

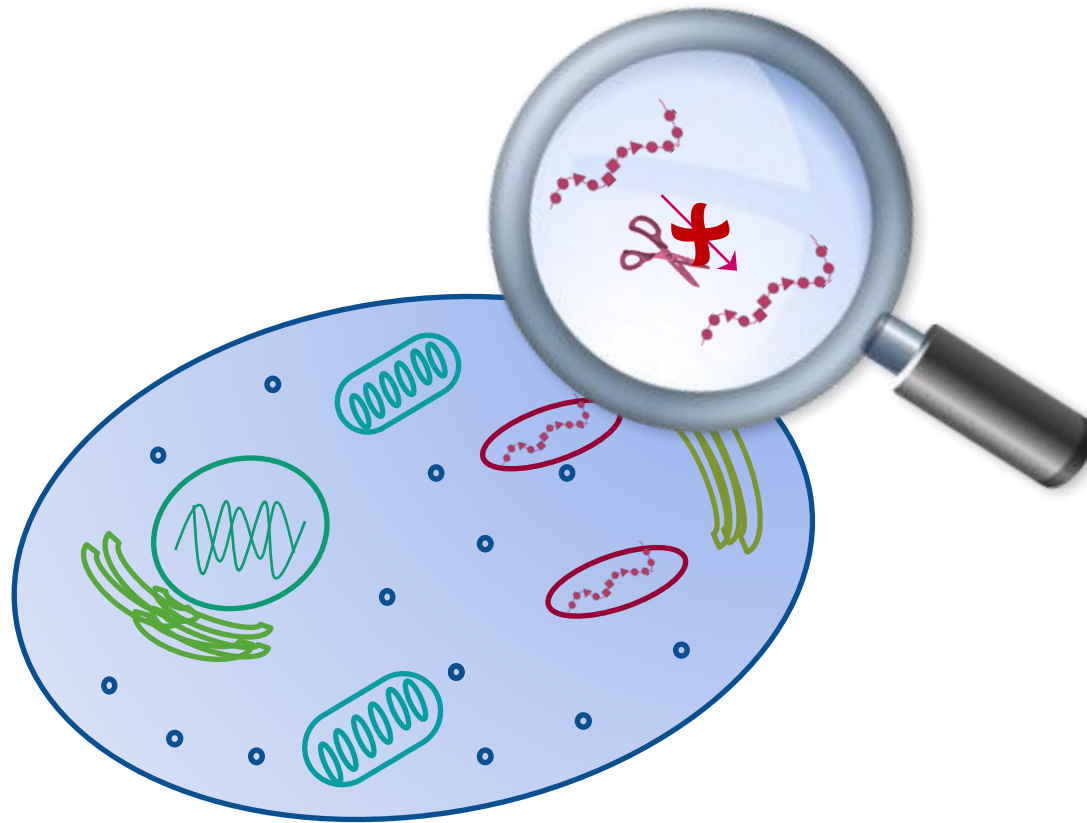


# TERAPIAS INOVADORAS NAS DLS'S: AS POSSIBILIDADES (ILIMITADAS) DA INVESTIGAÇÃO

**Maria Francisca Coutinho**

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

# O PROBLEMA...



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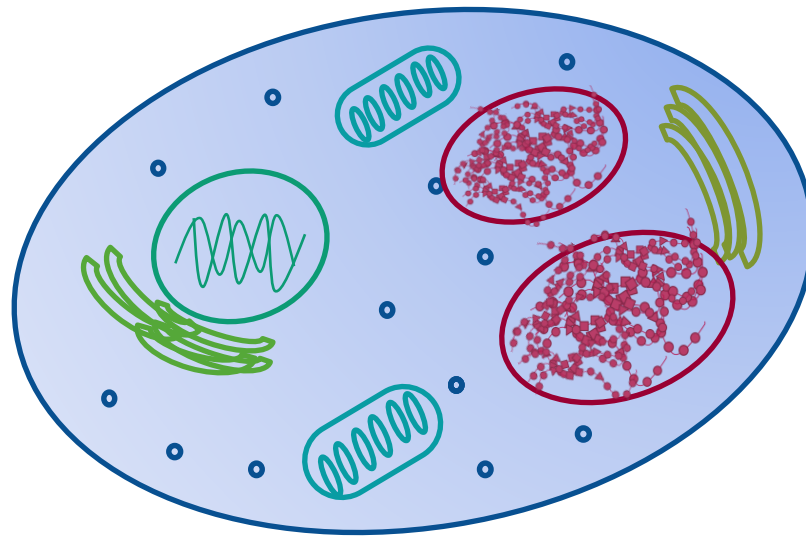
28 de Fevereiro, 2018

# O PROBLEMA...

- Acumulação progressiva



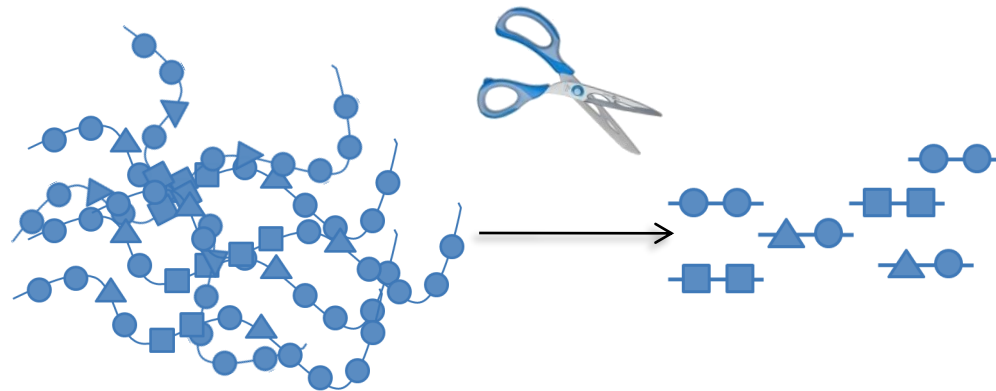
DOENÇA



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# A SOLUÇÃO?...



substituição enzimática



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# TERAPIA DE SUBSTITUIÇÃO ENZIMÁTICA

## Pathology Available ERT

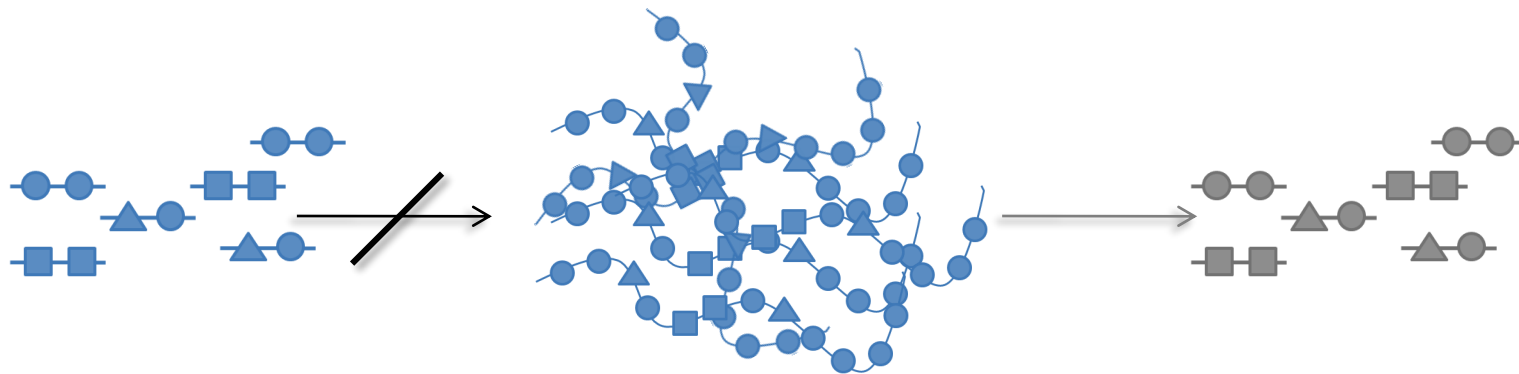
Gaucher	Cerezyme <sup>®</sup> (Imiglucerase; Genzyme)	VPRIV <sup>®</sup> (Velaglucerase alfa; Shire)	Elelyso <sup>®</sup> (Taliglucerase alfa; Pfizer)
Fabry	Replagal <sup>®</sup> (Agalsidase alfa; Shire)	Fabrazyme <sup>®</sup> (Agalsidase beta; Genzyme)	
MPS I	Aldurazyme <sup>®</sup> (Laronidase; Genzyme)		
MPS II	Elaprase <sup>®</sup> (Idursulfase; Shire)		
MPS IV A	Vimizim <sup>®</sup> (Elosulfase alfa; Biomarin)		
MPS VI	Naglazyme <sup>®</sup> (Galsulfase; Biomarin)		
Pompe	Myozyme <sup>®</sup> (Lumizyme, Alglucosidase alfa; Genzyme)		
Deficiência em LAL	Kanuma <sup>®</sup> (Sebelipase alfa; Alexion)		



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# A SOLUÇÃO?...



redução do substrato

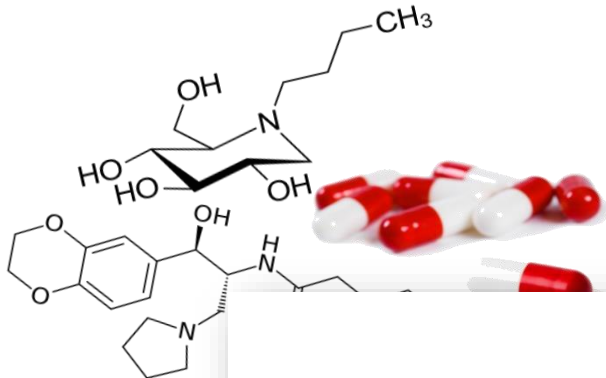
substituição enzimática



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# TERAPIA DE REDUÇÃO DO SUBSTRATO (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<sup>‡§</sup>, Gabrielle R. Neises<sup>¶</sup>, Raymond A. Dwek<sup>‡</sup>, and Terry D. Butters<sup>‡</sup>

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

THE JOURNAL OF BIOLOGICAL CHEMISTRY  
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## N-Butyldeoxyjirimycin Is a Novel Inhibitor of Glycolipid Biosynthesis but Does Not Affect the Maturation of Lysosomal Enzymes

Frances M. Platt<sup>‡§</sup>, Terry D. Butters<sup>‡</sup>, Gabrielle R. Neises<sup>¶</sup>, Raymond A. Dwek<sup>‡</sup>, and James A. Harris<sup>‡</sup>  
From the Glycobiology Institute, Department of Biochemistry, 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 maturation of lysosomal enzymes. In this study, we have investigated the effect of increasing concentrations of NB-DGJ on the maturation of lysosomal enzymes in the N-methyl and N-ethyl glycosylated substrates. However, N-propyl and N-butyl glycosylated substrates were not affected.

The galactose analogue NB-DGJ was found to be a novel inhibitor of the maturation of lysosomal enzymes in the N-methyl and N-ethyl glycosylated substrates. However, N-propyl and N-butyl glycosylated substrates were not affected.

The effect of increasing concentrations of NB-DGJ on the maturation of lysosomal enzymes in the N-methyl and N-ethyl glycosylated substrates was investigated. However, N-propyl and N-butyl glycosylated substrates were not affected.

## 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 therapeu-

~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

recognition processes and

ver, the n to be nction de ana result- ibit the ose to nthetic nd cyto d their efore of pounds ate gly- ential of

14669, 1999  
ed in U.S.A.

5, 1999)

e diphos- e, GlcCer ntified as ncluding ell recog- nyl-2-de- ts homo- e. These metabo- (2-6).

mologues decanoyl veness of th a pyr- amino-3- e inhibi- erivative group in- servation yl group ompound drawing ng P4 or vity. The



WORLD PARKINSON DISEASE DAY®

18th March / 18 de Fevereiro, 2018

# TERAPIA DE REDUÇÃO DO SUBSTRATO (SRT)

---

<b>Doença</b>	<b>SRT aprovada</b>
Gaucher	Zavesca <sup>®</sup> (Miglustat; Actelion)  Cerdelga <sup>®</sup> (Eliglustat tartrate; Genzyme)
Niemann-Pick C	Zavesca <sup>®</sup> (Miglustat; Actelion)

---



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MPS IVB Wolman Pycnodysostose  
Beta-mannosidose

Sandhoff Leucodistrofia Danon  
metacromática

Tay Sachs MPS IVA  
dase  
cosidose

Mucopolisacaridose  
tipo II + 60 doenças!

*maioria sem tratamento*

Niemann

tipo C ML III MPS IIIC Deficiência Múltipla

MPS IIIA Sialidose em sulfatases

Alfa- Krabbe MPS IIIB  
mannosidose

Galactosialidose



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# TANTO POR FAZER...

- Grande parte das DLS não tem tratamento
- Os tratamentos existentes têm várias limitações
  - Custo
  - obrigam a administrações recorrentes ao longo de toda a vida
  - não actuam sobre o SNC

...anticorpos?

...efeitos secundários?



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# AS POSSIBILIDADES (ILIMITADAS) DA INVESTIGAÇÃO...

- Abordagens mais ‘abrangentes’

*i.e. 1 terapia para 1 doença*

*(ou +!)*

- Abordagens mais ‘personalizadas’

*i.e. 1 terapia para cada mutação/*

*tipo de mutação*

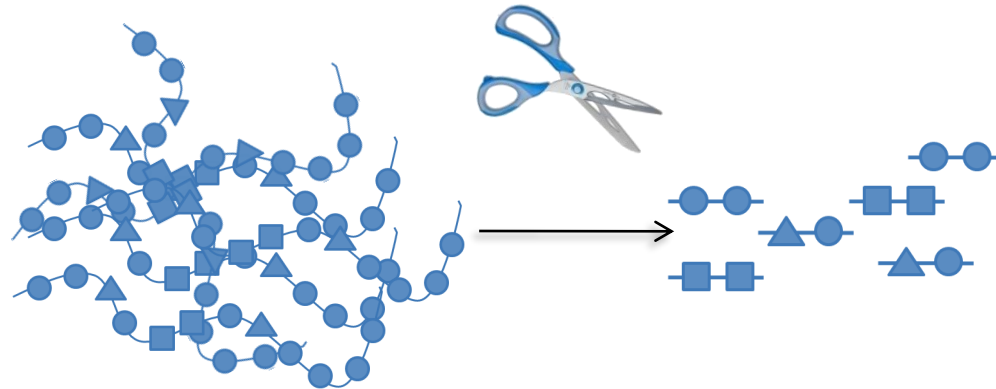


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# AS POSSIBILIDADES (ILIMITADAS) DA INVESTIGAÇÃO...

- Abordagens mais ‘abrangentes’



*substituição enzimática*

*+ eficaz*

*capaz de atravessar a BBB*

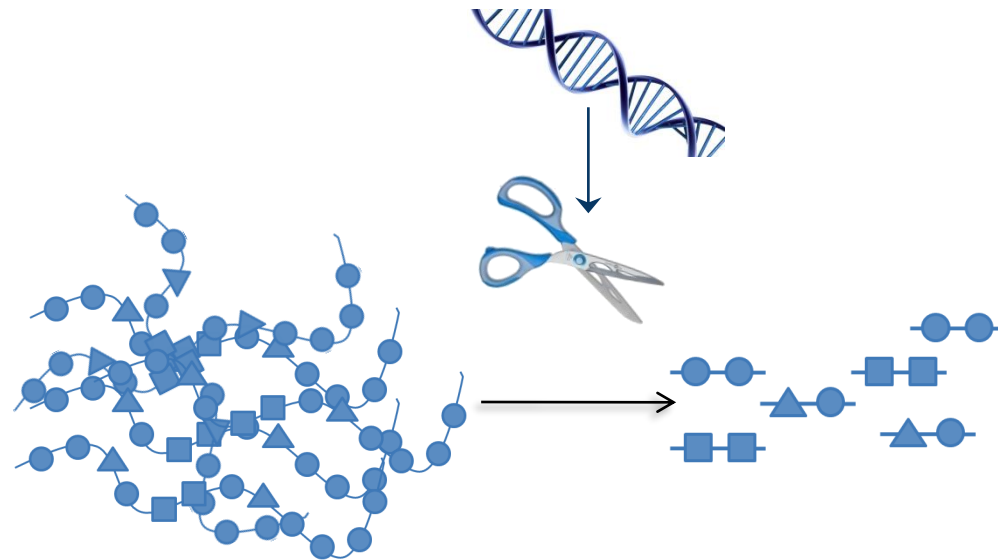


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# AS POSSIBILIDADES (ILIMITADAS) DA INVESTIGAÇÃO...

- Abordagens mais ‘abrangentes’



*terapia génica*

*Clinical trials:*

Leucodistrofia metacromática



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# AS POSSIBILIDADES (ILIMITADAS) DA INVESTIGAÇÃO...

- Abordagens mais ‘abrangentes’

*Objectivo final: cérebro*

*Como?*



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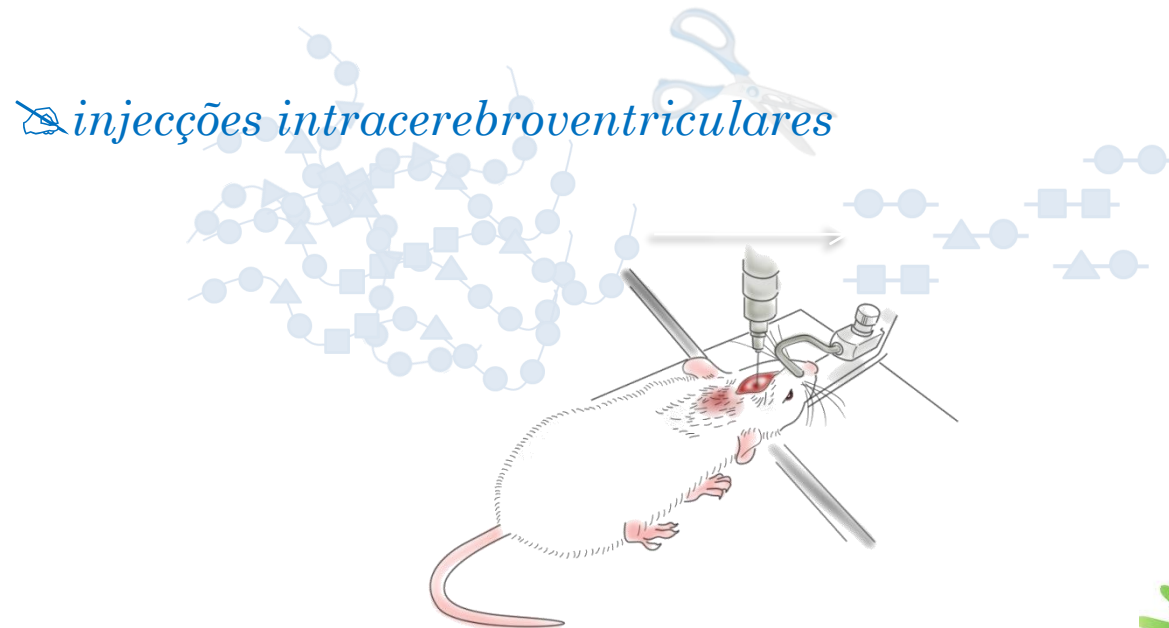
28 de Fevereiro, 2018

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- Abordagens mais ‘abrangentes’

*Objectivo final: cérebro*

*Como?*



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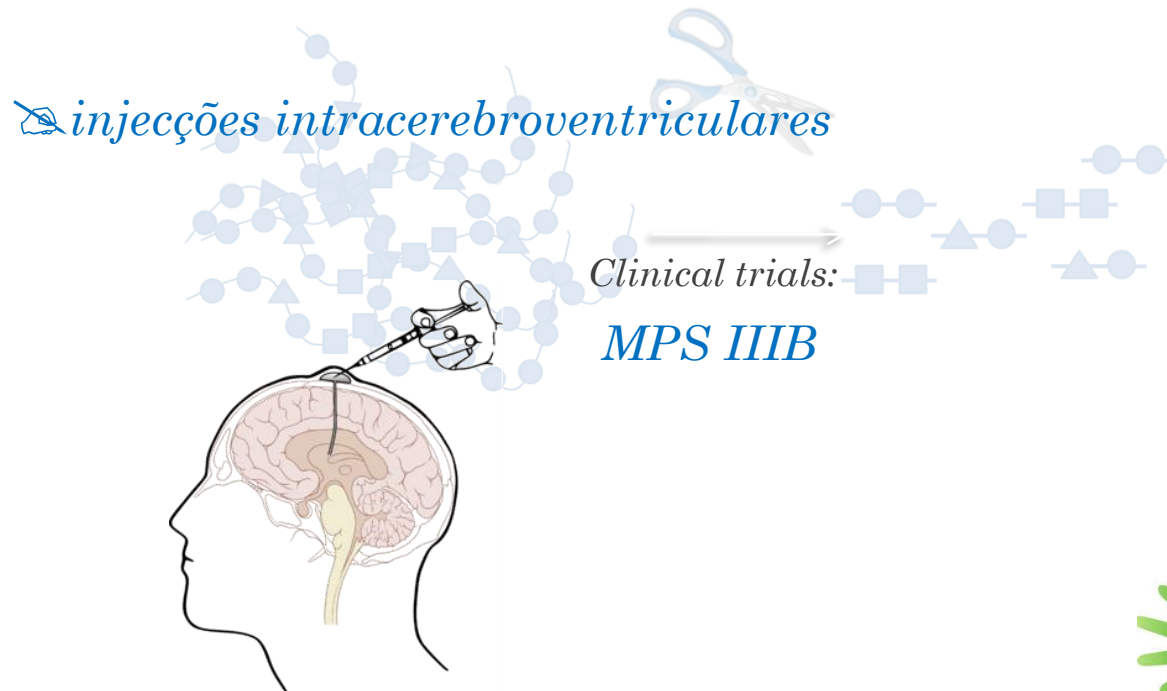
28 de Fevereiro, 2018

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- Abordagens mais ‘abrangentes’

*Objectivo final: cérebro*

*Como?*



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# AS POSSIBILIDADES (ILIMITADAS) DA INVESTIGAÇÃO...

- Abordagens mais ‘abrangentes’

*Objectivo final: cérebro*

*Como?*



*infeções*

*procedimentos cirúrgicos*



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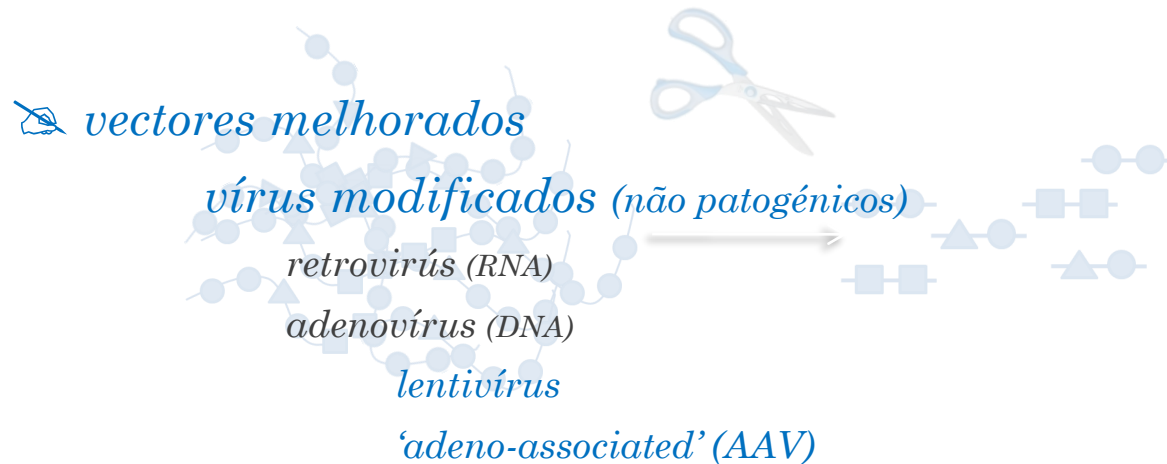
28 de Fevereiro, 2018

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- Abordagens mais ‘abrangentes’

*Objectivo final: cérebro*

*Como?*



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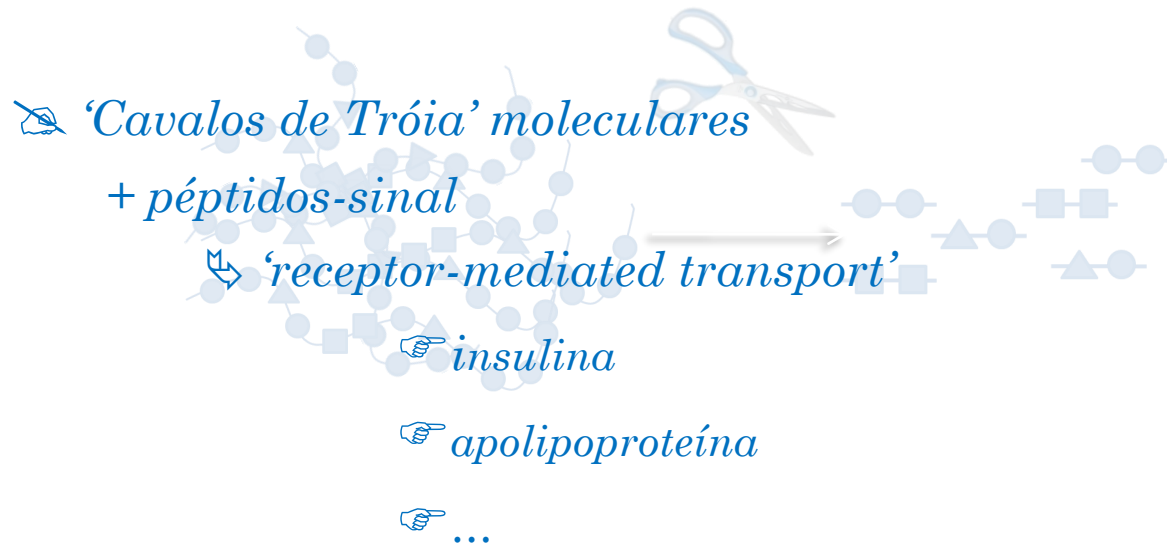
28 de Fevereiro, 2018

# AS POSSIBILIDADES (ILIMITADAS) DA INVESTIGAÇÃO...

## ○ Abordagens mais ‘abrangentes’

*Objectivo final: cérebro*

*Como?*

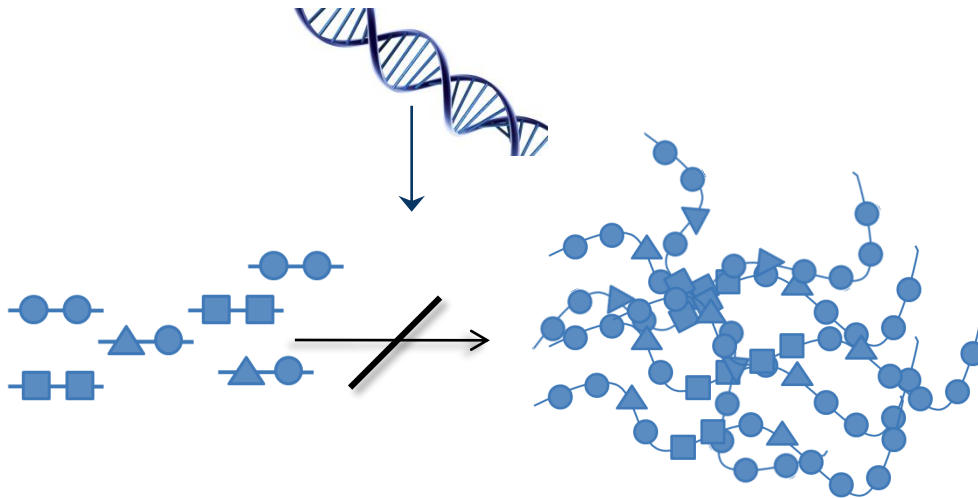


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# AS POSSIBILIDADES (ILIMITADAS) DA INVESTIGAÇÃO...

- Abordagens mais ‘abrangentes’



*redução do substrato genética*



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# AS POSSIBILIDADES (ILIMITADAS) DA INVESTIGAÇÃO...

## ○ Abordagens mais ‘personalizadas’

*Objectivo final:*

*correção / melhoria de* uma mutação  
um tipo de mutações *em particular*

*Como?*



*estabilizam proteínas mutantes*

*promovem o seu transporte até ao lisossoma*



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# AS POSSIBILIDADES (ILIMITADAS) DA INVESTIGAÇÃO...

## ○ Abordagens mais ‘personalizadas’

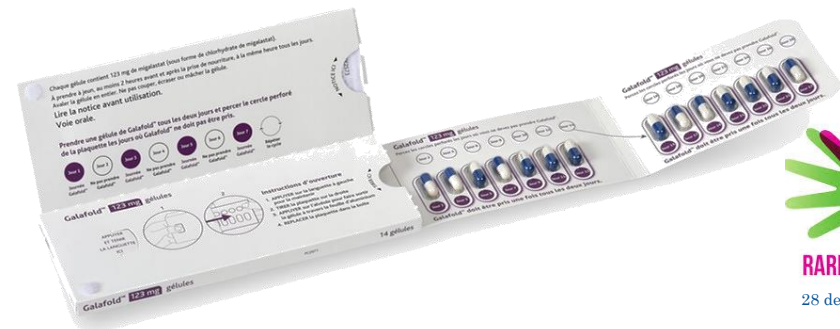
*Objectivo final:*

*correção / melhoria de* uma mutação  
um tipo de mutações *em particular*

*Como?*

 *chaperones moleculares*

**Galafold™**  
(Migalastat; Amicus Therapeutics)



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# AS POSSIBILIDADES (ILIMITADAS) DA INVESTIGAÇÃO...

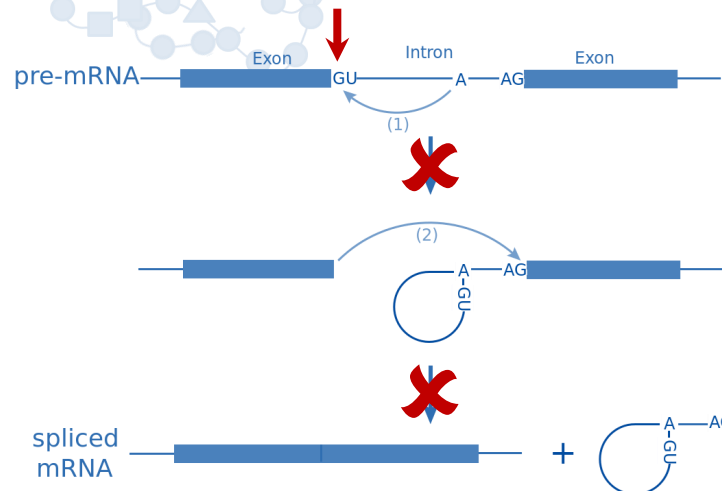
## ○ Abordagens mais ‘personalizadas’

*Objectivo final:*

*correção / melhoria de* uma mutação  
um tipo de mutações *em particular*

*Como?*

*✍ correção de mutações de splicing*



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# O nosso trabalho no INSA...

- Abordagens mais ‘abrangentes’
  - gSRT para MPS
  
- Abordagens mais ‘personalizadas’
  - correcção de mutações de *splicing* em diferentes DLS

Denominador comum:

Terapias de base genética

*RNA-based*

‘Fáceis’ de testar a nível celular – 1<sup>a</sup> fase



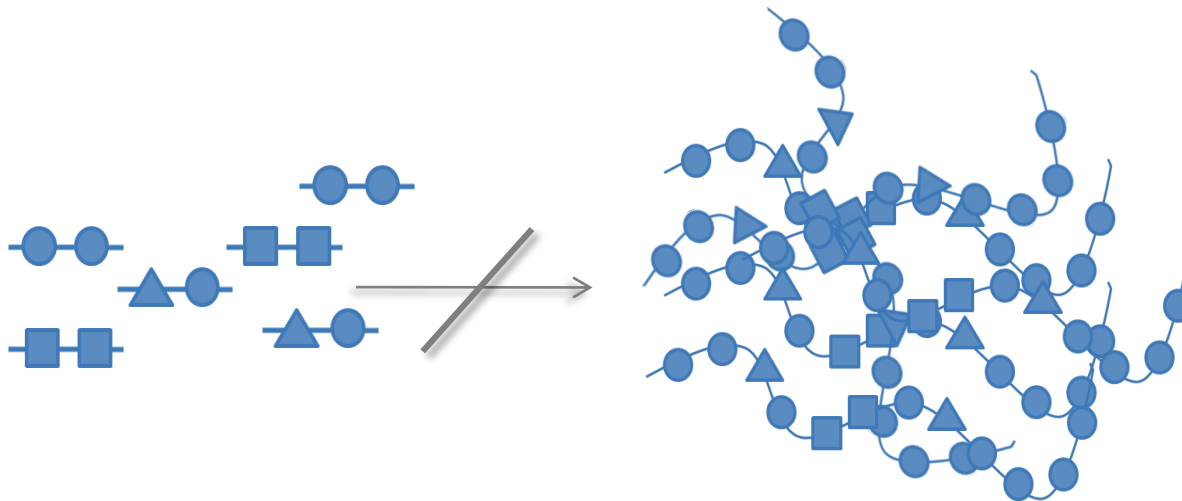
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# gSRT PARA MUCOPOLISSACARIDOSES



- Abordagem ‘abrangente’
- Alvos: *genes da biossíntese dos GAGs*

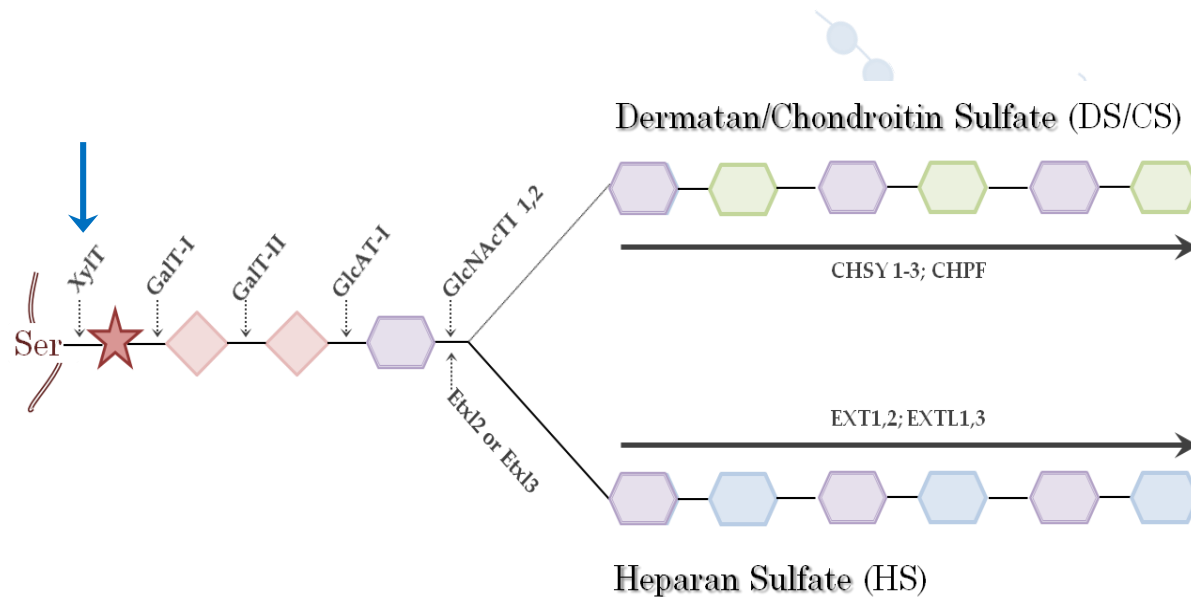


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# gSRT PARA MUCOPOLISSACARIDOSES

- Abordagem ‘abrangente’
- Alvos: *genes da biossíntese dos GAGs*

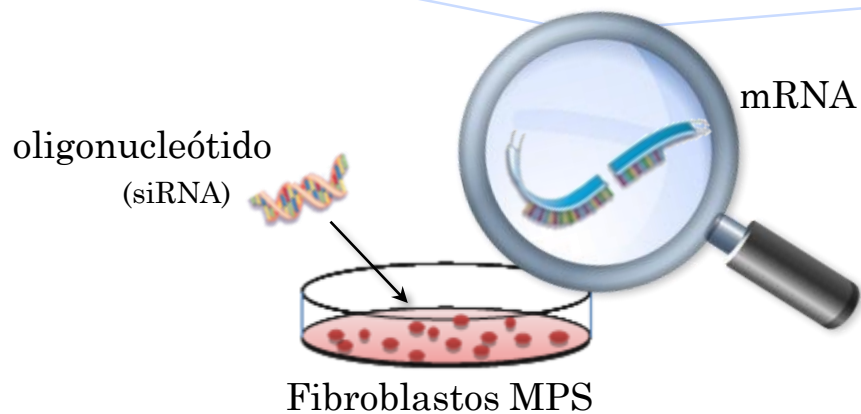
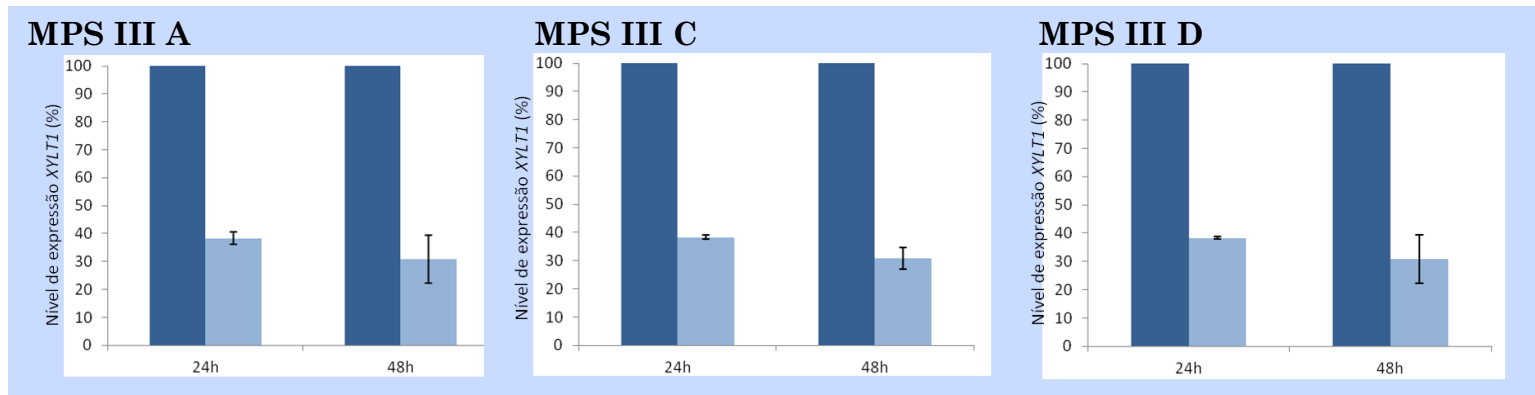


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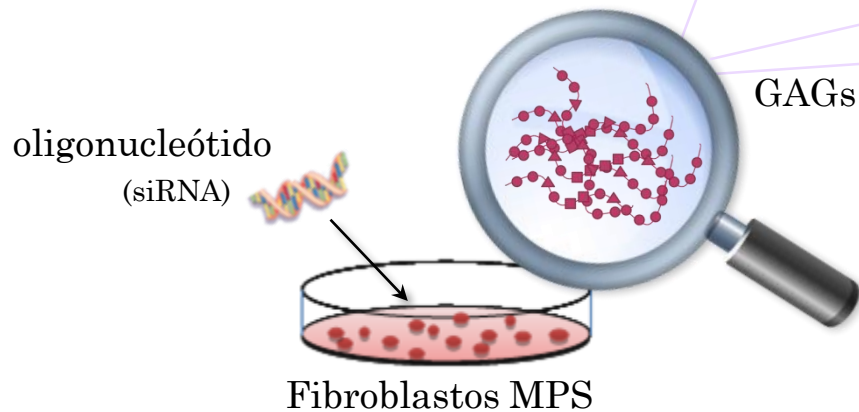
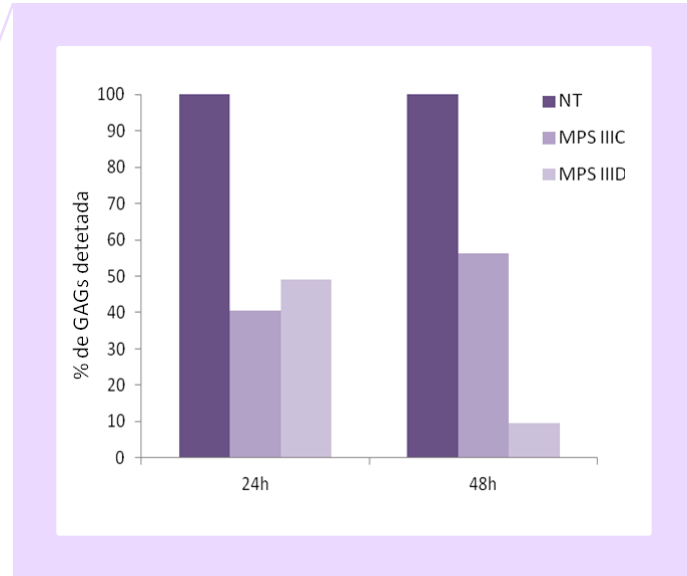
# gSRT PARA MUCOPOLISSACARIDOSES

- O exemplo das MPS III



# gSRT PARA MUCOPOLISSACARIDOSES

- O exemplo das MPS III



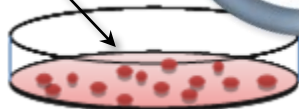
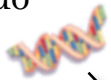
# gSRT PARA MUCOPOLISSACARIDOSES

- O exemplo das MPS III

*Resultados promissores!*

*Vale a pena continuar a estudar...*

oligonucleótido  
(siRNA)

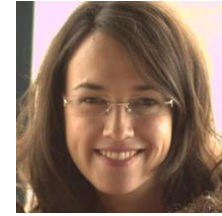


Fibroblastos MPS



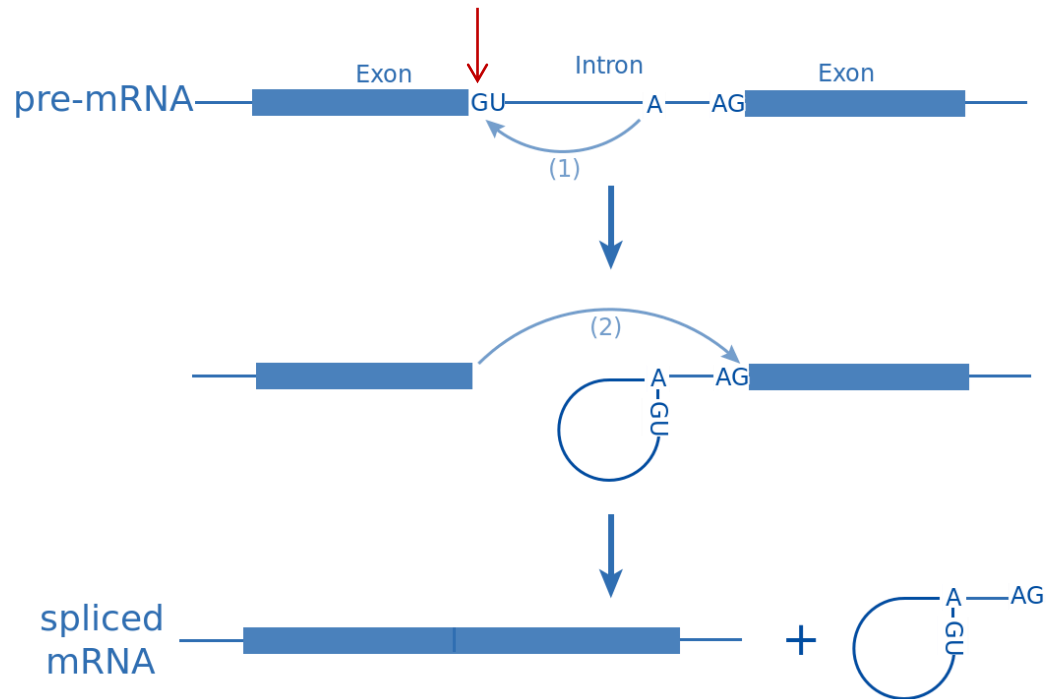
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# Terapia de *Splicing* PARA DLS

- Abordagem ‘personalizada’  
*...desde 2008*



# Terapia de *Splicing* PARA DLS

- O exemplo das MPS
- MPS III C
  - Mutações de *splicing* → frequentes (~20%)
    - ↳ 1 bastante frequente em doentes

Portugueses

Espanhóis

Marroquinos



**c.234+1G>A**



*bons motivos para testar uma  
terapia!*

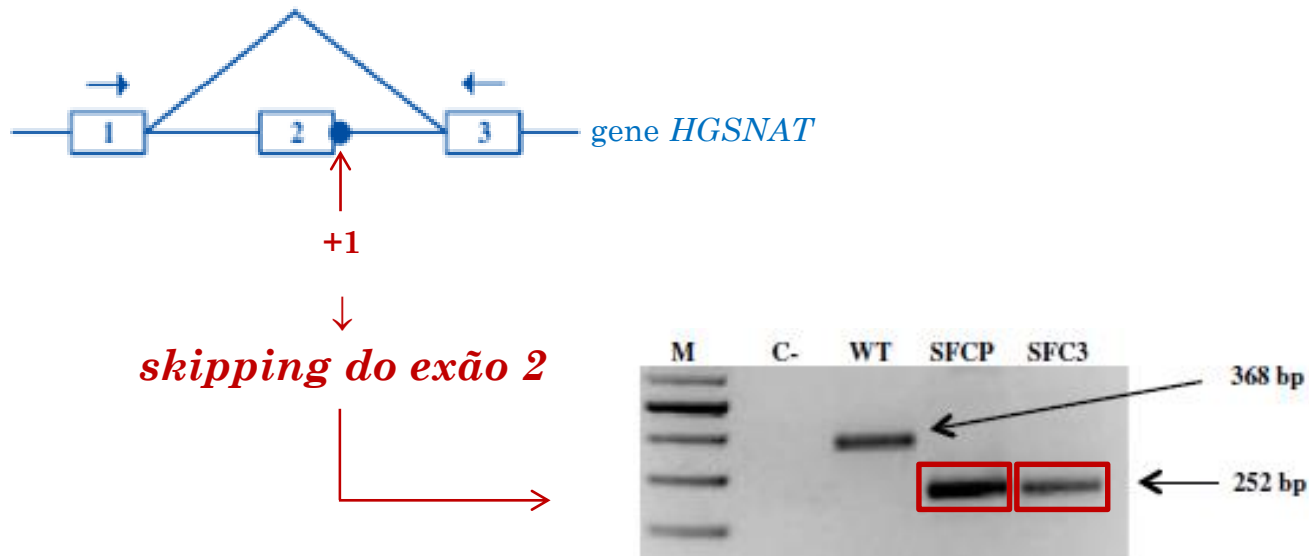


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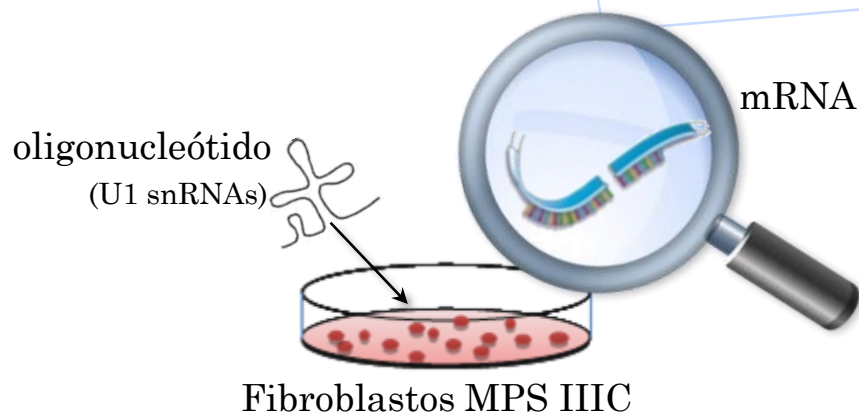
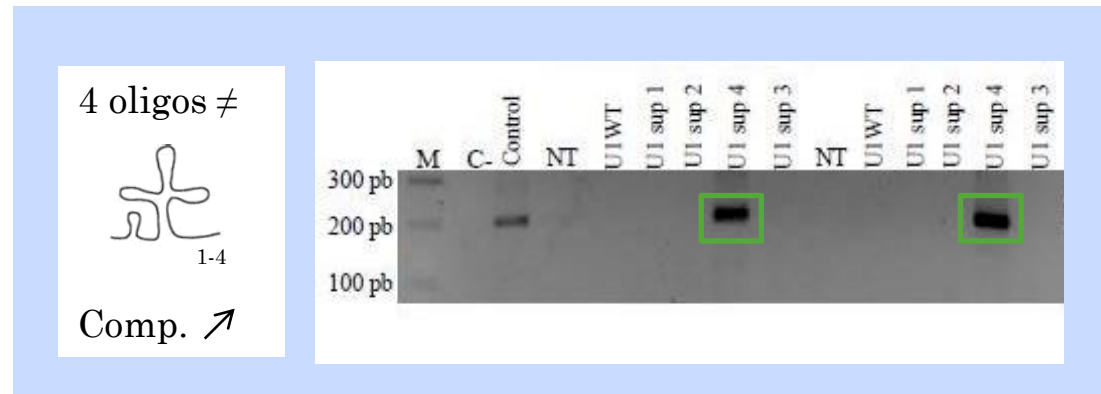
# Terapia de *Splicing* PARA DLS

- O exemplo das MPS
- MPS III C



# Terapia de *Splicing* PARA DLS

- O exemplo das MPS
- MPS III C



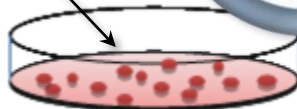
# Terapia de *Splicing* PARA DLS

- O exemplo das MPS
- MPS III C

*Resultados promissores!*

*Vale a pena continuar a estudar...*

oligonucleótido  
(U1 snRNAs)



Fibroblastos MPS IIIC



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# DAQUI EM DIANTE...

- Estudos *in vivo*



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# DAQUI EM DIANTE...

- Estudos *in vivo*



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# DAQUI EM DIANTE...

- Estudos *in vivo*

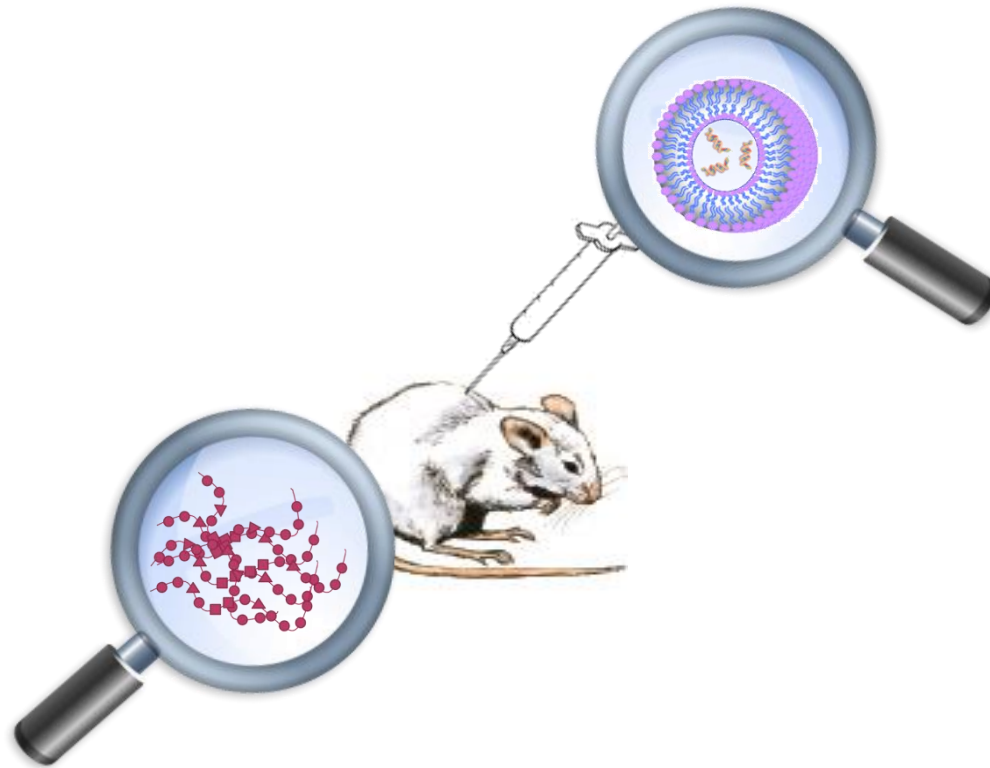


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# DAQUI EM DIANTE...

- Estudos *in vivo*



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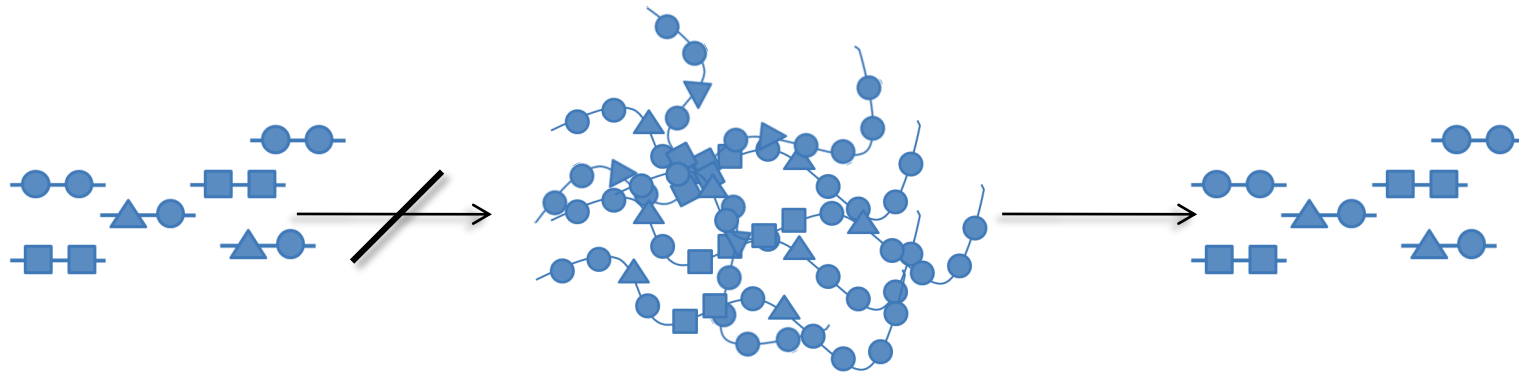
28 de Fevereiro, 2018

# DAQUI EM DIANTE...

- Estudos *in vivo*



# O(s) FUTURO(S)...



gSRT

+

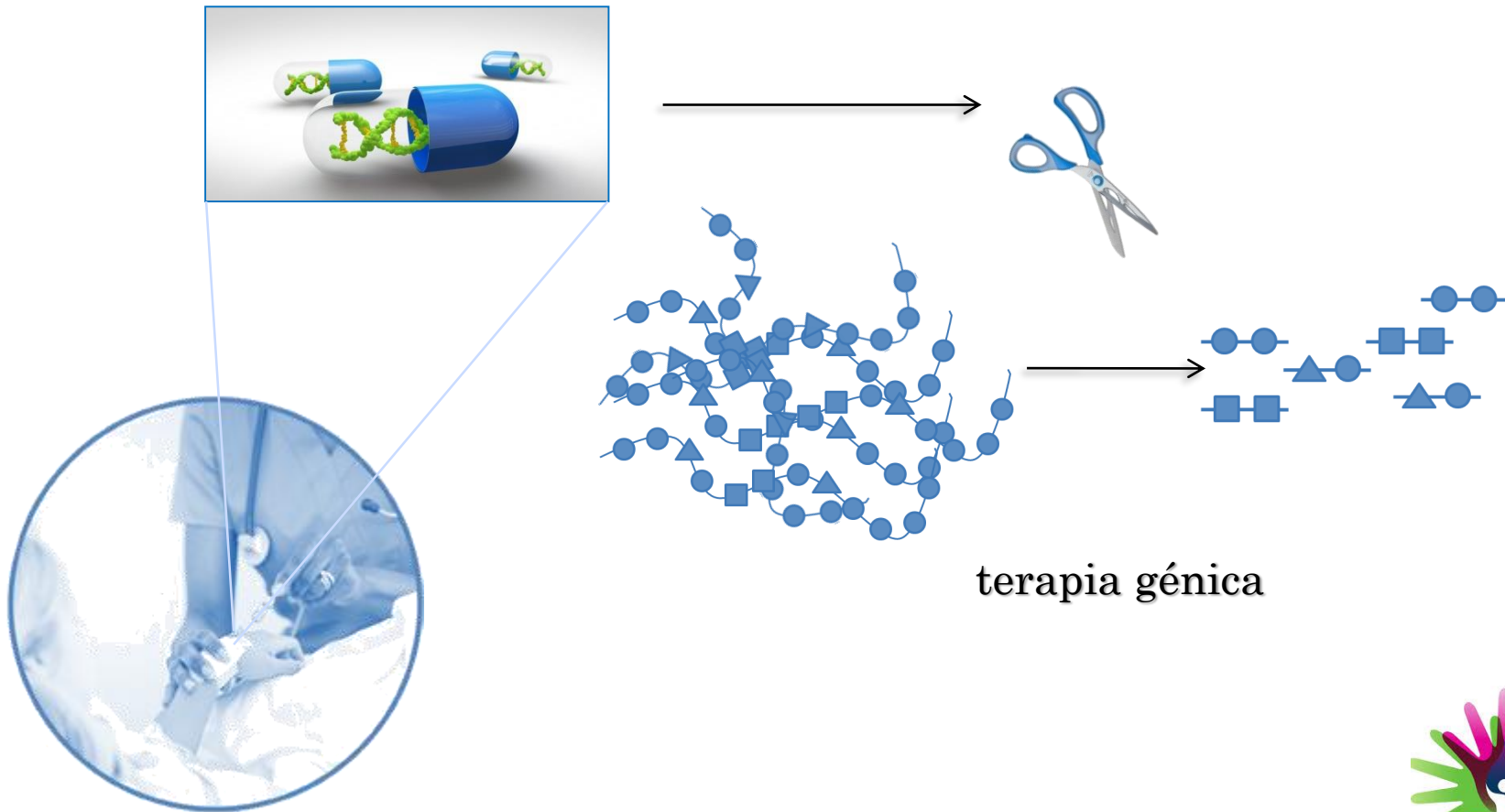
substituição enzimática



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# O(s) FUTURO(S)...

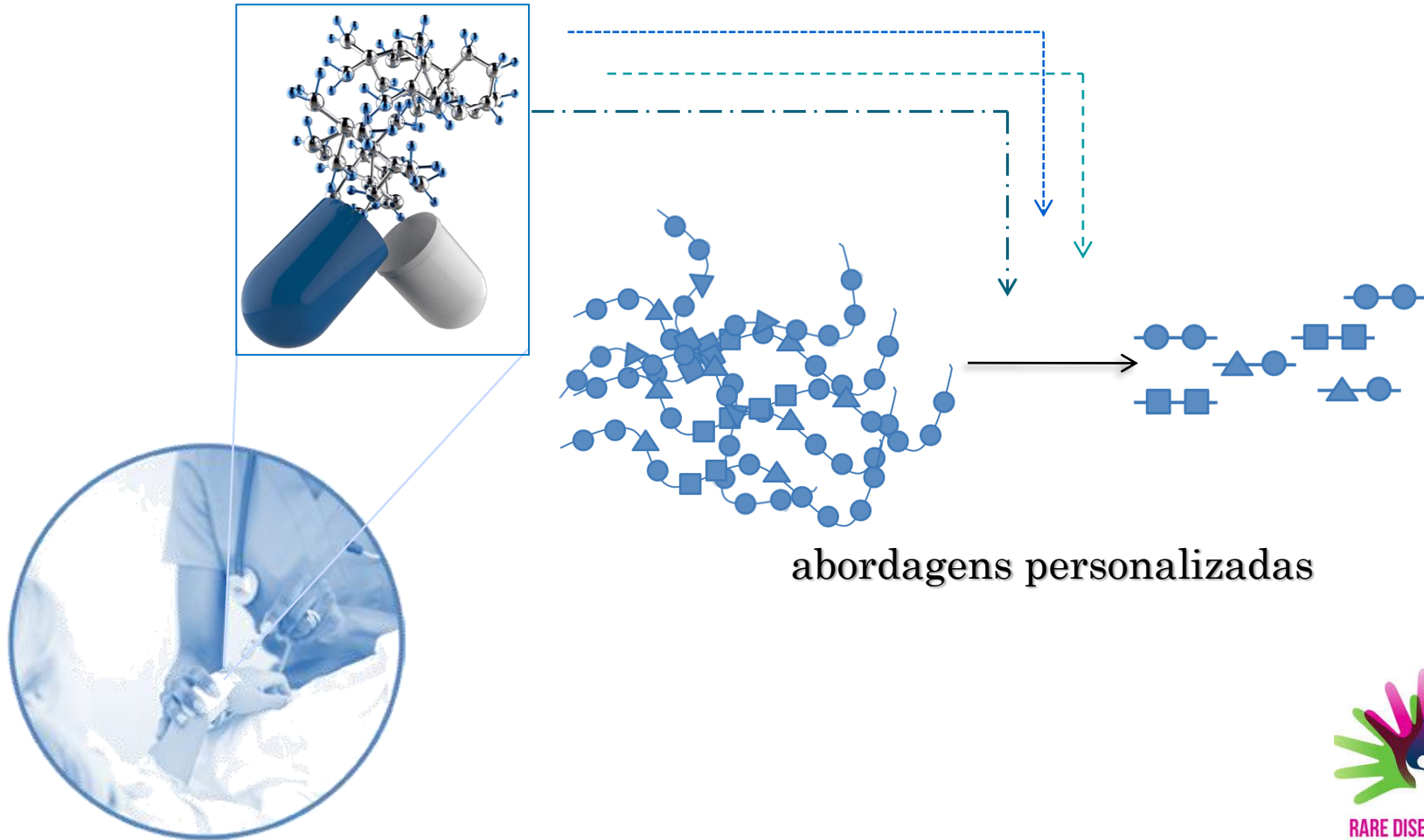


terapia génica



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# O(s) FUTURO(S)...



abordagens personalizadas



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# AGRADECIMENTOS

**Dr. Sandra Alves**

**Prof. M<sup>a</sup> João Prata**

**Juliana Inês Santos**

Paulo Gaspar

**Liliana Matos**

Regina Vilela

## FCT

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MINISTÉRIO DA CIÊNCIA, INOVAÇÃO E DO ENSINO SUPERIOR

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F U N D A Ç Ã O  
**Millennium**  
bcp

bcp/LIM/DGH/Dz2015



**António Reis ♥**

# MUITO OBRIGADA!



**RARE DISEASE DAY®**  
16 de Fevereiro, 2018