

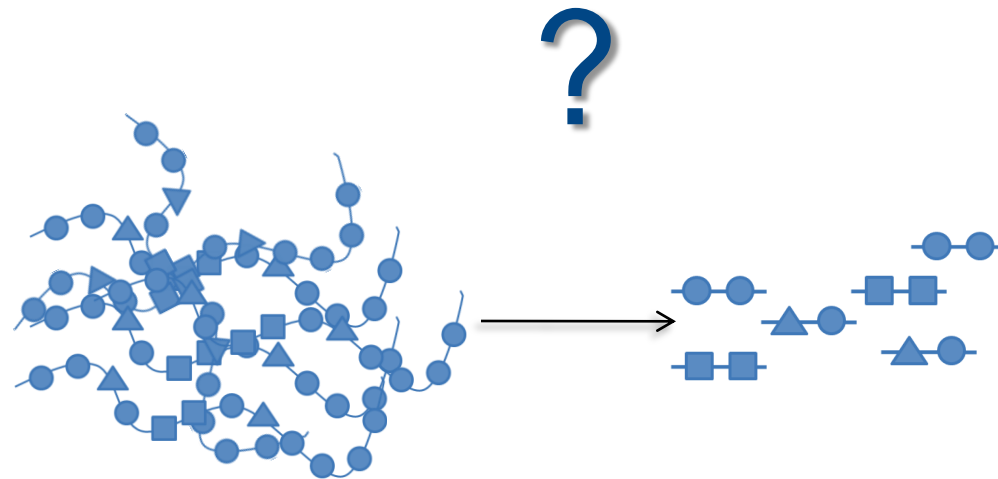


GENETICALLY MODULATED SUBSTRATE REDUCTION THERAPY

Maria Francisca Coutinho

Lysosomal Storage Disorders Group
Research & Development Unit,
Department of Human Genetics,
INSA

THE ENZYME AS A DRUG?



enzyme replacement

THE ENZYME AS A DRUG?

- 1996
- Norman Radin



Glycoconjugate Journal (1996) **13**: 153–157

Treatment of Gaucher disease with an enzyme inhibitor

NORMAN S. RADIN

Mental Health Research Institute and Division of Nephrology MSRBII, University of Michigan, 1550 W. Medical Centre Drive Ann Arbor, MI 48109-0676, USA

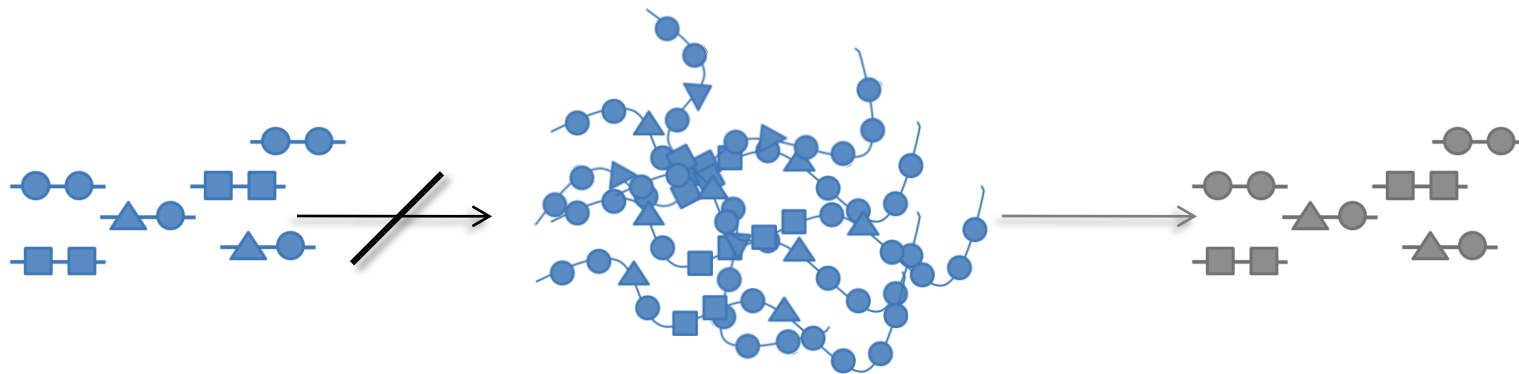
Received 23 May 1995, revised 20 June 1995

The hypothesis is offered predicting that Gaucher patients could be treated with a drug that slows the synthesis of glucosylceramide, the lipid that accumulates in this disorder. The present therapeutic approach involves augmenting the defective enzyme, glucosylceramide β -glucosidase, with exogenous β -glucosidase isolated from human tissue. This spectacularly expensive mode of treatment should be replaceable with a suitable enzyme inhibitor that simply slows formation of the lipid and matches the rate of synthesis with the rate of the defective, slowly working β -glucosidase. Several drugs that possess this ability are available, the best known of which is 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), a designer inhibitor that resembles the synthase's substrate and product. PDMP has been found to be effective in mice, rats, fish, and a wide variety of cultured cells. Its use, at suitable dosages, seems to be harmless, although long-term tests have not been made. The lack of suitable animal models of Gaucher disease has made it difficult to test the hypothesis adequately, but PDMP does rapidly lower the levels of glucosylceramide in normal animal tissues and the animals evidently do well with the lowered levels of glucosylceramide and its more complex glycolipid metabolites.

BRAINS
FOR
BRAIN



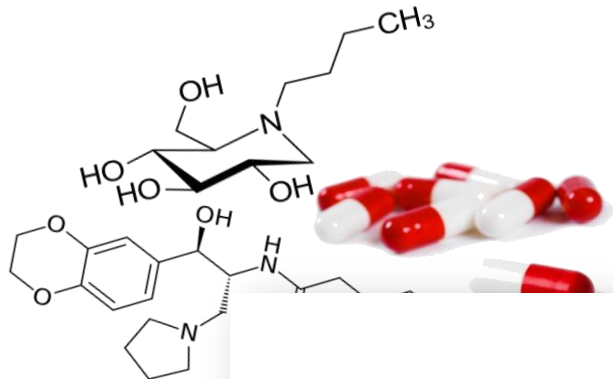
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substrate reduction

enzyme replacement

SUBSTRATE REDUCTION THERAPY (SRT)



THE JOURNAL OF BIOLOGICAL CHEMISTRY
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Vol. 269, No. 11, Issue of March 18, pp. 8362-8365, 1994
Printed in U.S.A.

N-Butyldeoxyjirimycin Is a Novel Inhibitor of Glycolipid Biosynthesis*

(Received for publication, October 1, 1993, and in revised form, November 15, 1993)

Frances M. Platt[§], Gabrielle R. Neises[¶], Raymond A. Dwek[‡], and Terry D. Butters[‡]

From the [‡]Glycobiology Institute, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, United Kingdom and the [¶]Monsanto Company, St. Louis, Missouri 63198

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N-Butyldeoxyjirimycin Is a Novel Inhibitor of Glycolipid Biosynthesis but Does Not Affect the Activity of the Enzyme α -Glucosidase I

Frances M. Platt[§] and Terry D. Butters[‡]
From the Glycobiology Institute, University of Oxford, South Parks Road, Oxford OX1 3QU, United Kingdom

We have previously reported that N-butyldeoxyjirimycin (NB-DGJ) is a novel inhibitor of the N-linked oligosaccharide biosynthesis but does not affect the activity of the enzyme α -glucosidase I. In this study, we have evaluated the effect of NB-DGJ on the maturation of the enzyme α -glucosidase I in lysosomal glucocerebrosidase-deficient fibroblasts. NB-DGJ was found to be a potent inhibitor of the maturation of α -glucosidase I in these cells. The effect of increasing NB-DGJ concentration on the maturation of α -glucosidase I was studied in the presence of the N-methyl and N-ethyl derivatives of NB-DGJ. However, N-propyl and N-butyl derivatives of NB-DGJ were found to be more potent inhibitors of the maturation of α -glucosidase I than the N-methyl and N-ethyl derivatives.

Safety, Tolerability, and Pharmacokinetics of Eliglustat Tartrate (Genz-112638) After Single Doses, Multiple Doses, and Food in Healthy Volunteers

M. Judith Peterschmitt, MD, MMSc, Amy Burke, MPH, Larry Blankstein, PhD, Sharon E. Smith, MD, Ana Cristina Puga, MD, PhD, William G. Kramer, PhD, James A. Harris, BSc, MRSC, David Mathews, MD, and Peter L. Bonate, PhD

Three phase 1 studies of eliglustat tartrate (Genz-112638), an oral inhibitor of glucosylceramide synthase under development for treating Gaucher disease type 1 (GD1), evaluated the safety, tolerability, and pharmacokinetics in healthy volunteers after escalating single doses ($n = 99$), escalating multiple doses ($n = 36$), and food ($n = 24$). Eliglustat tartrate was well tolerated at single doses ≤ 20 mg/kg and multiple doses ≤ 200 mg bid, with 50 mg bid producing plasma concentrations in the predicted ther-

apeutic range. The plasma concentration-time profile was characterized by a biphasic increase in plasma concentration, reaching a peak concentration ~ 2 hours, followed by a monophasic decline with a ~ 6 -hour terminal half-life. Unchanged drug in 8-hour urine collections was $<1.5\%$ of administered doses. Food did not significantly affect the rate or extent of absorption. Multiple-dose pharmacokinetics was nonlinear, showing higher than expected plasma drug concentrations. Steady state was reached ~ 60 hours after bid dosing. Higher drug exposure occurred in slower CYP2D6 metabolizers. Based on favorable results in healthy participants, a phase 2 trial

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WorkShop
1st April, 2017

SUBSTRATE REDUCTION THERAPY (SRT)

Pathology	Approved SRT
Gaucher	Zavesca [®] (Miglustat; Actelion) Cerdelga [®] (Eliglustat tartrate; Genzyme)
Niemann-Pick C	Zavesca [®] (Miglustat; Actelion)



SUBSTRATE REDUCTION THERAPY (SRT)

1. NAME OF THE MEDICINAL PRODUCT

Zavesca 100 mg hard capsules

4.4 Special warnings and precautions for use

Tremor

Approximately 37% of patients in clinical trial in Niemann-Pick type C disease reported these tremors were described as an exaggerated tremor. These tremors began within the first month, and in many cases within 3 months. Dose reduction may ameliorate the tremors. Dose reduction or treatment with anti-tremor treatment may sometimes be required.

Gastrointestinal disturbances

Gastrointestinal events, mainly diarrhoea, have been observed from the outset of treatment or intermittently during treatment. These events are likely inhibition of intestinal disaccharidases leading to reduced absorption of dietary disaccharides. In clinical trial, gastrointestinal events have been observed to respond to reduction of sucrose, lactose and other carbohydrates. Treatment with anti-diarrhoeal medicinal products such as loperamide may be necessary. Patients with chronic diarrhoea who do not respond to these interventions should be treated with anti-diarrhoeal medicinal products. These events have not been evaluated in patients with a history of inflammatory bowel disease.

Effects on spermatogenesis

Male patients should maintain reliable contraception during treatment. It has been shown that miglustat adversely affects sperm count and fertility (see sections 4.6 and 5.3). Until the effects on fertility in male patients should cease Zavesca and treatment should be continued for 3 months.



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

EMA/39261/2015
EMA/H/C/003724

[EPAR summary for the public](#)

Cerdelga
eliglustat

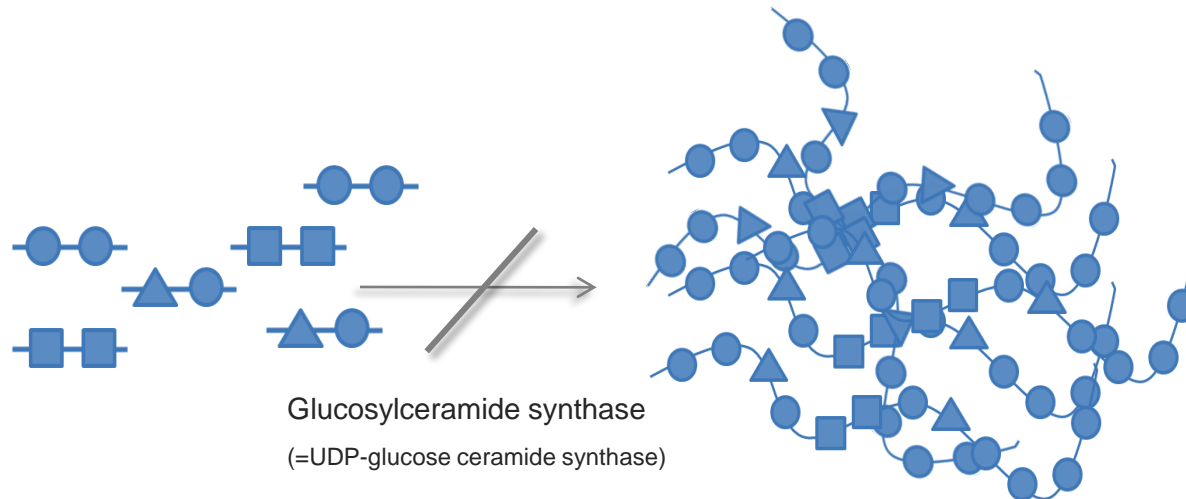
What are the risks associated with Cerdelga?

The most common side effect seen with Cerdelga (which may affect more than 1 in 10 people) is **diarrhoea**, in approximately 6 out of 100 patients. The majority of side effects are mild and transient. For the full list of all side effects reported for Cerdelga, see the package leaflet.

Cerdelga must not be taken together with certain medicines that can interfere with the ability of the body to break down the medicine, which may affect the levels of Cerdelga in blood. For the full list of restrictions, see the package leaflet.

gSRT PROOF OF PRINCIPLE - GD

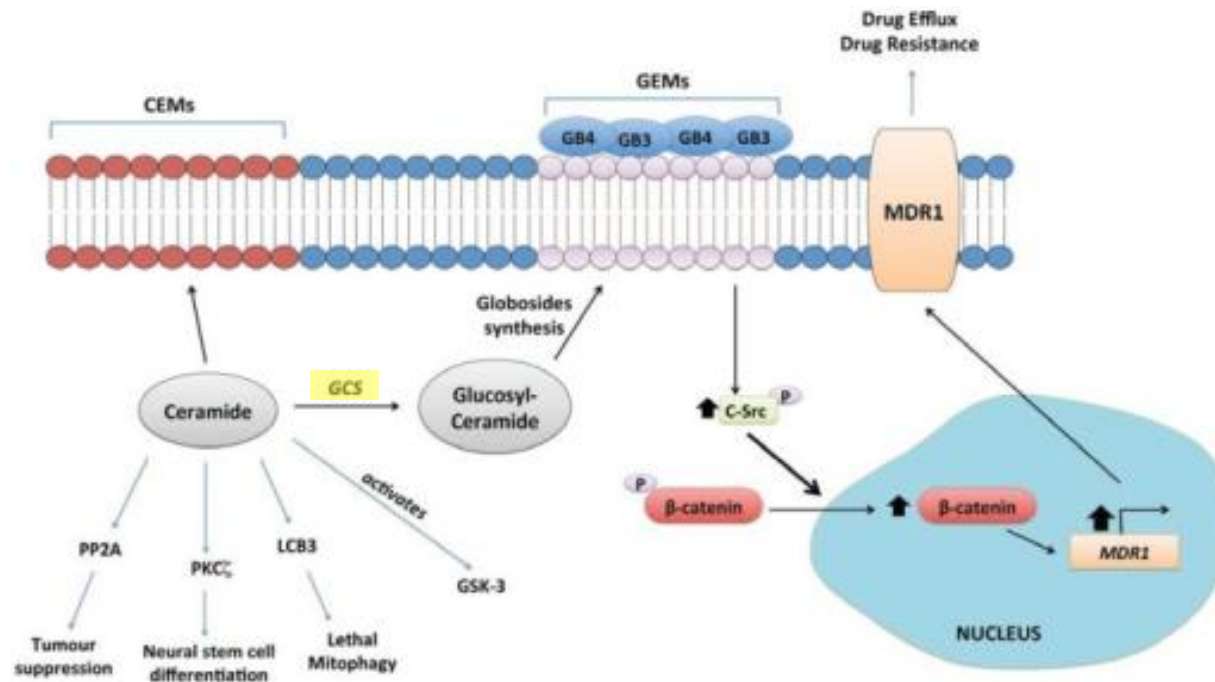
- Spanish group
- Target: GCS



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gSRT PROOF OF PRINCIPLE - GD

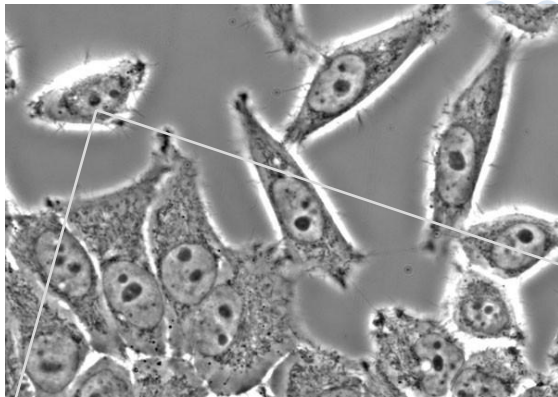
- Spanish group
- Target: GCS



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gSRT PROOF OF PRINCIPLE - GD

- Target: *GCS*
- Test in: HeLa cells



Human *GCS* gene

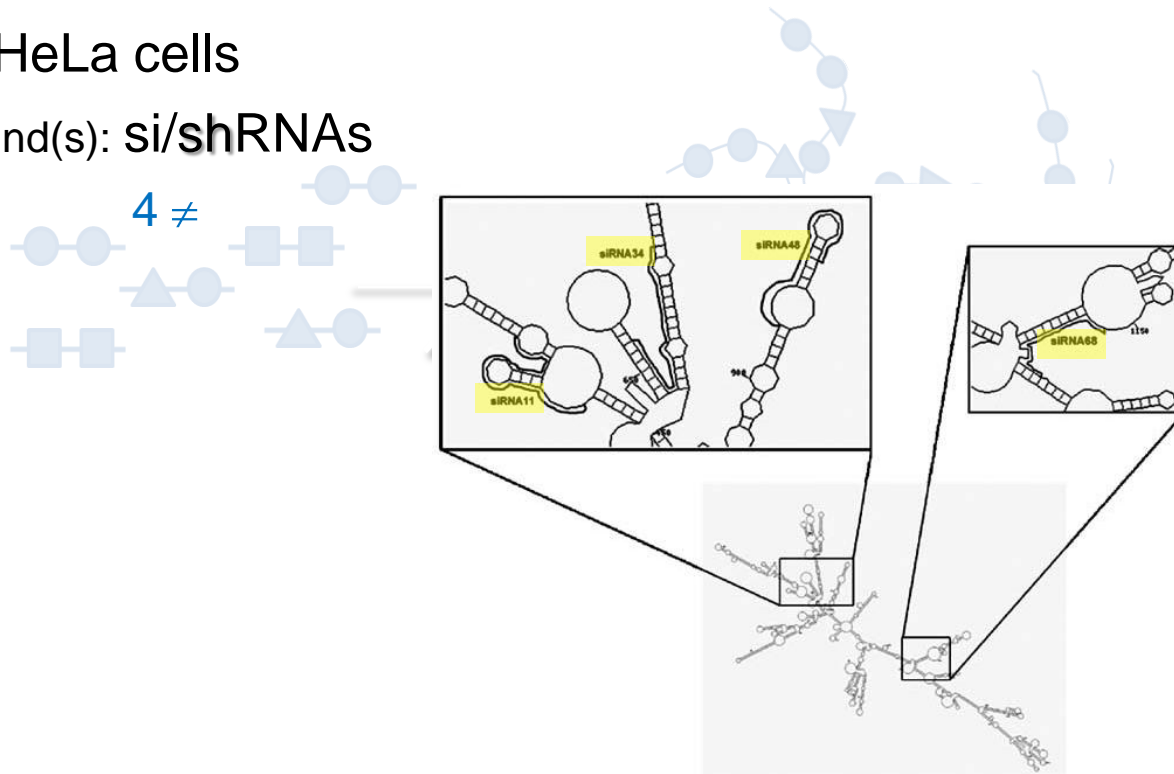


http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000133937;r=14:94768216-94770230;t=ENST00000238558 (adapted)



gSRT PROOF OF PRINCIPLE - GD

- Target: *GCS*
- Test in: HeLa cells
- Compound(s): si/shRNAs



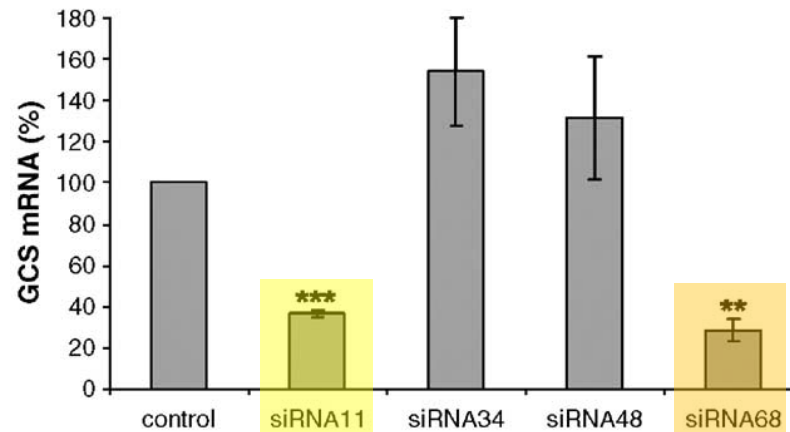
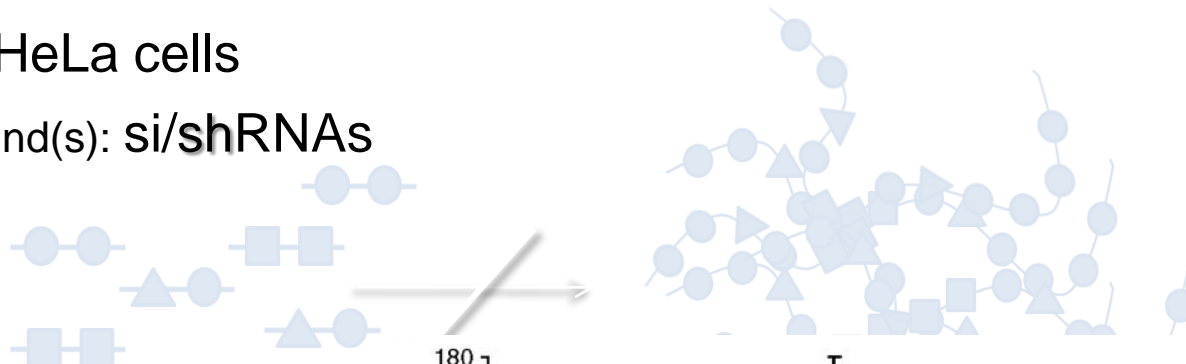
Diaz-Font *et al.*, (2006)



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gSRT PROOF OF PRINCIPLE - GD

- Target: *GCS*
- Test in: HeLa cells
- Compound(s): si/shRNAs



Diaz-Font *et al.*, (2006)

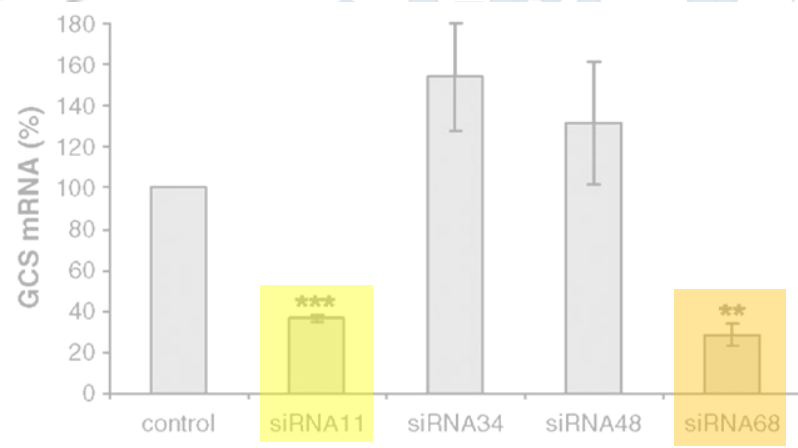
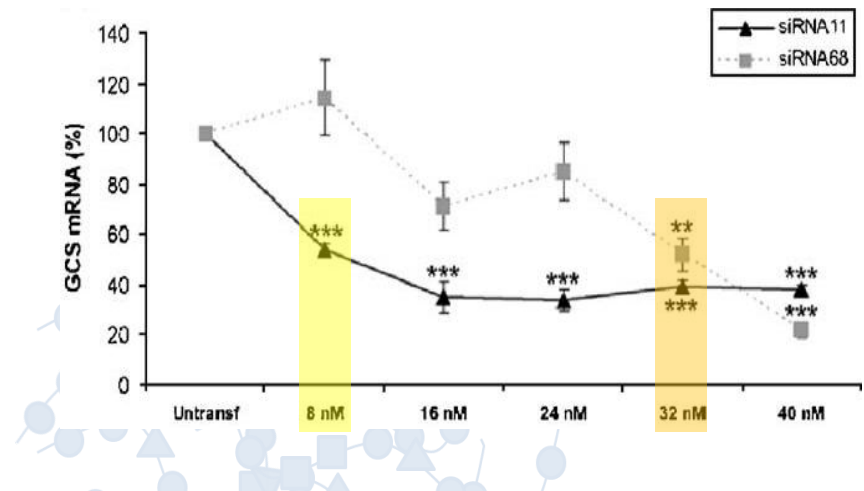
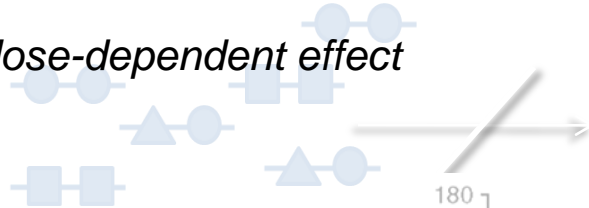


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gSRT PROOF OF PRINCIPLE - GD

- Target: GCS
- Test in: HeLa cells
- Compound(s): si/shRNAs

dose-dependent effect



Diaz-Font *et al.*, (2006)

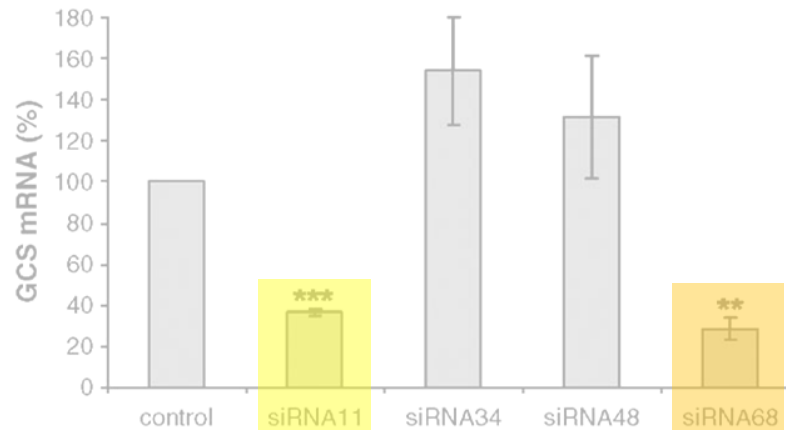
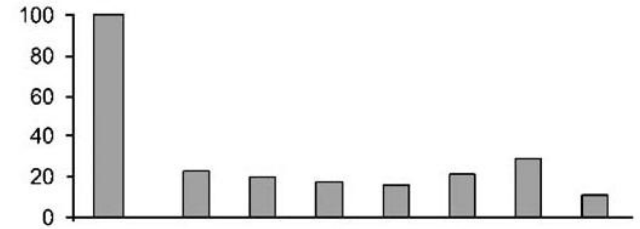
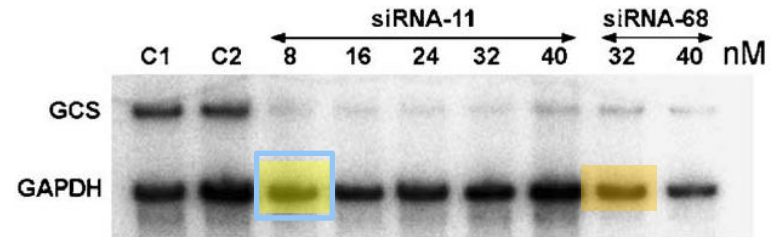


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gSRT PROOF OF PRINCIPLE - GD

- Target: *GCS*
- Test in: HeLa cells
- Compound(s): si/shRNAs

dose-dependent effect



Diaz-Font *et al.*, (2006)



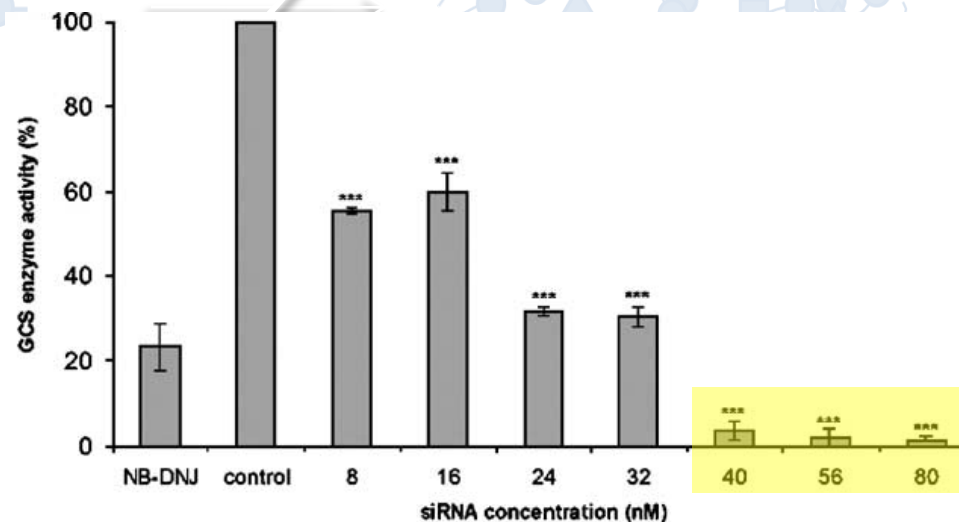
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gSRT PROOF OF PRINCIPLE - GD

- Target: *GCS*
- Test in: HeLa cells
- Compound(s): si/shRNAs

effect on *GCS* activity

(72h)



Diaz-Font *et al.*, (2006)



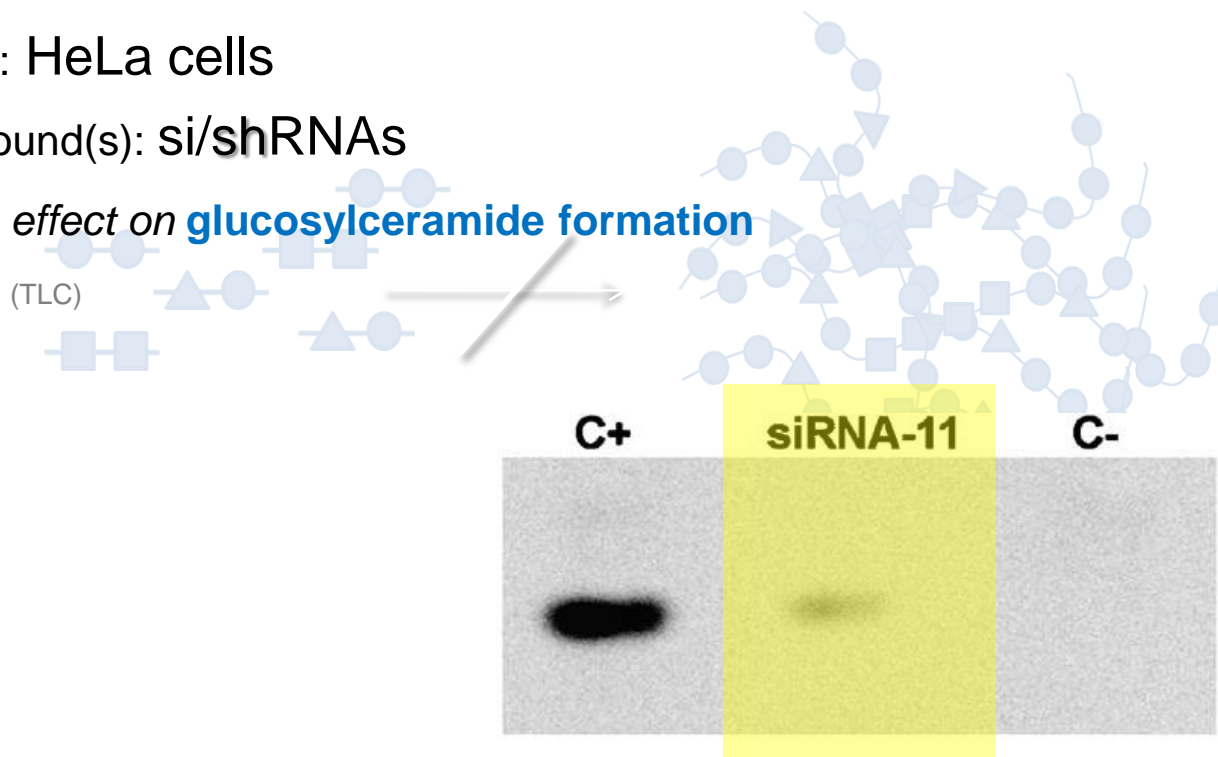
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gSRT PROOF OF PRINCIPLE - GD

- Target: *GCS*
- Test in: HeLa cells
- Compound(s): si/shRNAs

effect on **glucosylceramide formation**

(TLC)



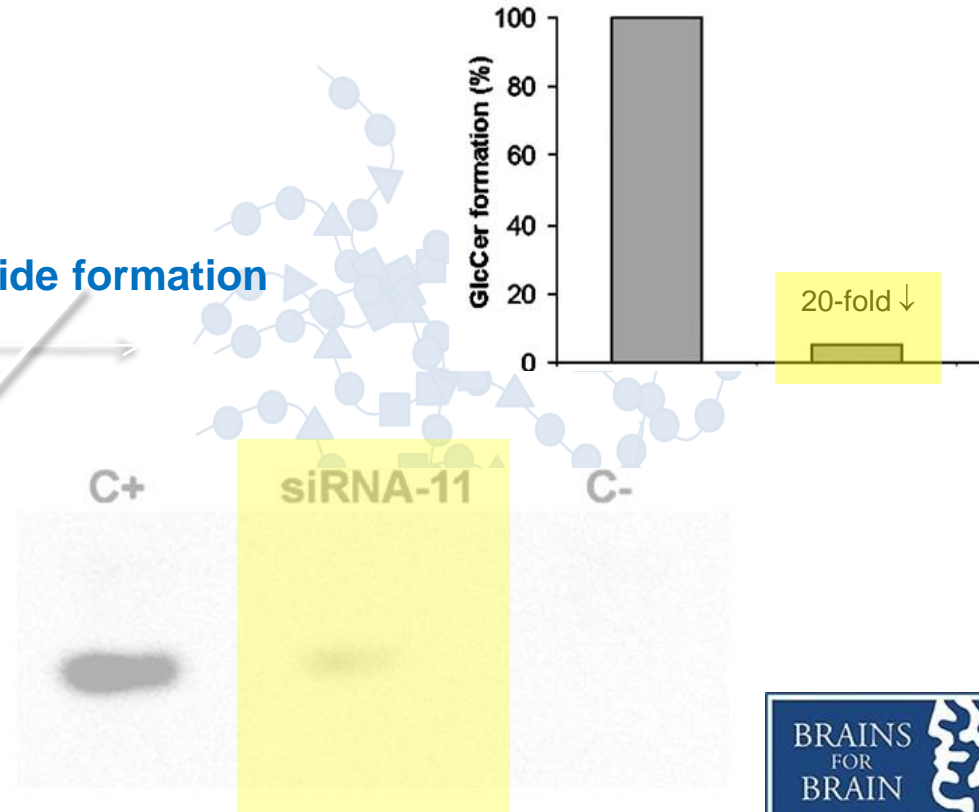
Diaz-Font *et al.*, (2006)

gSRT PROOF OF PRINCIPLE - GD

- Target: *GCS*
- Test in: HeLa cells
- Compound(s): si/shRNAs

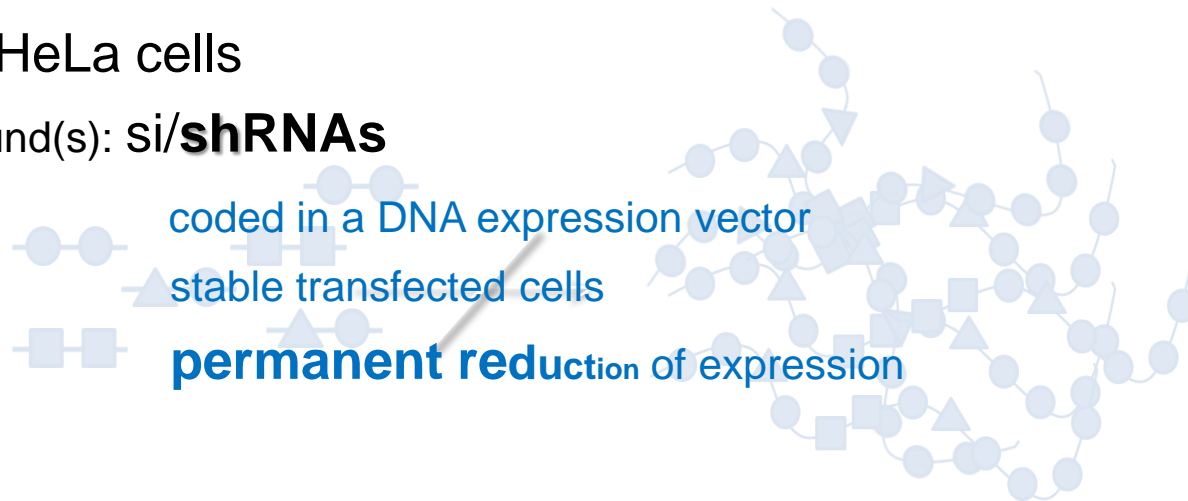
effect on **glucosylceramide formation**

(TLC)



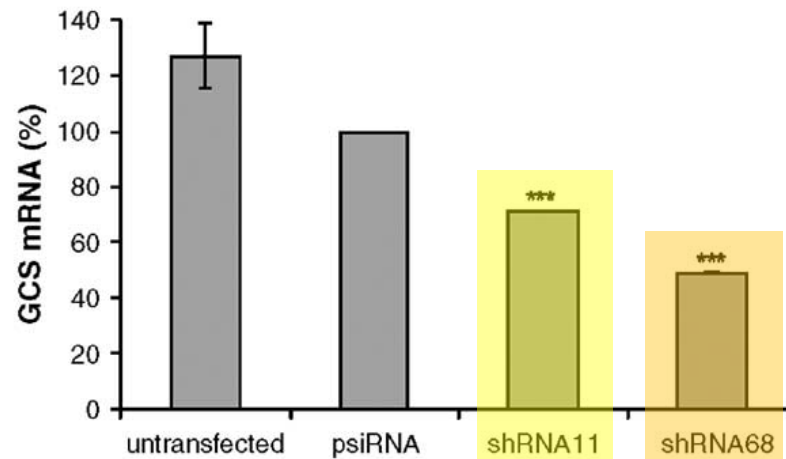
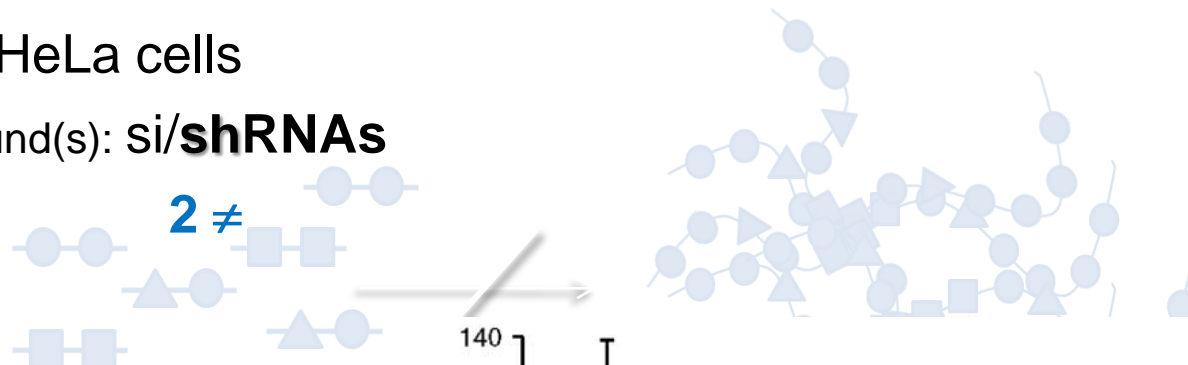
gSRT PROOF OF PRINCIPLE - GD

- Target: *GCS*
- Test in: HeLa cells
- Compound(s): si/**shRNAs**



gSRT PROOF OF PRINCIPLE - GD

- Target: *GCS*
- Test in: HeLa cells
- Compound(s): si/shRNAs



Diaz-Font *et al.*, (2006)

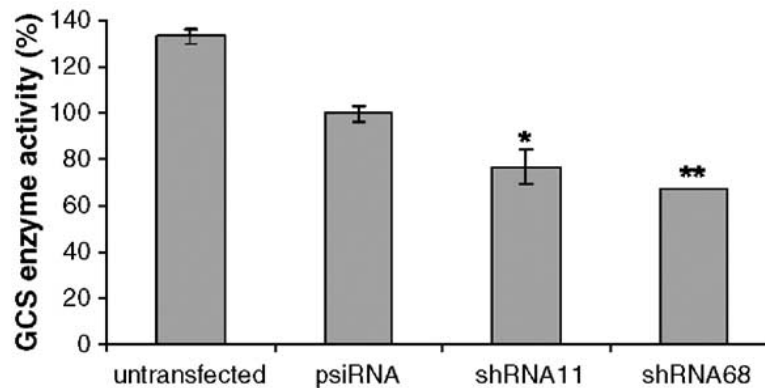


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gSRT PROOF OF PRINCIPLE - GD

- Target: **GCS**
- Test in: HeLa cells
- Compound(s): si/**shRNAs**

effect on **GCS activity**



Diaz-Font *et al.*, (2006)

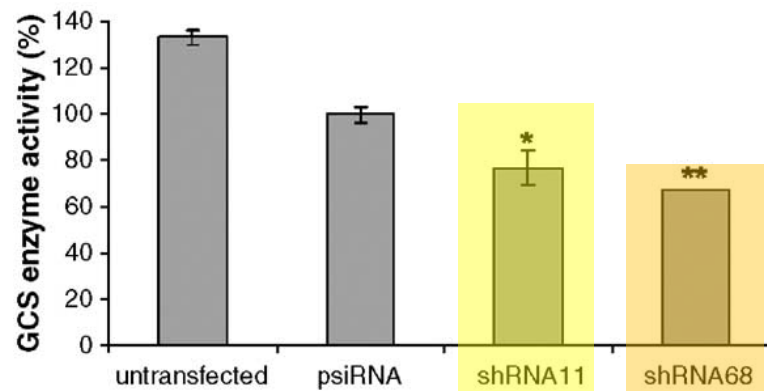


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gSRT PROOF OF PRINCIPLE - GD

- Target: **GCS**
- Test in: HeLa cells
- Compound(s): si/**shRNAs**

effect on **GCS activity**



Diaz-Font *et al.*, (2006)



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gSRT PROOF OF PRINCIPLE - GD

SUMMARY

si/shRNAs

- ↓ GCS mRNA levels
- ↓ GCS enzyme activity
- ↓ glucosylceramide Synthesis



MPS IVB Wolman Pycnodysostosis
Beta-mannosidosis
Sandhoff Metachromatic Danon
leukodystrophy
Tay-Sachs MPS IVA
Mucopolysaccharidosis
Mucopolysaccharidosis Fucosidosis
Mucopolysaccharidosis type I Fabry
type II Gaucher Farber
Niemann-Pick Pompe MPS II
type C ML III MPS IIIC Multiple sulfatase
MPS IIIA Sialidosis deficiency
Alpha-Krabbe MPS IIIB
mannosidosis Galactosialidosis



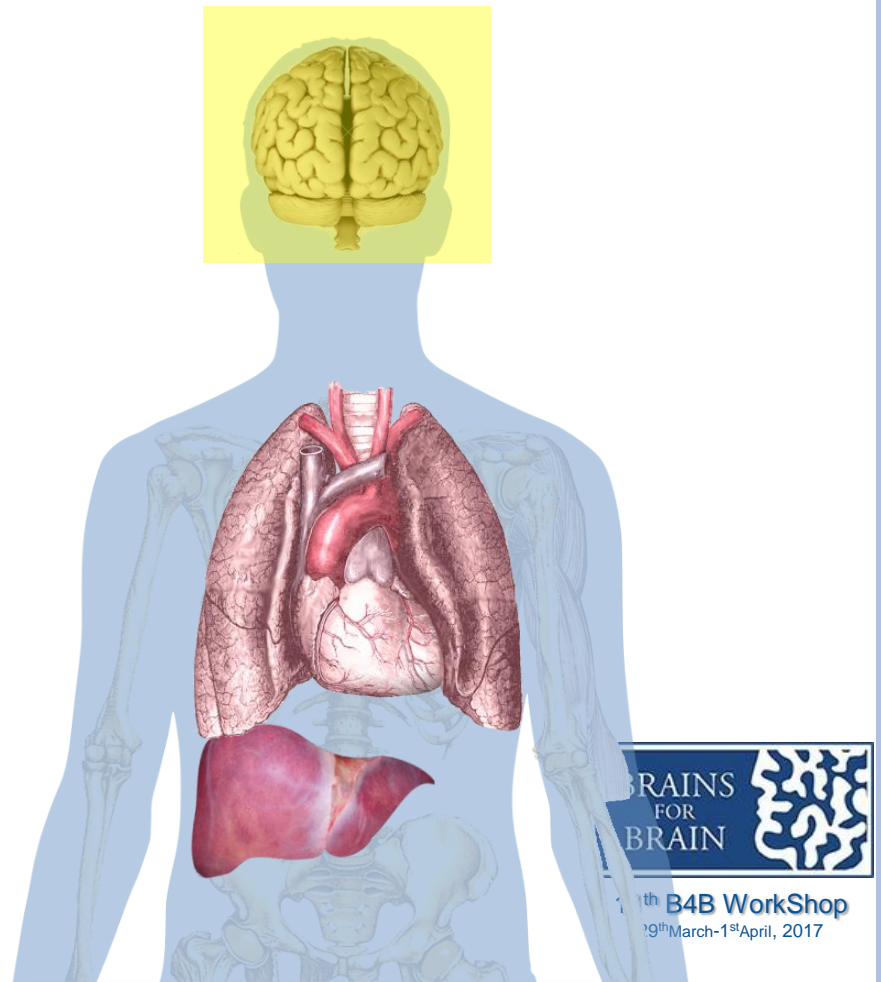
MUCOPOLYSACCHARIDOSES (MPS)

- Chronic
- Progressive
- Large spectrum of severity
& symptoms

MPS III

(= Sanfilippo Syndrome)

A, B, C and D



BRAINS
FOR
BRAIN



with B4B WorkShop
29th March - 1st April, 2017

AVAILABLE THERAPIES

○ Enzyme Replacement Therapy

- Only available for 4 MPSs!
- No effect on neurological symptoms!

... antibodies!



AVAILABLE THERAPIES

- Enzyme Replacement Therapy

**Perfect Target
for
Substrate Reduction
Approaches!**

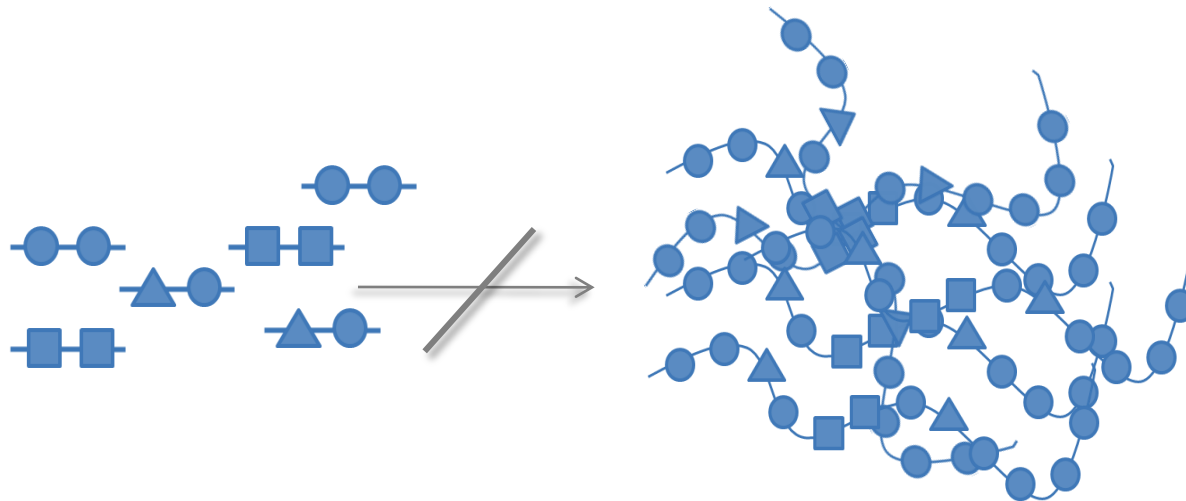
BRAINS
FOR
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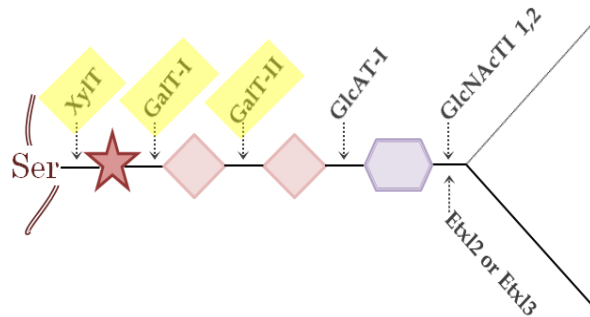
gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Polish group
- Targets: *XYLT1*, *XYLT2*, *GALTI*, *GALTII*



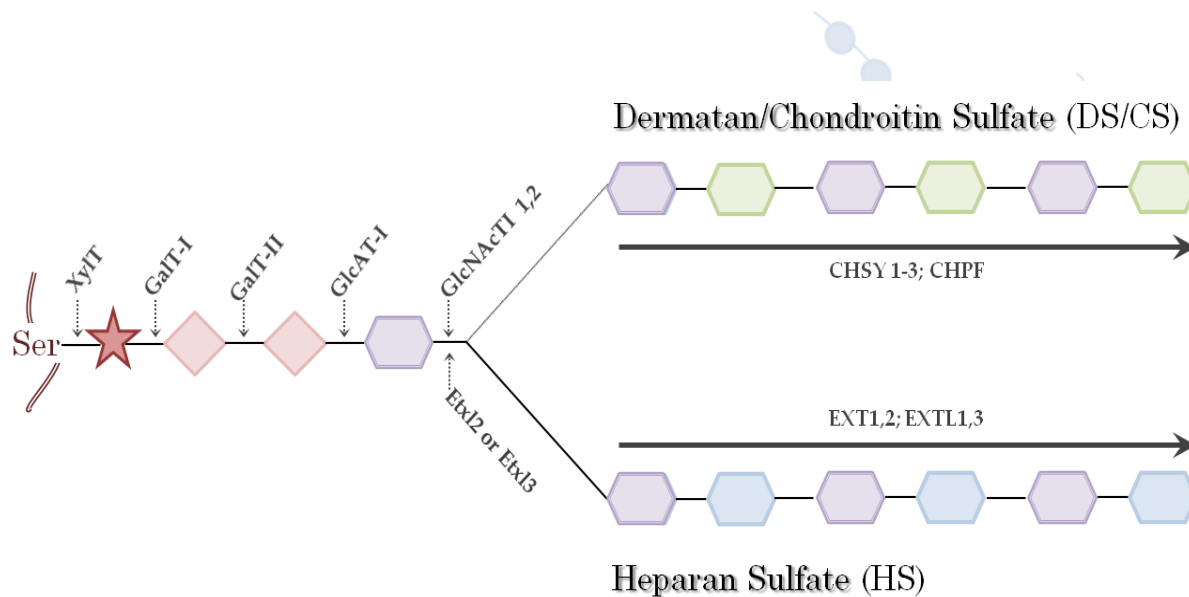
gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Polish group
- Targets: *XYLT1*, *XYLT2*, *GALTI*, *GALTII*



gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Polish group
- Targets: *XYLT1*, *XYLT2*, *GALTI*, *GALTI1*



gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Polish group
- Targets: *XYLT1*, *XYLT2*, *GALTI*, *GALTI1*
- Test in: MPS IIIA fibroblasts



<http://www.sciencellonline.com/products-services/primary-cells/human-dermal-fibroblasts-adult.html>

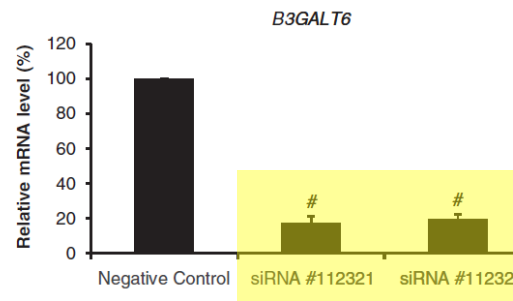
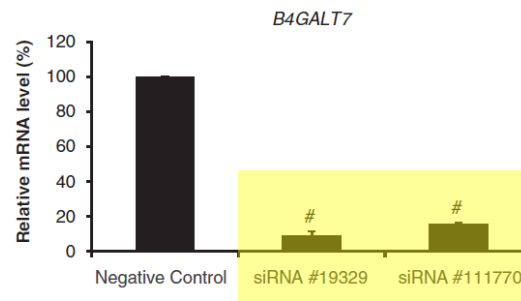
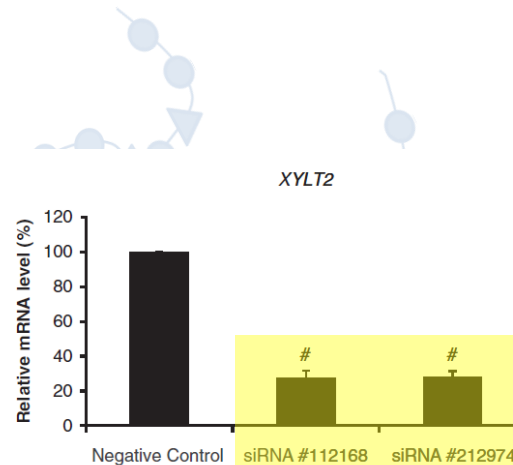
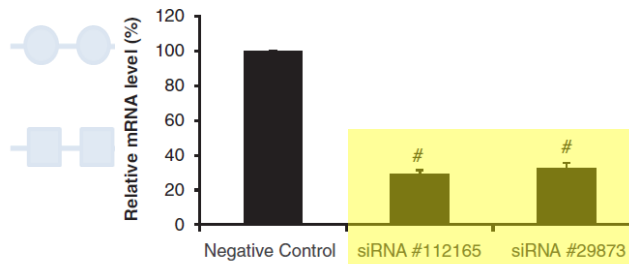


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gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

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

8 ≠



Dziedzic *et al.*, (2010)

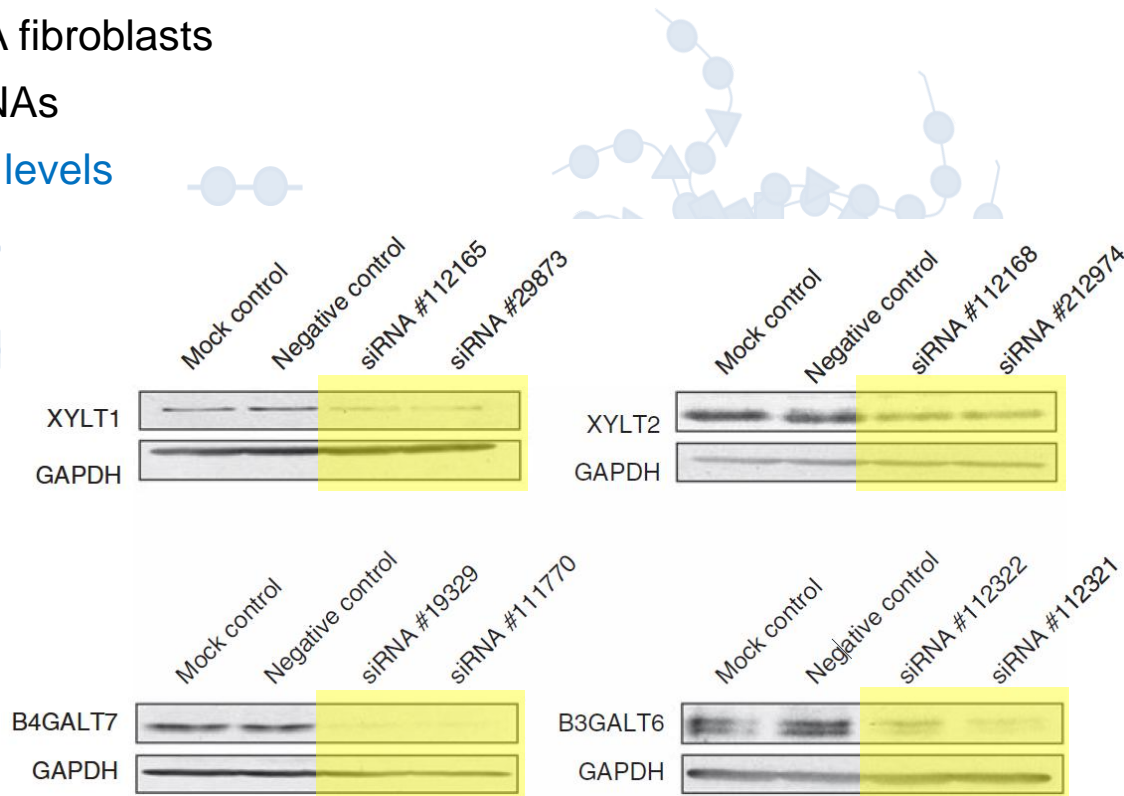


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gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

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

protein levels



Dziedzic *et al.*, (2010)

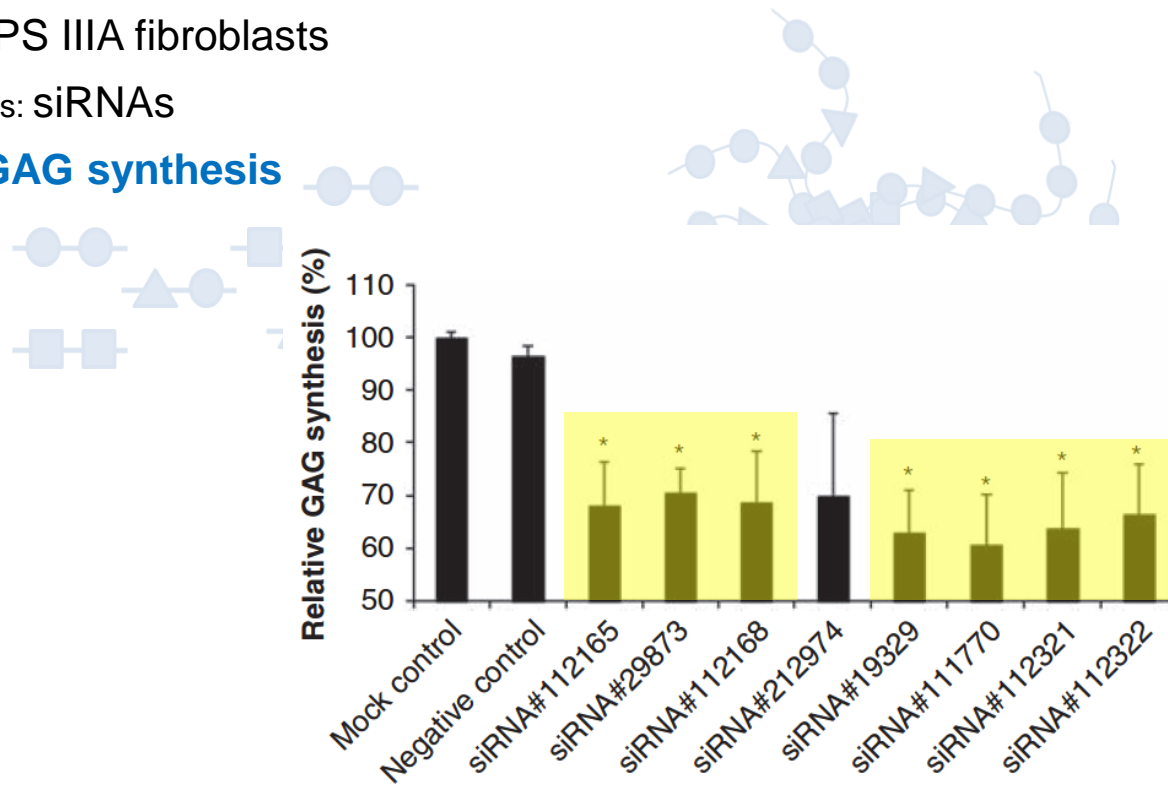


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gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

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

GAG synthesis



Dziedzic et al., (2010)



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gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

SUMMARY

 “early stages” **GAGs biosynthesis genes**

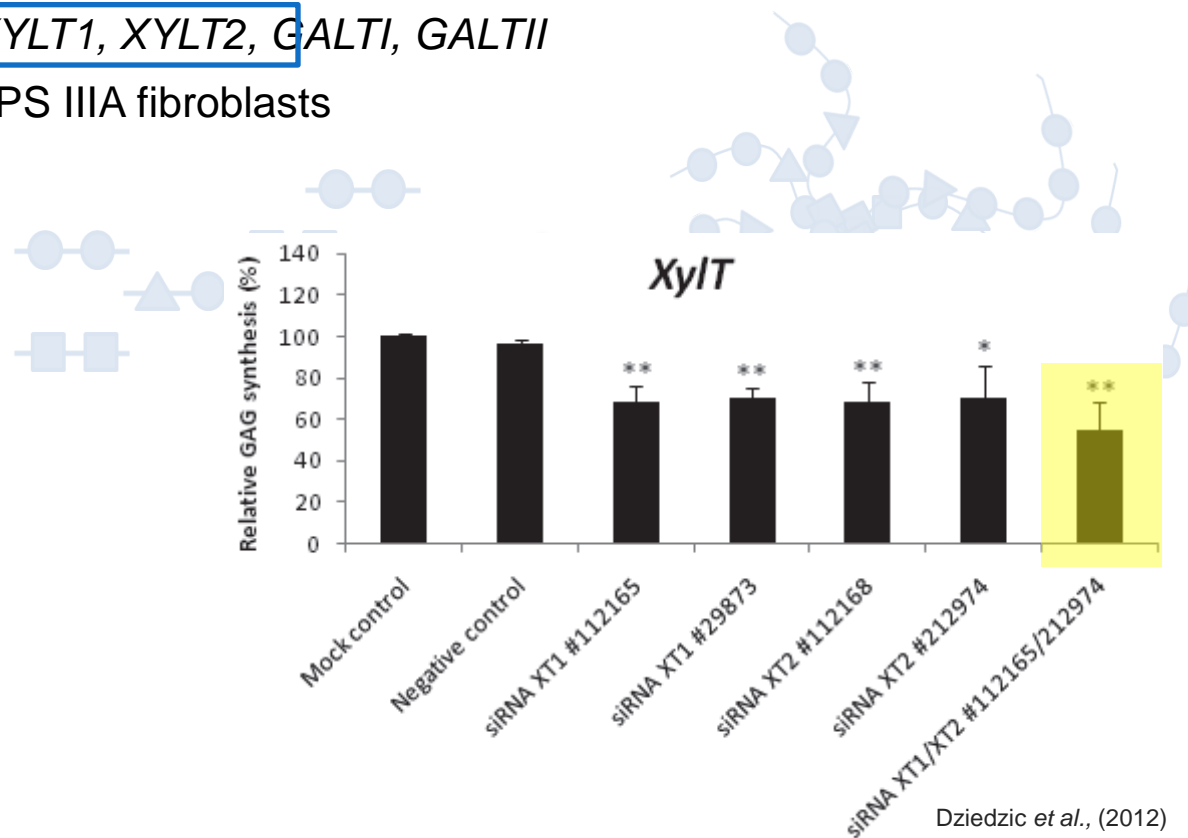
↓ **Synthesis**

BRAINS
FOR
BRAIN



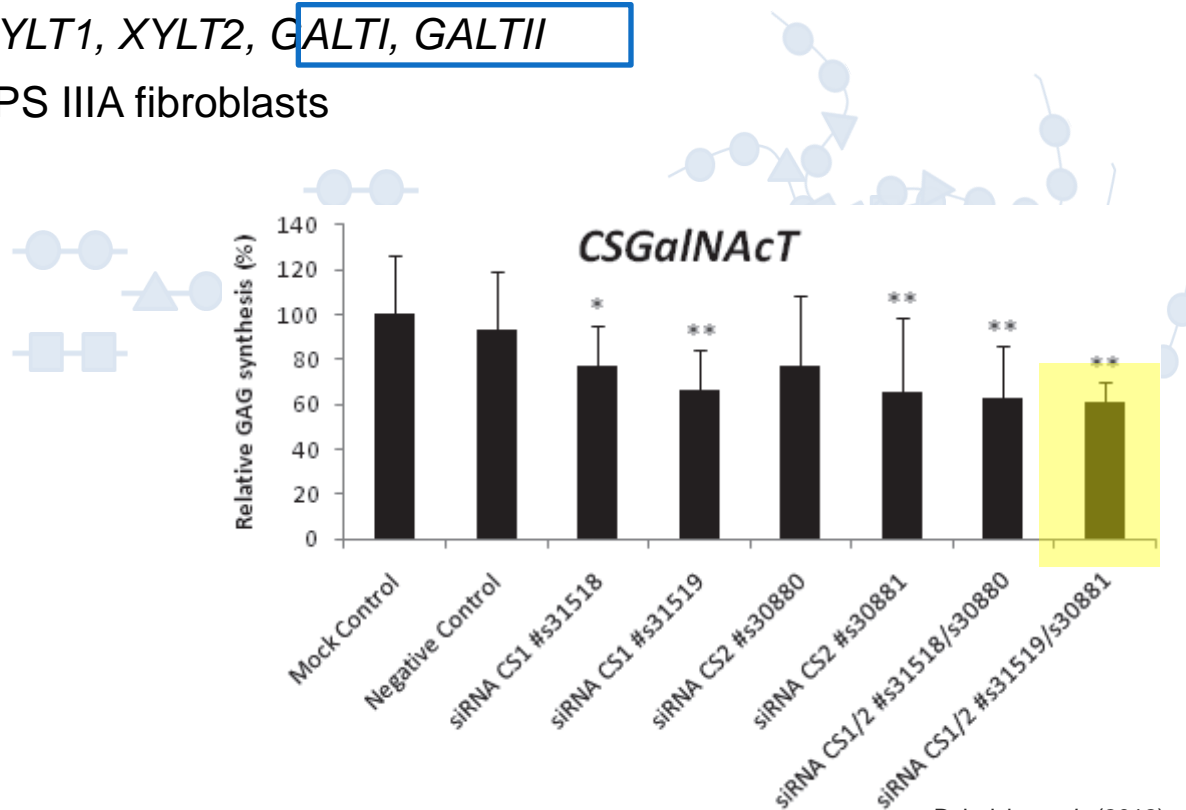
gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Targets: **XYLT1, XYLT2, GALT1, GALTII**
- Test in: MPS IIIA fibroblasts



gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Targets: *XYLT1*, *XYLT2*, *GALTI*, *GALTII*
- Test in: MPS IIIA fibroblasts



Dziedzic *et al.*, (2012)

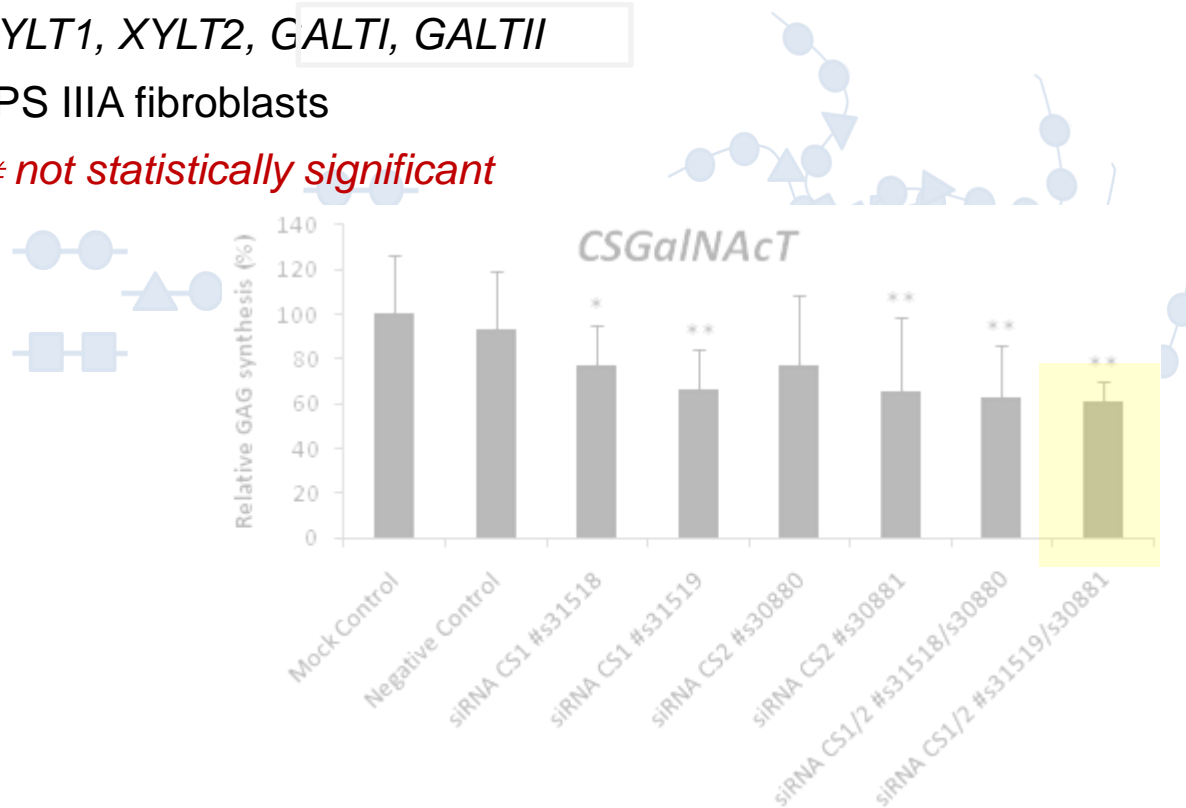


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gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Targets: *XYLT1*, *XYLT2*, *GALTI*, *GALTII*
- Test in: MPS IIIA fibroblasts

≠ not statistically significant



Dziedzic *et al.*, (2012)

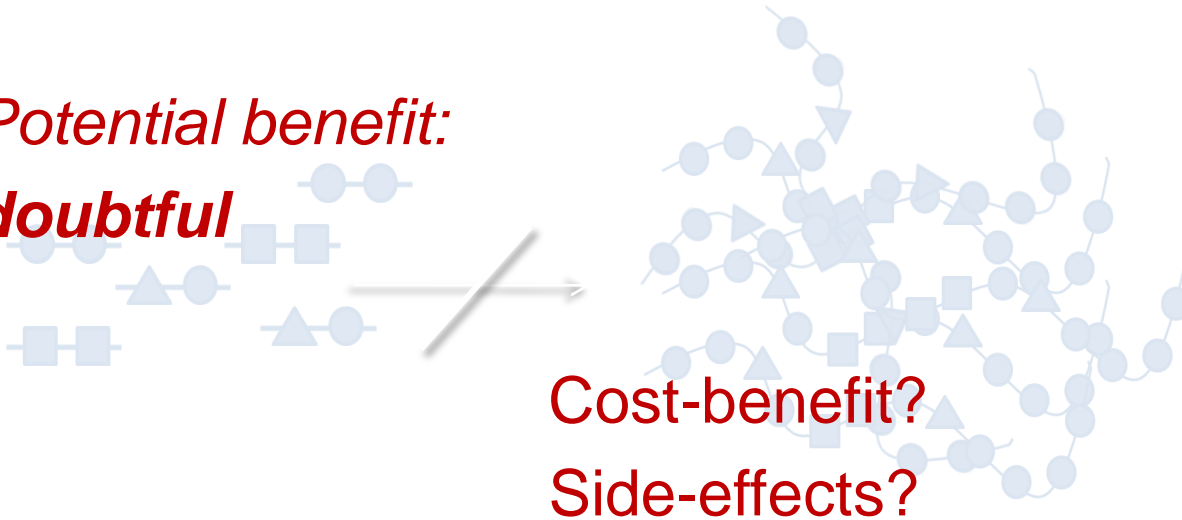


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gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Would the simultaneous use of 2 species of siRNAs be more effective than the use of single siRNAs?

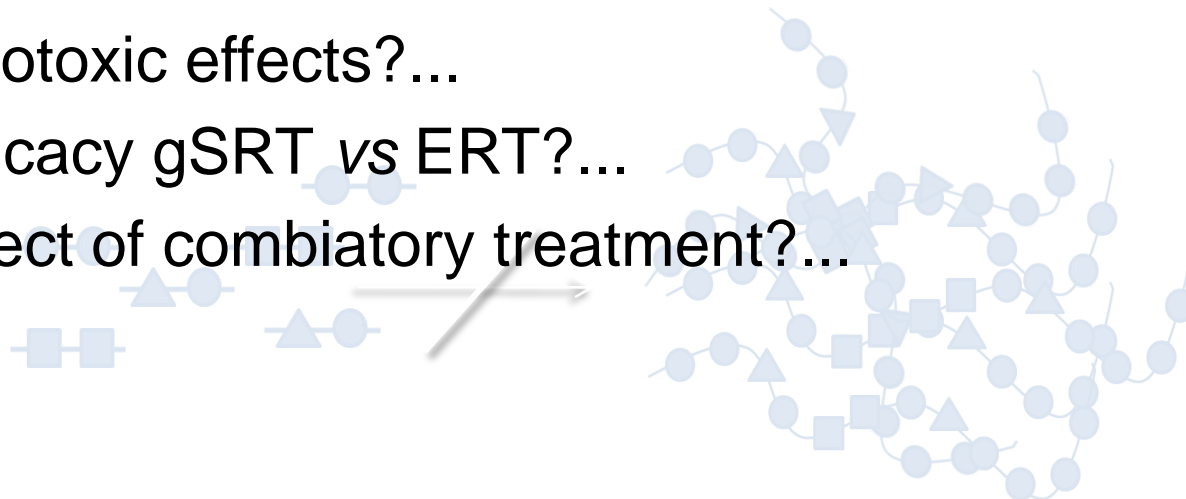
*Potential benefit:
doubtful*



gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

○ What about...

- cytotoxic effects?...
- efficacy gSRT vs ERT?...
- effect of combiatory treatment?...



MPS I MPS IIIA and MPS IIIB

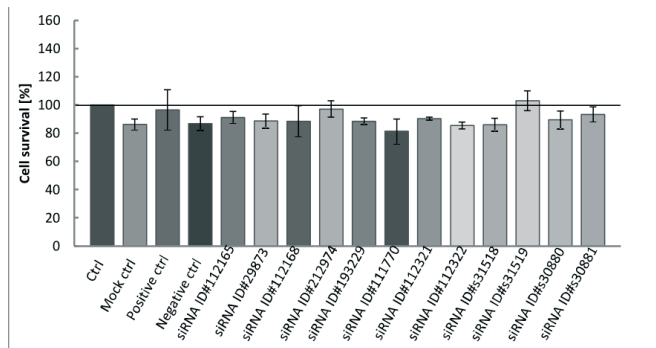


gSRT FOR OTHER LSDs - MUcOPOLYSACCHARIDOSES

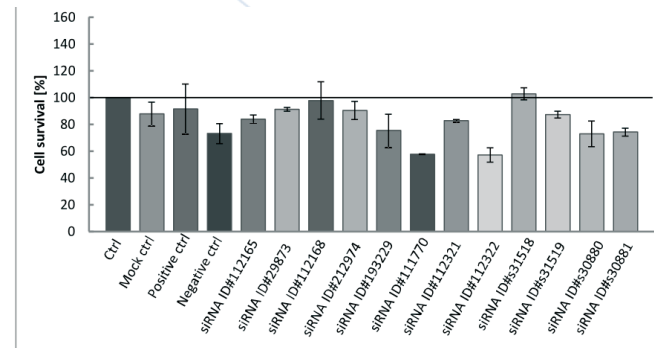
○ Cytotoxic effects?...

(cell survival %)

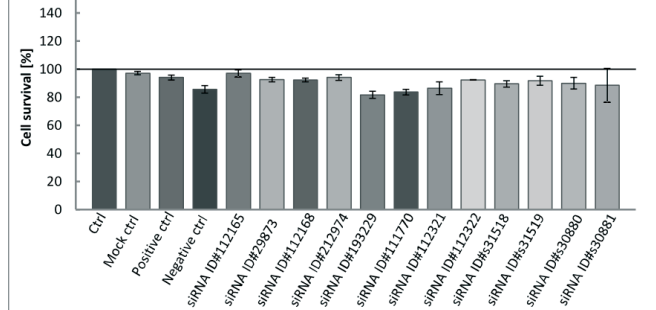
24h



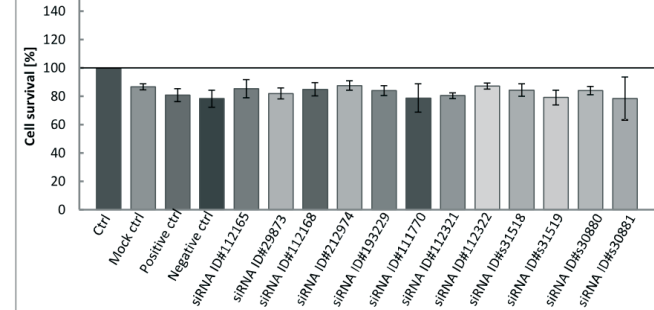
48h



24h



48h



Chmielarz *et al.*, (2012)

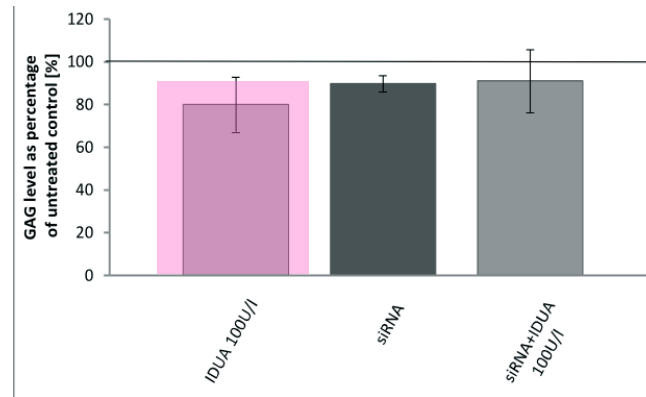


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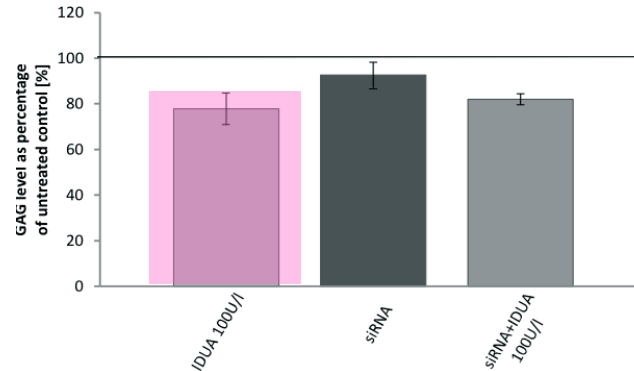
gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Efficacy gSRT vs ERT and effect of combiatory treatment?...

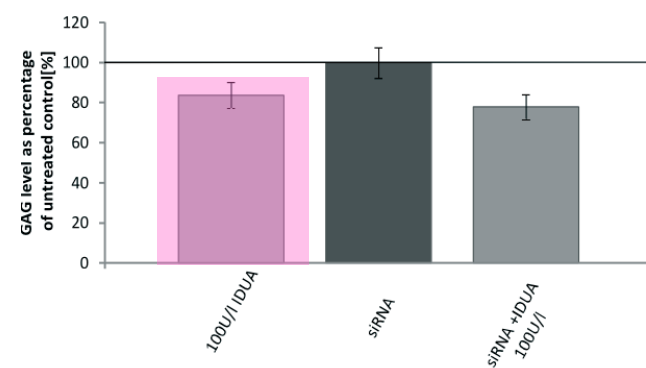
MPS I – cell line 1



MPS I – cell line 2



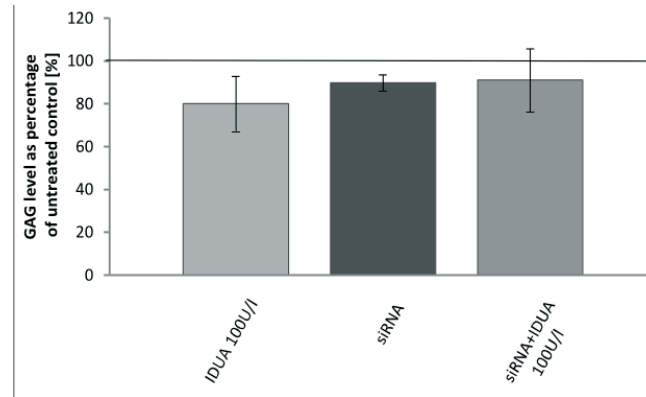
MPS I – cell line 3



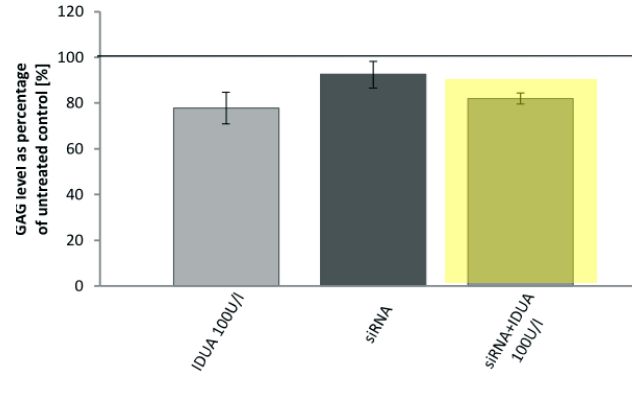
gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Efficacy gSRT vs ERT and effect of combiatory treatment?...

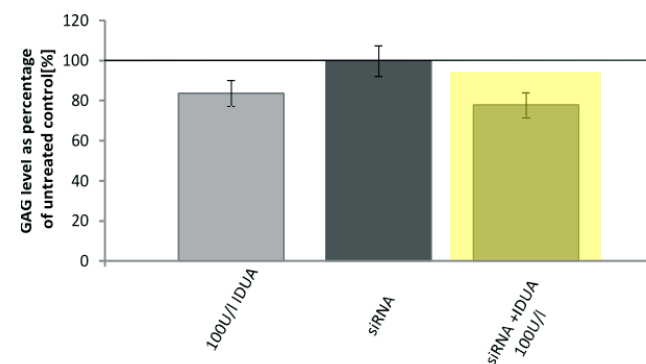
MPS I – cell line 1



MPS I – cell line 2



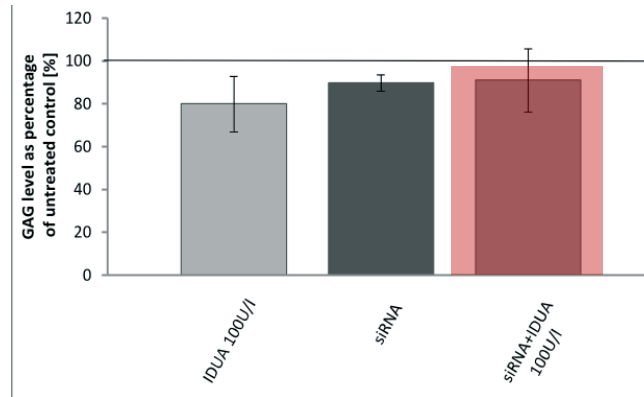
MPS I – cell line 3



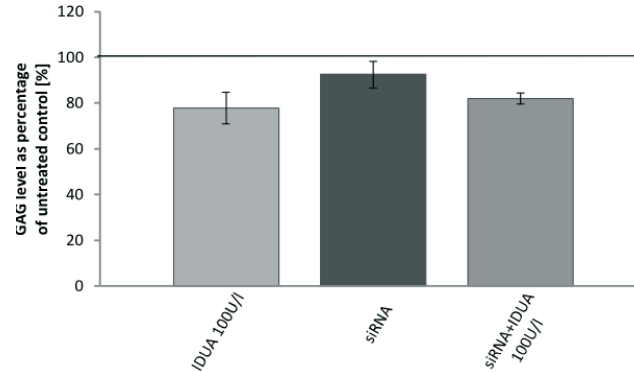
gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Efficacy gSRT vs ERT and effect of combiatory treatment?...

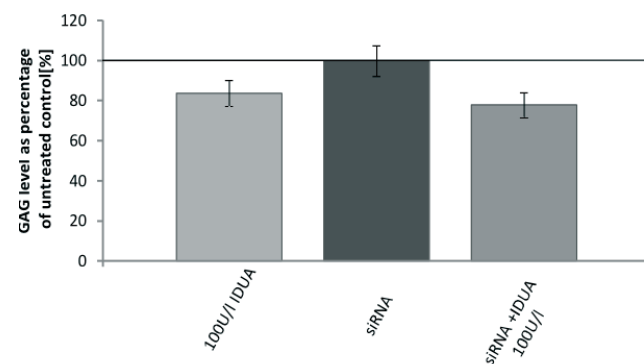
MPS I – cell line 1



MPS I – cell line 2



MPS I – cell line 3



gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Cytotoxic effects?...

No!

However,

some cytotoxic effects could occur in particular cell lines

- Efficacy gSRT vs ERT and effect of combiatory treatment?...

Depends on specific features of each cell line

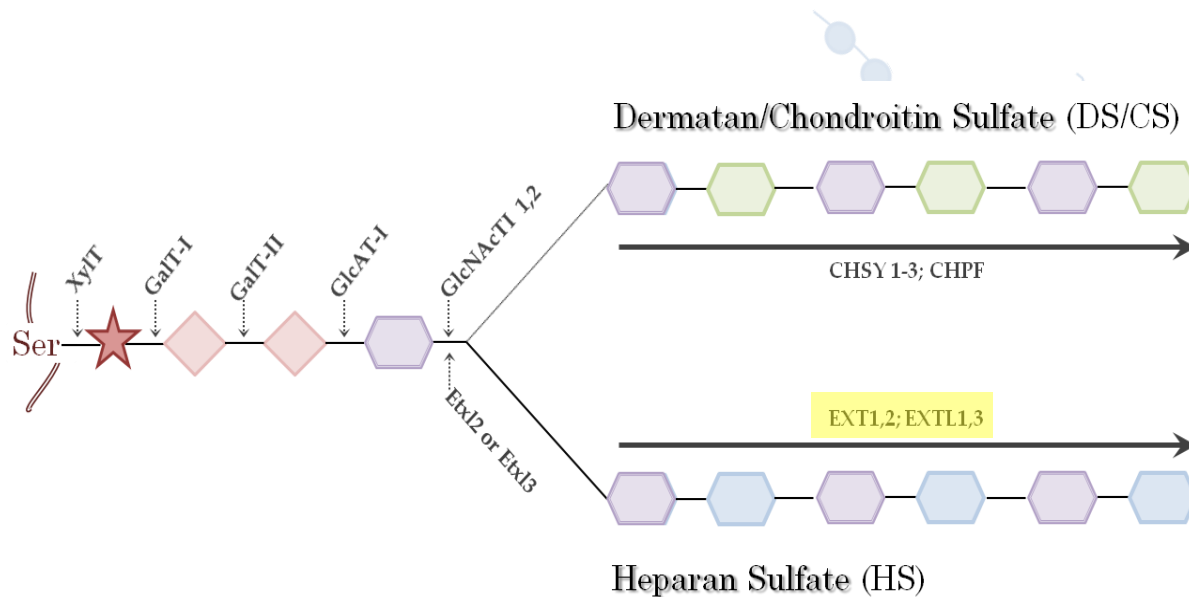
Thus,

the combined therapy might potentially result in various effects in different patients



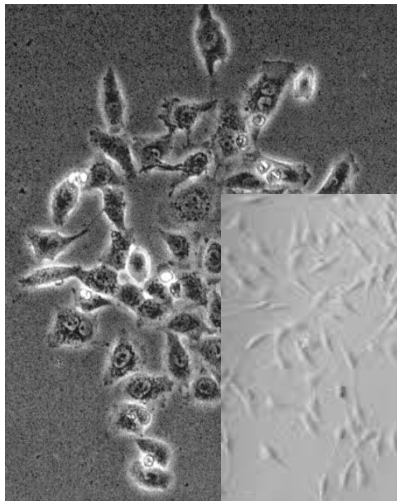
gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Australian group
- Targets: *EXTL2* and *EXTL3*

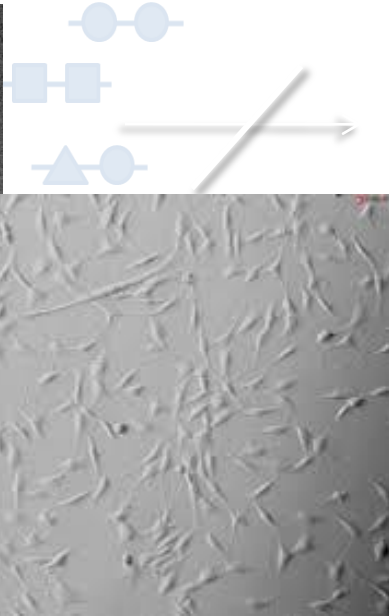


gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

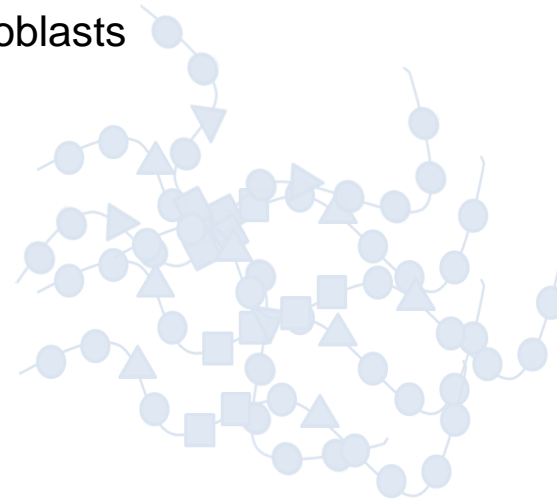
- Australian group
- Targets: *EXTL2* and *EXTL3*
- Test in: 293T cells; MPS I and MPS IIIA fibroblasts



https://upload.wikimedia.org/wikipedia/commons/3/3c/3_cells_grown_in_tissue_culture_medium.jpg



<http://www.sciencellonline.com/products-services/primary-cells/human-dermal-fibroblasts-adult.html>



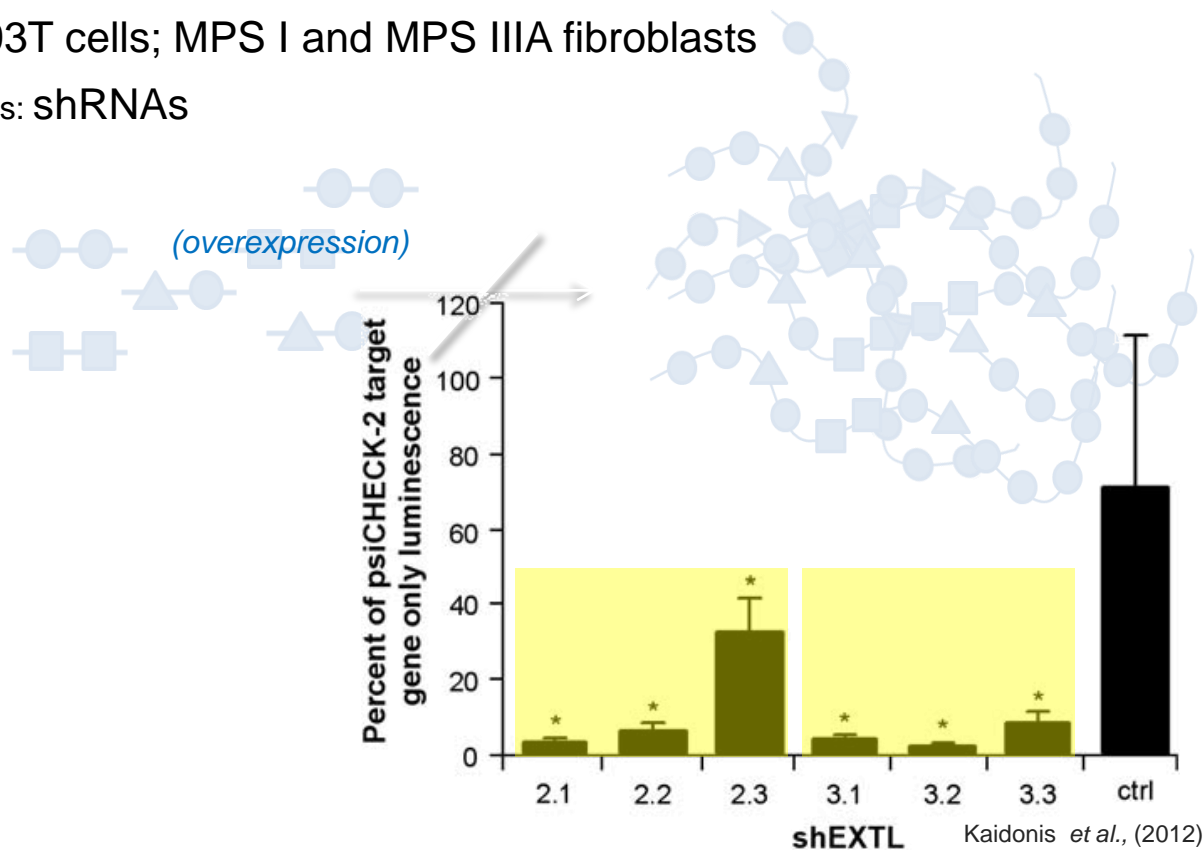
Kaidonis *et al.*, (2012)



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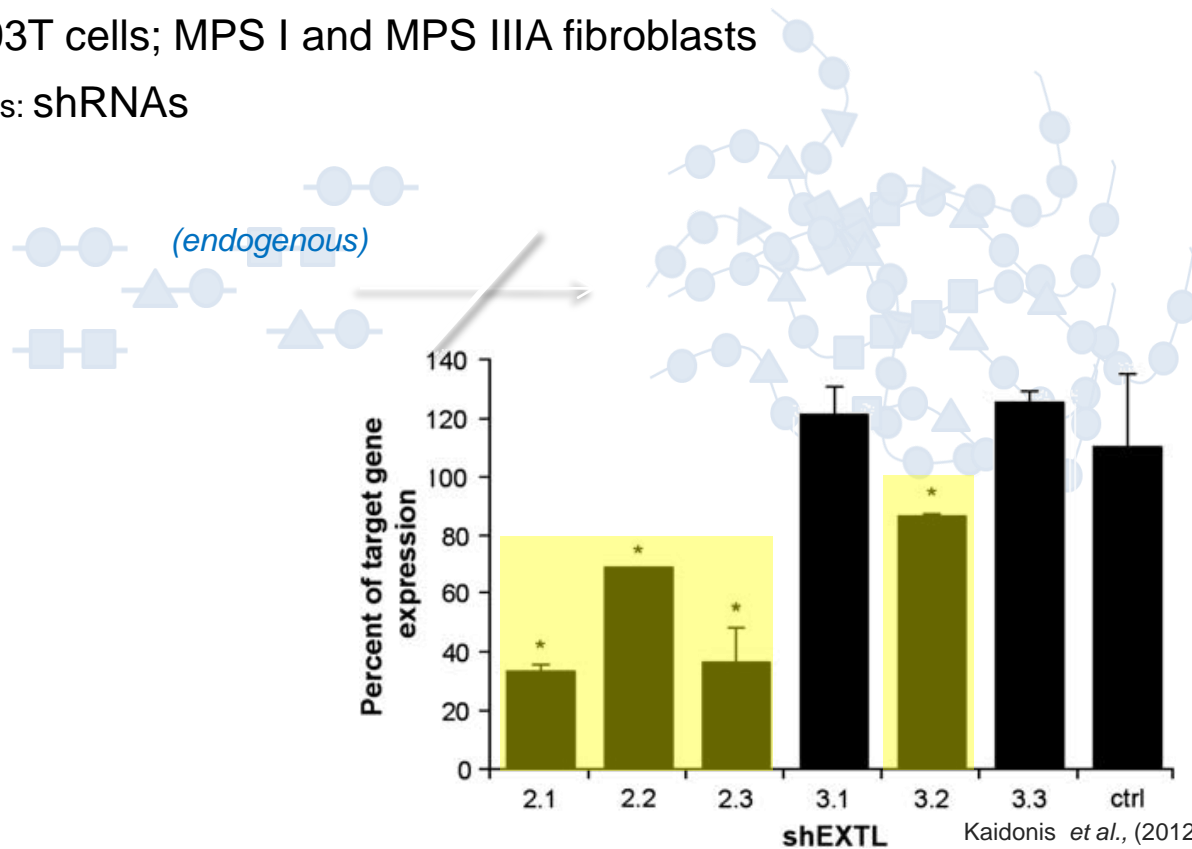
gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

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



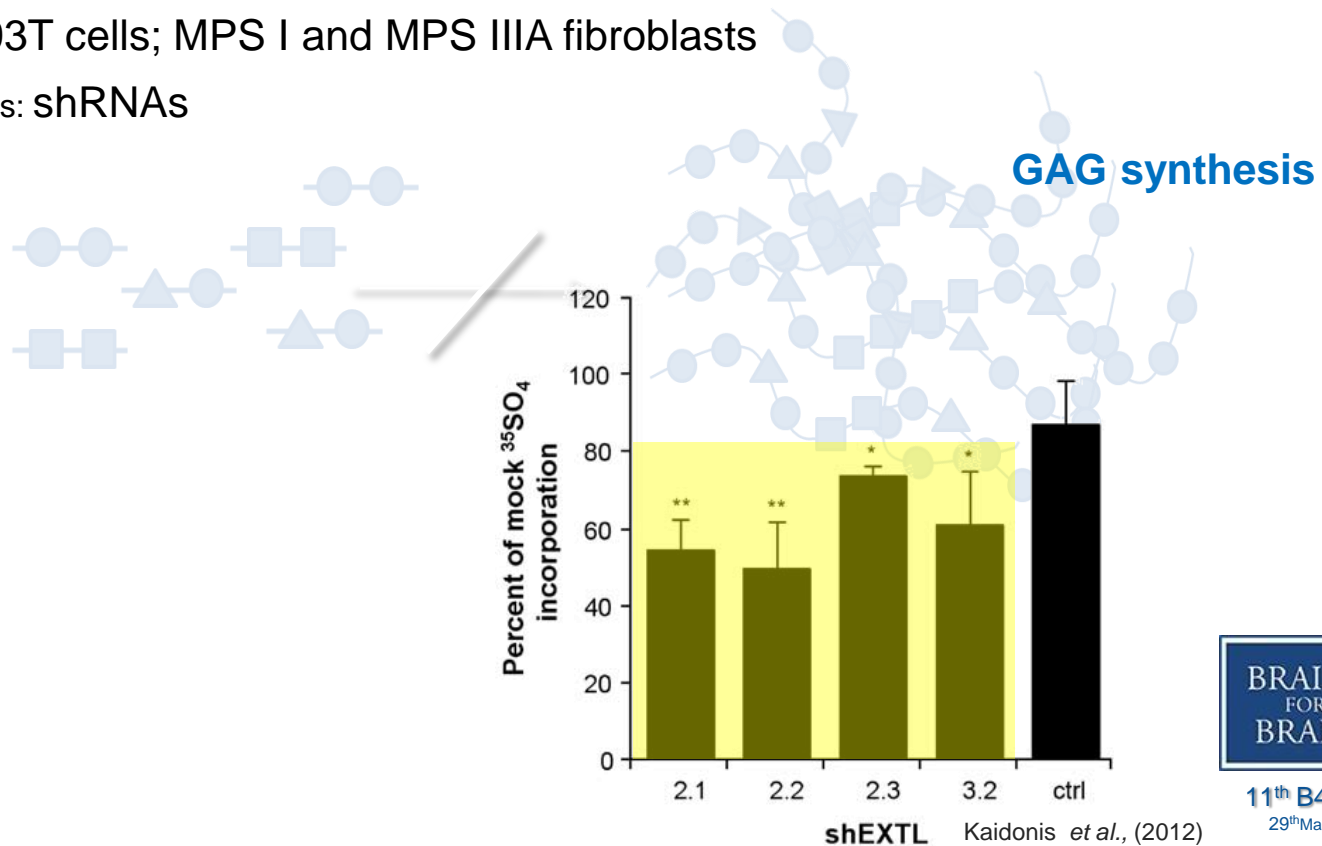
gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

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



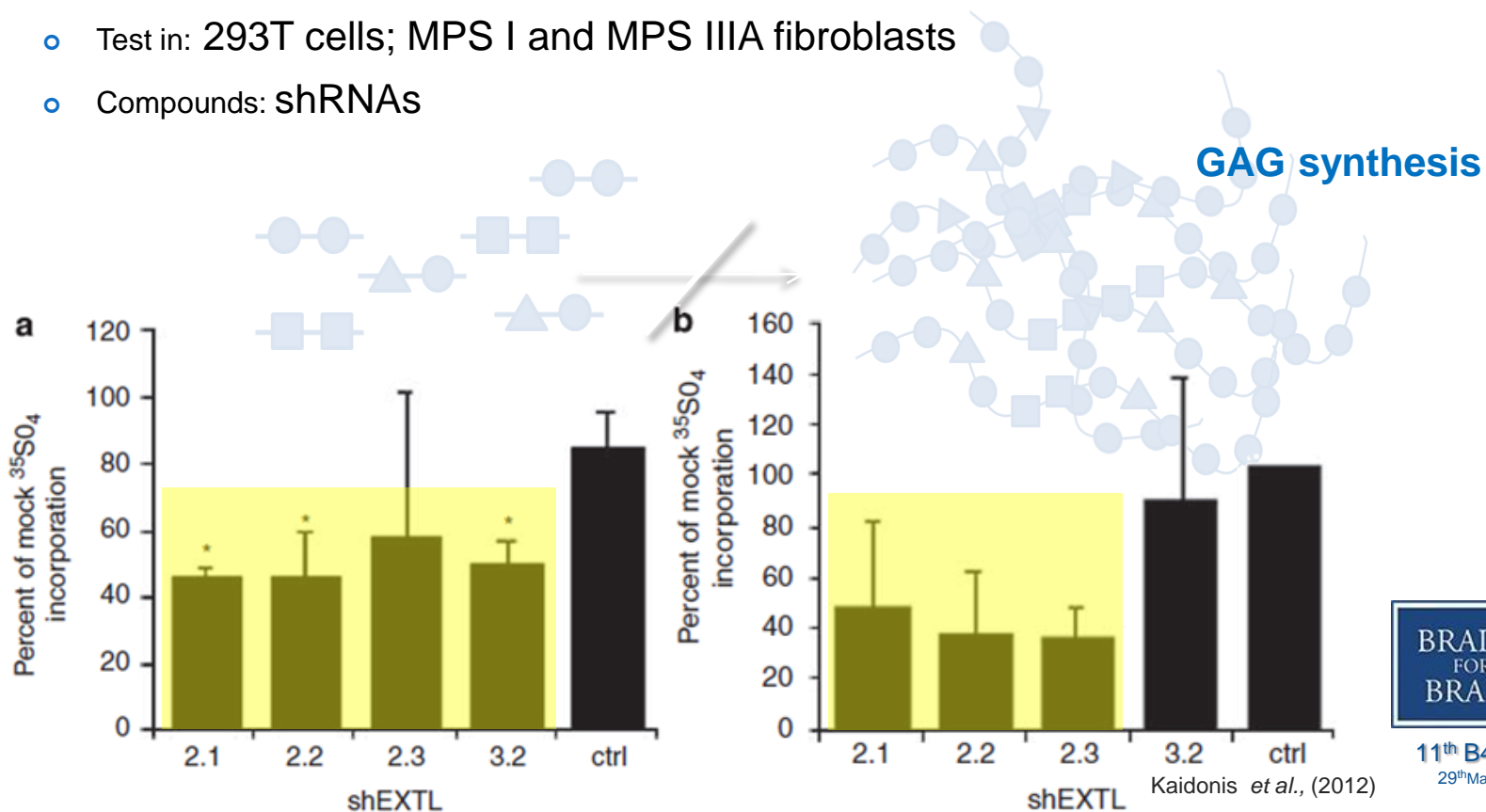
gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

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



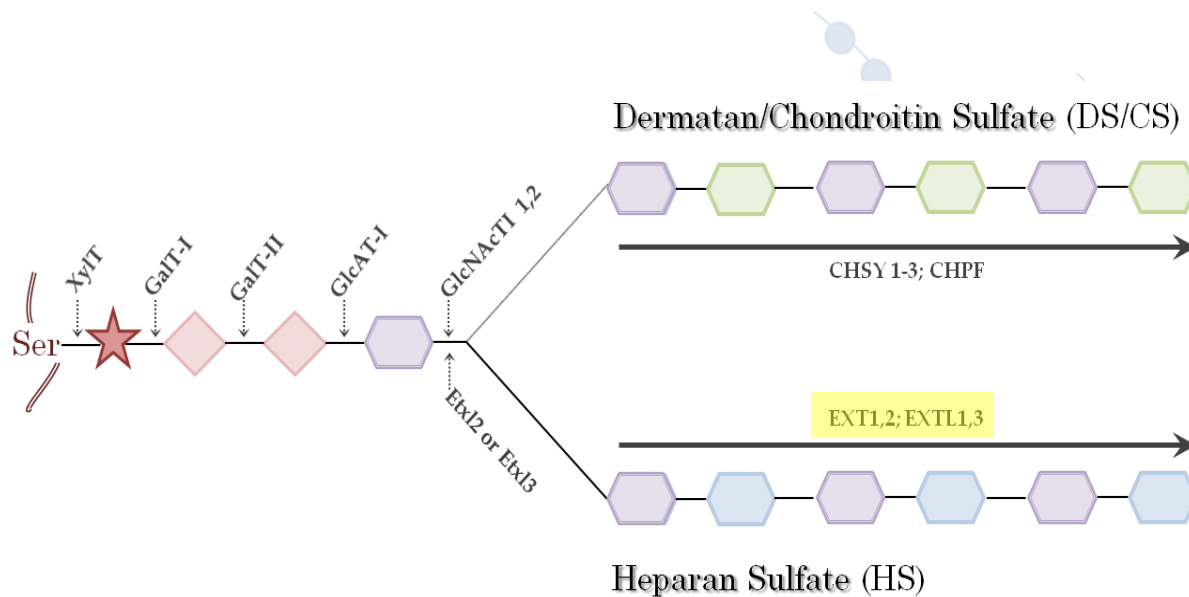
gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

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



gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Spanish group
- Targets: *EXTL2* and *EXTL3*



gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Targets: *EXTL2* and *EXTL3*
- Test in: MPS IIIC fibroblasts 2 ≠



<http://www.sciencellonline.com/products-services/primary-cells/human-dermal-fibroblasts-adult.html>



gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Targets: *EXTL2* and *EXTL3*
- Test in: MPS IIIC fibroblasts 2 ≠
- Compounds: siRNAs

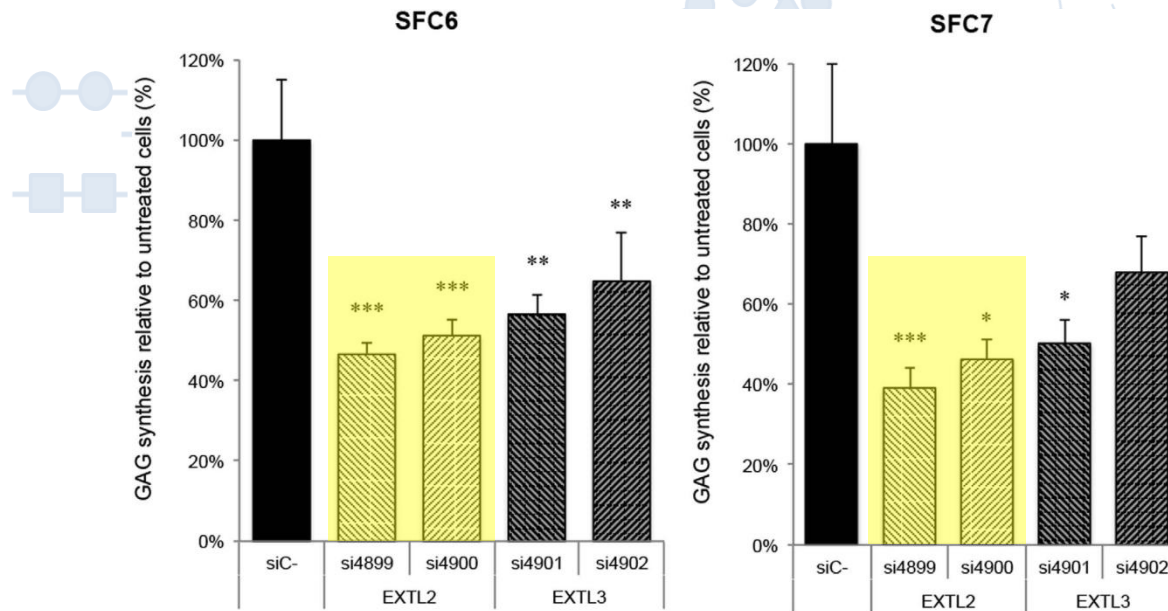


<http://www.sciencellonline.com/products-services/primary-cells/human-dermal-fibroblasts-adult.html>



gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Targets: *EXTL2* and *EXTL3*
- Test in: MPS IIIC fibroblasts
- Compounds: siRNAs



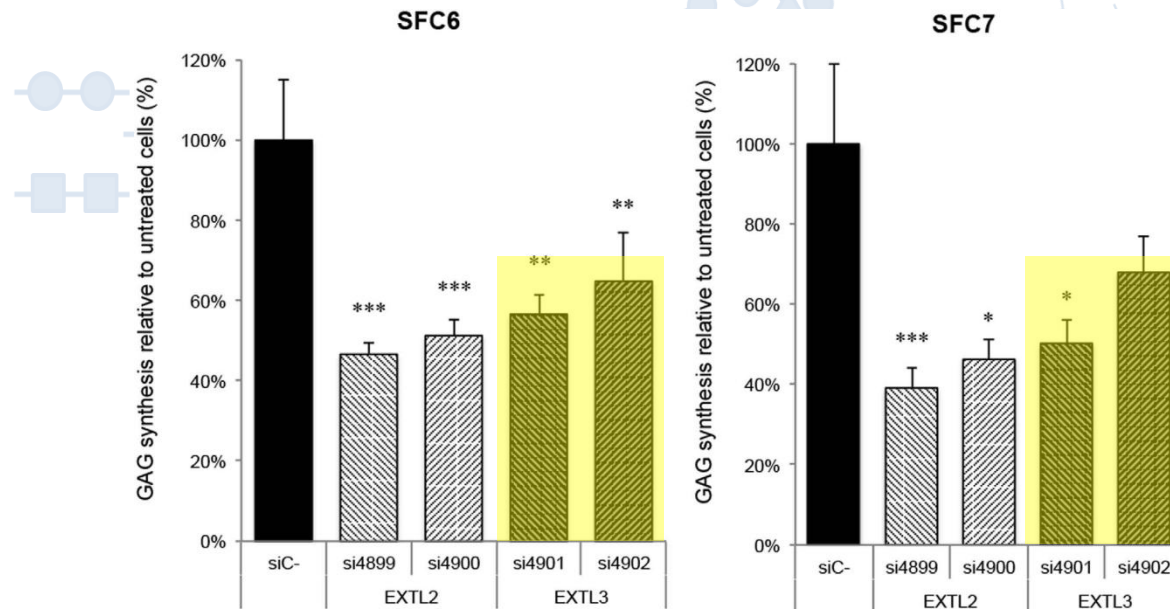
Canals *et al.*, (2015)



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gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Targets: *EXTL2* and *EXTL3*
- Test in: MPS IIIC fibroblasts
- Compounds: siRNAs



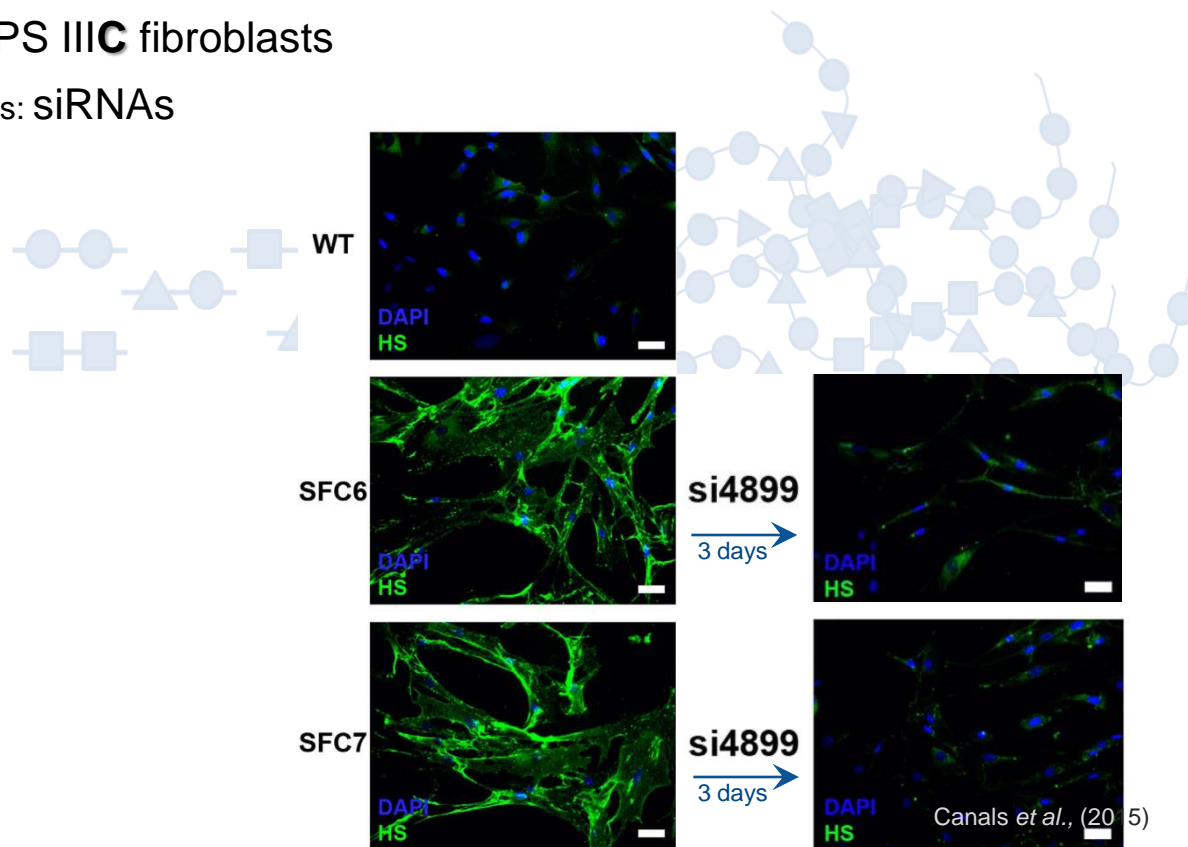
Canals *et al.*, (2015)



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gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

- Targets: *EXTL2* and *EXTL3*
- Test in: MPS IIIC fibroblasts
- Compounds: siRNAs



gSRT FOR OTHER LSDs - MUCOPOLYSACCHARIDOSES

SUMMARY

si/shRNAs

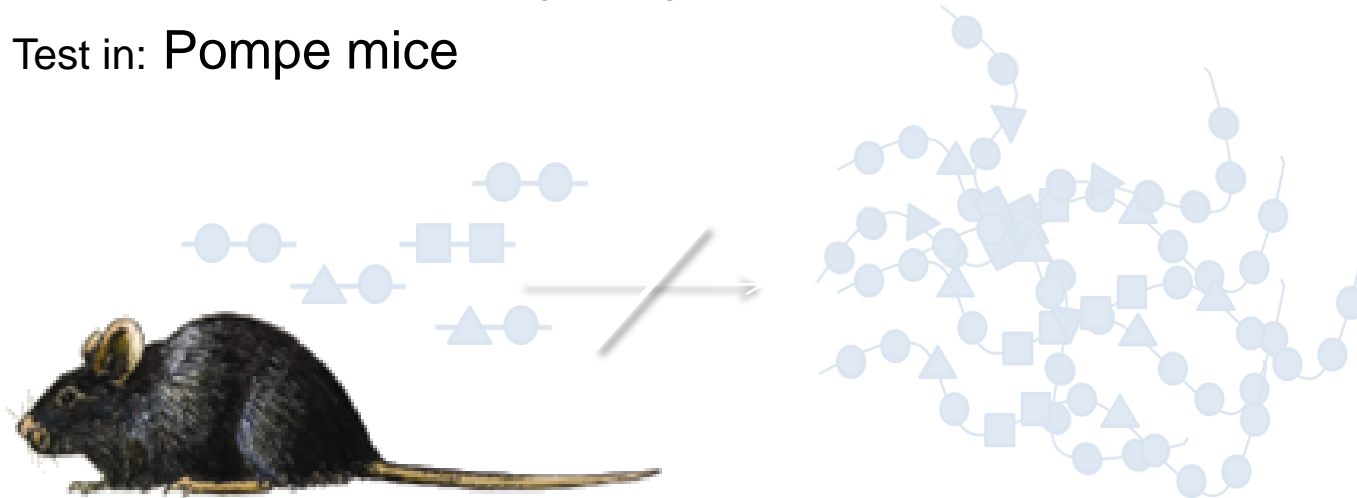
- ↓ mRNA levels
- ↓ enzyme activity
- ↓ **GAGs** synthesis

**Reverse the cellular
phenotype!!!**



gSRT FOR OTHER LSDs - POMPE

- American group
- Target: *muscle specific glycogen synthase (Gys1)*
- Test in: Pompe mice



$GAA^{-/-}$ (Charles River Laboratories, Wilmington, MA)

gSRT FOR OTHER LSDs - POMPE

- American group
- Target: *muscle specific glycogen synthase (Gys1)*
- Test in: Pompe mice
- Compound(s): GS-PPMO

phosphorodiamidate morpholino oligonucleotide
conjugated to a cell penetrating peptide

≈ therapy for Duchene muscular dystrophy



gSRT FOR OTHER LSDs - POMPE

- American group
- Target: *muscle specific glycogen synthase (Gys1)*
- Test in: Pompe mice
- Compound(s): GS-PPMO

phosphorodiamidate morpholino oligonucleotide
conjugated to a cell penetrating peptide



http://www.ensembl.org/Mus_musculus/Transcript/Summary?db=core;g=ENSMUSG00000003865;r=7:45434844-45456619;t=ENSMUST00000003964 (adapted)

Exon skipping

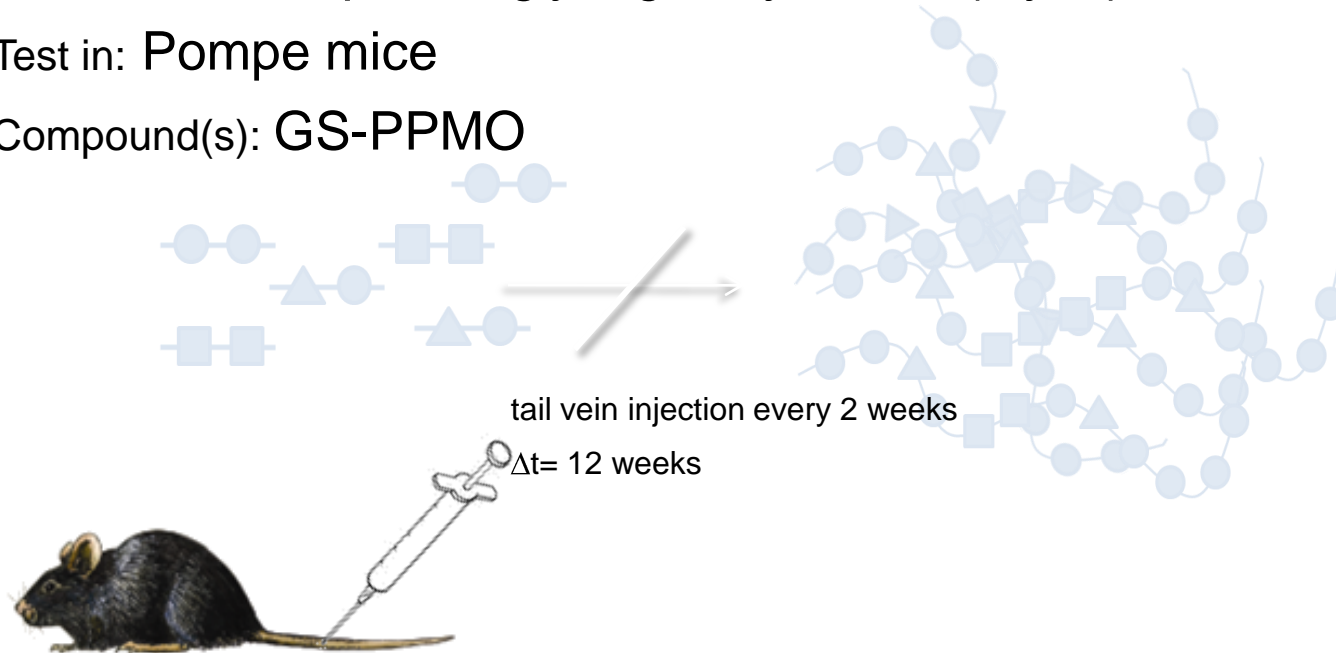
PTC



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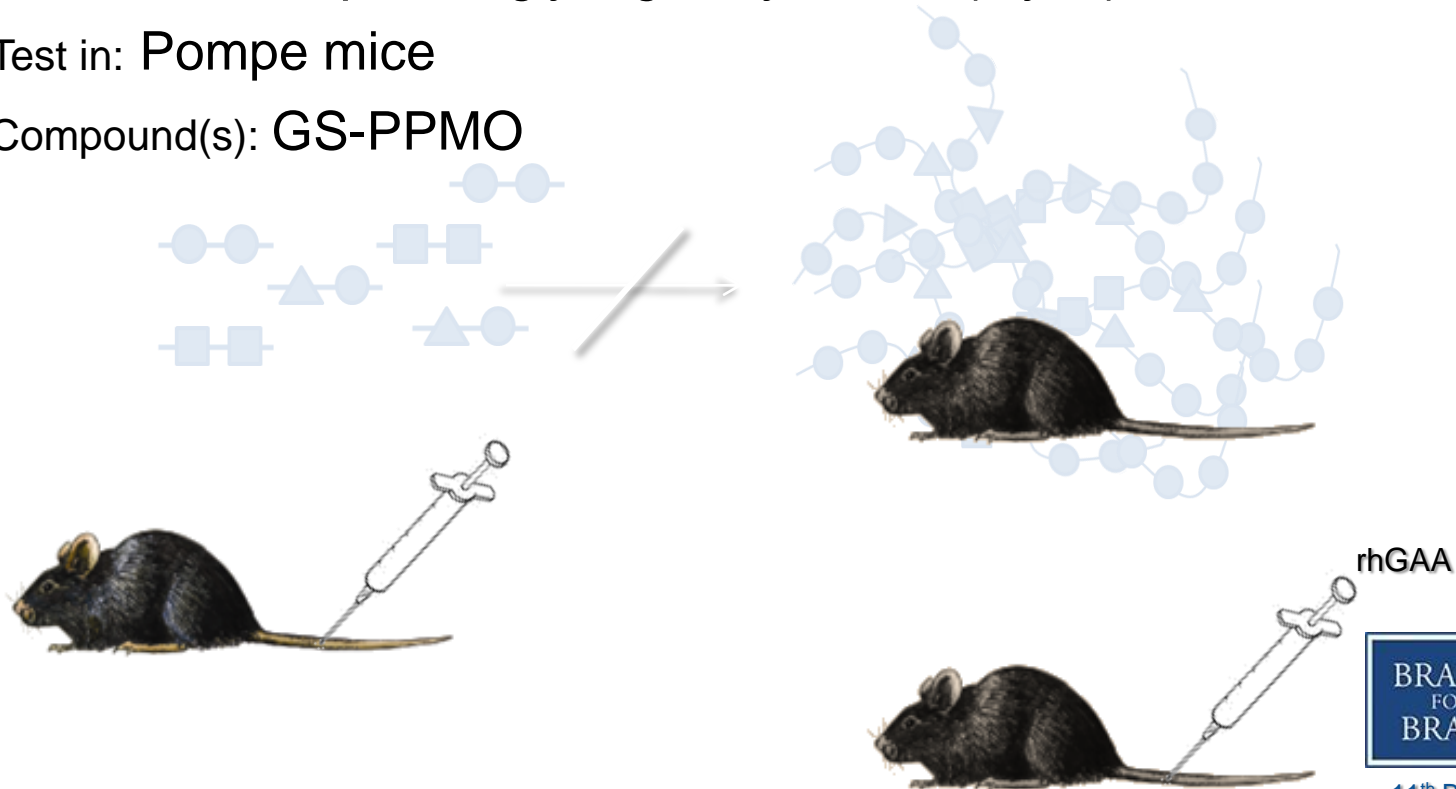
gSRT FOR OTHER LSDs - POMPE

- Target: *muscle specific glycogen synthase (Gys1)*
- Test in: Pompe mice
- Compound(s): GS-PPMO



gSRT FOR OTHER LSDs - POMPE

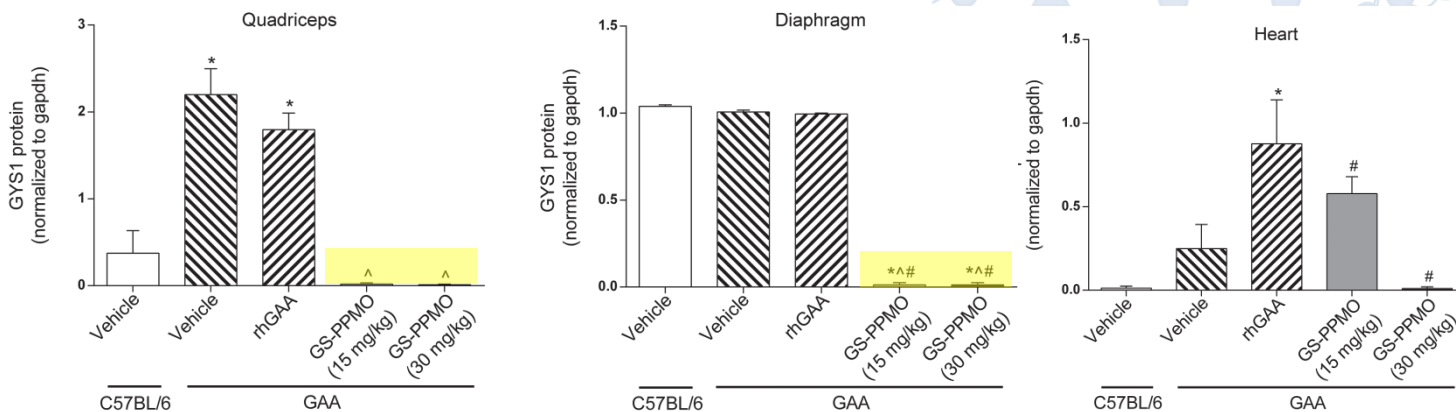
- Target: *muscle specific glycogen synthase (Gys1)*
- Test in: Pompe mice
- Compound(s): GS-PPMO



gSRT FOR OTHER LSDs - POMPE

- Target: *muscle specific glycogen synthase (Gys1)*
- Test in: Pompe mice
- Compound(s): GS-PPMO

skeletal muscles



Clayton *et al.* (2014)

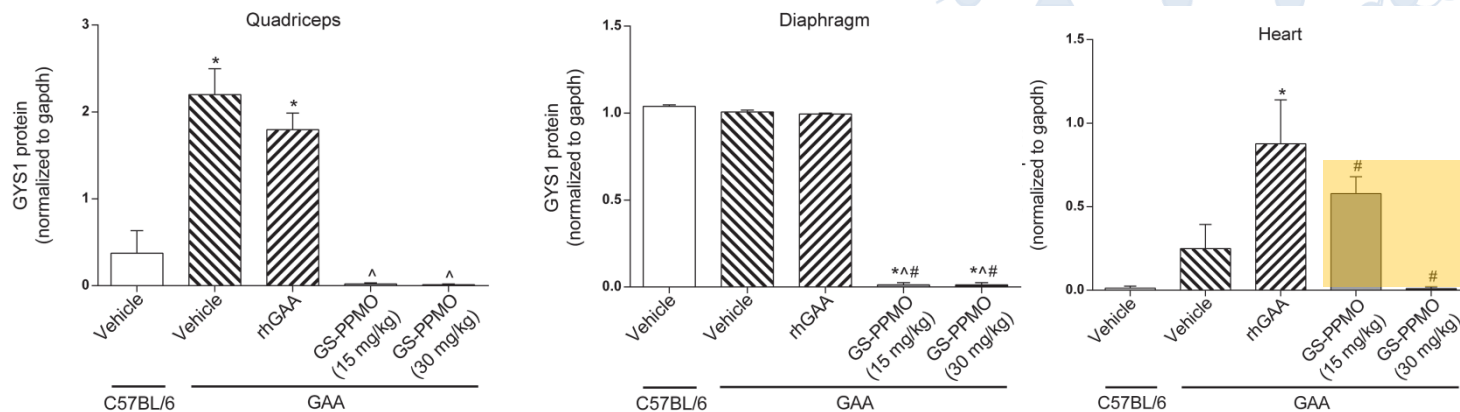


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gSRT FOR OTHER LSDs - POMPE

- Target: *muscle specific glycogen synthase (Gys1)*
- Test in: Pompe mice
- Compound(s): GS-PPMO

cardiac muscles



Clayton et al. (2014)

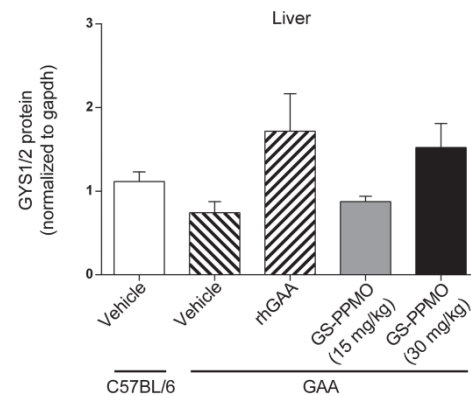


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gSRT FOR OTHER LSDs - POMPE

- Target: *muscle specific glycogen synthase (Gys1)*
- Test in: Pompe mice
- Compound(s): GS-PPMO

tissue-specific effect



Clayton *et al.* (2014)

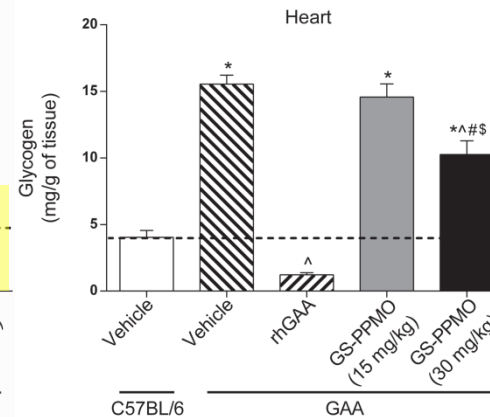
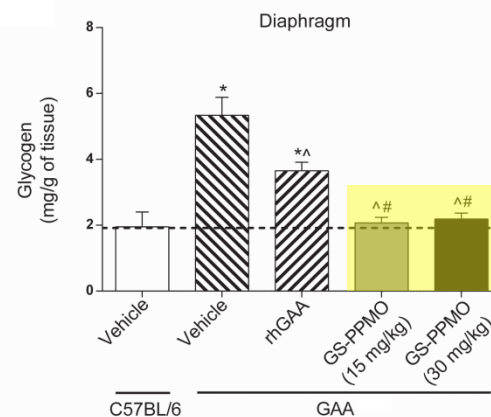
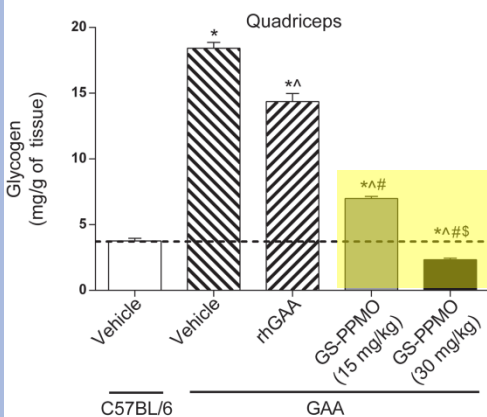


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gSRT FOR OTHER LSDs - POMPE

- Target: *muscle specific glycogen synthase (Gys1)*
- Test in: Pompe mice
- Compound(s): GS-PPMO

lysosomal glycogen accumulation



gSRT FOR OTHER LSDs - POMPE

SUMMARY

- ↓ Target protein levels
- ↓ Iglycogen levels

Tissue-specific effect



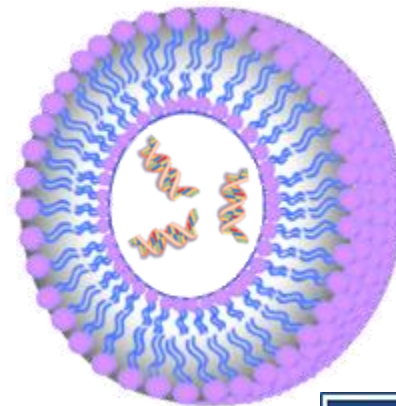
A LOOK FORWARD...

- Vector design & si/shRNA encapsulation
 - ↑ bioavailability of siRNAs;
 - protection from degradation
 - control of
 - circulation time
 - release rate

Lipid nanoparticles?

siRNA-peptide conjugates?

Cell-penetrating peptides (CPP)



BRAINS
FOR
BRAIN



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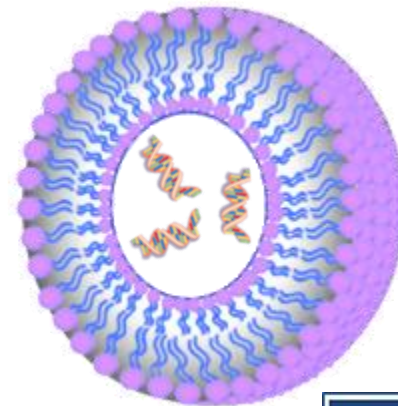
A LOOK FORWARD...

- Vector design & si/shRNA encapsulation

- ↑ bioavailability of siRNAs;
- protection from degradation
- control of
 - circulation time
 - release rate

- Genotoxic evaluation of the si/shRNA+nanocarrier formulation(s)

- internalization
- cell viability
- genotoxic potential



A LOOK FORWARD...

- *In vivo* studies



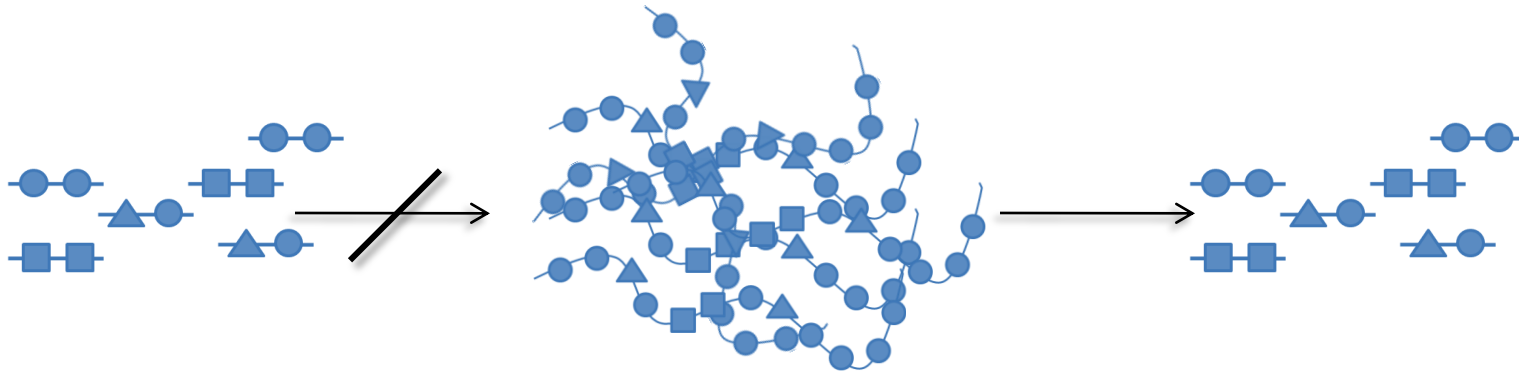
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THE OPEN QUESTIONS:

- Delivery
 - Appropriate vector
- Animal models
 - Recapitulate human disease
 - *In vivo* testing
- Mode of administration
 - local?
 - Systemic?
 - Intravenous?
 - Intrathecal?



THE FUTURE...



genetic substrate reduction + enzyme replacement



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ACKNOWLEDGMENTS

Dr. Sandra Alves

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Paulo Gaspar

FCT

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F U N D A Ç Ã O
Millennium
bcp

bcp/LIM/DGH/Dz2015



THANK YOU!

