AL4AnimalS 2023 Internal Project report template

(fill in English)

1. Project Reference and Title

Reference: AL4A-PROJ-LT3.5

Title: Addressing a challenging enzyme in vitro: proof of principle on the therapeutic potential of an antisense oligonucleotide approach for ML II

2. Summary of work developed (Max 3000 characters)

(Objectives and results in relation to objectives, their relevance and innovation and advancement to knowledge. If necessary, attach tables and/or figures if applicable. MAX 2 A4 pages)

1. To confirm the specificity of selected ASOs to skip the GNPTAB exon 19, a scramble ASO (expected to have no effect on GNPTAB gene) was transfected in WT & ML II patient fibroblasts. RT-PCR results showed that this ASO did not changed exon 19 splicing pattern proving the specificity of GNPTAB ASOs (Fig1A). Also, the enzymatic activity of various hydrolases was analysed in the same transfected cells. Results showed that the scramble ASO seems to interfere, even if little, with hydrolases activities of treated WT & ML II fibroblasts as shown by the differences in activities observed (Fig1B). The experiments number need to be increased to confirm these results.

2. To analyse the effect of exon 19 skipping on the GlcNAc-PT, a WT construct with the full GNPTAB cDNA (pGNPTAB_WT) and a mutant without exon 19 (pGNPTAB_delEx19) were tested in HEK293T cells. RT-PCR results after transfection showed the expected transcript pattern in both constructs (Fig2A). Moreover, GNPTAB protein expression was analysed by Western Blot (WB) using an antibody (Ab) specific for a myc-His tag located in constructs downstream the GNPTAB insert. Both constructs expressed a band corresponding to the α/β precursor, with or without exon 19. However, regarding the cleaved β-subunit the results are not so clear since we observed 2 bands (~48KDa) for the WT construct and further analysis is needed to understand if any of them corresponds to the target (Fig2B).
Both constructs were also transfected in ML II fibroblasts and cDNA analysis showed the expected transcript pattern. The activity of hydrolases will also be assessed to check if it increases with the delEx19 construct expression, suggesting a potential therapeutic effect.

3. We generated a novel Ab for the GlcNAc-PT β-subunit (rabbits). We expected to detect the protein in WT but not in ML II fibroblasts. However, WB results showed a band with the expected protein size in both cases (Fig3A). So, to test the Ab specificity different assays were done. The pre-bleed serum of the immunized rabbits was tested by WB and no target band was detected confirming that animals did not have Abs against the human protein (Fig3B). Also, the 2 synthetic β-subunit peptides used to immunize the animals were detected by WB confirming the specificity of the produced Abs (Fig3C). Protein sequencing (mass spectrometry) was also performed in extracts of WT & ML II fibroblasts after SDS-Page to search for the GNPTAB protein. As our Ab target has 45KDa, bands within 37-50KDa were analysed but the protein was not detected not even in the WT, suggesting that the protein amount used was insufficient for target protein

detection. Finally, the Ab was tested by WB in HEK293T cells transfected with both constructs and the expected protein pattern at least for the α/β precursor was observed (Fig3D). This last experiment needs to be repeated, however results obtained in the various assays suggest that our Ab is specific for GNPTAB protein detection.

3. Outputs (publications, communications, advanced studies; Max 3000 characters)

Communications (2023 and 2024):

Poster

- Gonçalves M*, Gaspar P, Encarnação M, Moreira L, Santos JI, Coutinho MF, Prata MJ, Omidi M, Pohl S, Silva F, Oliveira P, Matos L§, Alves S§. Restoring N-Acetylglucosamine-1-Phosphotransferase Function in Mucolipidosis II: Antisense Oligonucleotide Exon-skipping Therapeutic Approach. 28th Annual Meeting of the Portuguese Society of Human Genetics (SPGH), Porto – Portugal, December 2024. §These authors contributed equally to the work. *Presenting author.

- Matos L§*, Gonçalves M§, Ribeiro I, Gaspar P, Encarnação M, Santos JI, Coutinho MF, Prata MJ, Omidi M, Pohl S, Oliveira P, Silva F, Alves S. A Personalized Antisense Oligonucleotide Exon-skipping Therapeutic Approach for Mucolipidosis II. Annual Symposium of the Society for the Study of Inborn Errors of Metabolism (SSIEM), Porto – Portugal, September 2024. §These authors contributed equally to the work. *Presenting author.

- Gonçalves M*, Ribeiro I, Gaspar P, Encarnação M, Moreira L, Santos JI, Coutinho MF, Prata MJ, Omidi M, Pohl S, Oliveira P, Silva F, Matos L§, Alves S§. The cellular route of N-acetylglucosamine-1-phosphotransferase and its disruption in Mucolipidosis II: RNA therapy with antisense oligonucleotides as a possible solution. III Meeting AL4animalS, Porto – Portugal, May 2024. §These authors contributed equally to the work. *Presenting author.

- Matos L§*, Gonçalves M§, Santos JI, Coutinho MF, Prata MJ, Oliveira P, Omidi M, Pohl S, Alves S. Exploring an antisense oligonucleotide exon-skipping therapeutic strategy for Mucolipidosis II. 19th Annual Meeting of the Oligonucleotide Therapeutics Society, Barcelona – Spain, October 2023. §These authors contributed equally to the work. *Presenting author.

- Gonçalves M§*, Matos L§, Santos JI, Coutinho MF, Prata MJ, Pires MJ, Oliveira P, Alves S. Developing models and an RNA-based therapy for a rare disease. 2nd Meeting of the Associate Laboratory for Animal and Veterinary Sciences (AL4animalS), Vila Real – Portugal, May 2023. §These authors contributed equally to the work. *Presenting author.

Oral

- Matos L. Addressing a Challenging Enzyme in Vitro: Proof of Principle on the Therapeutic Potential of an Antisense Oligonucleotide Approach for Mucolipidosis II. III Meeting AL4animalS, Porto – Portugal, May 2024.

- Gonçalves M§*, Matos L§, Santos JI, Coutinho MF, Prata MJ, Pires MJ, Oliveira P, Omidi M, Pohl S, Alves S. RNA-Based therapies and disease models for Mucolipidosis II. AL4AnimalS Thematic Meeting, Comparative and Translational Medicine and Biotechnology, Porto – Portugal, October 2023. §These authors contributed equally to the work. *Presenting author.

Principal Investigator (PI) Name

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