

# Antisense oligonucleotide exon-skipping as a therapeutic approach for Mucopolidosis type II $\alpha/\beta$ : *in vitro* and *in vivo* studies

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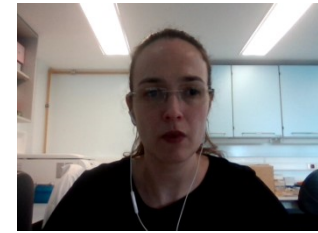
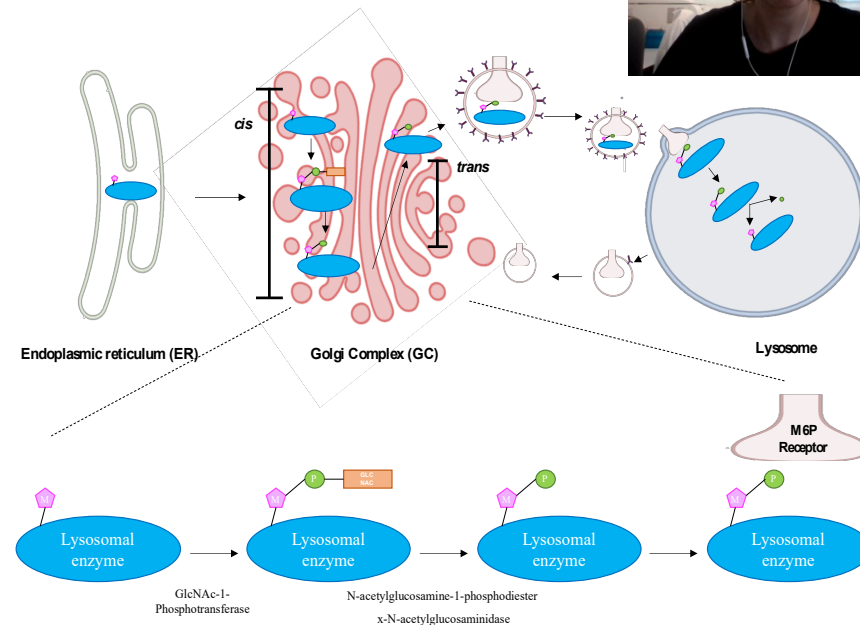
# Background

## Mucopolipidosis type II $\alpha/\beta$ (ML II $\alpha/\beta$ )

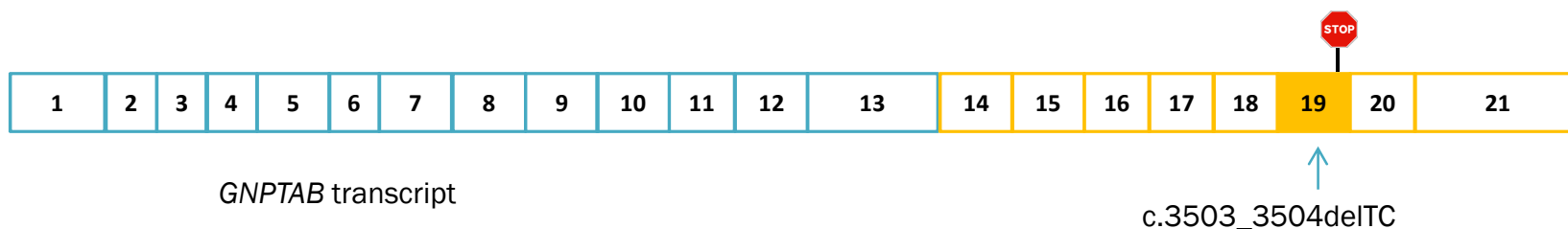
Rare and severe Lysosomal Storage Disorder

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

Involved in lysosomal enzymes traffic to lysosome



- In ML II  $\alpha/\beta$ , 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



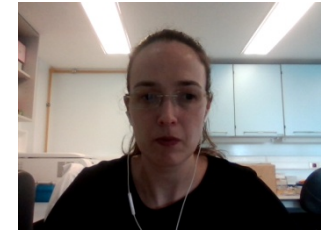
Absence of GlcNAc-1-phosphotransferase activity

# Background

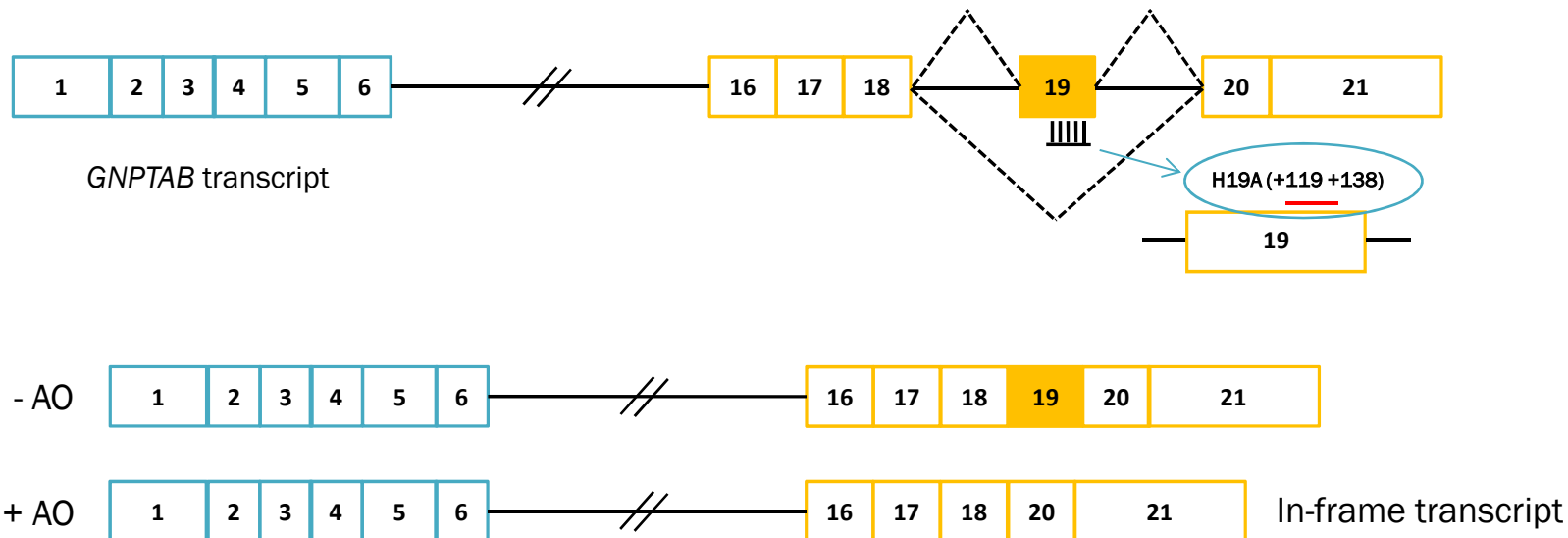
> Hum Gene Ther. 2020 Jul;31(13-14):775-783. doi: 10.1089/hum.2020.034. Epub 2020 May 20.

## Development of an Antisense Oligonucleotide-Mediated Exon Skipping Therapeutic Strategy for Mucopolipidosis II: Validation at RNA Level

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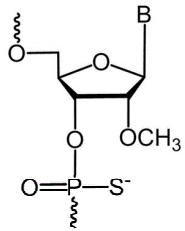
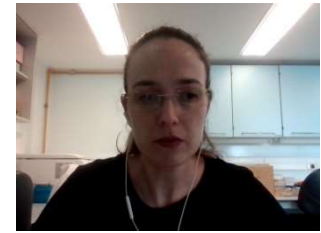


- In a previous *in vitro* study in ML II  $\alpha/\beta$  patient fibroblasts, Antisense Oligonucleotides (AOs) were used to promote the exon 19 skipping from the *GNPTAB* pre-mRNA, resulting successfully in the production of an in-frame mRNA

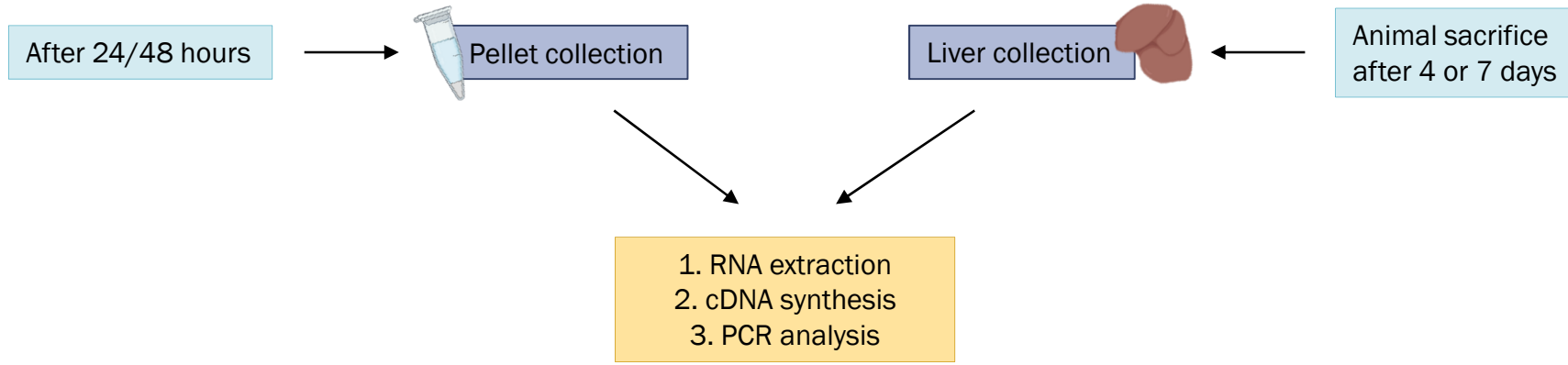
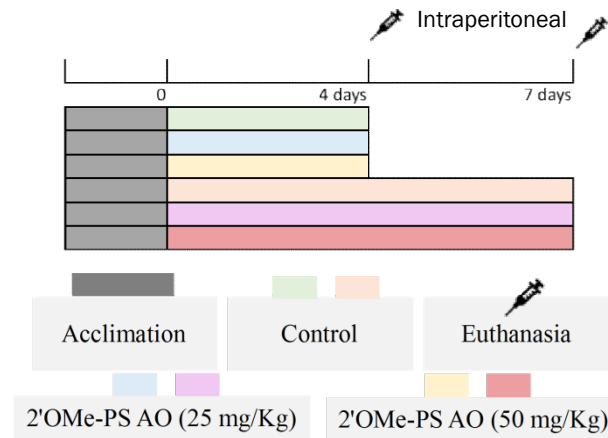


→ Currently, our goal is to evaluate the therapeutic potential of this approach, both *in vitro* in C57BL/6 fibroblasts and *in vivo* in C57BL/6 mice

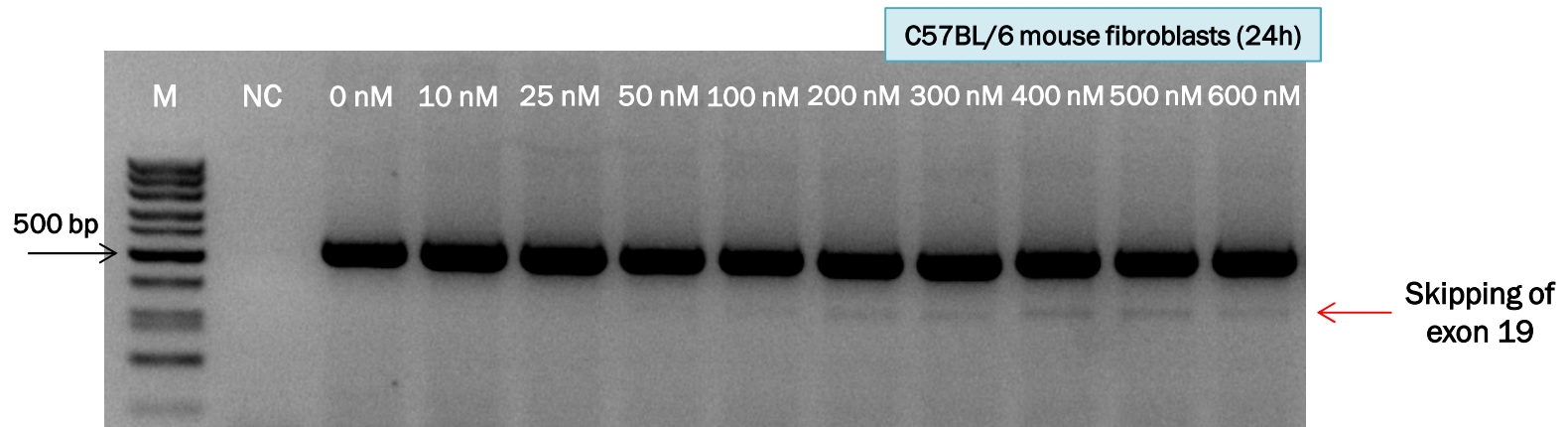
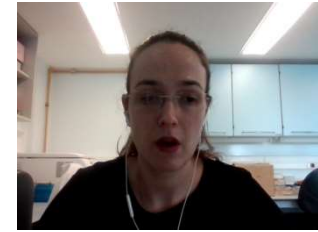
# Methodology



► 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



# Results

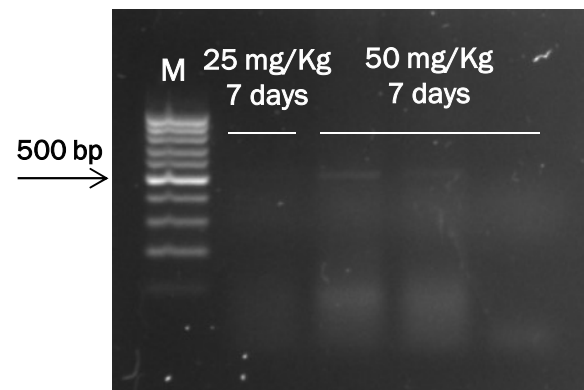
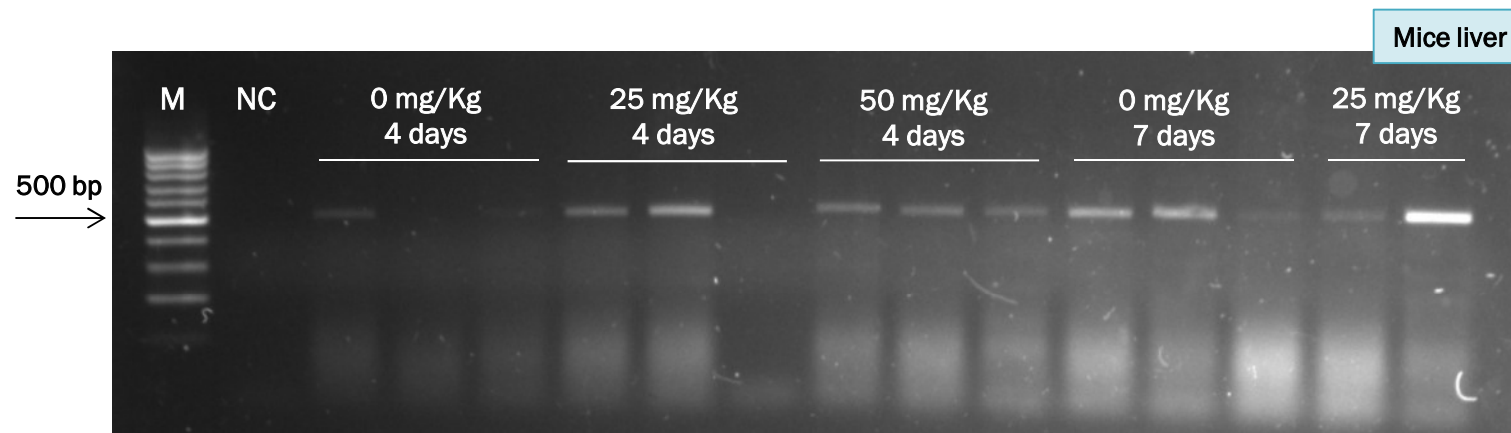
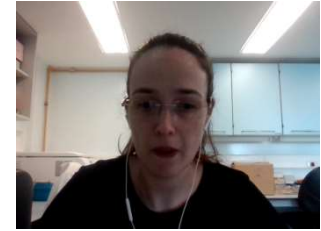


✓ After sequencing, the skipping of exon 19 was verified at concentrations above 100 nM, although in small amounts

→ The 2'OMe-PS A0 was able to modulate exon 19 splicing

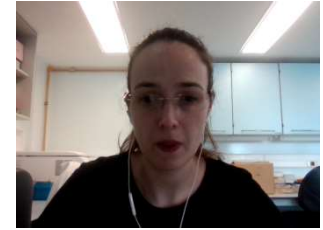
→ Similar results were observed at 48 hours

# Results



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

# Conclusions



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



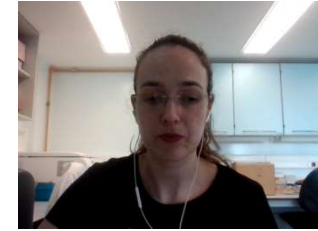
▶ To explain these first *in vivo* results, we can theorize that :



- ✓ 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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Hamburg-Eppendorf



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