
marisa.encarnacao@insa.min-saude.pt

ACKNOWLEDGMENTS

Dr. Sandra Alves
Prof. M^a João Prata
Juliana Inês Santos
Paulo Gaspar

Prof. Maria C. Pedroso de Lima
Prof. Amália S. Jurado
Liliana S. Mendonça



SPM Sociedade Portuguesa de Doenças Metabólicas
 Bolsa de apoio à investigação
Dr. Aguiñaldo Cabral
 2019/00011/2020/SPDM2019/ALD

Sanfilippo Portugal
Millennium
 bsp/LM/DGH/Ed2015

FCT
 Fundação de Amparo à Pesquisa em Saúde
 3013/2018/0101/2018/014
 3013/2018/0101/2018/014

Sanfilippo Children's Hospital
 Fundação de Amparo à Pesquisa em Saúde
 2019/00011/2020/SPDM2019/ALD

António Reis ♥

[PUB]

Dr. Sandra Alves
Prof. M^a João Prata
Juliana Inês Santos
Paulo Gaspar

Prof. Maria C. Pedroso de Lima
Prof. Amália S. Jurado



PO-34
From bench to bioterium and back again:
DEVELOPMENT OF A U1 shRNA-BASED
THERAPEUTIC STRATEGY FOR
MUCOPOLYSACCHARIDOSIS IIIC

16th INTERNATIONAL SYMPOSIUM ON THE PATHOPHYSIOLOGY AND THERAPY OF MUCOPOLYSACCHARIDOSIS
 11th - 13th November 2020
 VIRTUAL SYMPOSIUM

THANK YOU!

