

# TRANS (SPLICING) IS BETTER

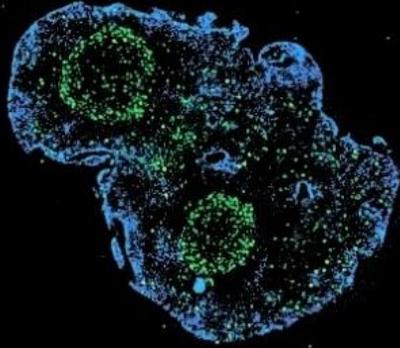
An innovative strategy to treat laminopathies

**Prof.ssa Isabella Saggio**

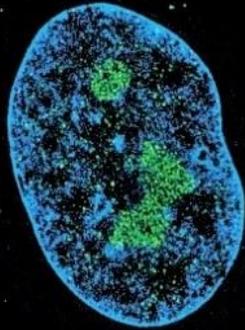
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Clara Salciccia  
Elisabetta Botticelli  
Giovanni D'Uva**

**A.A. 2019 - 2020**

**Mutant nucleus**

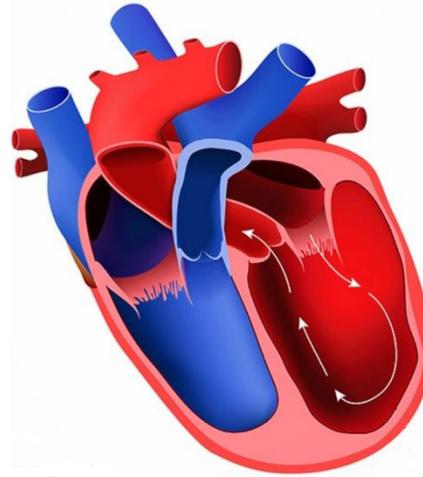


**Healthy nucleus**



- **LMNA locus mutated**
- **Delayed or decreased gene expression**
  - **Awry nucleus**

**Dilated**



**Cardiomyopathy**

- **Dilatation of the left ventricle**
  - **Heart failure**
- **Arrhythmias and/or abnormal conduction**



- **Muscular dystrophy (EDMD in our model)**  
*EDMD: Emery-Dreifuss muscular dystrophy*
  - **Premature aging**
  - **Lipodystrophy**

# GOALS



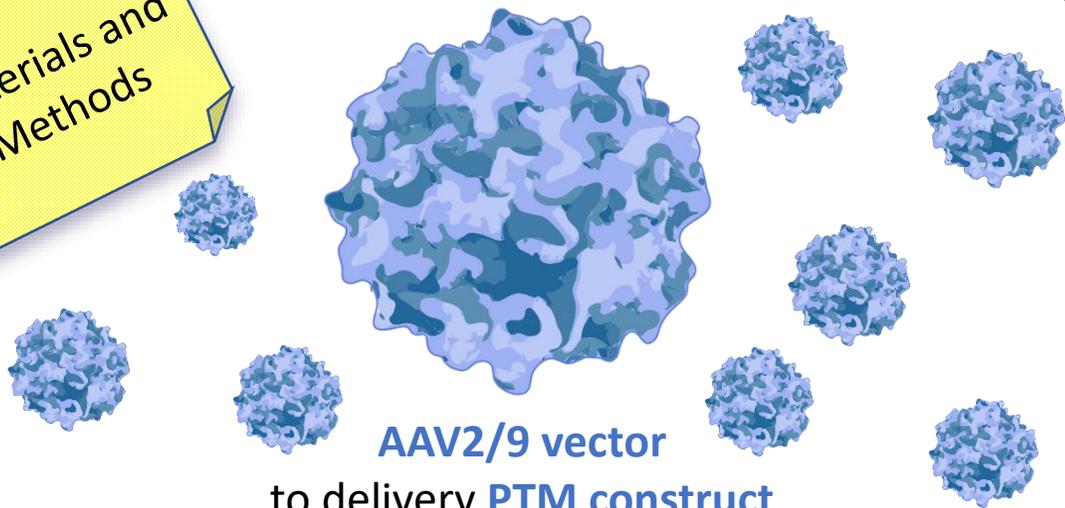
- **Redirecting** splicing of LMNA gene to correct protein expression
- **Restoring** WT phenotype
  1. Expression of WT protein
  2. Normal nuclear shape
  3. Blocking DCM (dilated cardiomyopathy) development
  4. Rescuing locomotory abilities

# STRATEGY

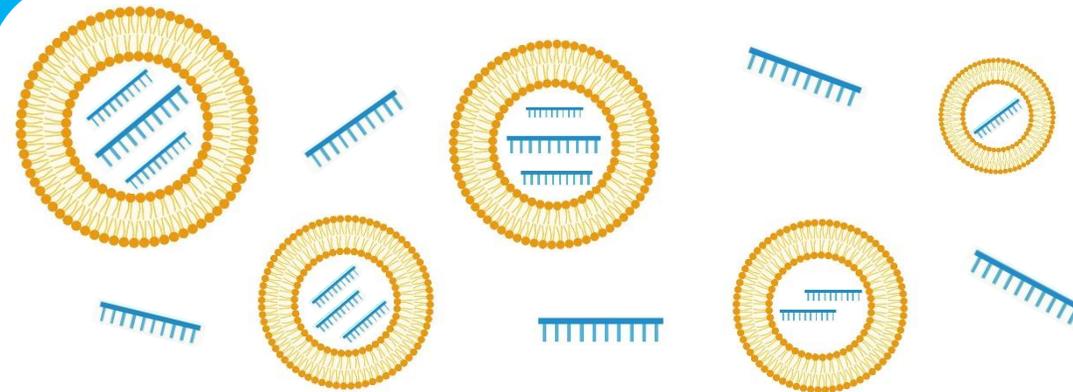


- **Trans-splicing + ASOs** (antisense oligonucleotides) in:
  1. **In vitro** model (primary culture)
  2. **In vivo** mouse model (LmnaH222P/H222P)

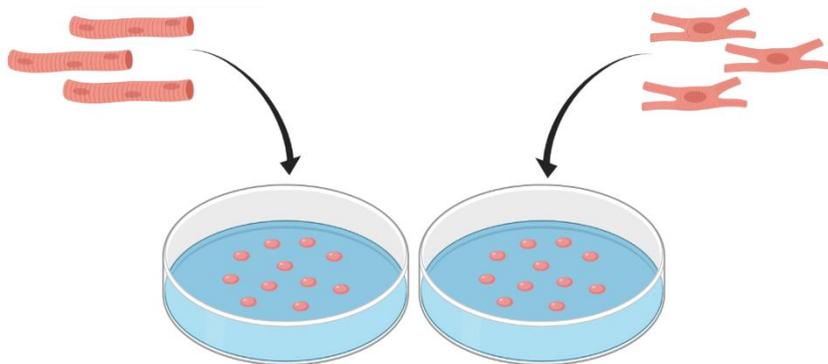
Materials and Methods



**AAV2/9 vector**  
to delivery **PTM construct**  
*PTM: pre trans-spliced molecule*



**TAG LIPOSOMES FOR HEART (PCM+TAT) AND SKELETAL MUSCLE ( $\alpha$ -SARCOGLYCAN) and ASOs to target cis-splicing sites and promote trans-splicing**



- **Primary culture** from skeletal muscle and heart from our mouse model
- **Techniques:** MTS, PCR, qRT-PCR, Immunofluorescence



Normal localization of lamins A/C and Emerin



Abnormal Weight



Abnormal conduction



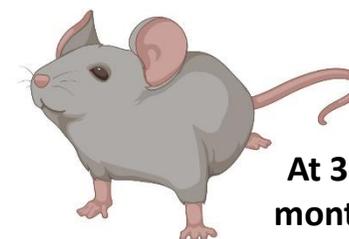
DCM Development



Abnormal locomotion



Dystrophic pattern of skeletal muscles (EDMD)

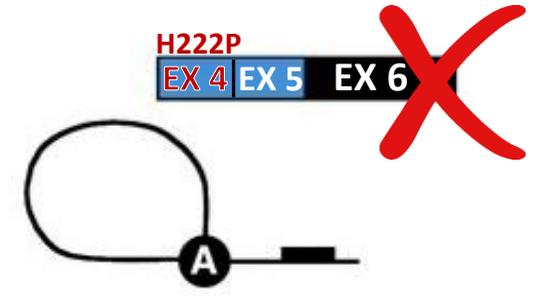
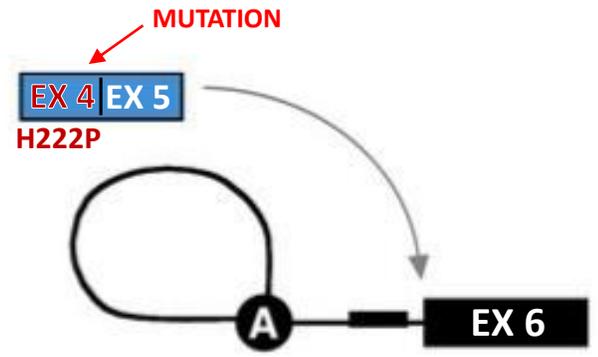
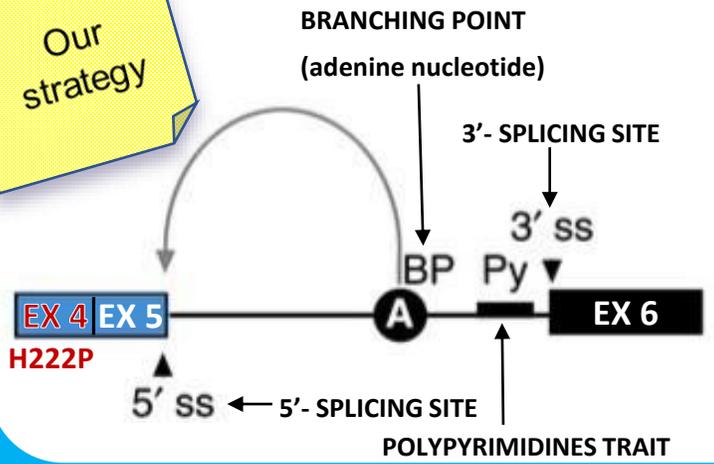


At 3-6 months

**Lmna**<sup>H222P/H222P</sup>

Our strategy

Adapted from Garcia-Blanco 2003



\*\*\*EX 4 EX 5 and EX 6 of LMNA pre-mRNA

CIS-SPLICING = MUTATED PROTEIN ☹️

### WHY TRANS-SPLICING?

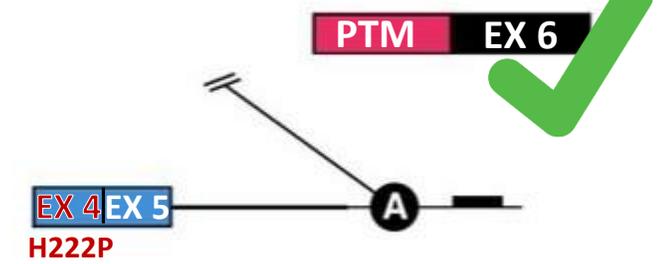
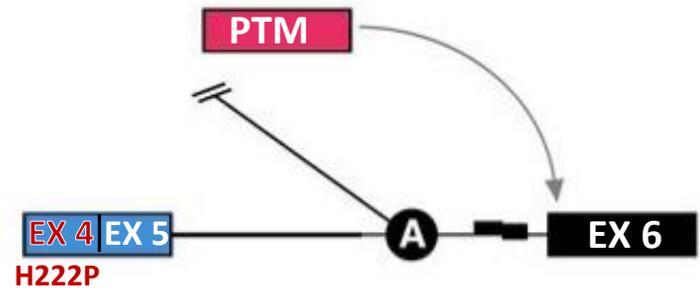
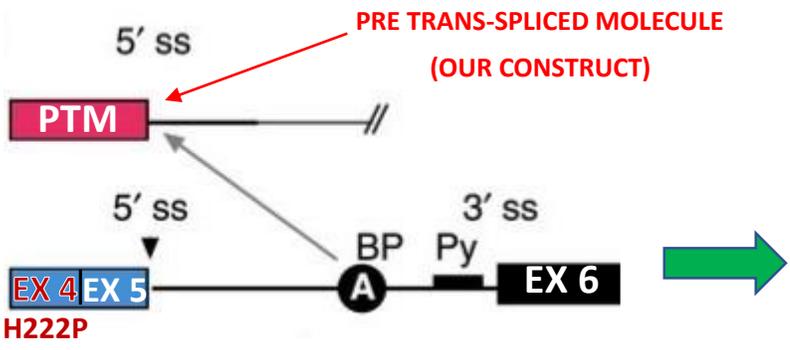


## IT'S AN ENDOGENOUS MECHANISM



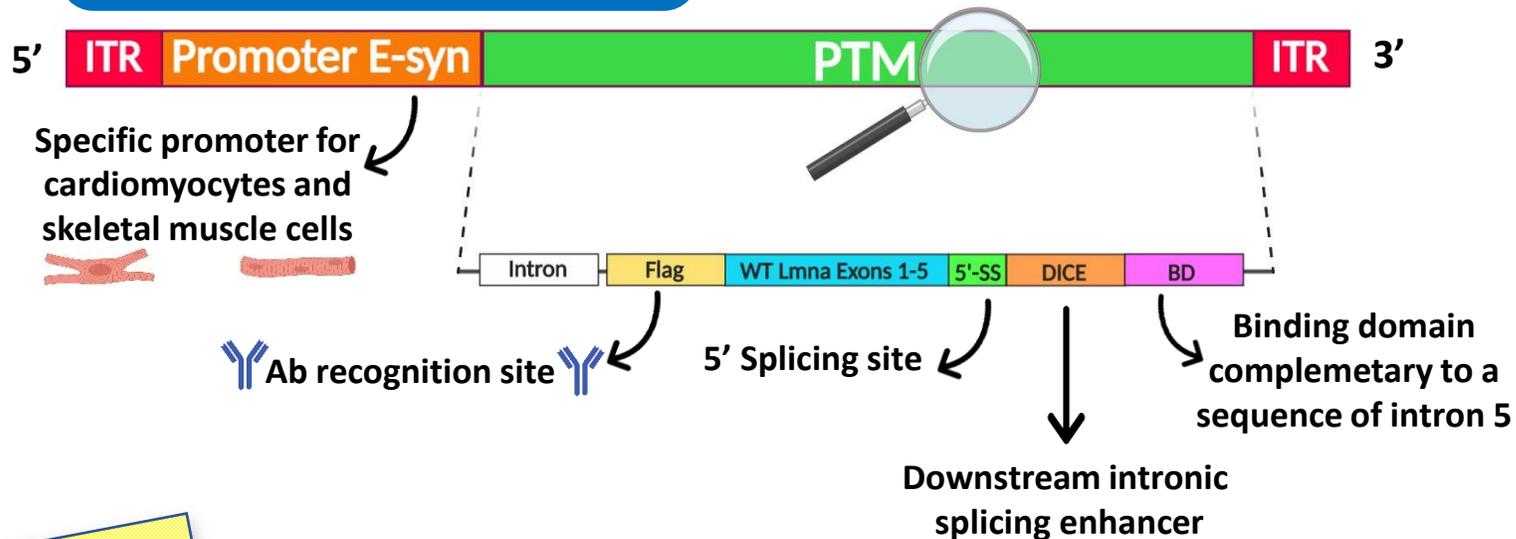
FINE TUNING!

Adapted from Garcia-Blanco 2003

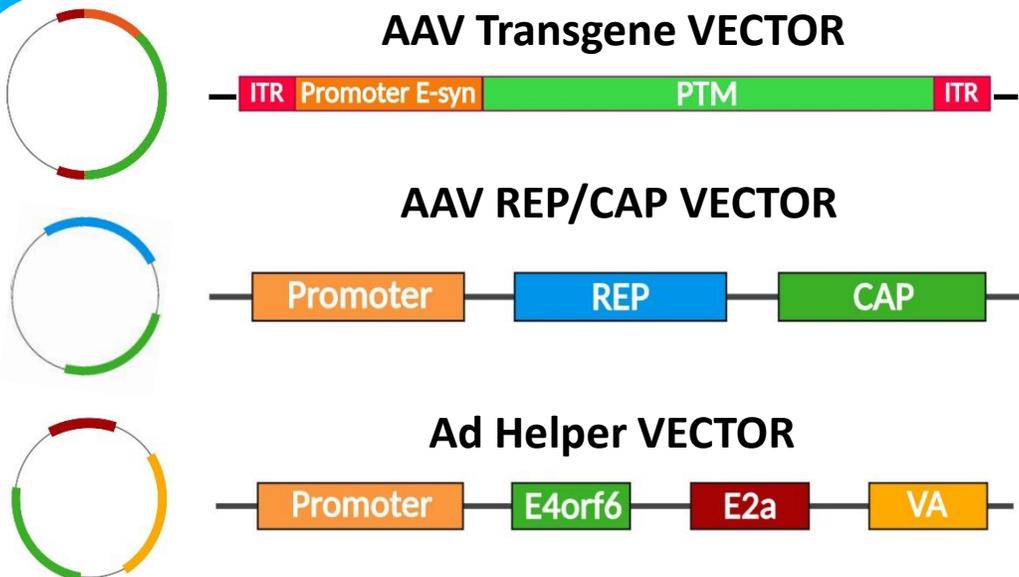


TRANS-SPLICING = WT PROTEIN 😊

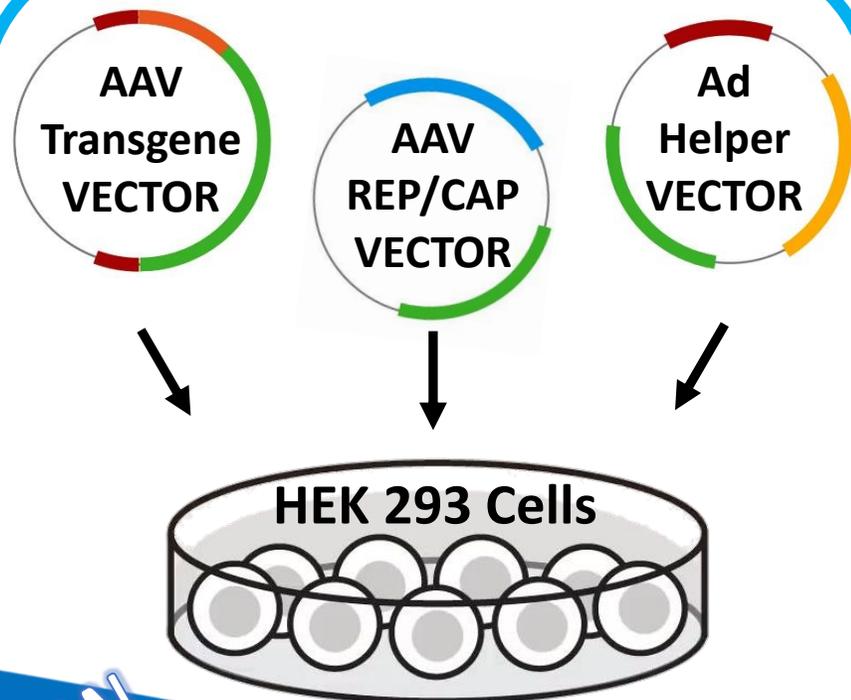
## PRE TRANS-SPICED MOLECULE



PTM Construct + Vector Production

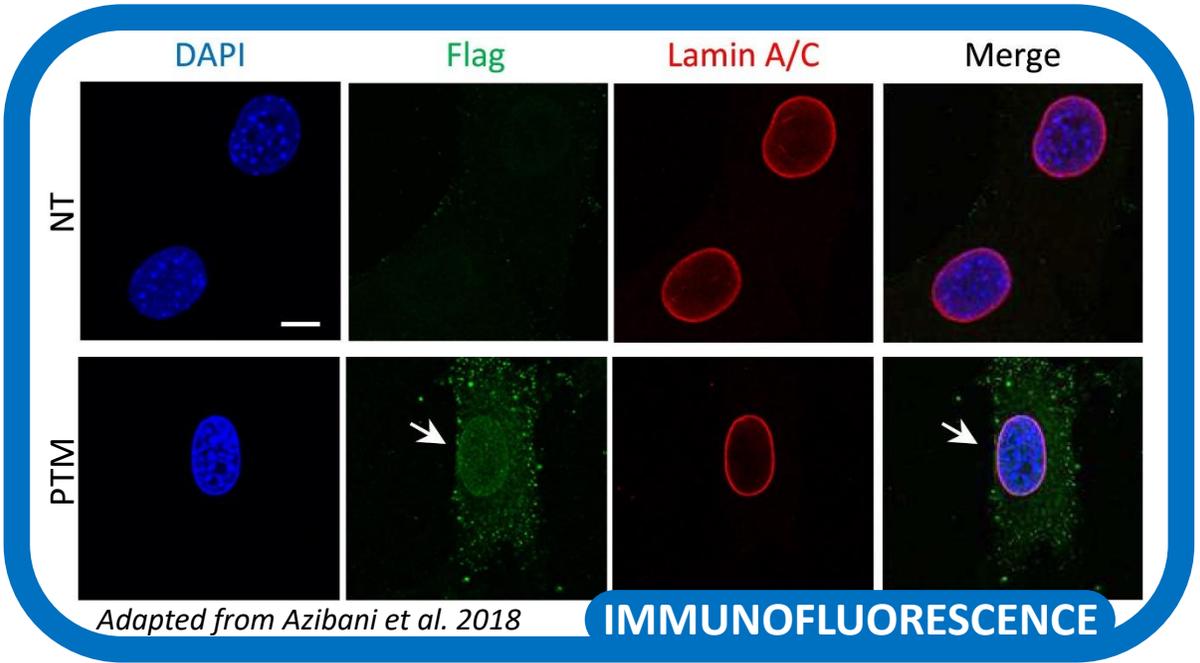
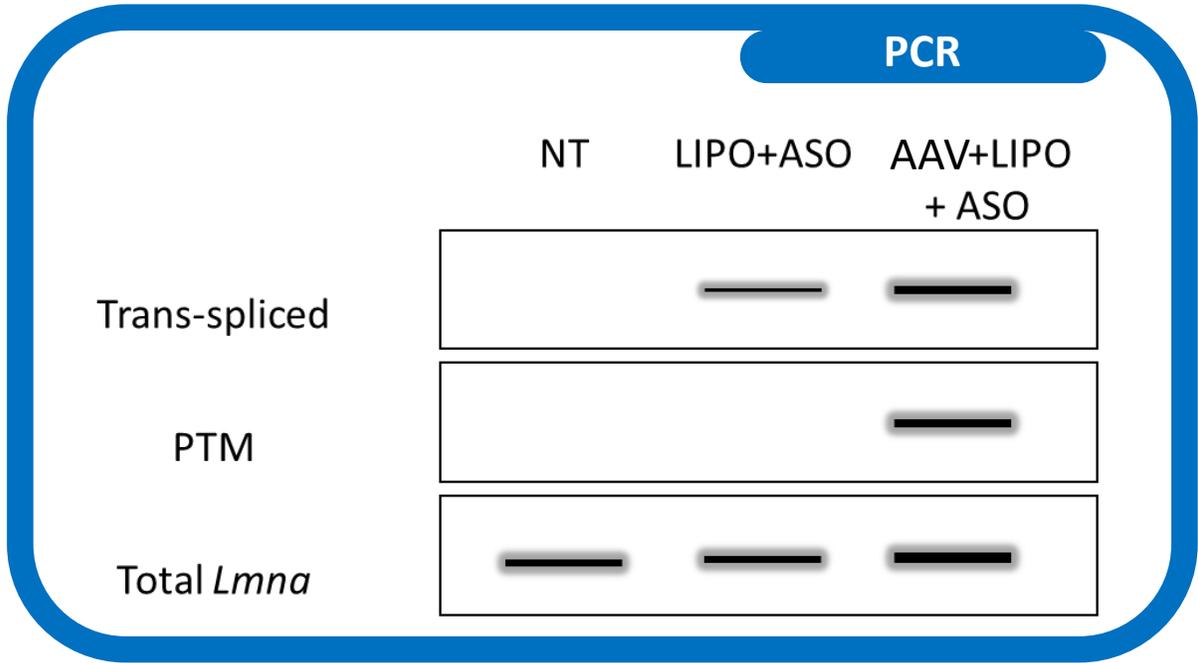
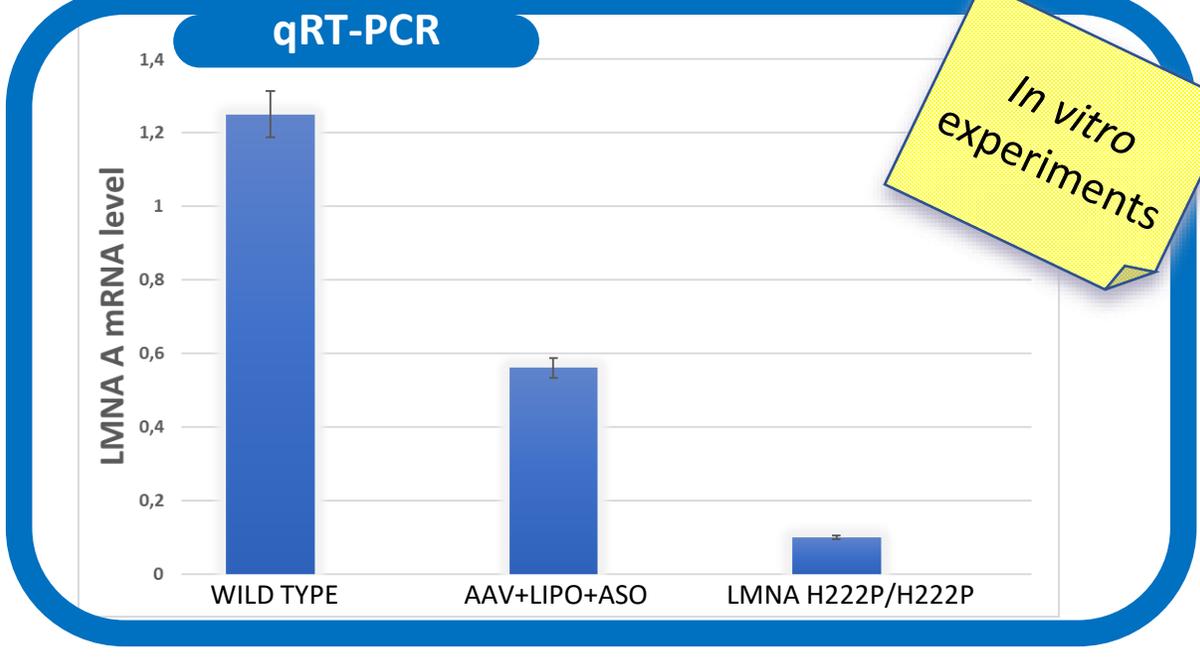
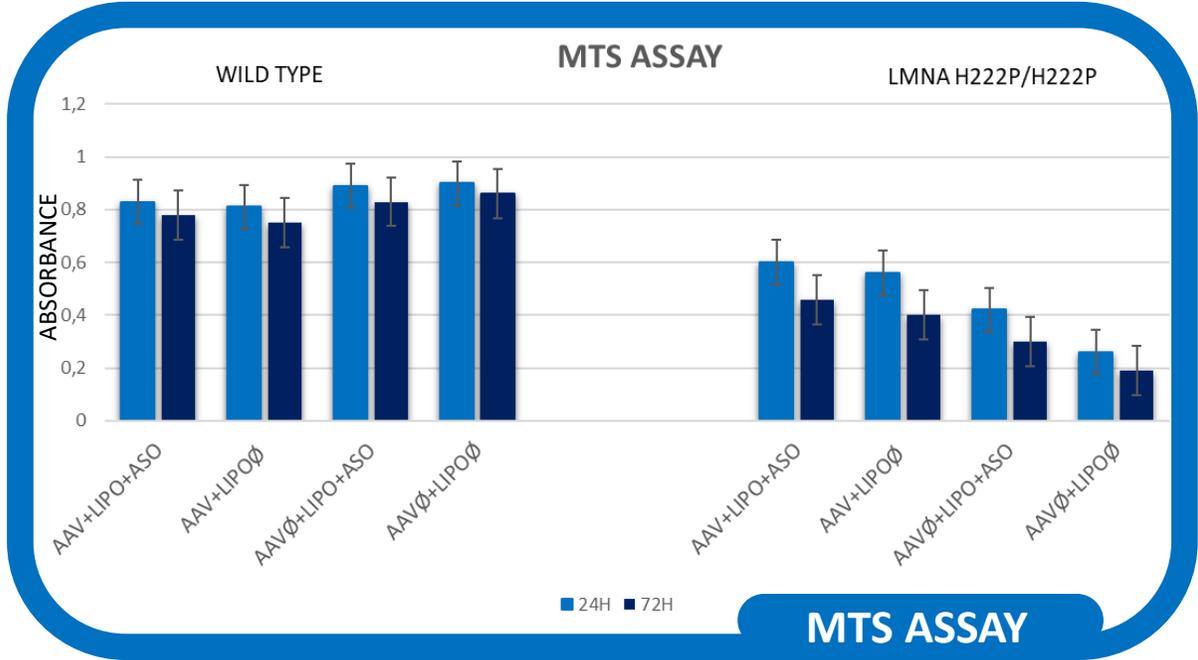


PRODUCTION

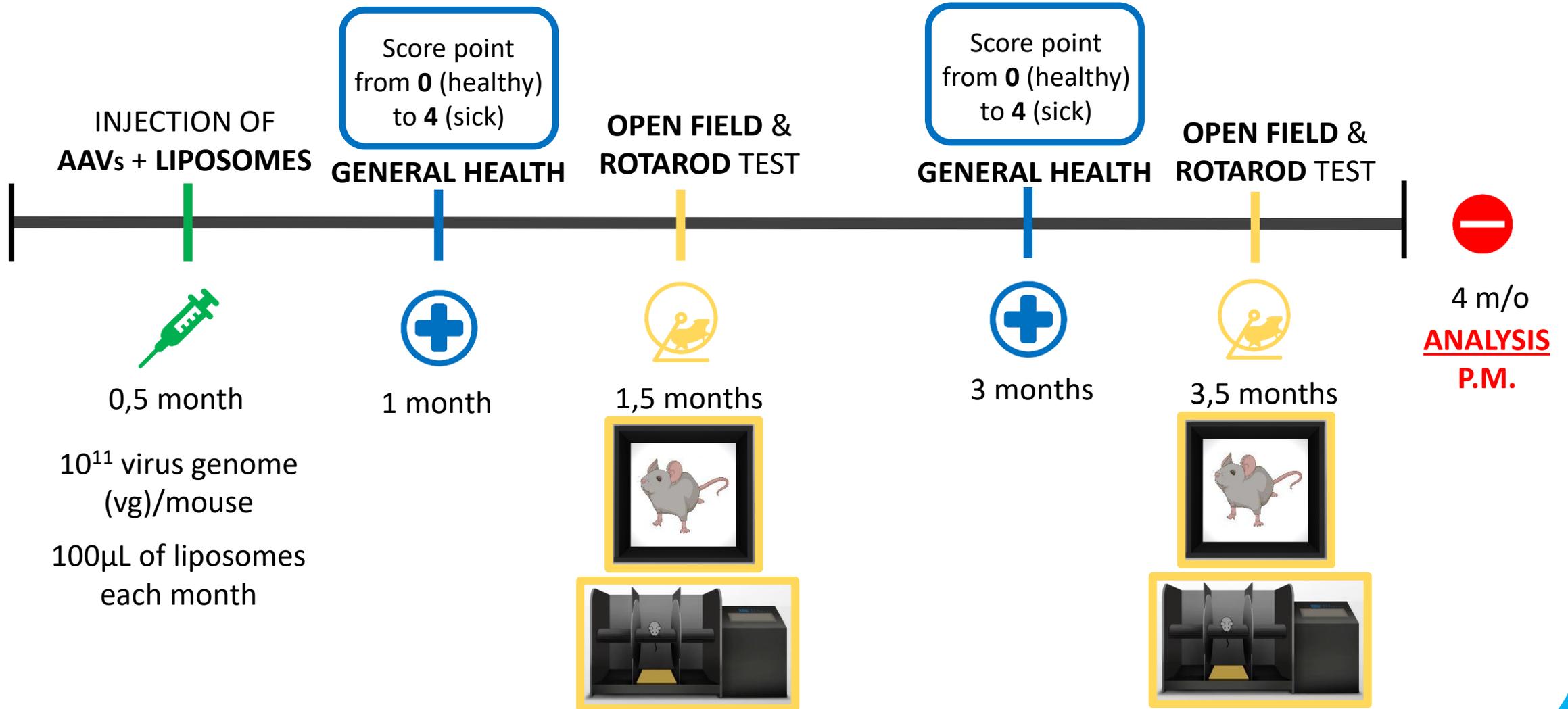


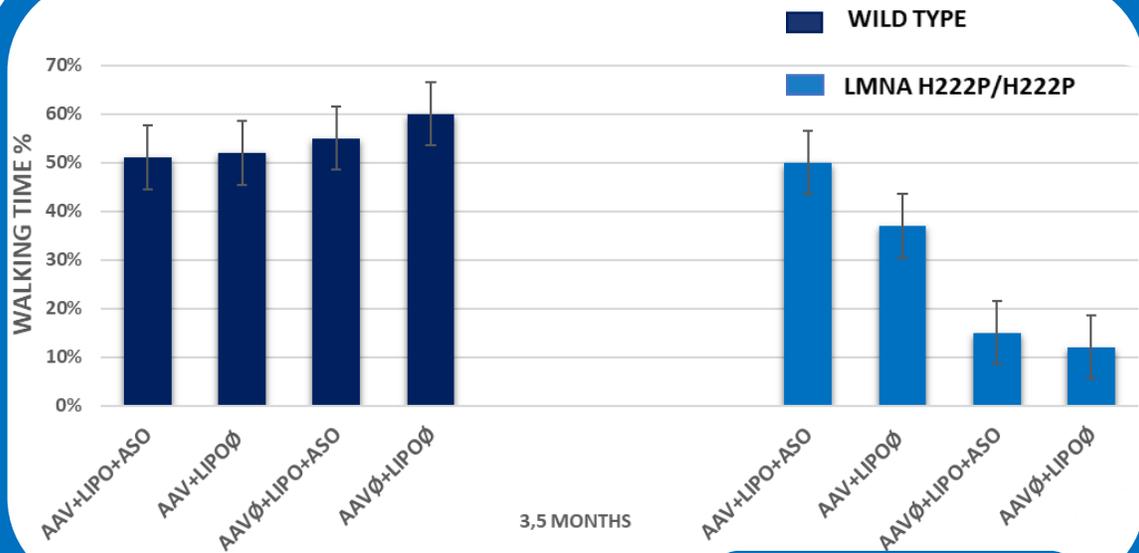
AAVs ready to transfect!



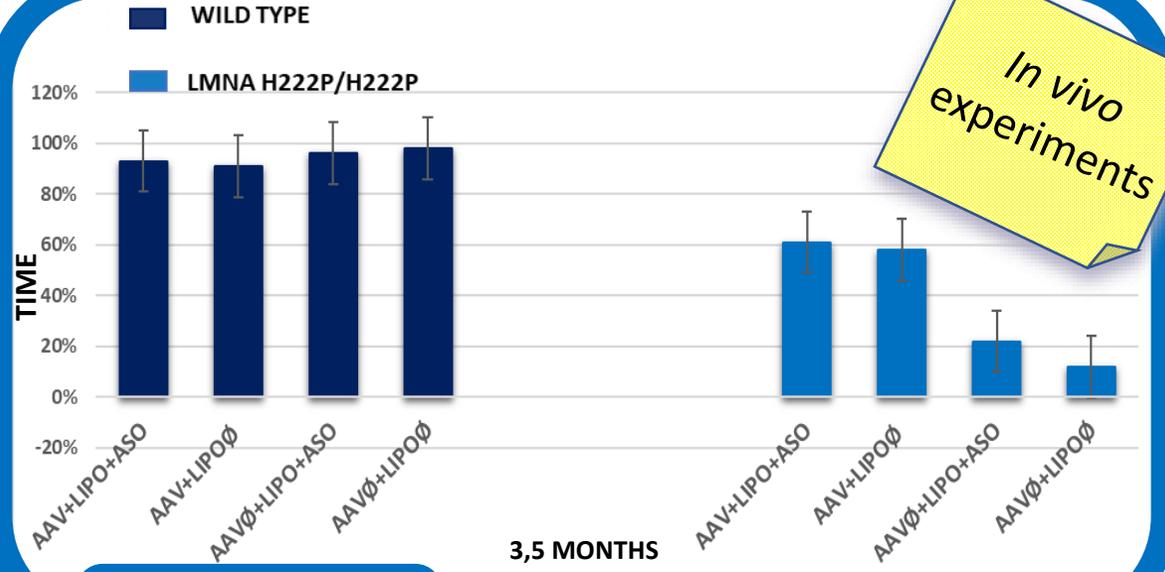


# Experimental design - *Lmna*<sup>H222P/H222P</sup> ♂ mice

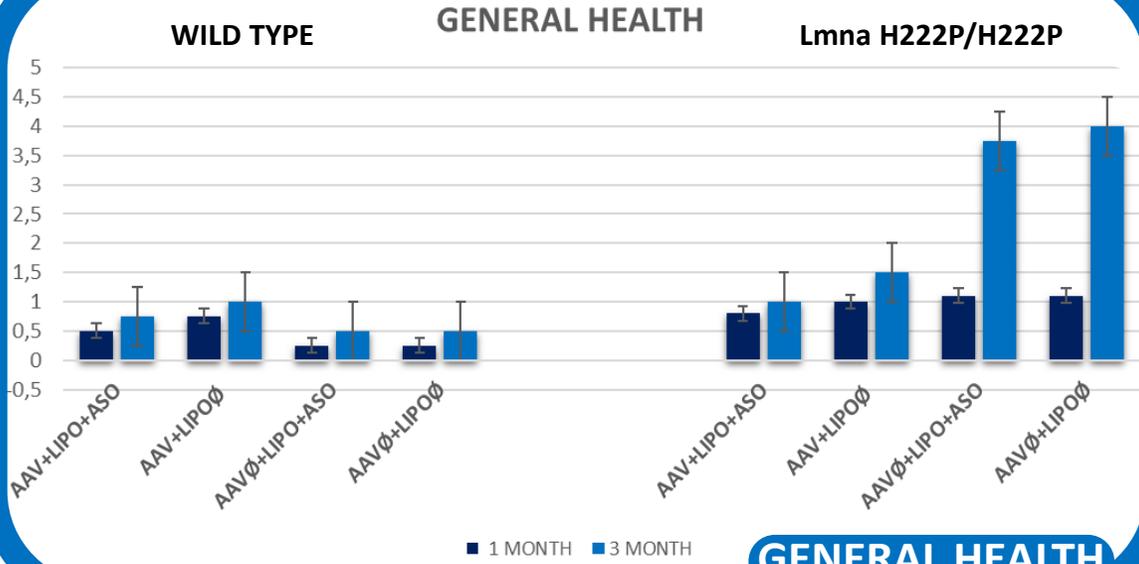




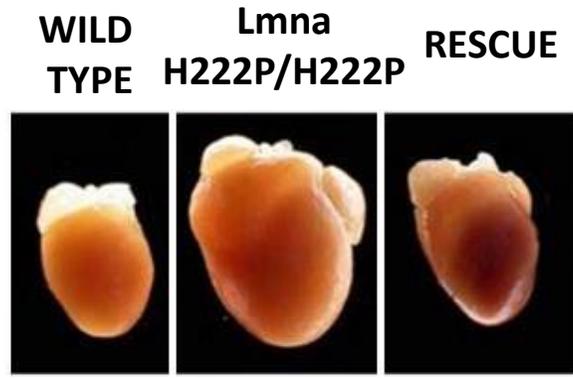
**OPEN FIELD**



**ROTAROD**



**GENERAL HEALTH**

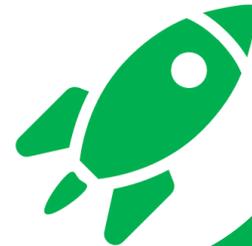


*Adapted from Arimura et al. 2004*

**P.M. ANALYSIS**

# FUTURE PERSPECTIVES

- Apply the same protocol on Lmna H222P/H222P female mice
- Use lentiviral vectors to treat proliferative sick tissues
- Confirm obtained results on stabilized cell line in order to analyze long term effects
- Clinical trials on Lmna H222P/H222P human patients (mouse→homozygosis vs human→heterozygosis)
- Screen available drugs that could enhance further trans splicing



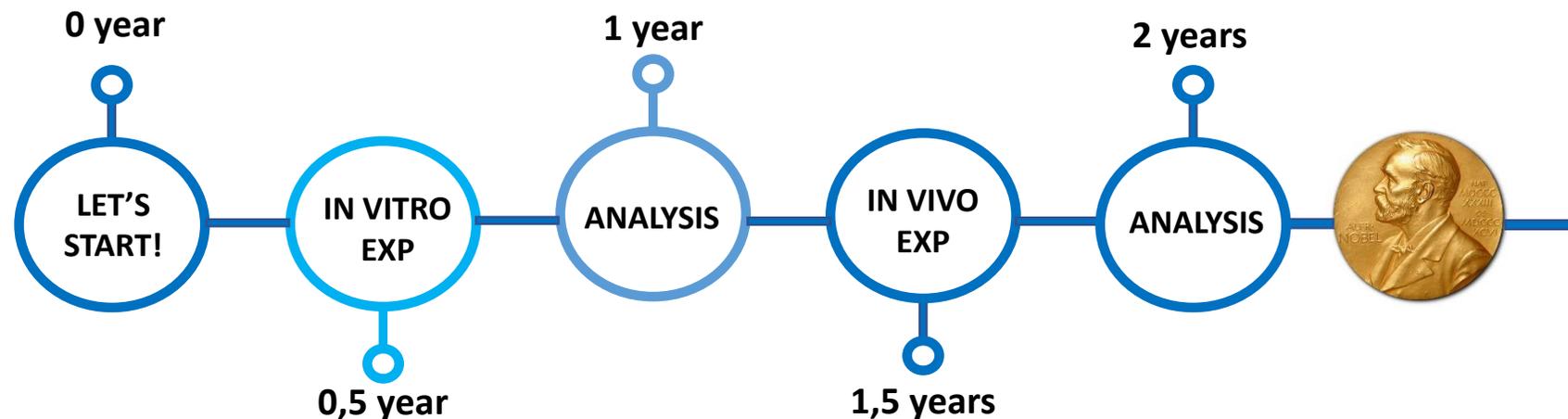
# Project timeline + costs

- Kit Western blot: 2500 €
- Tissue PCR kit: 2380 €
- Antibody I: 800 €
- Antibody II: 300 €
- Cell culture materials: 8864 €
- MTS: 247 €
- Rotarod (6 sets): 5995 €
- Open field (4 sets): 1790 €
- 40 x (WT) mice: 1200 €
- 50 x (LMNA H222P/H222P) mice: 2365 € (x mouse)
- Stabulation costs: 2800 € (700 € x month)
- Lipofectamine<sup>®</sup> transfection reagent: 906 € (1,5 ml)
- ASO: 1.000 €
- AAV vector : 800 €
- Researcher's salary: 1250 € (x month x 5 researchers)
- **TOTAL COST: 298 852 €**



**SIGMA-ALDRICH**

**ThermoFisher**  
SCIENTIFIC



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