Bolsa SPDM de apoio à investigação
Dr. Aguinaldo Cabral

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], Liliana Matos [1,2], Maria João Prata [3,6], Amália S. Jurado[7], Maria C. Pedroso de Lima [4] and Sandra Alves [1,2]

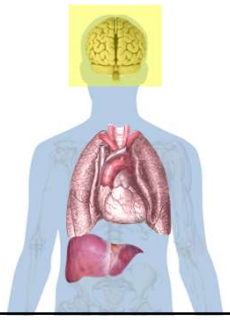
MUCOPOLYSACCHARIDOSIS (MPS) TYPE III

- 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 promissing results

AVAILABLE THERAPIES

None!

…only symptomatic!

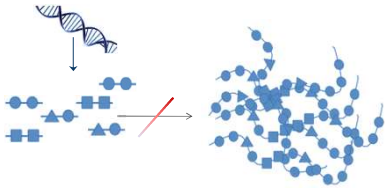
Perfect Target for Substrate Reduction Approaches!

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

⇓

Still, it's being attempted with some promissing results

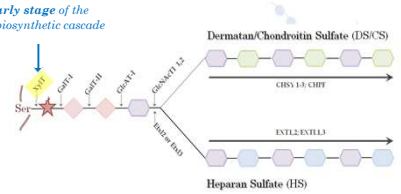
gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III



genetic substrate reduction

gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III

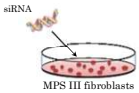
***early stage** of the HS biosynthetic cascade*



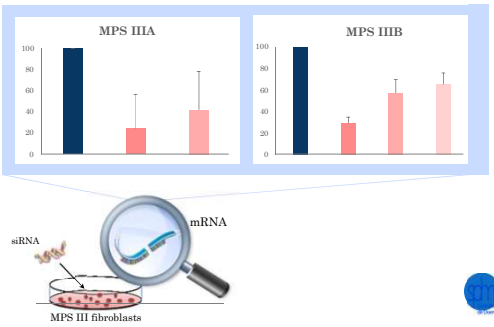
gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III

naturally occurring post-ranscriptional gene silencing process

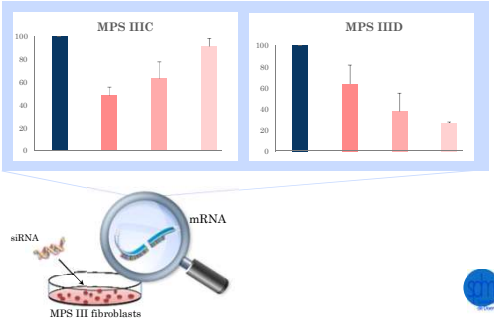
Designed to induce RNAi



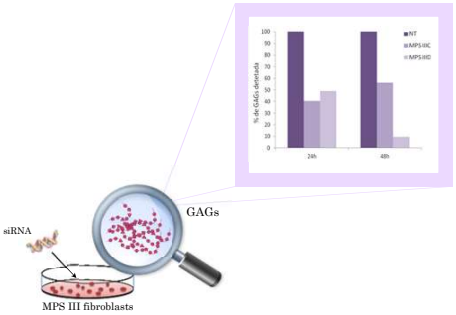
gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III



gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III



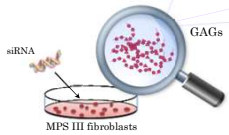
gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III



gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III

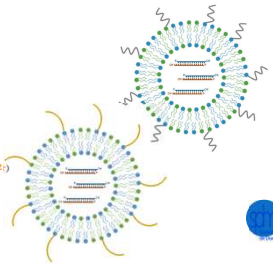
Further validation:

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

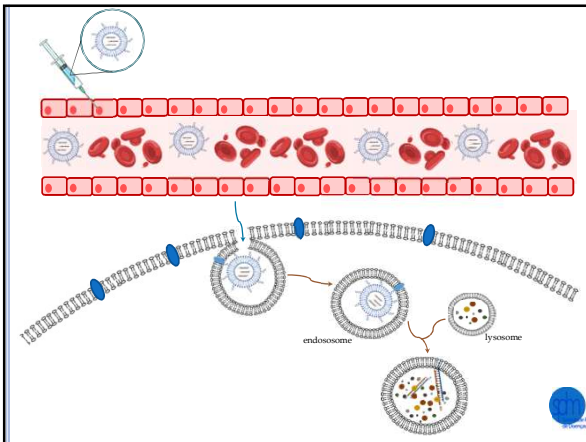


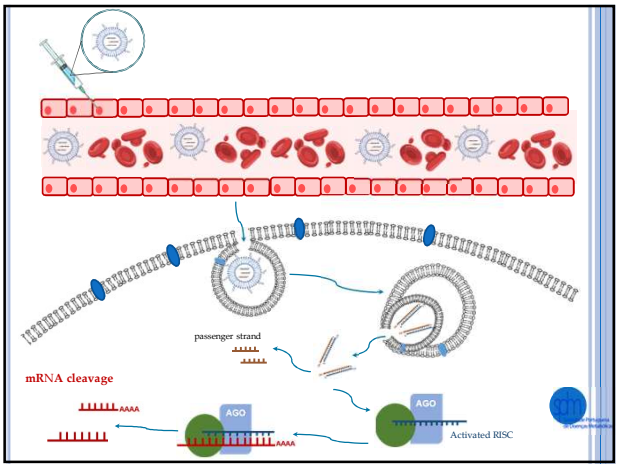
A LOOK FORWARD…

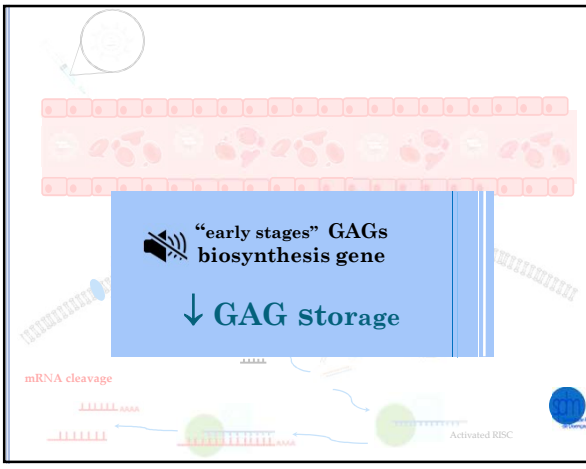
- Vector design & siRNA encapsulation into liposomes
  - ↑ bioavailability of siRNAs;
  - protection from degradation
  - control of
    - circulation time
    - release rate
- Coupling of specific ligands to siRNA-carrying liposomes
  - Transferrin (Tf)
  - Rabies virus peptide derivative (RGV-2$_9$)
- Efficiency assessment + Targeting of brain cells

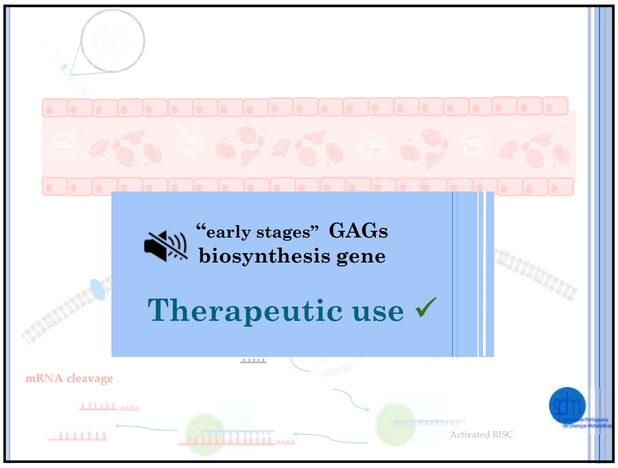


JUST A LITTLE INSIGHT…

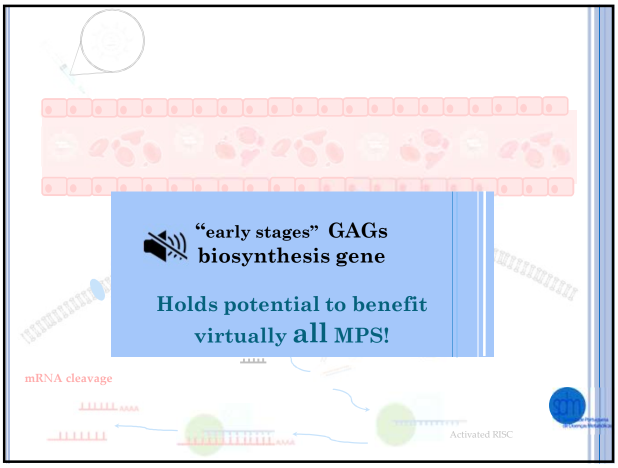




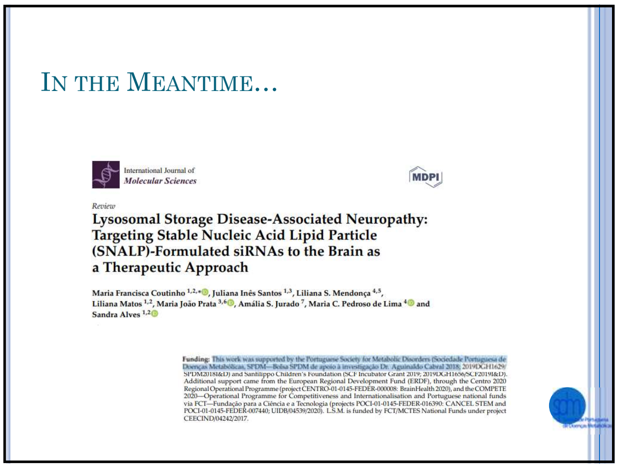




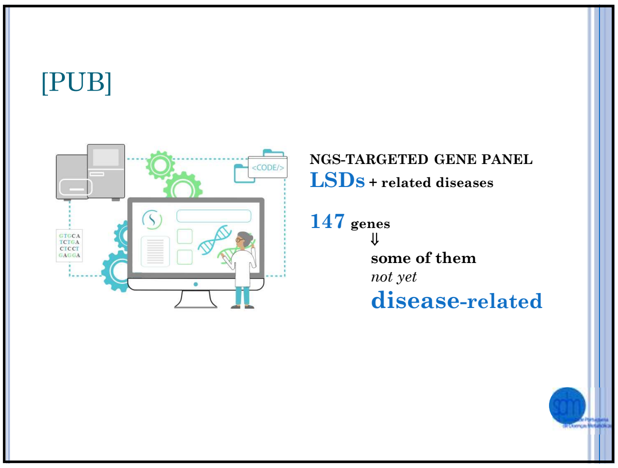
"early stages" GAGs biosynthesis gene
Therapeutic use ✓
mRNA cleavage
Activated RISC



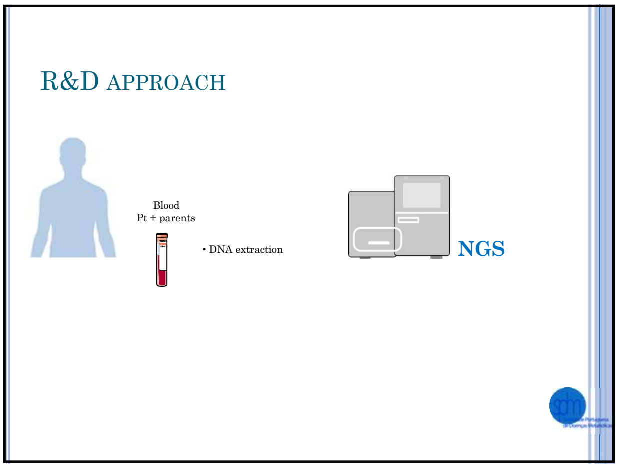
"early stages" GAGs biosynthesis gene
Holds potential to benefit virtually all MPS!
mRNA cleavage
Activated RISC



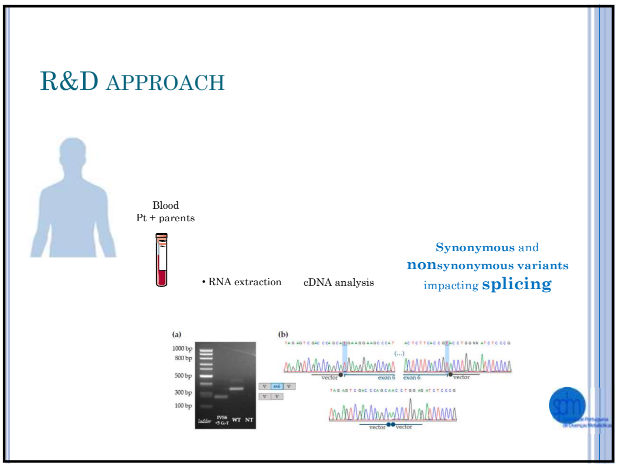
In the Meantime…
International Journal of Molecular Sciences
MDPI
Review
Lysosomal Storage Disease-Associated Neuropathy: Targeting Stable Nucleic Acid Lipid Particle (SNALP)-Formulated siRNAs to the Brain as a Therapeutic Approach
Maria Francisca Coutinho 1,2,*, Juliana Inês Santos 1,3, Liliana S. Mendonça 4,5, Liliana Matos 1,2, Maria João Prata 3,6, Amália S. Jurado 7, Maria C. Pedroso de Lima 4 and Sandra Alves 1,2
Funding: This work was supported by the Portuguese Society for Metabolic Disorders (Sociedade Portuguesa de Doenças Metabólicas, SPDM—Bolsa SPDM de apoio à investigação Dr. Aguinaldo Cabral 2018; 2019DGH1629/SPDM2018I&D) and Sanfilippo Children's Foundation (SCF Incubator Grant 2019; 2019DGH1656/SCF2019I&D). Additional support came from the European Regional Development Fund (ERDF), through the Centro 2020 Regional Operational Programme (project CENTRO-01-0145-FEDER-000008: BrainHealth 2020), and the COMPETE 2020—Operational Programme for Competitiveness and Internationalisation and Portuguese national funds via FCT—Fundação para a Ciência e a Tecnologia (projects POCI-01-0145-FEDER-016390: CANCEL STEM and POCI-01-0145-FEDER-007440; UIDB/04539/2020). L.S.M. is funded by FCT/MCTES National Funds under project CEECIND/04242/2017.



[PUB]
<CODE/>
NGS-TARGETED GENE PANEL
LSDs + related diseases
147 genes
⇓
some of them
not yet
disease-related

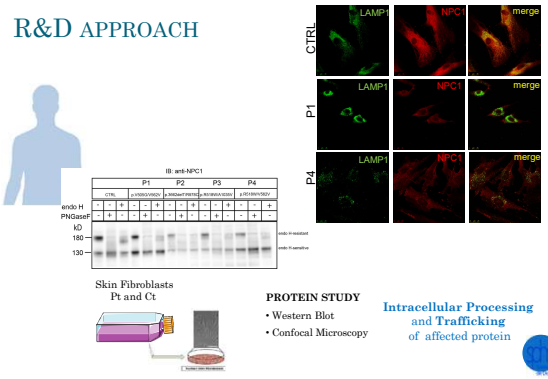


R&D approach
Blood
Pt + parents
• DNA extraction
NGS



R&D approach
Blood
Pt + parents
• RNA extraction
cDNA analysis
Synonymous and nonsynonymous variants impacting splicing
(a)
(b)

R&D APPROACH



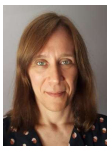
Skin Fibroblasts
Pt and Ct

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. Mª João Prata
Juliana Inês Santos
Paulo Gaspar

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

Bolsa de apoio à investigação
Dr. Aguinaldo Cabral
2019DGH1629/SPDM2018I&D

Millennium bcp
bcp/LIM/DGH/Dez2015

FCT
SFRH/BPD/101965/2014
SFRH/BD/124372/2016

2019DGH1656/SCF2019I&D

António Reis ♥

[PUB]

Dr. Sandra Alves
Prof. Mª João Prata
Juliana Inês Santos
Paulo Gaspar

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

Bolsa de apoio à investigação
Dr. Aguinaldo Cabral
2019DGH1629/SPDM2018I&D

THANK YOU!