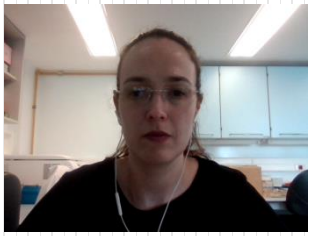

Antisense oligonucleotide exon-skipping as a therapeutic approach for Mucolipidosis type II α/β: *in vitro* and *in vivo* studies

Liliana Matos[a,b*], Mariana Gonçalves[a,d*], Juliana I. Santos[a,b,c], Mª Francisca Coutinho[a,b], Mª João Prata[c,e], Mª João Pires[d], Paula Oliveira[d], Sandra Alves[a,b]

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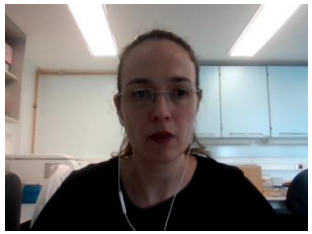
* These authors contributed equally to this work

25 ANOS Virtual ANNUAL MEETING SOCIEDADE PORTUGUESA DE GENÉTICA HUMANA 18-19 NOV 2021

Background



Mucolipidosis type II α/β (ML II α/β)

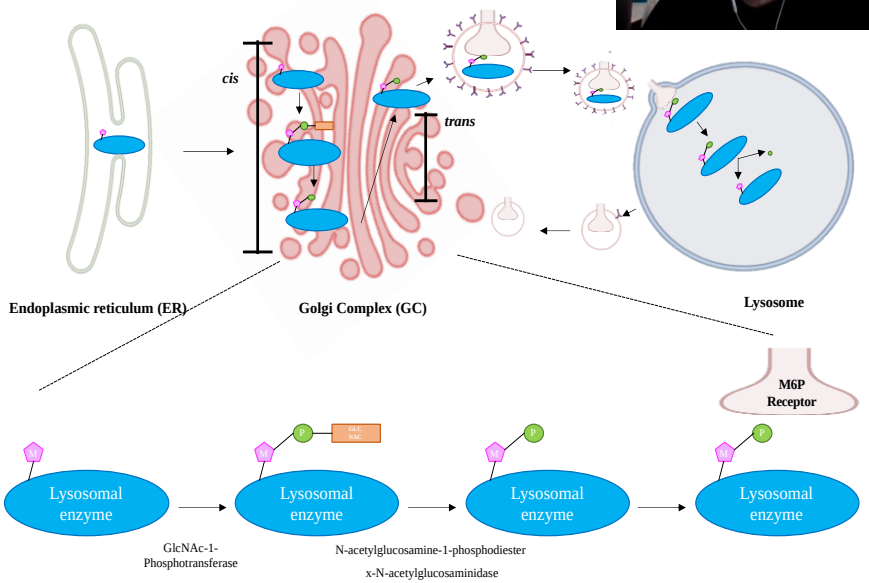
→ Rare and severe Lysosomal Storage Disorder

↓

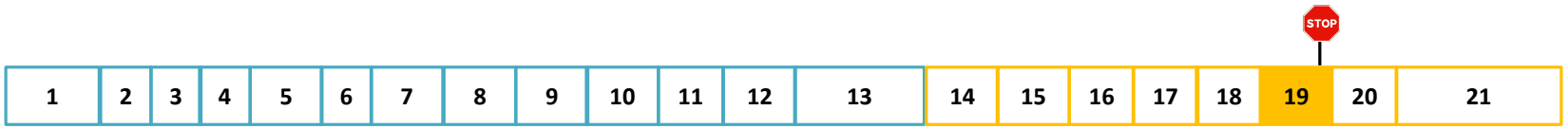
Caused by the deficiency of the enzyme GlcNAc-1-phosphotransferase

↓

Involved in lysosomal enzymes traffic to lysosome



- In ML II α/β, the mutation (c.3503_3504del) in *GNPTAB* exon 19 is the most frequent, making it a good target for a specific mutation therapy as there is no therapy for this disease



GNPTAB transcript

c.3503_3504delTC

↓

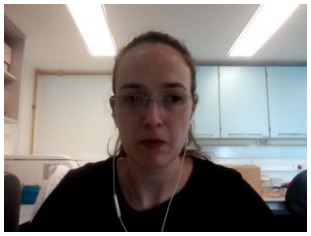
Absence of GlcNAc-1-phosphotransferase activity

Background

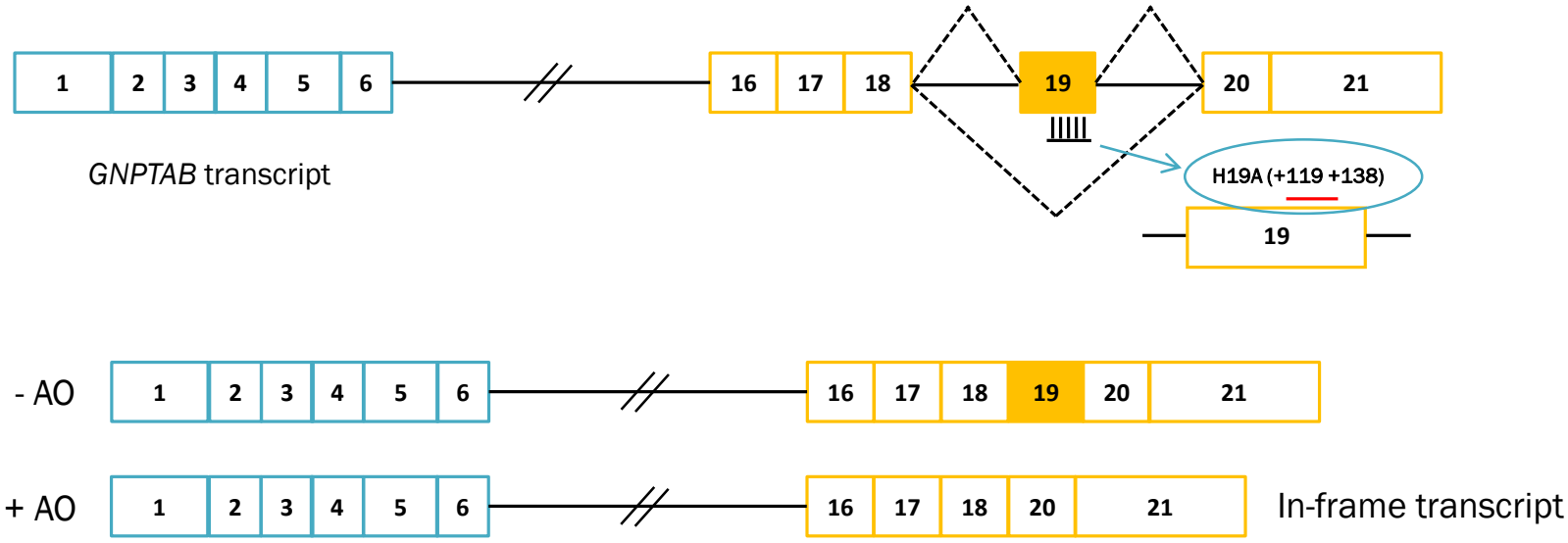
> Hum Gene Ther. 2020 Jul;31(13-14):775-783. doi: 10.1089/hum.2020.034. Epub 2020 May 20.

Development of an Antisense Oligonucleotide–Mediated Exon Skipping Therapeutic Strategy for Mucolipidosis II: Validation at RNA Level

Liliana Matos [1,2], Regina Vilela [1], Melissa Rocha [1], Juliana I Santos [1,3], Maria Francisca Coutinho [1,2], Paulo Gaspar [4], Maria João Prata [3,5], Sandra Alves [1,2]

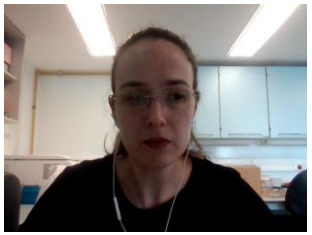


- In a previous *in vitro* study in ML II α/β patient fibroblasts, Antisense Oligonucleotides (AOs) were used to promote the exon 19 skipping from the *GNPTAB* pre-mRNA, resulting successfully in the production of an in-frame mRNA



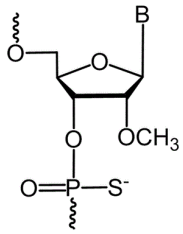
→ Currently, our goal is to evaluate the therapeutic potential of this approach, both *in vitro* in C57BL/6 fibroblasts and *in vivo* in C57BL/6 mice

Methodology

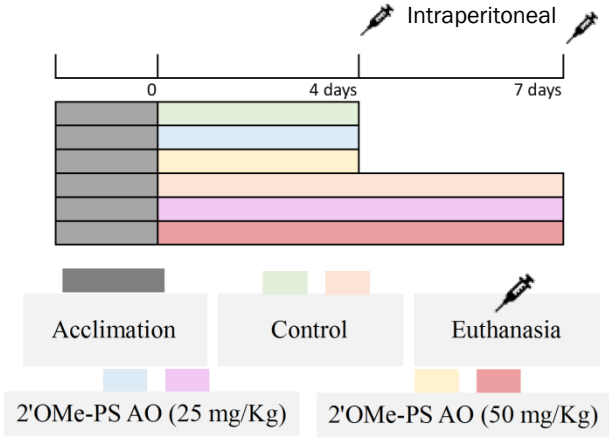


In vitro

In vivo



- **C57BL/6 mouse fibroblasts** were seeded in 6-well plates and subsequently **transfected** with concentrations of **2'OMe-PS AO** (targeting exon 19) ranging from 10 nM to 600 nM

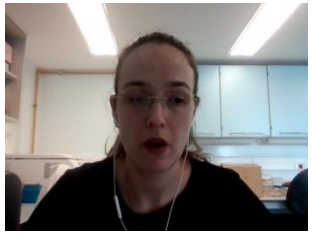


After 24/48 hours → Pellet collection

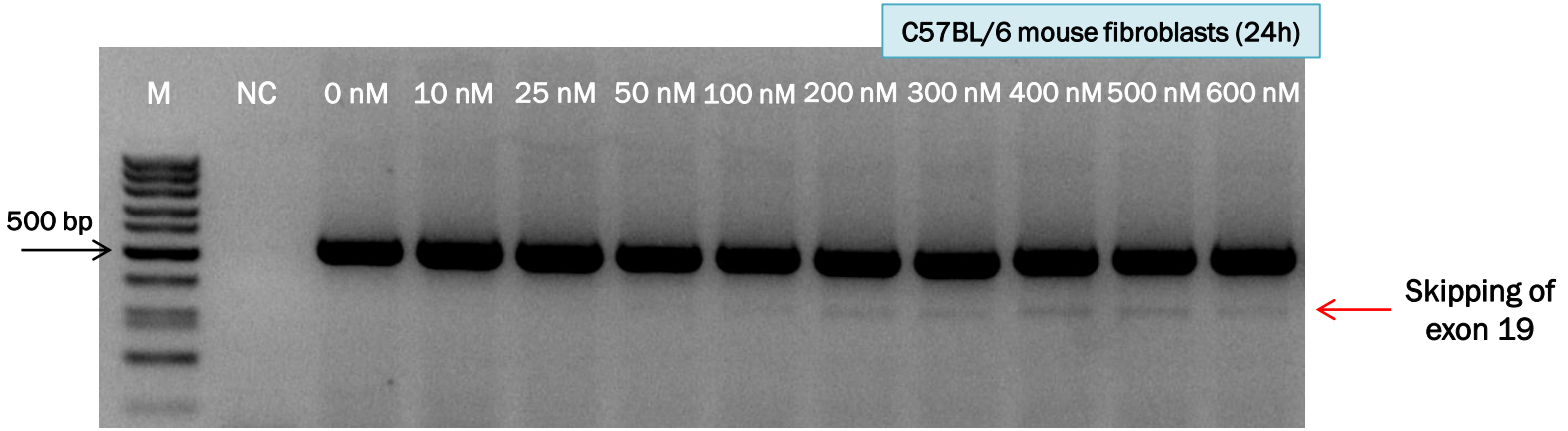
Liver collection ← Animal sacrifice after 4 or 7 days

1. RNA extraction
2. cDNA synthesis
3. PCR analysis

Results



In vitro

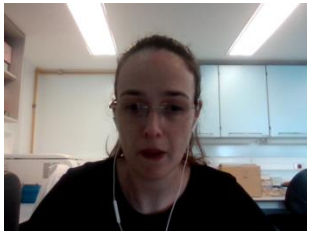


After sequencing, the skipping of exon 19 was verified at concentrations above 100 nM, although in small amounts

→ The 2'OMe-PS AO was able to modulate exon 19 splicing

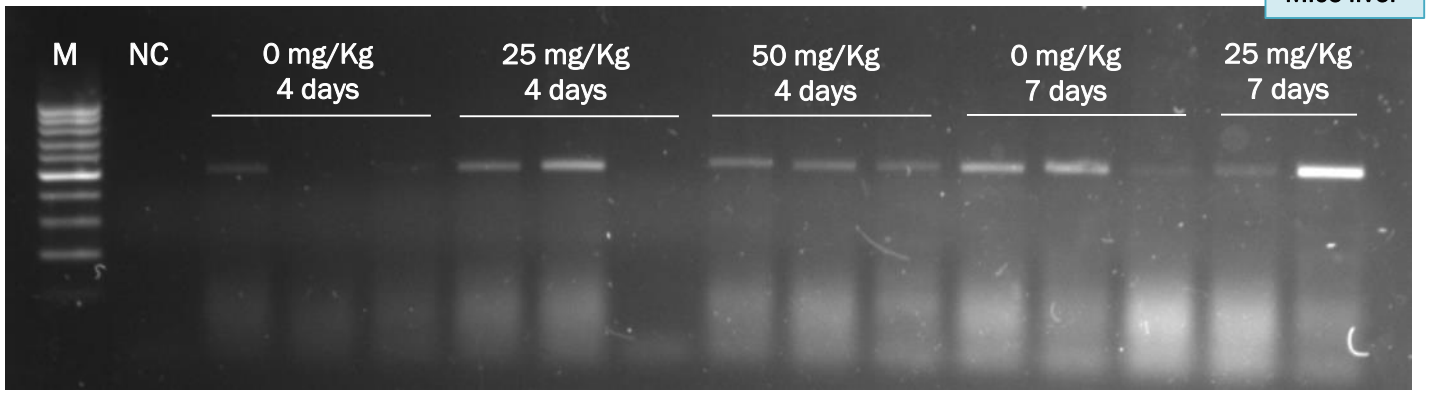
→ Similar results were observed at 48 hours

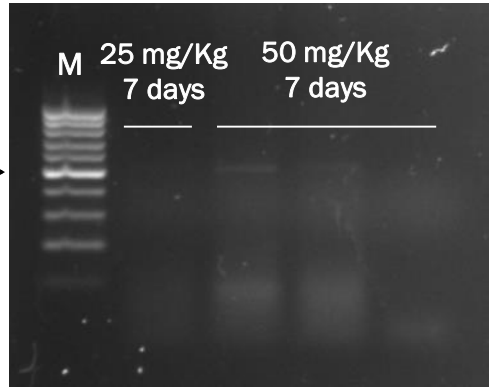
Results



In vivo

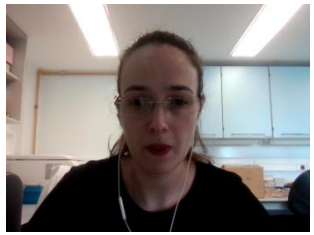
Mice liver





After molecular analysis, exon 19 skipping was not observed using either of the tested doses or incubation periods

Conclusions



In vitro

In vivo

▶ The 2'OMe-PS AO targeting a region on the 2nd half of mouse exon 19 was able to remove exon 19 from the final *GNPTAB* mRNA

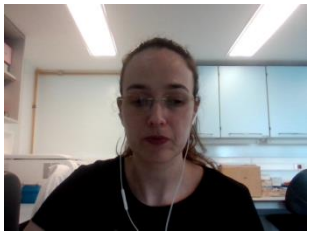
▶ To explain these first *in vivo* results, we can theorize that :

✓ The doses administered were not sufficient to achieve a response

✓ The AO may have had a high clearance rate

→ Other experiments will be done in the near future to overcome these preliminary data

Acknowledgments



Lysosomal storage disorders research group

- Sandra Alves (PI)
- Mariana Gonçalves
- Francisca Coutinho
- Juliana Inês Santos

Former members:

- Regina Vilela
- Melissa Rocha

Collaborators:

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MINISTÉRIO DA CIÊNCIA, INOVAÇÃO E DO ENSINO SUPERIOR

SOCIEDADE PORTUGUESA DE DOENÇAS METABÓLICAS

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Asociación NOUR de A.N.M.I. Mucolipidosis