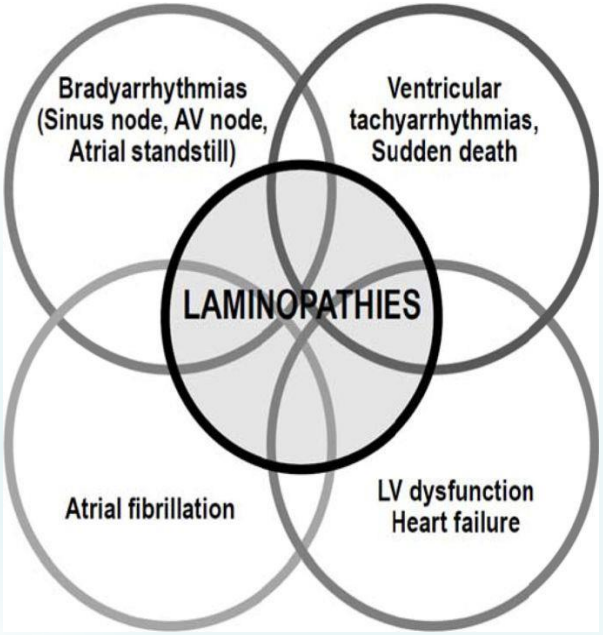
HGPS therapy with source-and-replace genome editing with liposome vector.

- A group of genetic diseases caused by alterations of the **nuclear protein lamina A / C** and some related proteins.

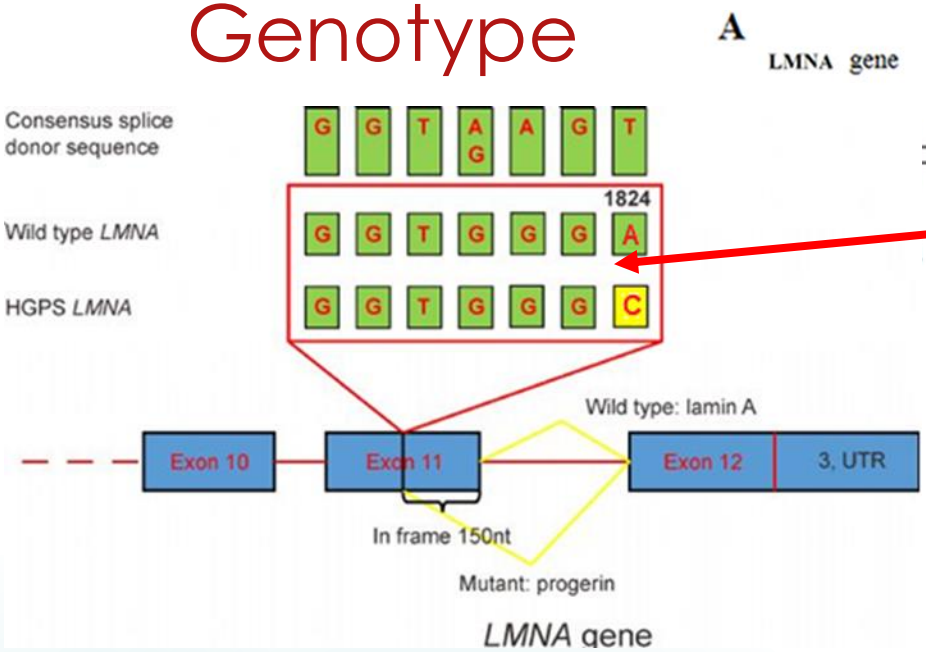


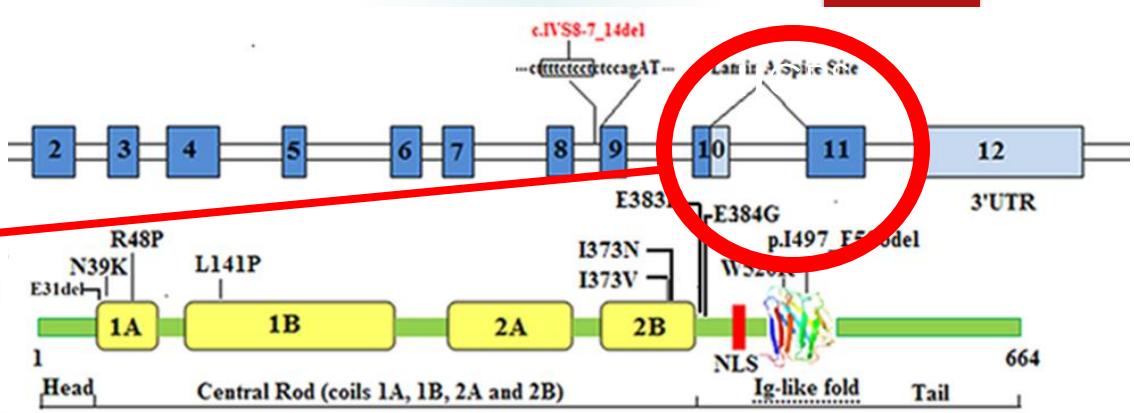
- Hutchinson-Gilford-progeria-syndrome

- Negative Consequences

- Hair Loss
- Osteoporosis
- Lipodystrophy
- Growth Delay
- Atherosclerosis
- Artheriosclerosis

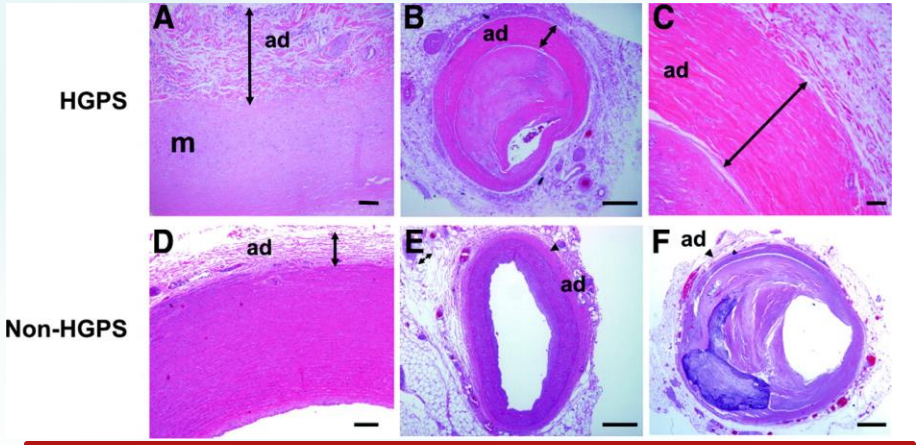
Genotype



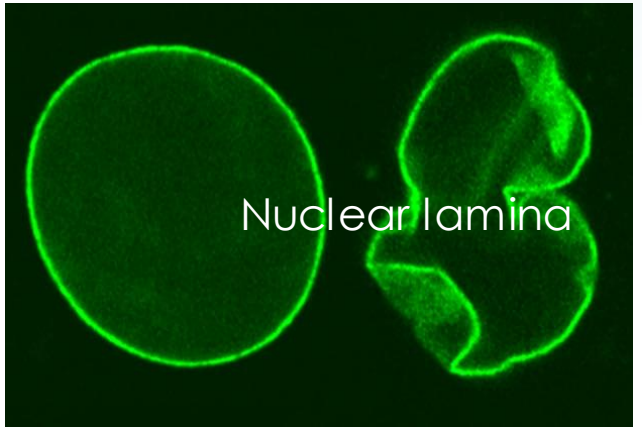


point mutation that carries the disease

Phenotype

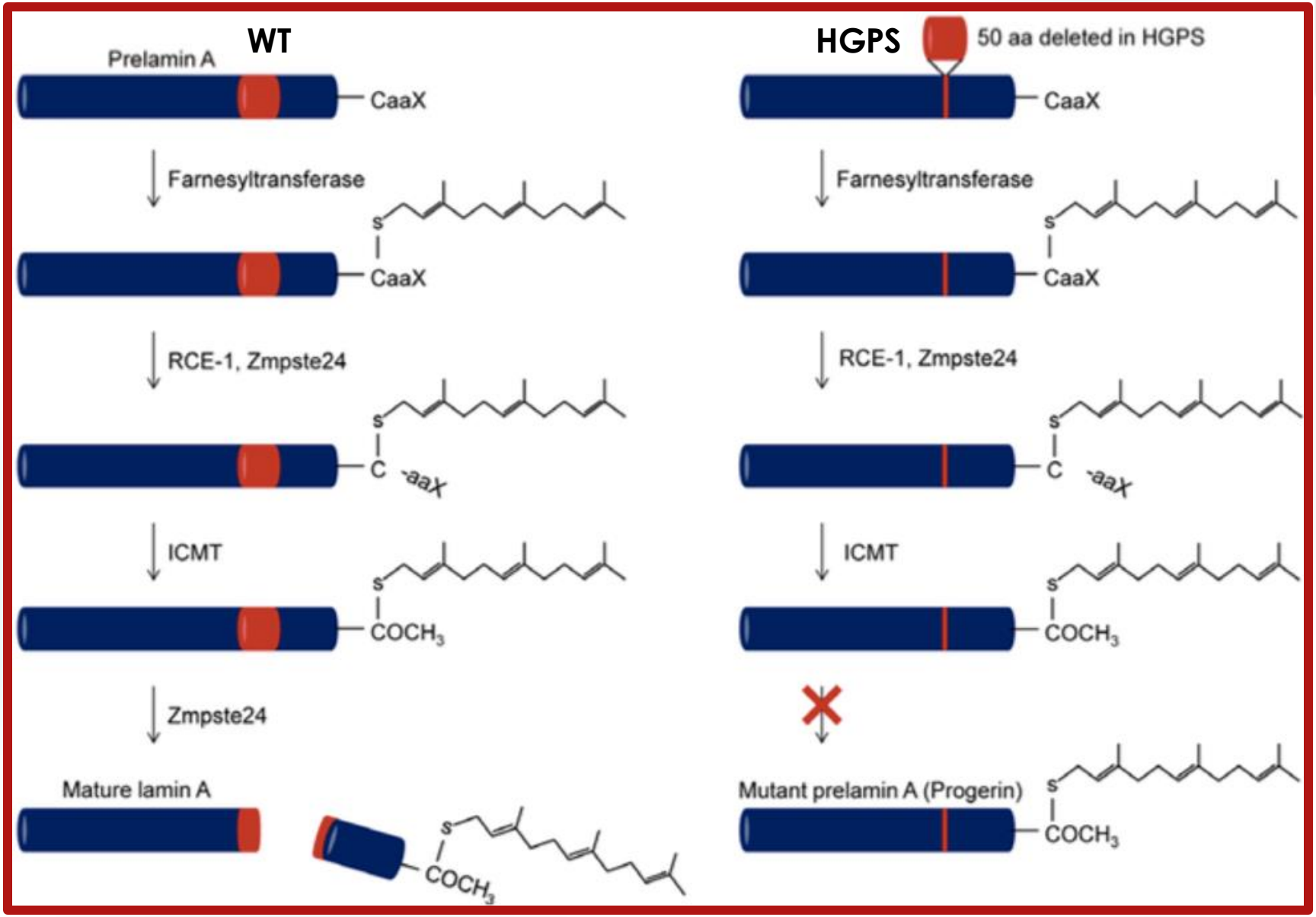


Hutchinson-Gilford progeria syndrome (HGPS) exhibit dramatically accelerated cardiovascular disease (CVD).



Progerin is what makes the nucleus to be unstable.

Post-Traslation modification



OUR GOAL IS?

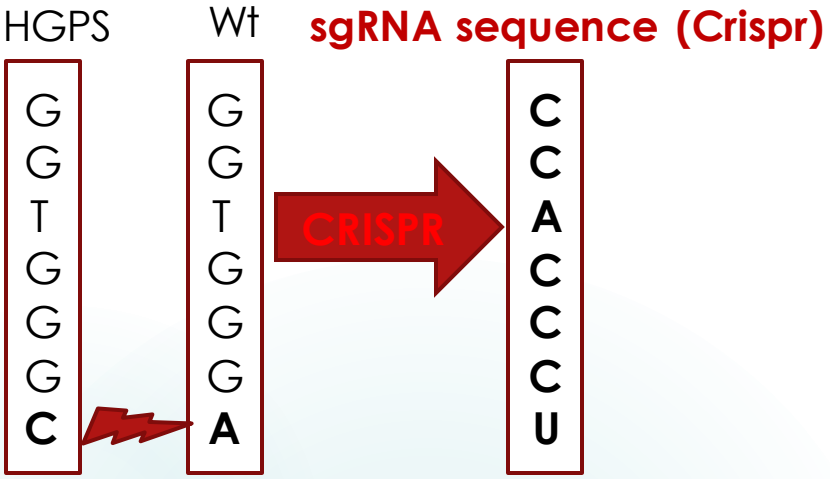
- Improve life expectancy;
- Reduce cardiovascular disease;
- Restablish wild type phenotype;
- Use of Non-invasive gene therapy;

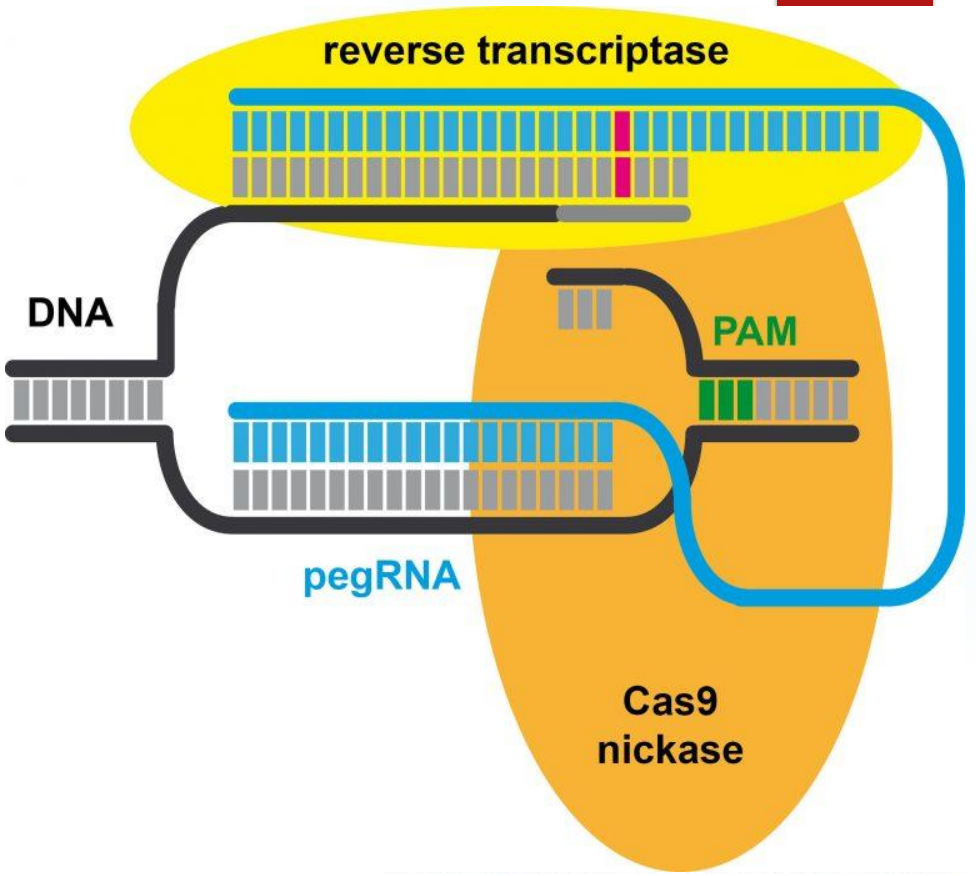
HOW?

- Cardiovascular cells treatment by **gene editing** search-and-replace
- Cells Treatment **in vitro** as proof of concept and **in vivo** (mice);

Methodologies and Protocol

Crispr- search and replace





- ✓ CrisprCas9 (nickase)
- ✓ sgRNA (8-15b)
- ✓ RT- polymerases

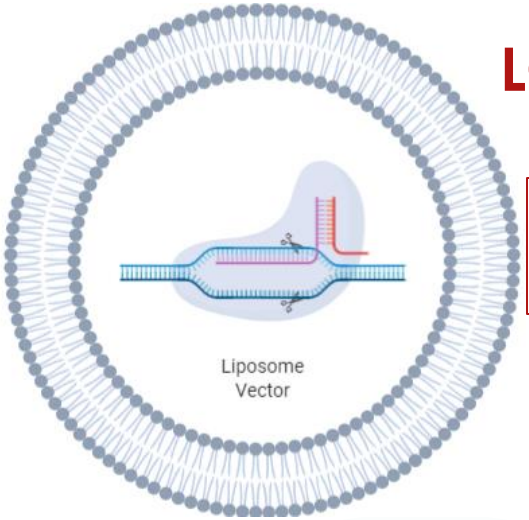
Range 7: 763 to 923 GenBank Graphics

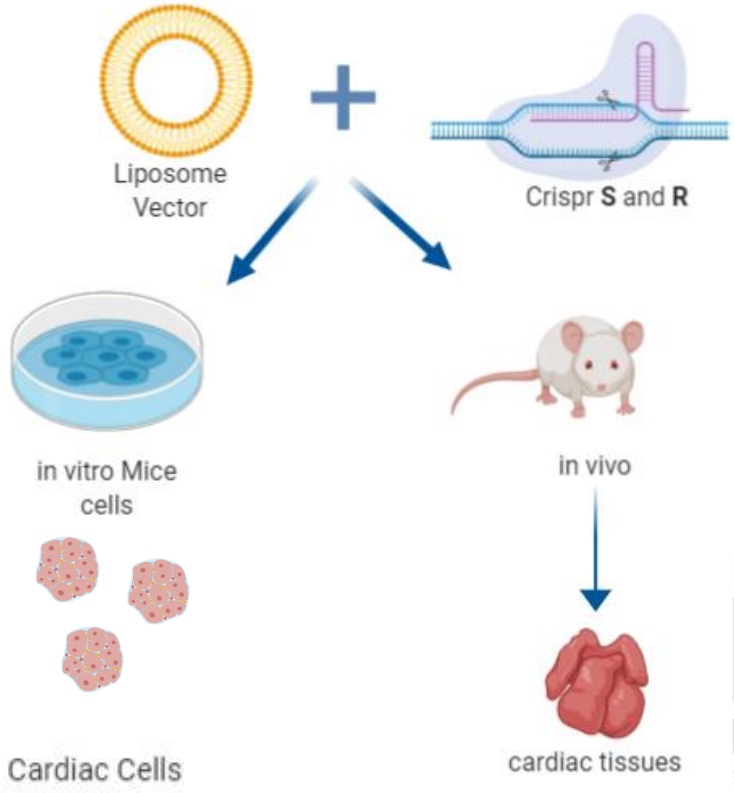
Score	Expect	Identities	Gaps	Strand
292 bits(158)	6e-75	160/161(99%)	0/161(0%)	Plus/Plus

Wild	21498	CTAGCAACACCAAGAAGGAGGGGGACTTGTTGGCTGCGCAGGCCCGGCTCAAGGACCTCG	21557
HGPS	763	CTCGCAACACCAAGAAGGAGGGGGACTTGTTGGCTGCGCAGGCCCGGCTCAAGGACCTCG	822

Experimental Protocol
LCPE (Liposome Crispr Prime Editing) treatment

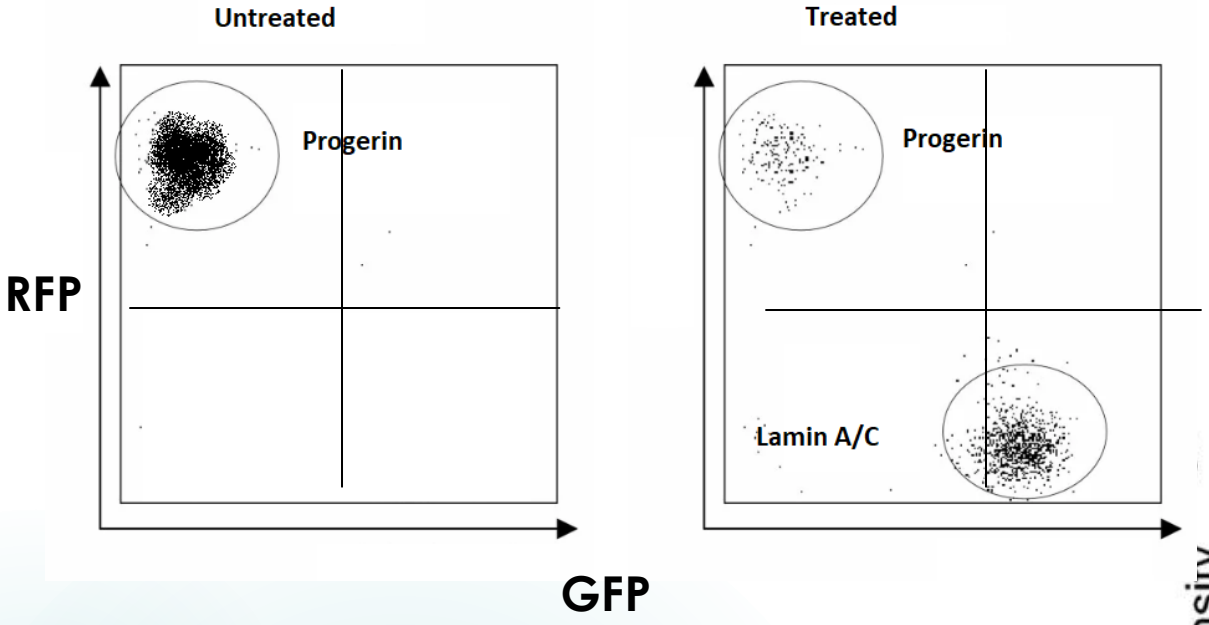
Liposome Vector + Crispr scearch and replace (bought assembled)

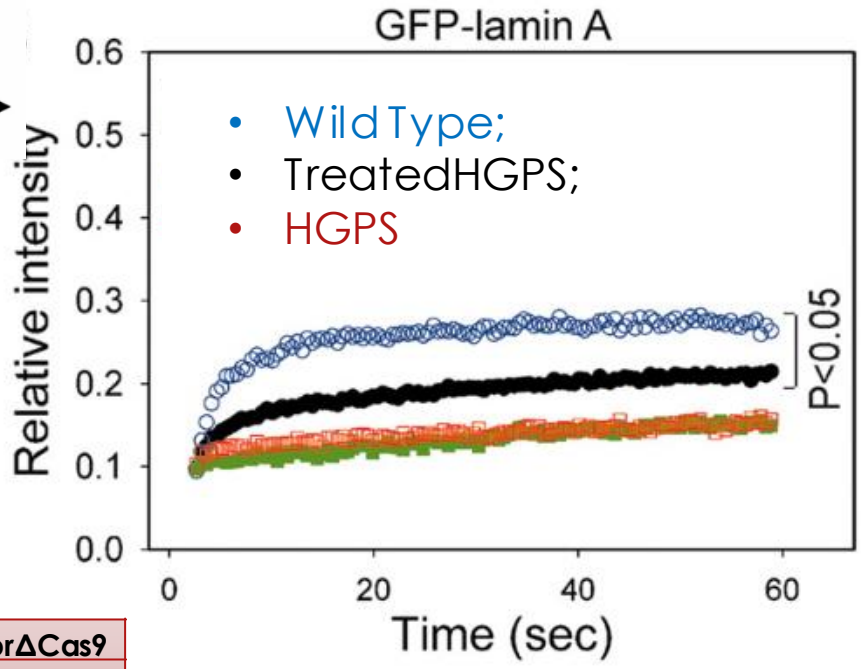




Intraperitoneal injection

Results in Vitro: Mice cells



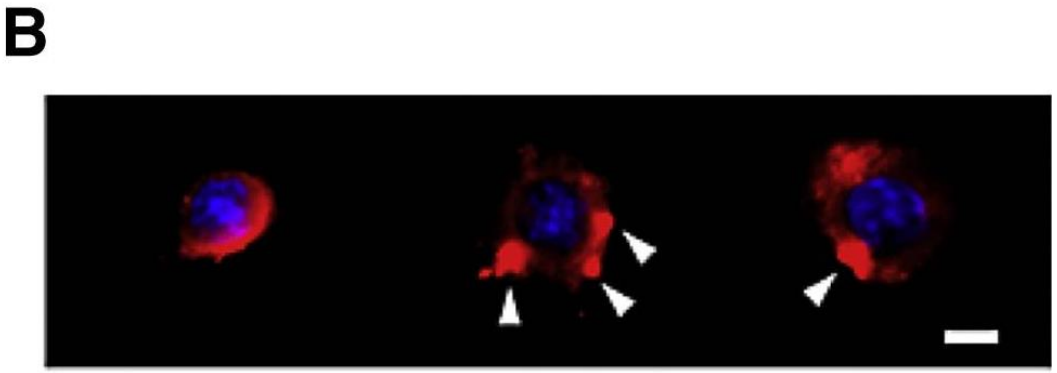


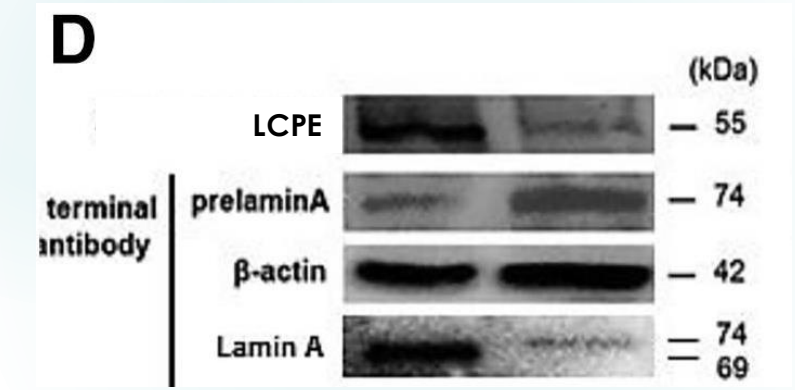
Mock treatment effects analysis (20mice)

	Liposome	Liposome+Crispr	Liposome+CrisprΔCas9
In Vitro Cardiac Cells (Wt)	no changes	1x10^(-8) error cut	no changes
In Vitro Cardiac Cells (HGPS)	no changes	1x10^(-9) error cut	no changes
In Vivo Cardiac Tissues (Wt)	no changes	1x10^(-12) error cut	no changes
In Vivo Cardiac Tissues (HGPS)	no changes	1x10^(-14) error cut	no changes

Results in Vitro: Mice cells

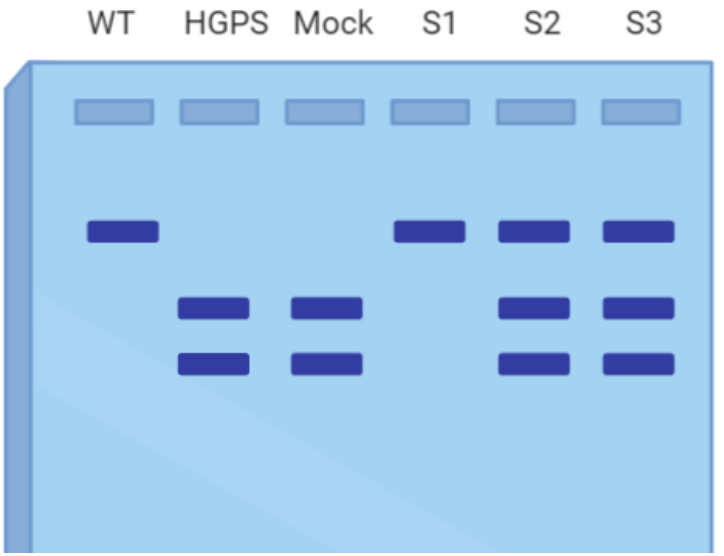
(B) Representative immunohistochemical staining with anti-lamin A antibodies. Original magnification, ×400. Scale bar, 10 μm. Arrowhead, abnormality in the nuclear membrane.



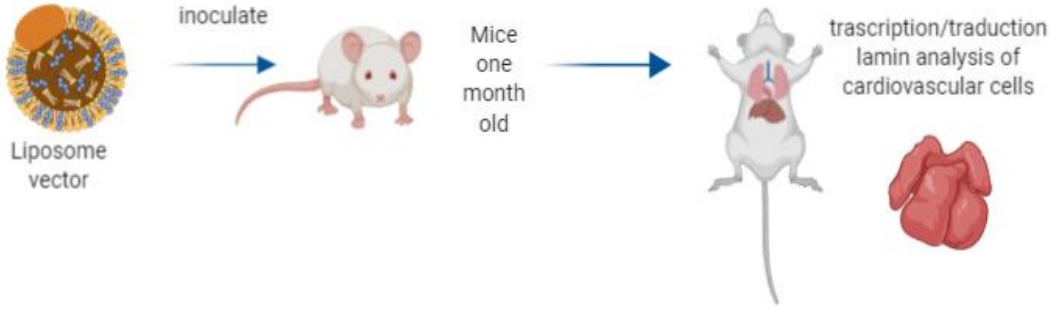


(D) Western blot analysis to measure the expression of the C-terminal prelamin A and lamin A in WT and Zmpste24−/− MDSPCs, relative to the expression of β-actin or vinculin.

RFLP

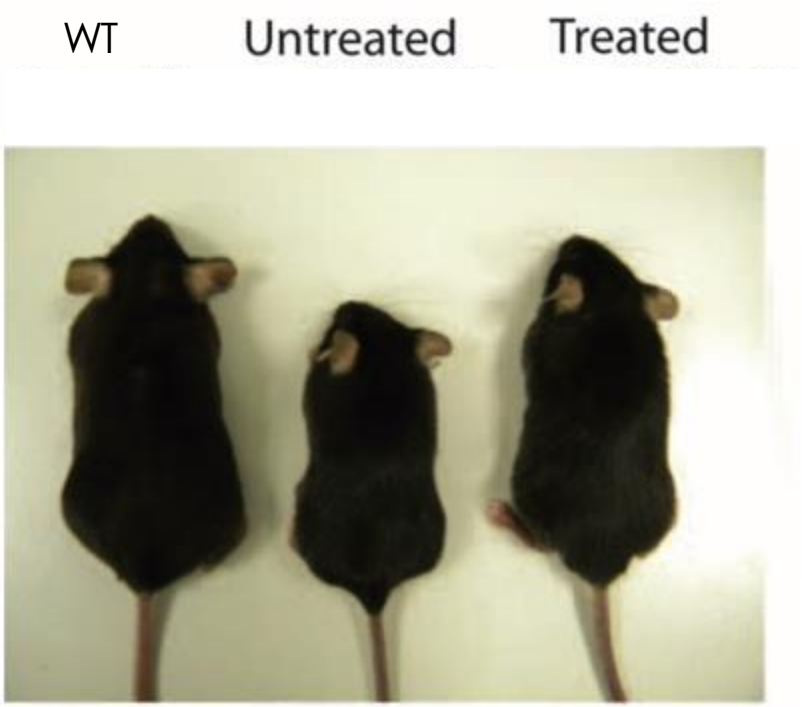


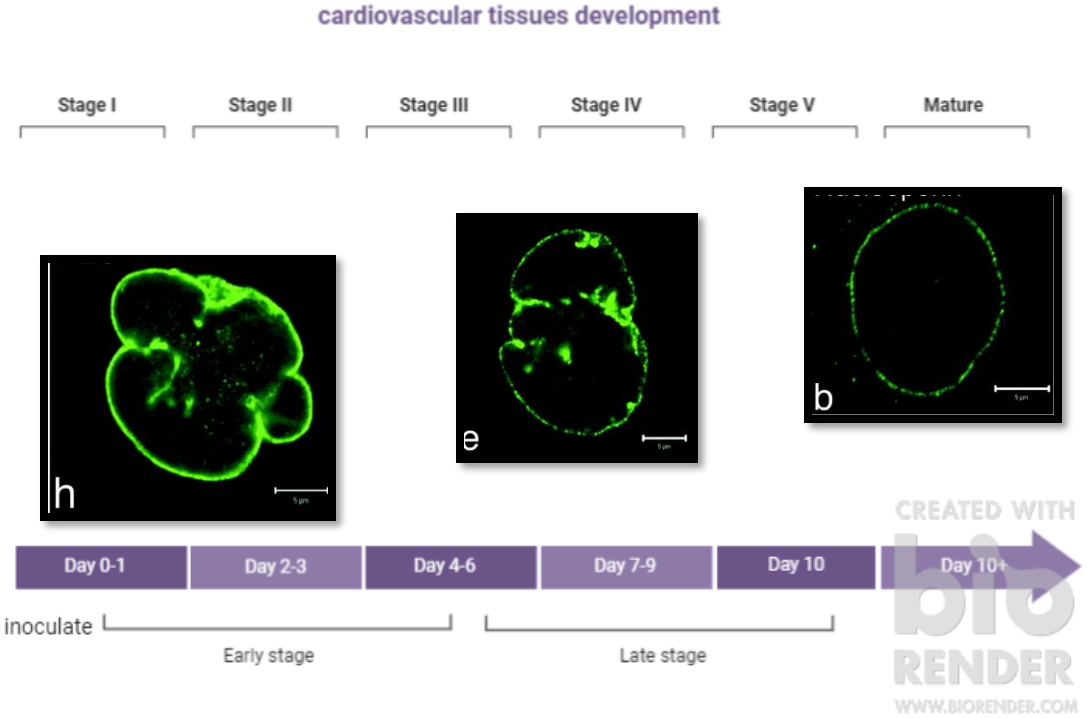
Results in Vivo



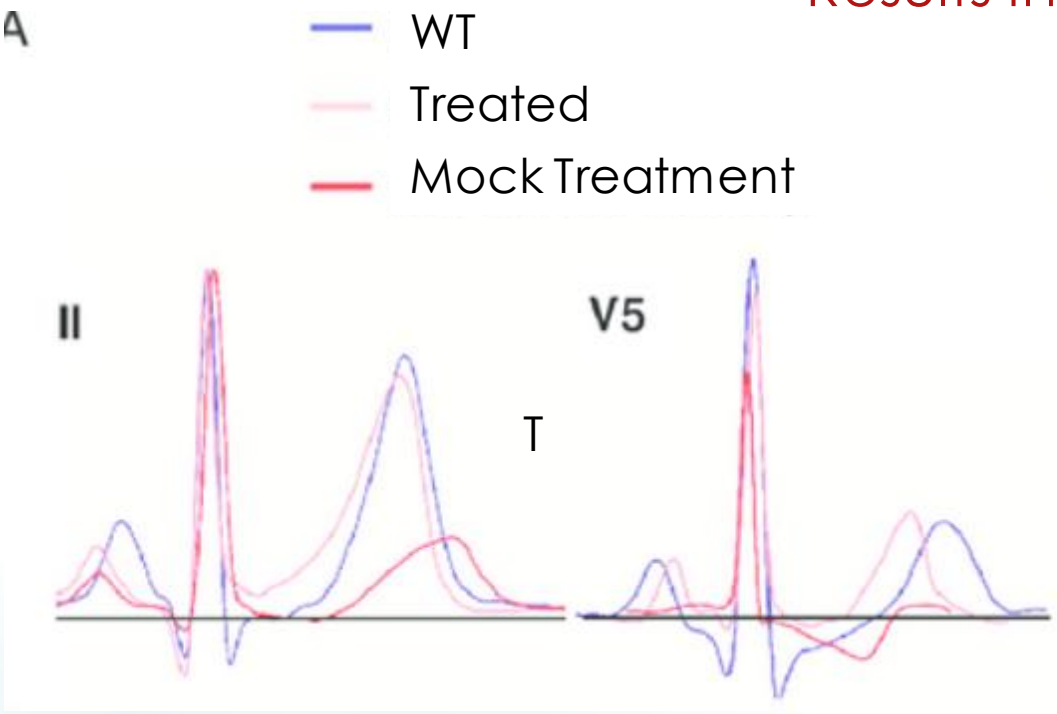
ImmunoFluorescence of nuclear lamina.

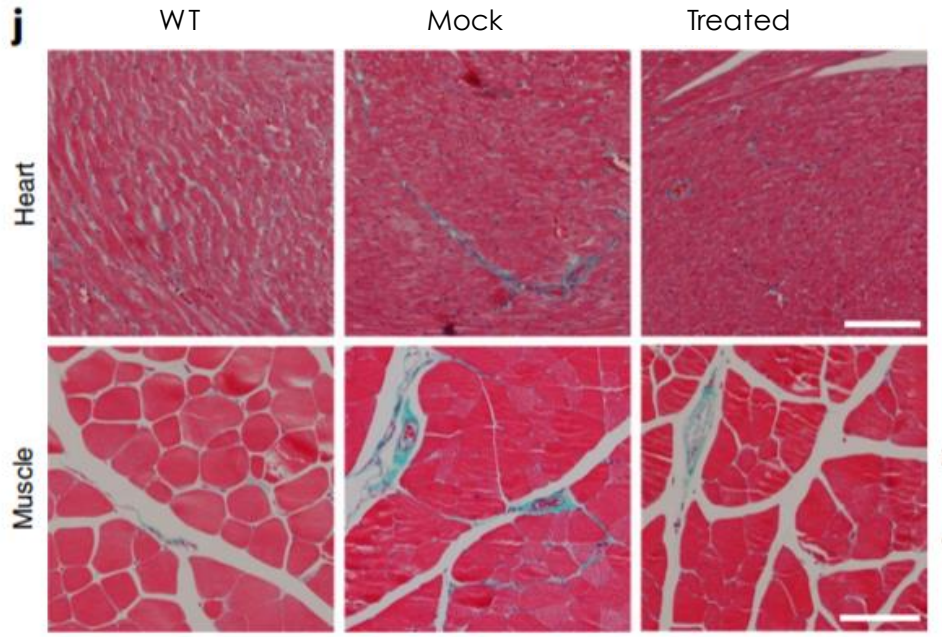
It is evident a partial recovery of wild type phenotype with **LCPE** treatment.





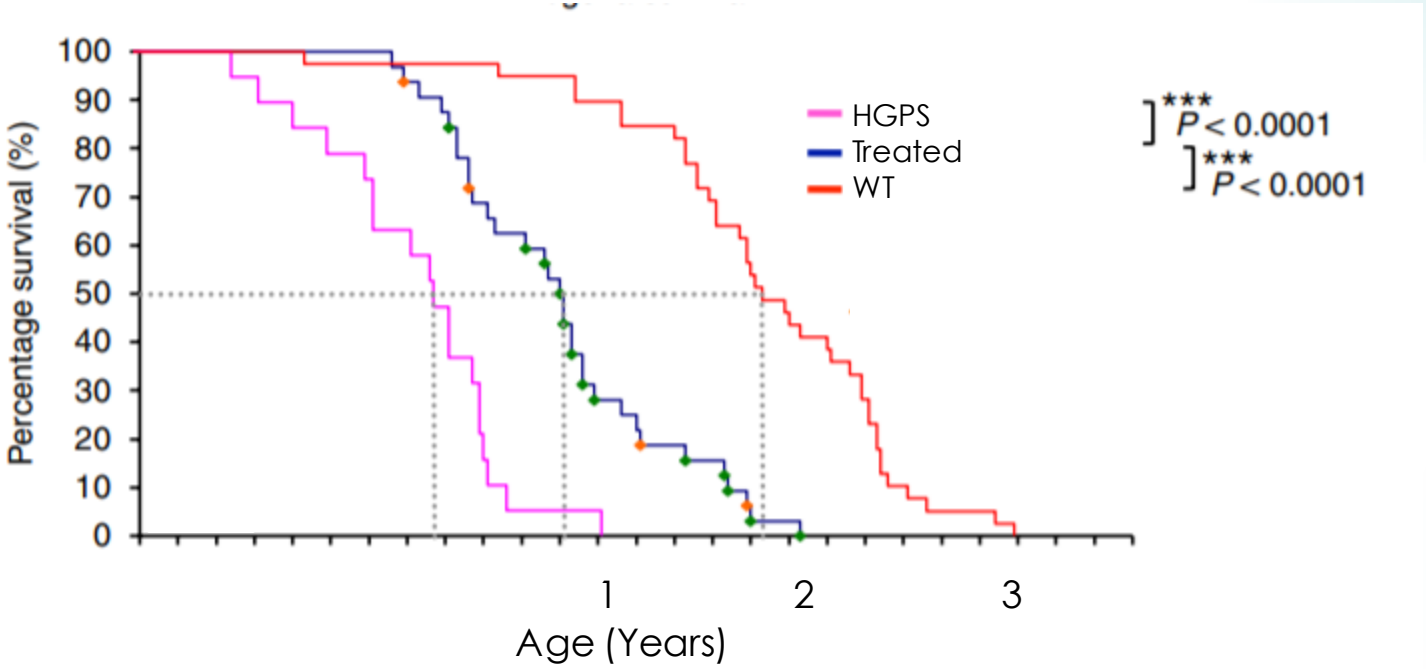
Results in Vivo

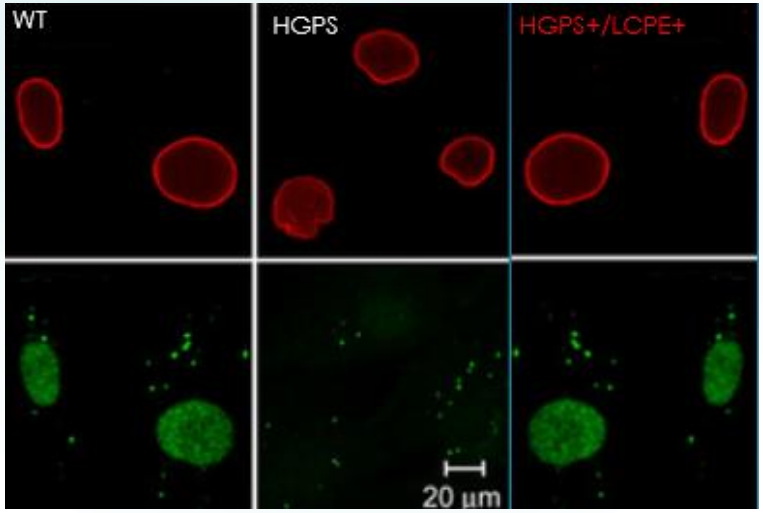




Significant QT interval prolungation = Higher risk of Ischemia

Results in Vivo

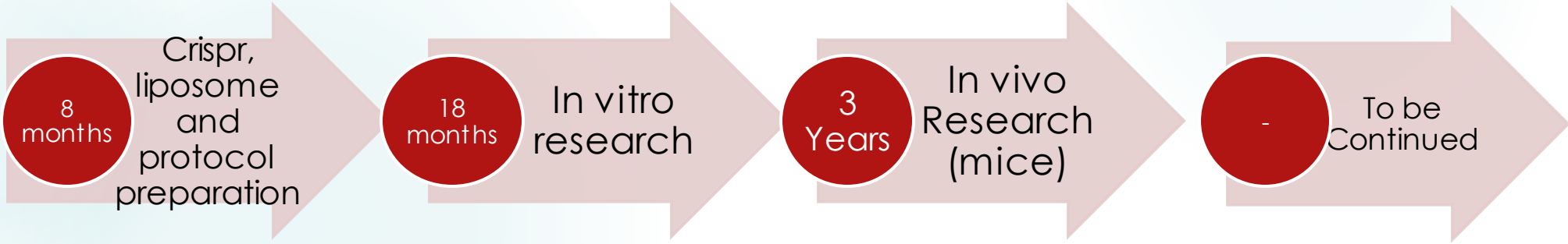




After LCPE treatment we observed restoration nuclear laminar of cardiovascular cells

Budget and Times

	Qt.	Inside storage	Outside storage
Mice Wt	20	4,8€ (one for die)	11,6€ (one for die)
Mice HGPS+	20	11,4€ (one for die)	16,8€ (one for die)
Mice HGPS+ .tr. LCPE	20	11,4€ (one for die	16,8€ (one for die)
Crispr Prime Kit	20	300€ (one Kit)	300€ (one Kit)
Staff	3+1	1500€ x3 (Phd) + 2500€(post Phd)	1500€ x3 (Phd) + 2500€(post Phd)
Liposome Kit	20	200€	200€
Various	/	10'000.00€	10'000.00€
cages for mice	60	8,80€ (one for die)	10,95€ (one for die)
Total	**3 years**	**230'000.00€**	**250'000.00€**



Pitfalls

- Possible oncogenic gene insertion
- High costs

Solutions

- Sequencing
- Long term trials
- More efficient protocols

https://ahajournals.org/journal/atvb
http://biomodel.uah.es/en/lab/cybertory/analysis/trans.htm
https://blast.ncbi.nlm.nih.gov/Blast.cgi
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