Emery-Dreifuss muscular dystrophy: Silencing and restoring of LMNA gene

Gene therapy
Professoressa I.Saggio
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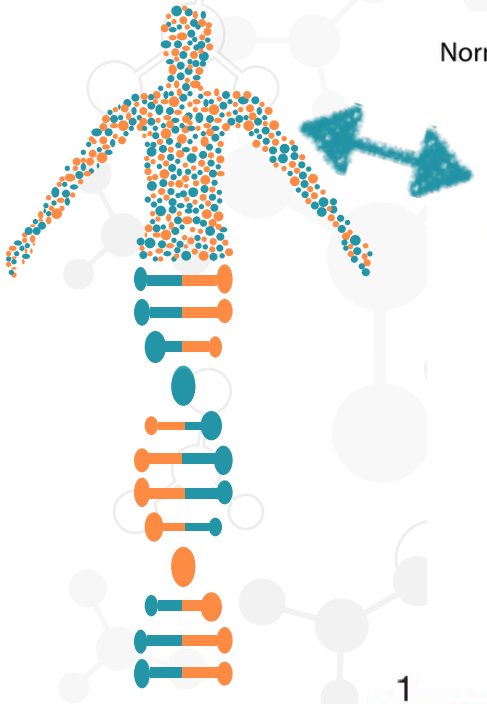
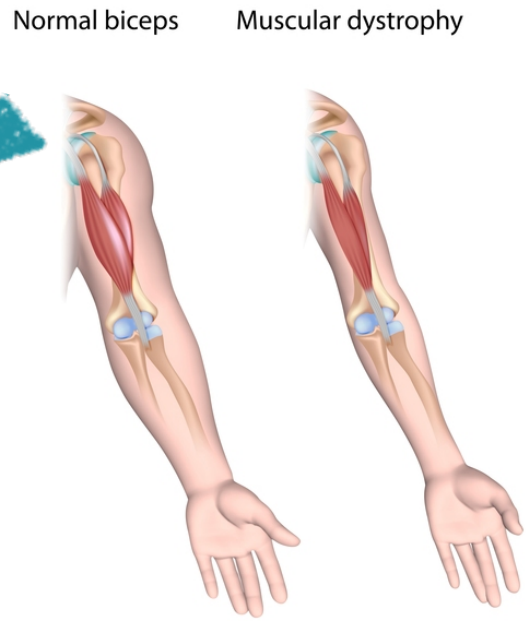
Lelli Susanna, Marchetti Claudia, Mantua Benedetta, Mazzetti Debora, Reccagni Alice

Emery-Dreifuss muscular dystrophy

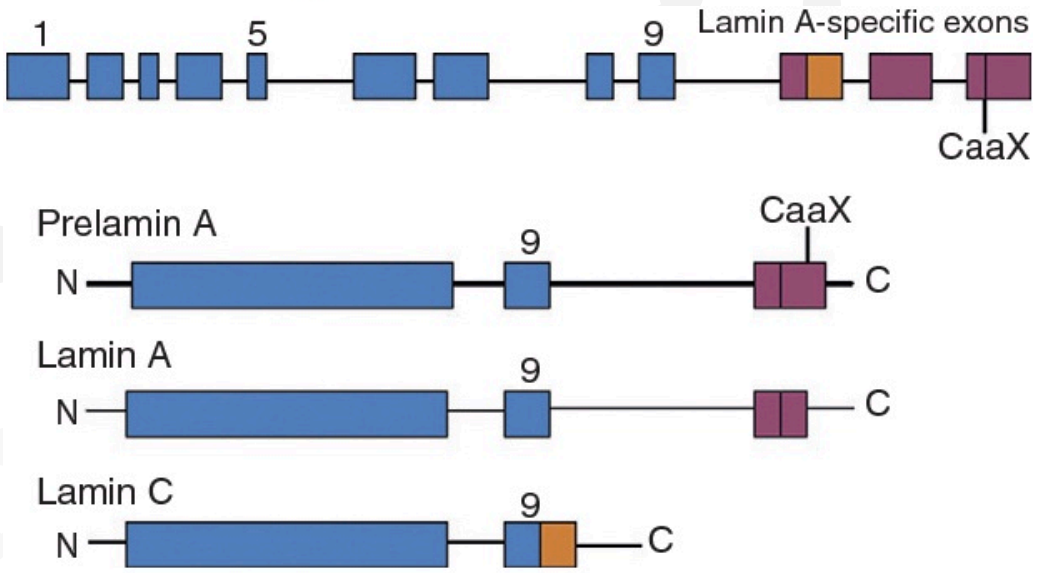




It is characterized by

- the triad of weakness of the shoulder and pelvic girdle muscles,
- contractures of the elbows, neck, and Achilles tendon,
- cardiac involvement, most commonly arrhythmias

LMNA gene



The mutation

EDMD2 is caused by heterozygous mutation in the gene encoding lamin A/C (LMNA on chromosome 1q22). 23 different mutations are distributed between exons 1 and 9 in the region of LMNA common to both lamins A and C.

Figure adapted from: "*Megan-J.-Puckelwartz et al.,2011*"

Aims of the project

1. SILENCING
the ineffective LMNA gene

2. RESTORING
the wild type phenotype

3. REDUCE
off-targets events

Strategy and why

01 **SILENCING LMNA**
CRISPR/Cas9 delivered using cell derived nanovesicles: GESICLES

02 **RESTORING WT LMNA**
Third-generation lentivirus vector

Why CRISPR /Cas9 gesicles?

- No persistent expression of Cas9;
- Glycoproteins on their surface mediate binding and fusion with skeleton muscles cells;
- Allows control of the dose and duration of the complex in the cell, reducing the chance of off-target effects.

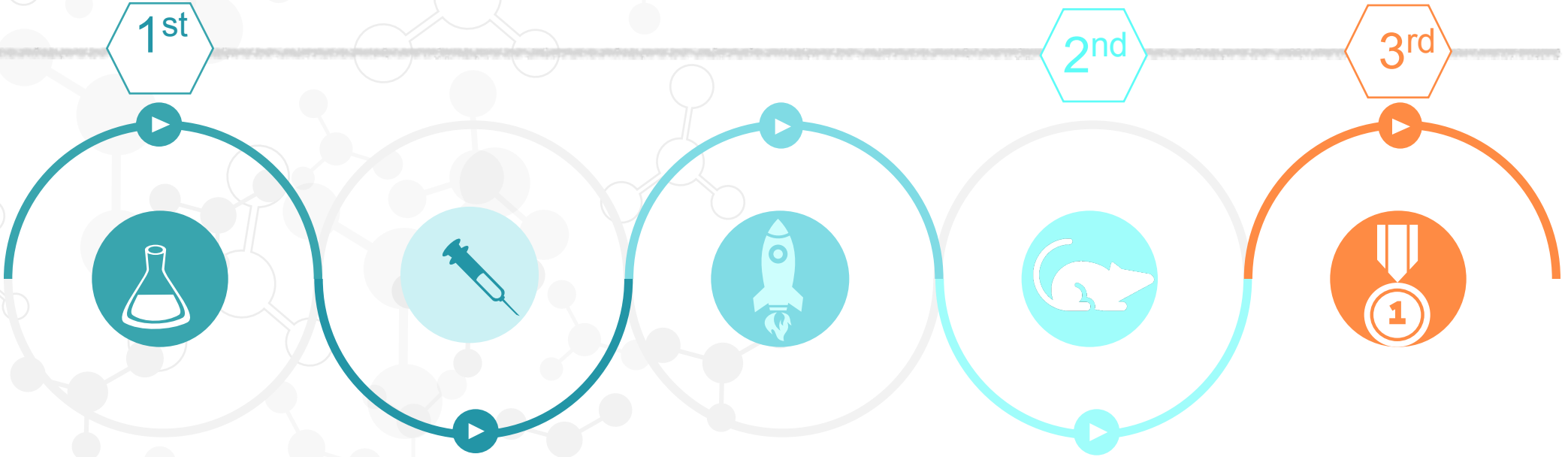
Why third-generation lentivirus vector?

Advantages:

- High packaging capacity,
- Stable gene expression in both dividing and post-mitotic cells,
- Low immunogenicity in the recipient organism.

Experimental plan

Years



Production of the vectors

Development of CRISPR/Cas9 gesicles and of third-generation lentivirus vectors with LMNA gene

In vitro experiment part 1

CRISPR/Cas9 transfection with gesicles for silencing the endogenous LMNA gene expression

In vitro experiment part 2

Third-generation lentiviral vectors delivery

In vivo experiment

Transfection of neonatal mice mutant for LMNA gene (H222P mice)

Results

Restoring of the WT phenotype and rescue of the muscles

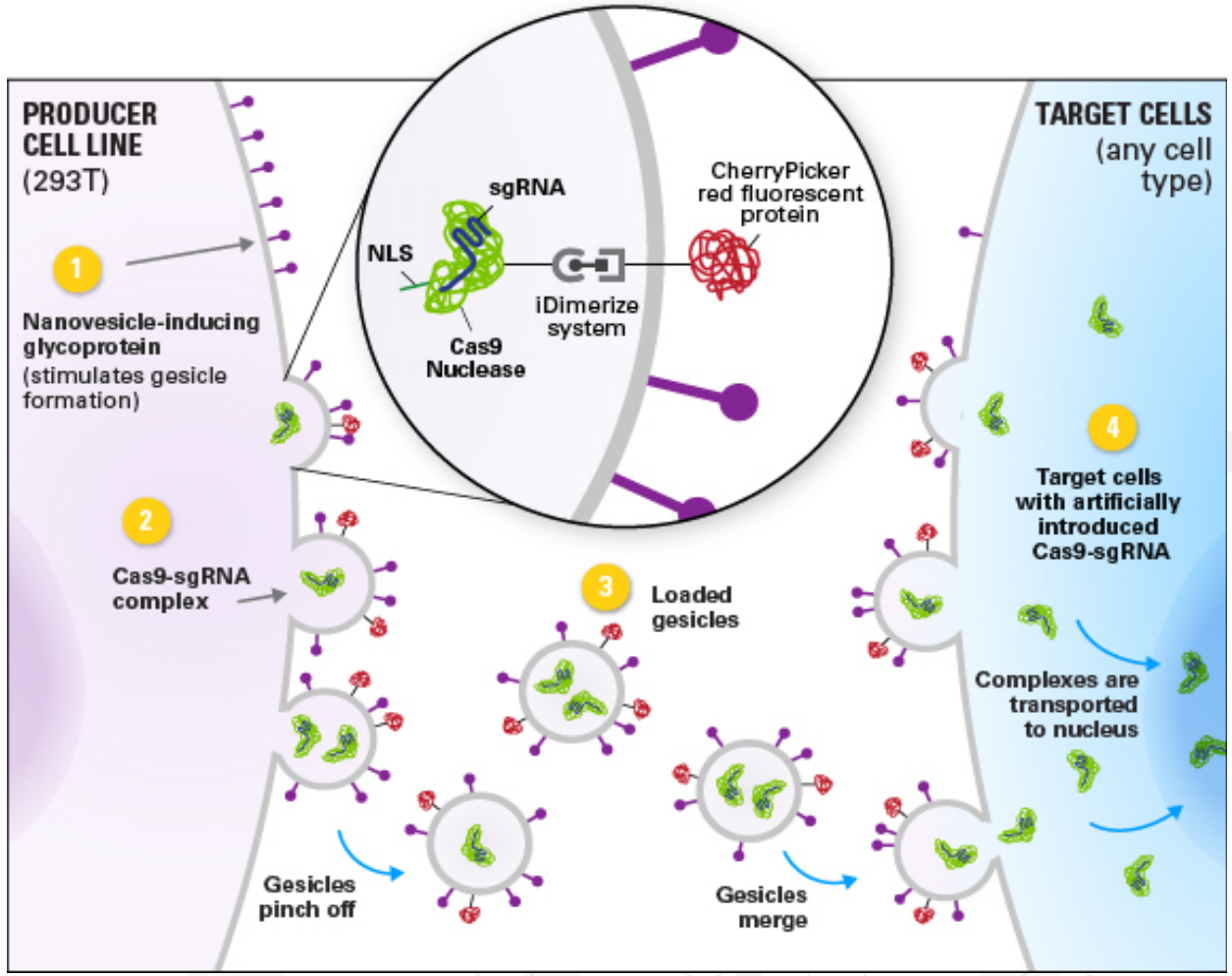


Figure B adapted from: *"www.takarabio.com"*

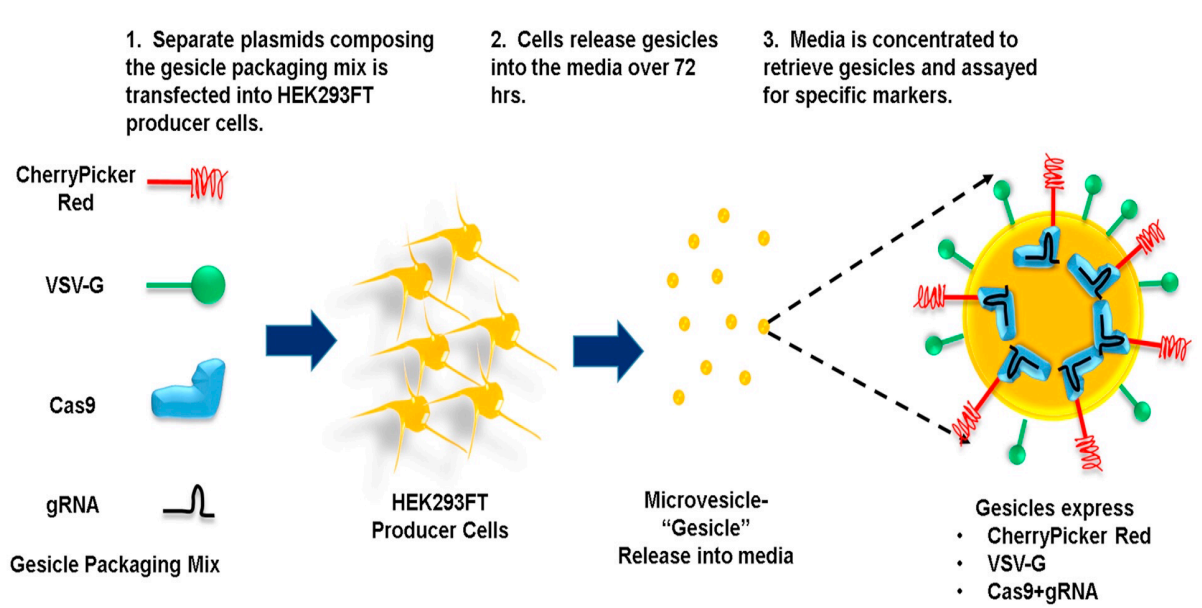


Figure A adapted from: *"Lee A. Campbell, et al., 2019"*

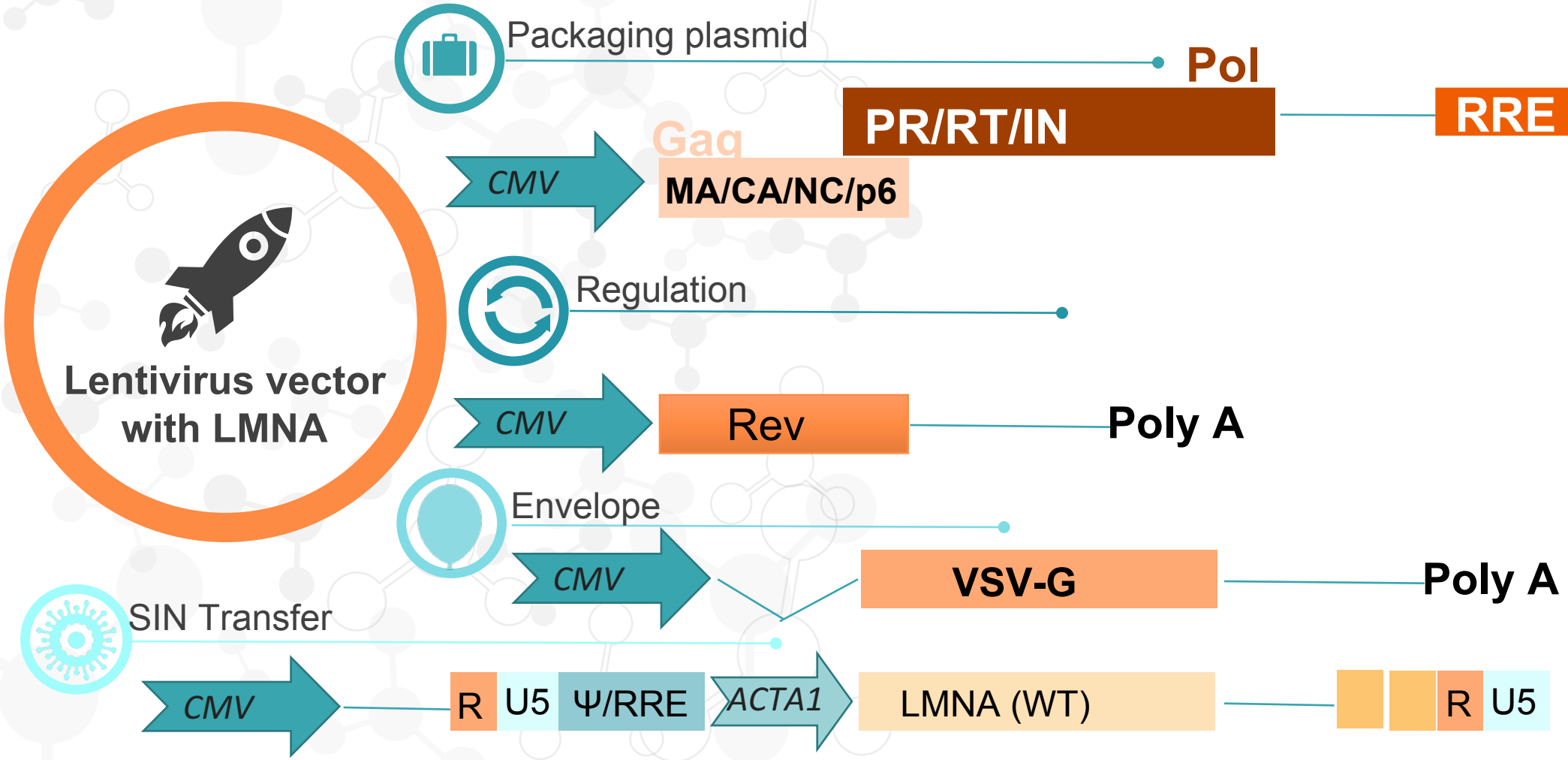
Delivering CRISPR-CAS9 with gesicles

Third-generation lentivirus vector

Using a specific promoter for skeleton muscles cells which is the one of Actin alpha 1 (ACTA1), which is expressed in skeletal muscle and target it with GFP fused to the gene. The generation of lentivirus vector will be in HEK cells.

1.

1 .LV-MAX Lentiviral Production System protocol overview



In vitro

Three different sgRNA

Three different sgRNAs have been tested in order to establish the best one and use it for the in vivo experiments.

Culture of skeleton-muscles cells from H222P mice

We chose the LMNA H222P missense mutation to create a faithful mouse model of this autosomal dominant Emery-Dreifuss muscular dystrophy.

Culture with H222P cells treated with sgRNA3 are injected with lentivirus

1st culture
with sgRNA1

2nd culture
with sgRNA2

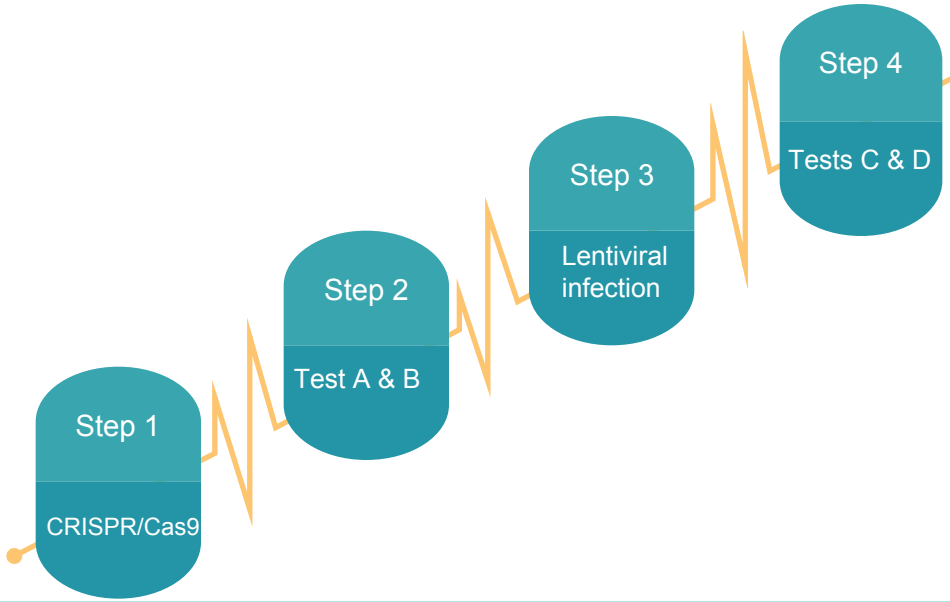
3rd culture
with sgRNA3

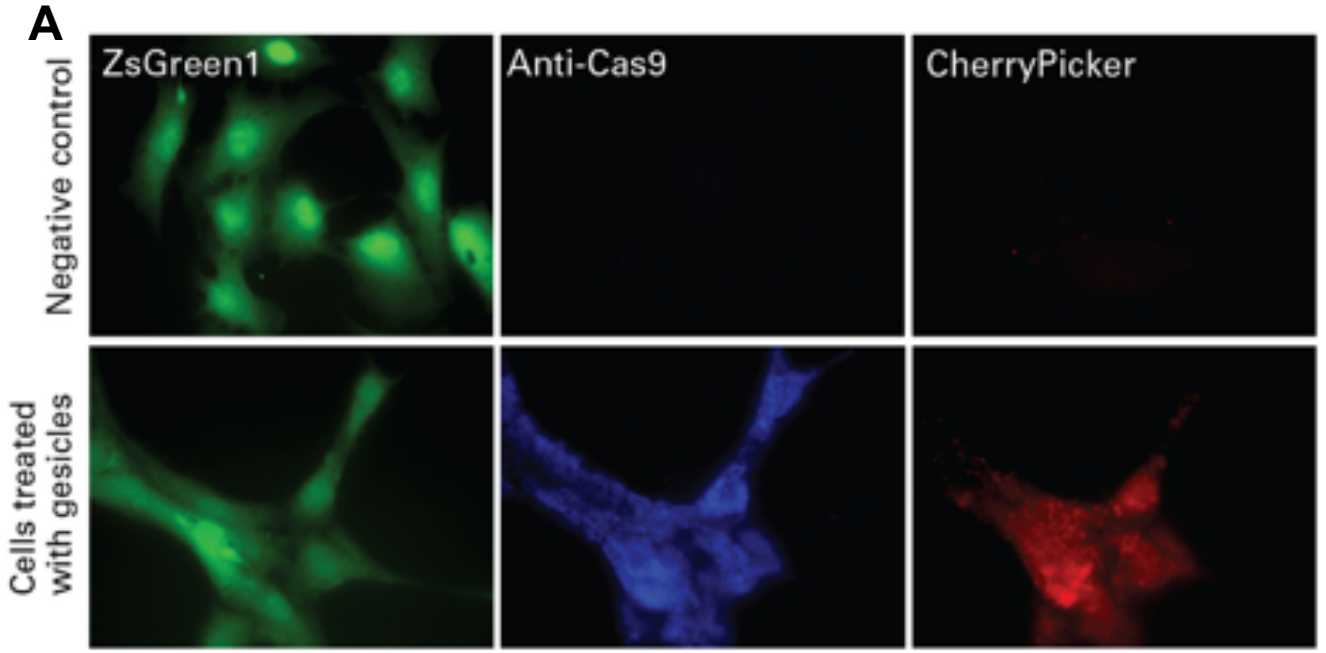
4th culture
Empty vector

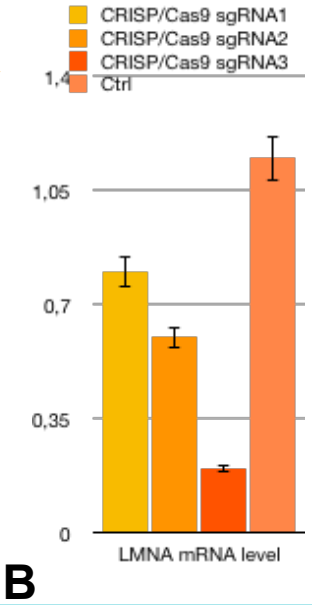
5th culture
Cells from WT mice

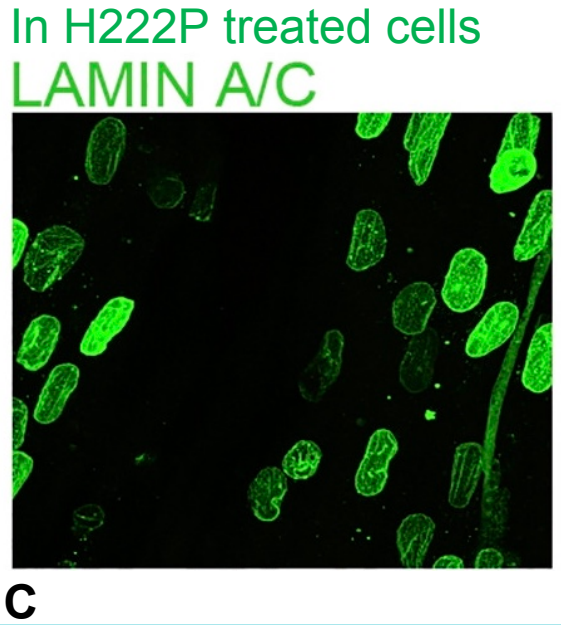
In vitro experiment

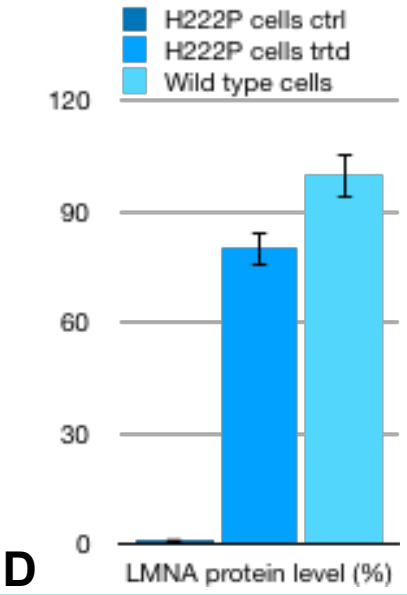
Analysis











In vivo

1 day
1st administration of gesicles

5days
2nd administration of lentivirus vector

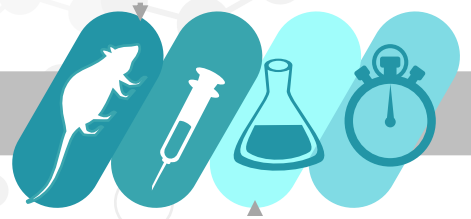
110 days
RotaRod test

4 months
Sacrifice

Timeline

Materials

Lmna H222P/H222P mice



IN VIVO EXPERIMENTS

IN VIVO RESULTS

Type of injection

Intraperitoneal injection which reaches faster the skeleton muscles cells and we divide it in two times: 1st administration of gesicles 2nd administration of lentivirus

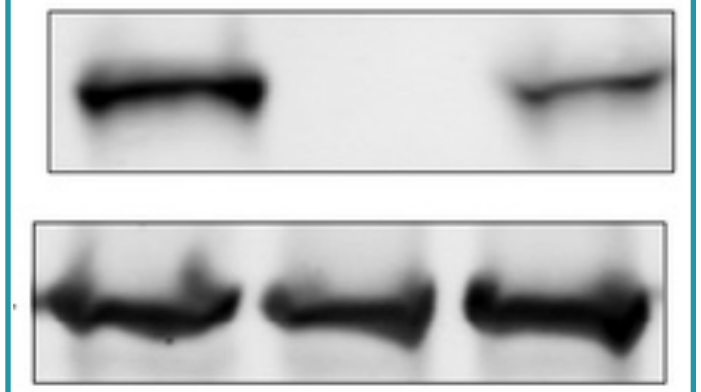
Quantity

As set in the in vitro analysis:

- Gesicles : 0,8 mg/kg
- Lentivirus vector : 1,3 mg/kg

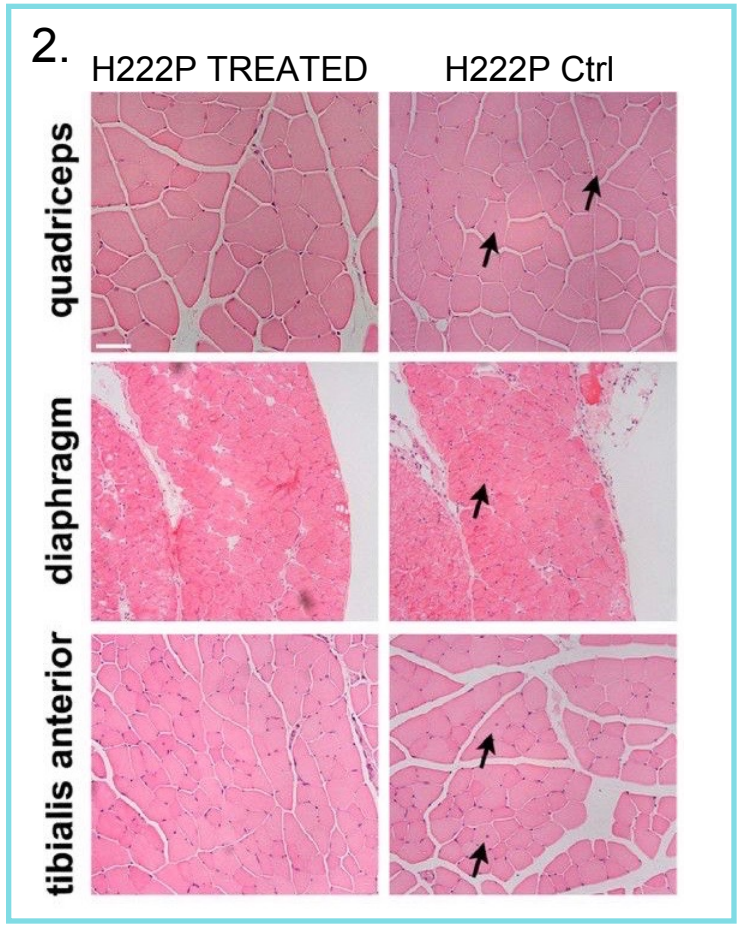
In vivo experiment results

1.WT H222P Ctrl H222p trtd

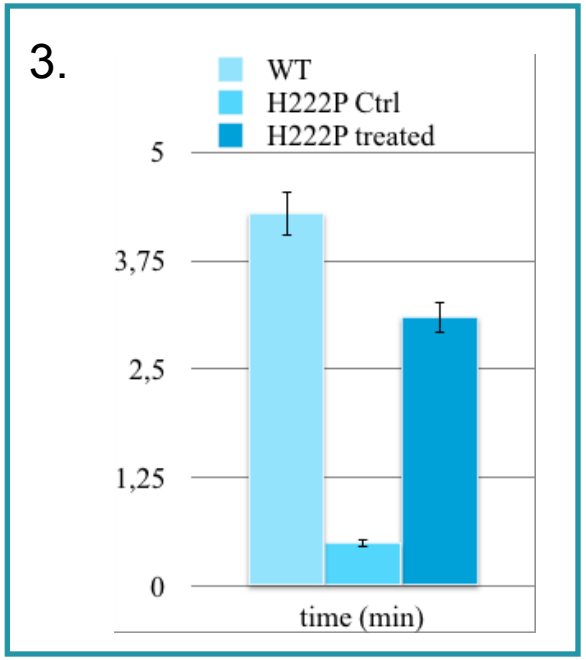


1. Western blot to analyze the production of LMNA after our treatment

2. Histological images to show how the phenotype can be reverted



3. RotaRod test is conduced after 110 days from birth and is used to evaluate the motor coordination of rodents.



The girls saved me thank you!

Materials & costs of production

1. Genscript CRISPR plasmid collection 500€
2. Cells cultures of skeletal muscle 500€
3. Immunofluorescence kit 520€ (ThermoFisher)
4. Lentiviral Packaging Mix: #VP100 400€ (AddGene)
5. Kit western Blot (reagents + instrumentation) 750€ (Thermo Fischer Scientific)
6. 30x (LMNA/H222P) mouse model 7200€/240€ each mouse (The Jackson Laboratory)
7. 15x (WT) mouse/1 free for each mouse model H222P (The Jackson Laboratory)
8. RotaRod test kit 320€ (PanLab)
9. Additional costs from basic lab manteinance and materials
10. Stabulation cost 1000€ each mouse for one year

Total cost : approximately **60.000€** without the salary cost of the researchers

References

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