


TARGETING SNALP-FORMULATED SIRNAS TO THE BRAIN – A THERAPEUTIC APPROACH IN LYOSOMAL STORAGE DISEASE-ASSOCIATED NEUROPATHY



the Sanfilippo Syndrome example

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 Liliana S. Mendonça^{4,5}, Liliana Matos^{1,5}, Maria João Prata^{3,6},
 Amália S. Jurado⁷, Maria C. Pedroso de Lima⁴ and Sandra Alves^{1,2}



WHO ARE WE...

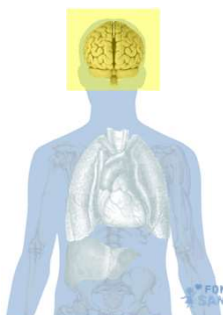
- Sandra Alves' R&D group on Lysosomal Storage Diseases
- Human Genetics Department
- National Health Institute Doutor Ricardo Jorge (@ Porto)



DISCLAIMER



SANFILIPPO SYNDROME (MPS III)



BRAIN



AVAILABLE THERAPIES

None!


...only symptomatic!

ameliorate symptoms
support disabled patients

ERT for neurodegenerative MPS requires the introduction of active enzyme into the CNS

⇓

EXTRA DIFFICULTIES



AVAILABLE THERAPIES

None!


...only symptomatic!

ameliorate symptoms
support disabled patients

ERT for neurodegenerative MPS requires the introduction of active enzyme into the CNS

⇓

Still, it's being attempted with some promising results



AVAILABLE THERAPIES

🔔 None!


...only symptomatic!

ameliorate symptoms
support disabled patients

ERT for neurodegenerative MPS requires the introduction of active enzyme into the CNS

↓

Still, it's being attempted
Dr. Anastasia Henry



AVAILABLE THERAPIES

🔔 None!

...only symptomatic!

**Perfect Target
for
Substrate Reduction
Approaches!**

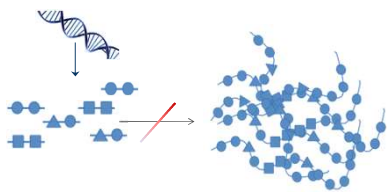
ERT for neurodegenerative MPS requires the introduction of active enzyme into the CNS

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Still, it's being attempted with some promising results



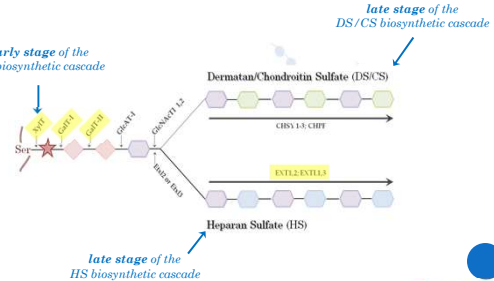
gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III



genetic substrate reduction




gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III



early stage of the HS biosynthetic cascade

late stage of the DS/CS biosynthetic cascade

late stage of the HS biosynthetic cascade

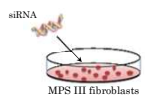


gSRT FOR MUCOPOLYSACCHARIDOSES


naturally occurring post-transcriptional gene silencing process

Designed to induce RNAi

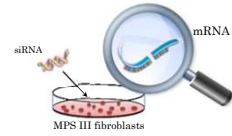
siRNA



MPS III fibroblasts




gSRT FOR MUCOPOLYSACCHARIDOSES



siRNA

mRNA

MPS III fibroblasts



gSRT FOR MUCOPOLYSACCHARIDOSES

siRNA
MPS III fibroblasts
GAGs

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gSRT FOR MUCOPOLYSACCHARIDOSES

- Polish group
- Targets: *XYLT1*, *XYLT2*, *GALT1*, *GALTII*
- Test in: MPS IIIA fibroblasts
- Compounds: siRNAs

8 ≠

Relative mRNA level (%)

XYLT1, XYLT2, B4GALT7, B4GALT6

Drizdovic et al., (2010)

gSRT FOR MUCOPOLYSACCHARIDOSES

- Polish group
- Targets: *XYLT1*, *XYLT2*, *GALT1*, *GALTII*
- Test in: MPS IIIA fibroblasts
- Compounds: siRNAs

protein levels

Mock control, Negative control, siRNA #112165, siRNA #22873, siRNA #112168, siRNA #112274, siRNA #112229, siRNA #111770, siRNA #112222, siRNA #112281

XYLT1, XYLT2, B4GALT7, B4GALT6

GAPDH

Drizdovic et al., (2010)

gSRT FOR MUCOPOLYSACCHARIDOSES

- Australian group
- Targets: *EXTL2* and *EXTL3*
- Test in: 293T cells; MPS I and MPS IIIA fibroblasts
- Compounds: shRNAs

6 ≠

(overexpression)

Percent of p53/CHK2 target gene only fluorescence

shEXTL2, shEXTL3

Kaidonis et al., (2012)

gSRT FOR MUCOPOLYSACCHARIDOSES

- Australian group
- Targets: *EXTL2* and *EXTL3*
- Test in: 293T cells; MPS I and MPS IIIA fibroblasts
- Compounds: shRNAs

(endogenous)

Percent of target gene expression

shEXTL2, shEXTL3

Kaidonis et al., (2012)

gSRT FOR MUCOPOLYSACCHARIDOSES

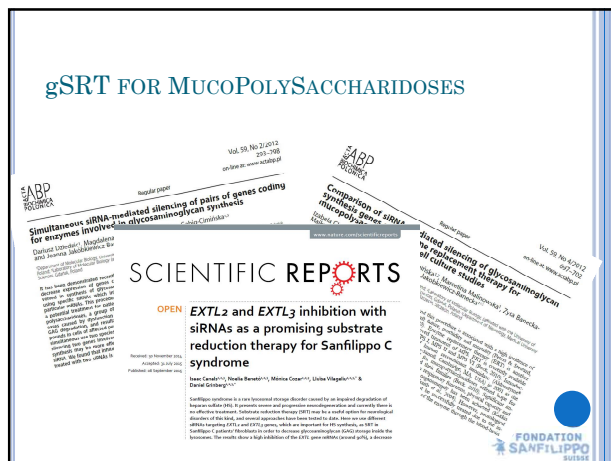
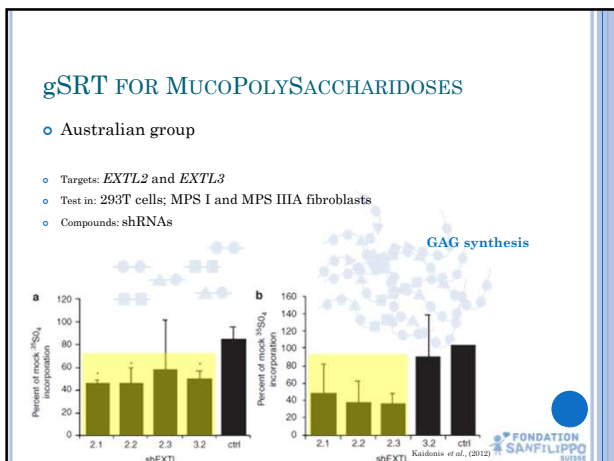
- Australian group
- Targets: *EXTL2* and *EXTL3*
- Test in: 293T cells; MPS I and MPS IIIA fibroblasts
- Compounds: shRNAs

GAG synthesis

Percent of mock 35S incorporation

shEXTL2, shEXTL3

Kaidonis et al., (2012)



gSRT FOR MUCOPOLYSACCHARIDOSES

The **good** news:

si/shRNAs

↓ mRNA levels
 ↓ enzyme activity
 ↓ GAGs synthesis

Reverse the cellular phenotype!!!

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gSRT FOR MUCOPOLYSACCHARIDOSES

The **bad** news:

si/shRNAs

🚫 limited cellular uptake
 🚫 low biological stability
 🚫 unfavourable pharmacokinetics

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gSRT FOR MUCOPOLYSACCHARIDOSES

DELIVERY

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gSRT FOR MUCOPOLYSACCHARIDOSES

Our bet:

Promote targeted **BRAIN-DELIVERY**
 using a *special class* of nanocarriers liposomes

↓

Stable Nucleic Acid Lipid Particles

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gSRT FOR MUCOPOLYSACCHARIDOSES

Mean size: ~100 nm
Charge: neutral

polyethylene glycol PEG
ionizable cationic lipid
non-ionizable lipid

gSRT FOR MUCOPOLYSACCHARIDOSES

15y
Efficient for *in vivo* delivery of siRNAs
↓
targeted gene-silencing

Several SNALP-formulated siRNA therapeutic molecules reached clinical trials and are under evaluation for efficacy.

+ **Patisiran**[®]
1st FDA-approved siRNA drug

gSRT FOR MUCOPOLYSACCHARIDOSES

In vivo proof of principle
↓
targeted gene-silencing of the hepatitis B virus (HBV)

siRNA dose	log ₁₀ HBV serum DNA (copies/ml)
Saline	~5.8
Control	~5.7
5 mg/kg	~4.5
3 mg/kg	~4.8
1 mg/kg	~5.2

IV injection

log₁₀ HBV serum DNA (copies/ml)

siRNA dose

Morrissey et al., (2005)

gSRT FOR MUCOPOLYSACCHARIDOSES

In vivo proof of principle
↓
targeted gene-silencing of the hepatitis B virus (HBV)

IV injection

Reduction
specific
dose-dependent
lasted up to 7d

Morrissey et al., (2005)

gSRT FOR MUCOPOLYSACCHARIDOSES

In vivo proof of principle
↓
targeted gene-silencing of the hepatitis B virus (HBV)

IV injection

↑ plasma half-life
↓ toxicity
↓ immunostimulatory side effects

Morrissey et al., (2005)

gSRT FOR MUCOPOLYSACCHARIDOSES

MAJOR ARTICLE

Postexposure protection of Guinea Pigs against a lethal Ebola challenge with RNA interference: a proof-of-concept study

Thomas W. Martin, Kathleen M. Martin, Janet S. Winger, et al.

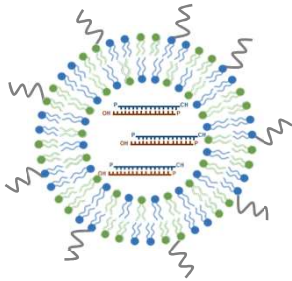
Background: We previously observed that small interfering RNAs (siRNAs) targeting the Zaire Ebola virus (ZEBV) RNA polymerase L protein formulated in cationic lipid nanoparticles (SNALPs) completely protected guinea pigs when administered shortly after a lethal ZEBV challenge. Although earlier results of ZEBV studies are useful for assessing prospective countermeasures, they are frequently not useful for prediction of efficacy in the more stringent nonhuman primate models. We therefore assessed the efficacy of modified nonimmunostimulatory siRNAs in a uniformly lethal non-human primate model of ZEBV hemorrhagic fever.

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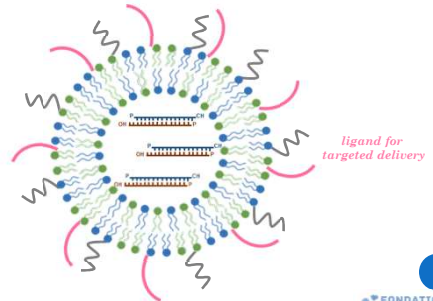
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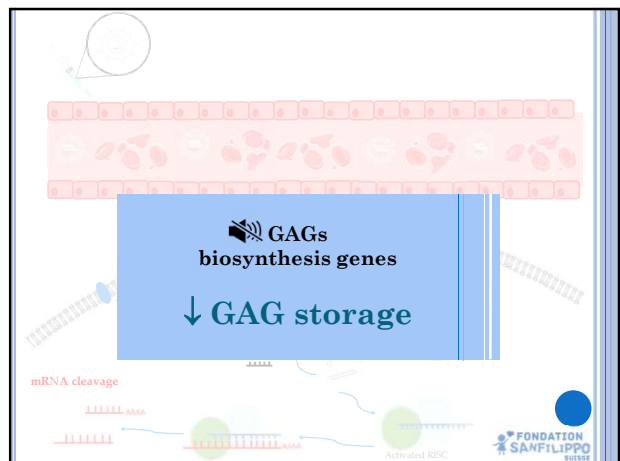
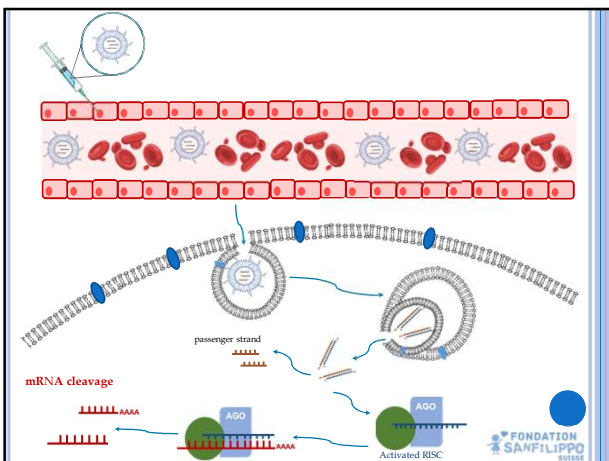
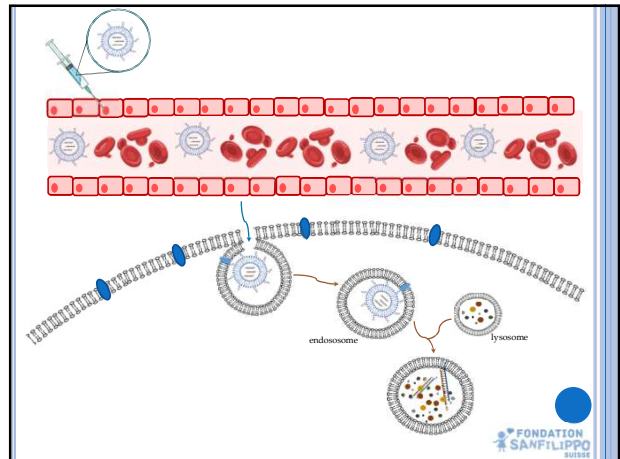
gSRT FOR MUCOPOLYSACCHARIDOSES



gSRT FOR MUCOPOLYSACCHARIDOSES



JUST A LITTLE INSIGHT...



GAGs biosynthesis genes

Therapeutic use ✓

mRNA cleavage

Activated RISC

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OUR OWN EFFORTS...

early stage of the HS biosynthetic cascade

Ser → GlcNAc6S → GalNAc6S → GalNAc6S6S → GlcNAc6S6S

↓

Dermatan/Chondroitin Sulfate (DS/CS) (via CHS1-L3, CHST)

↓

Heparan Sulfate (HS) (via EXT1-EXTL3)

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gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III

siRNA

mRNA

MPS III fibroblasts

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gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III

Condition	mRNA Level (%)
NT	100
siRNA anti-XYLT1	~25
siRNA anti-XYLT1	~45

Condition	mRNA Level (%)
NT	100
siRNA anti-XYLT1	~30
siRNA anti-XYLT1	~60
siRNA anti-XYLT1	~70

siRNA

mRNA

MPS III fibroblasts

NT ■

siRNA anti-XYLT1 ■

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gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III

Condition	mRNA Level (%)
NT	100
siRNA anti-XYLT1	~50
siRNA anti-XYLT1	~65
siRNA anti-XYLT1	~90

Condition	mRNA Level (%)
NT	100
siRNA anti-XYLT1	~65
siRNA anti-XYLT1	~40
siRNA anti-XYLT1	~25

siRNA

mRNA

MPS III fibroblasts

NT ■

siRNA anti-XYLT1 ■

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A LOOK FORWARD...

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

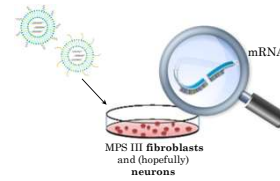
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OUR COLLABORATORS

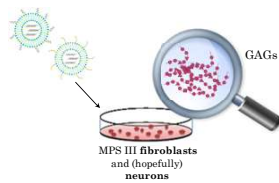
- M. Conceição Pedroso Lima' R&D group on Vectors and Gene Therapy
- Center for Neuroscience and Cell Biology
- University of Coimbra (@ Coimbra)



A LOOK FORWARD...



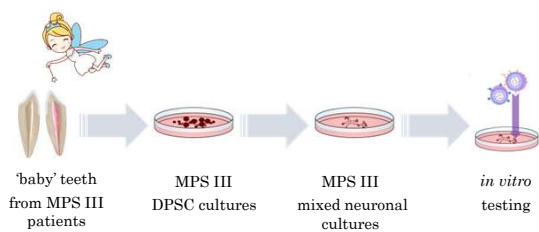
A LOOK FORWARD...



A LOOK FORWARD...



A LOOK FORWARD...



A LITTLE LOOK ON WHAT'S HAPPENING NOW...



A LITTLE LOOK ON WHAT'S HAPPENING NOW...

1 week later → 1 + 1/2 weeks later

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[PUB] 🍷 🦷 🧑 🧪

Contact us!

francisca.coutinho@insa.min-saude.pt
sandra.alves@insa.min-saude.pt

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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^{1,2}, Liliana S. Mendonça^{1,2}, Liliana Mateus^{1,2}, Maria João Prata^{3,4,5}, Amália S. Jurado⁷, Maria C. Pedroso de Lima^{6,8} and Sandra Alves^{1,2}

Funding: This work was supported by the Portuguese Society for Metabolic Disorders (Sociedade Portuguesa de Doenças Metabólicas, SPDM) through SPDM de apoio à investigação Dr. Aguiñaldo Cabral 2018, 2019 and 2020; SPDM 2019/2020; and Fundação Calisto Tanzi's Foundation (FCT) through Grant 2019. Additional support came from the European Regional Development Fund (ERDF) through the Centro 2020 Regional Operational Programme (POSDR) (POSDR/01-2015-0618-FEDER-00001: Smart Health 2020), and the COMPETE 2020—Operational Programme for Competitiveness and Internationalisation and Portuguese national funds via FCT—funding para a Ciência e Tecnologia projects POCI/01-018-FEDER-03006, CANCEL STEM and POCI/01-014-FEDER-00740; UIDB/04532/2020; USM is funded by FCT/ACTES National Funds under project CITEC/0004/2007.

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Juliana Inês Santos
Paulo Gaspar

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

Sanfilippo Portugal Millennium FCT Sanfilippo Portugal

António Reis ♥

THANK YOU!

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