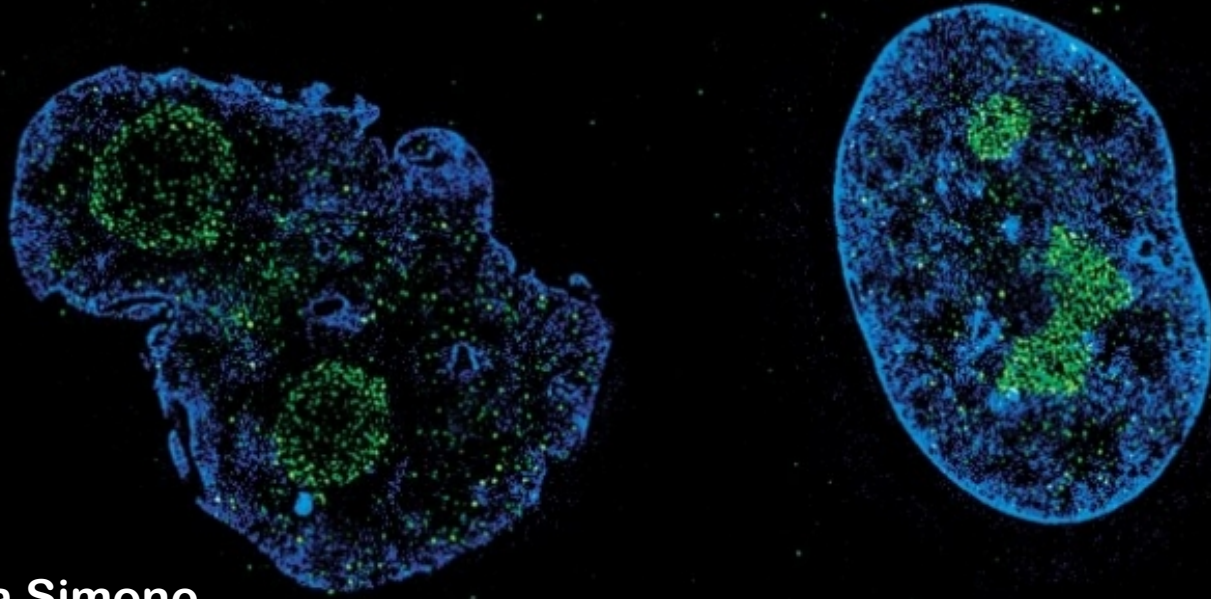


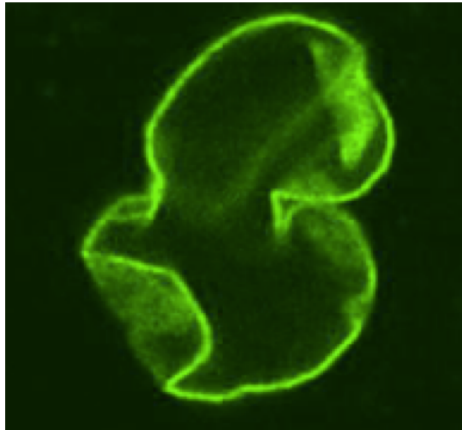
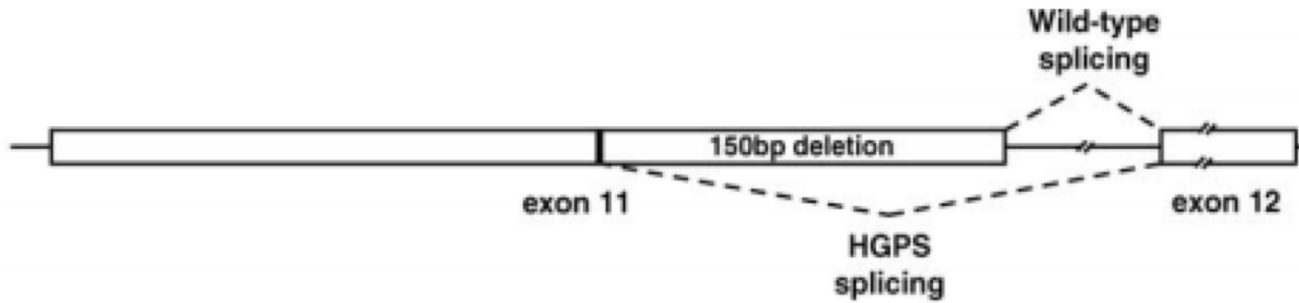
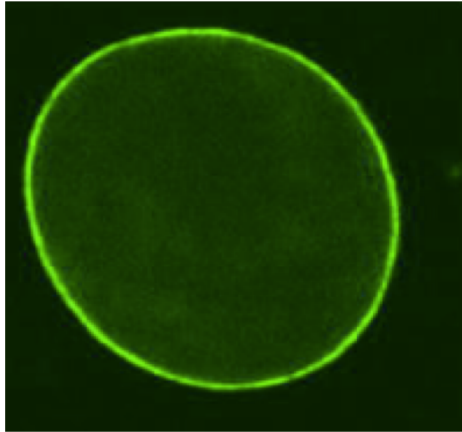
# Endogenous expression of ASO and siRNA for treating Hutchinson-Gilford Progeria Syndrome (HGPS)



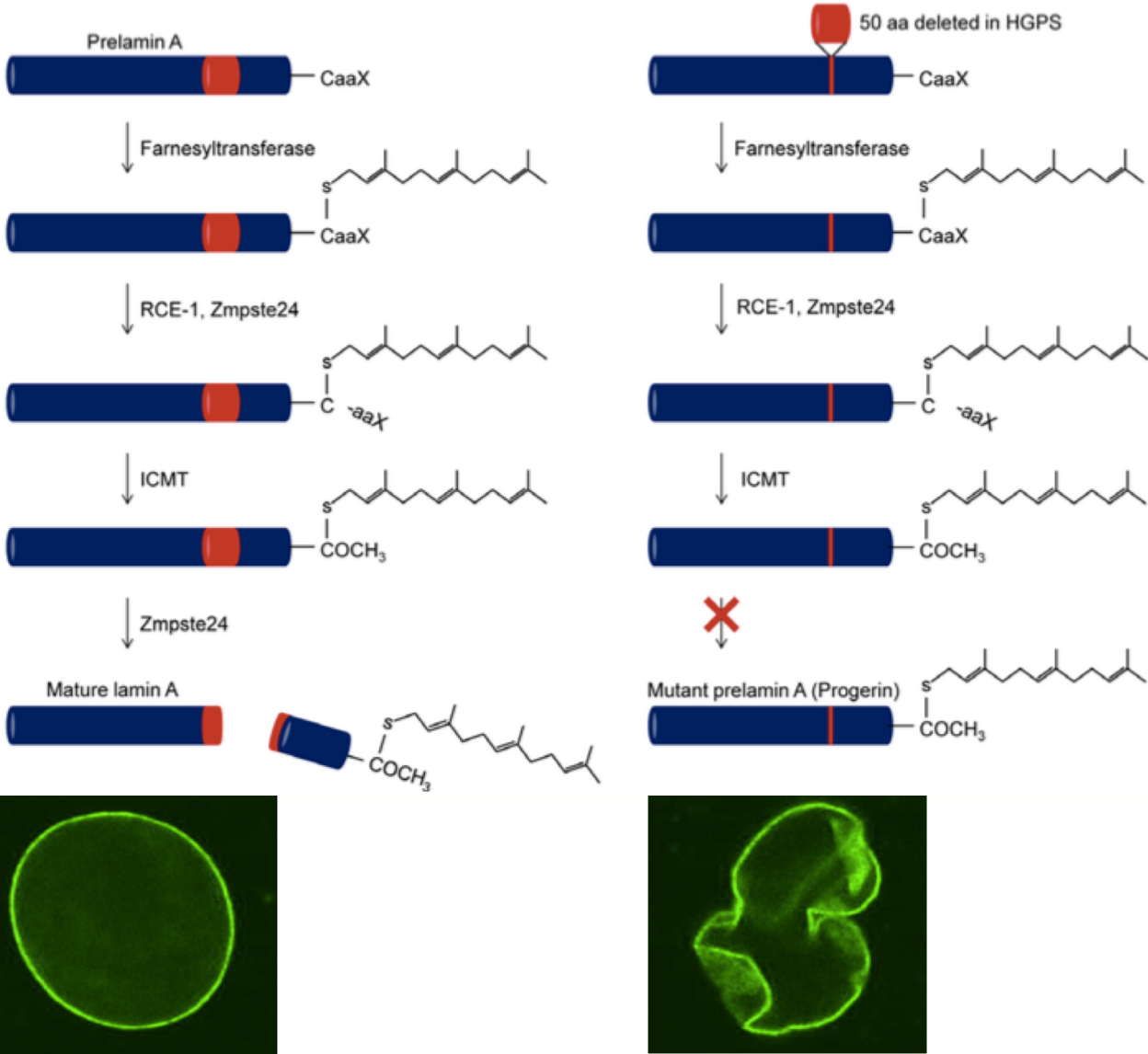
Frascolla Simone  
Moresi Fabiana  
Passeri Iacopo  
Pioppini Carlotta

Prof.ssa Isabella Saggio  
A.A. 2018/2019

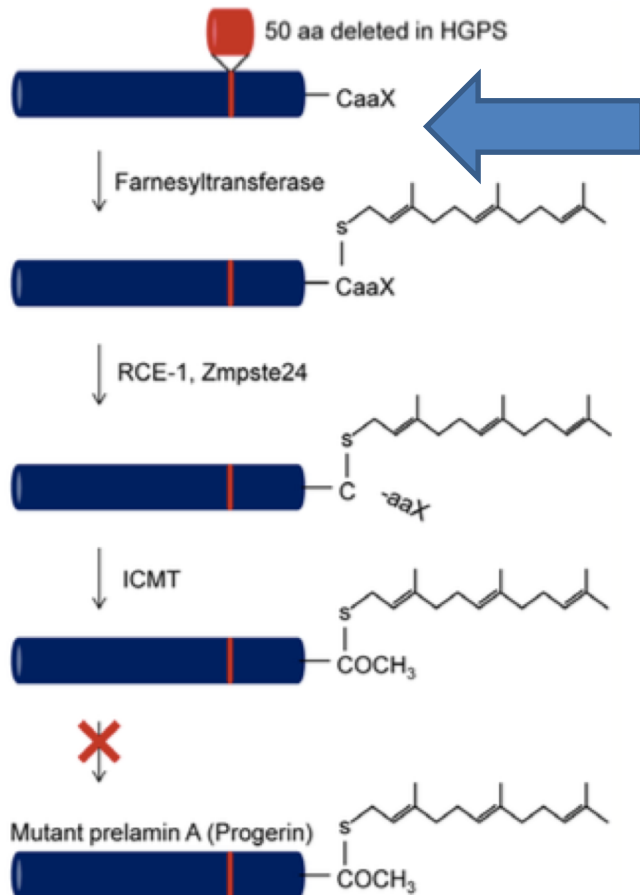
# HGPS MUTATION



# LAMIN vs PROGERIN MATURATION

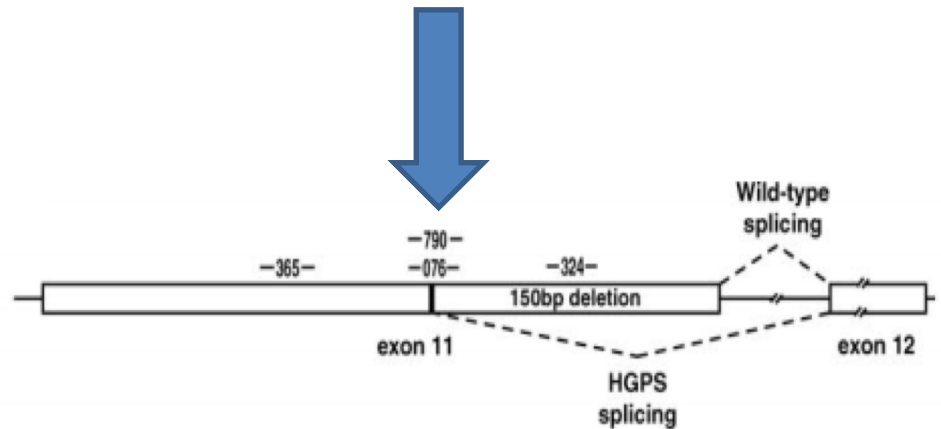


# RECENT THERAPY APPROACHES



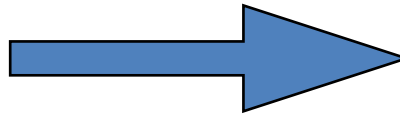
Farnesyltransferase Inhibitor (FTI)

AntiSense Oligonucleotides (ASOs)





**OUR  
GOAL**



**Treating  
HGPS**

**HOW?**

- Rescuing WT splice site
- Inhibiting farnesylation of progerin
- Reducing progerin levels

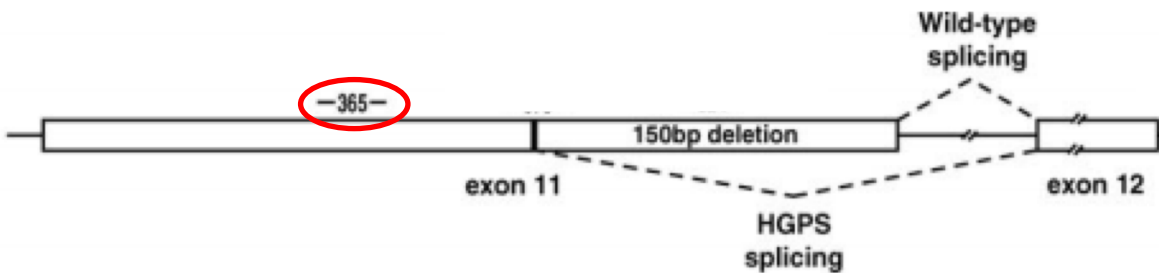
**WHEN?**

- As soon as HGPS is diagnosed  
( $\approx$ 2 years old)

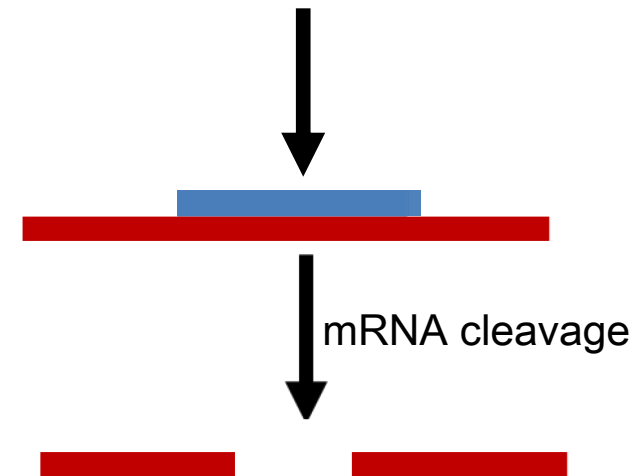
# OUR APPROACH: COMBINATORY THERAPY!

ASO 365

siRNA



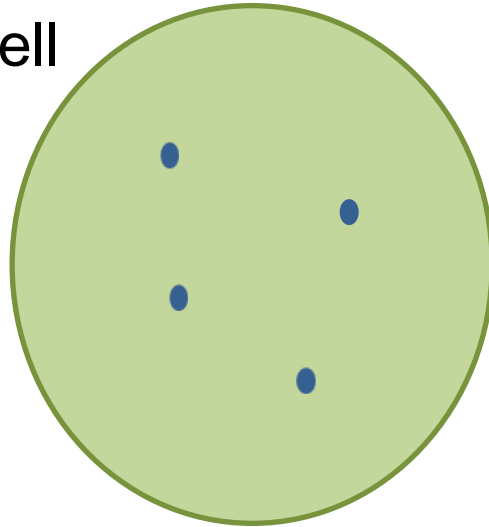
$\beta$  subunit mRNA of FTase



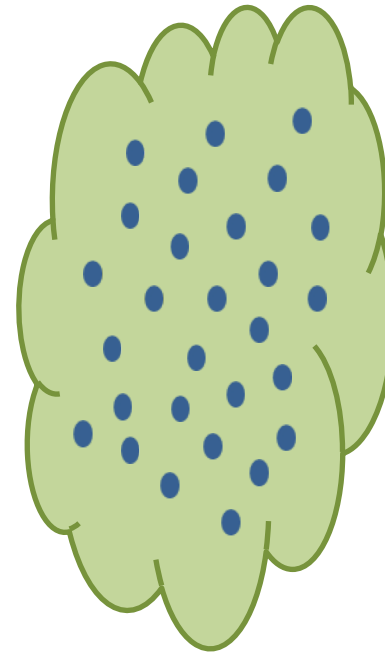
FTase doesn't work anymore

# THE REASON WHY

WT cell



HGPS cell



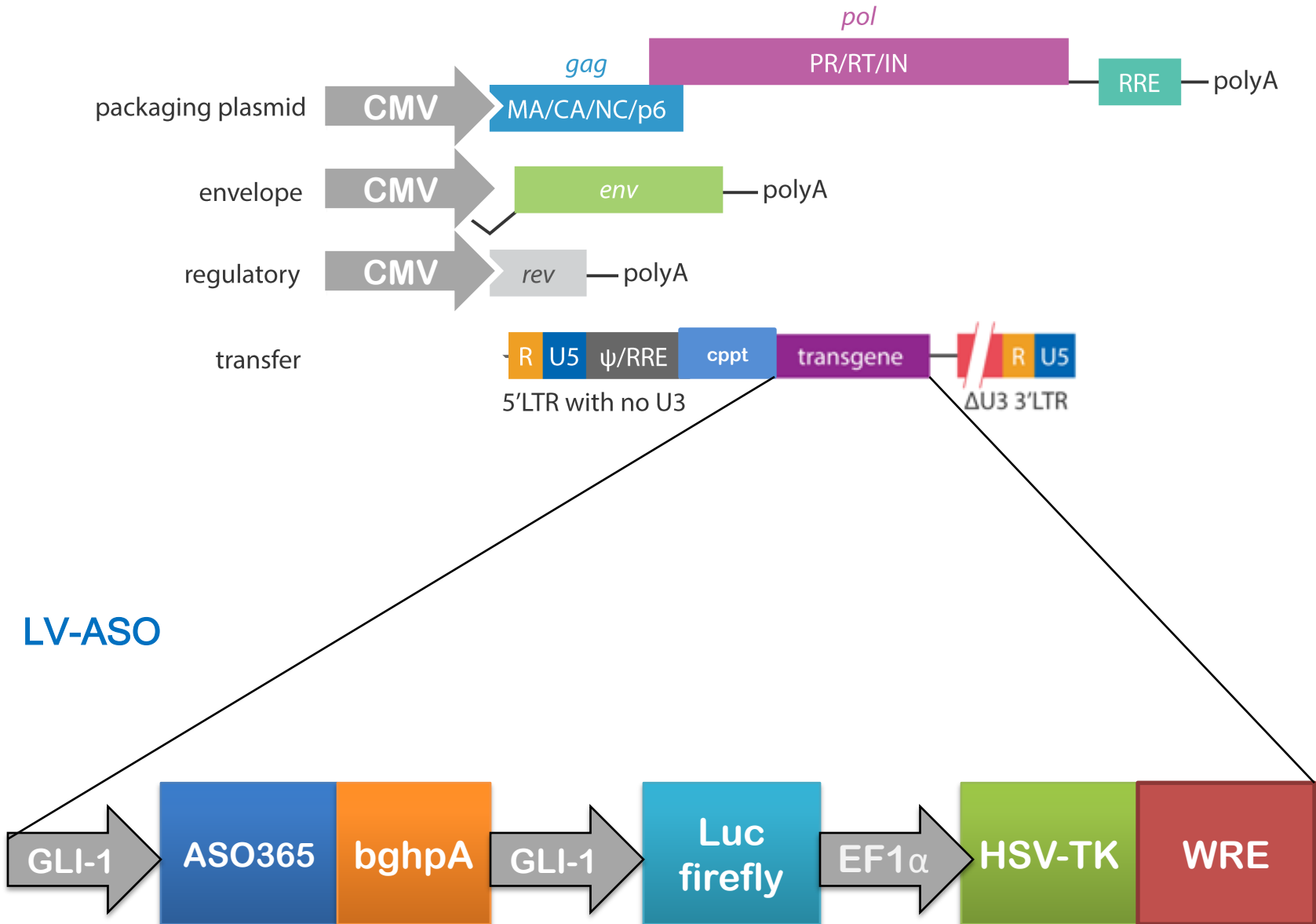
 MEOX2

Symbol	LocusLink	Change*	Gene designation
Transcription			
<b>MEOX2</b>	4223	<b>29.1</b>	Mesenchyme homeo box 2
FOXE1	2304	13.2	Forkhead box E1
OAZ	23090	5.2	OLF-1/EBF associated zinc finger
BTEB1	687	3.9	Basic transc. element binding 1
GATA6	2627	3.9	GATA binding protein 6
TOX	9760	3.6	Thymus high mobility group box
STAT1	6772	3.3	Signal transducer activator transc. 1

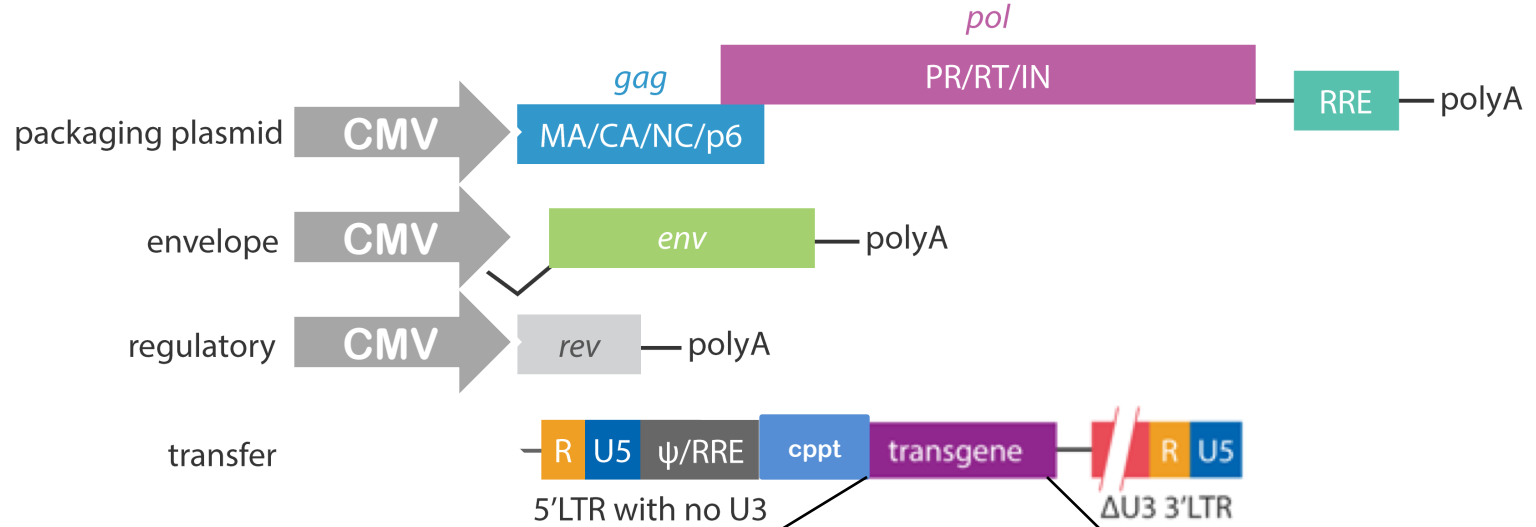
The GLI-1 gene promoter region is occupied by MEOX2, accompanied by transcriptionally active RNA Pol II

  
**MEOX2-dependent GLI-1  
gene expression**

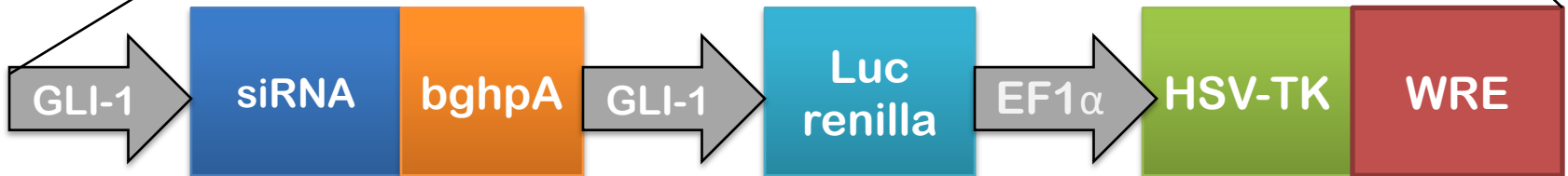
# OUR LENTIVECTOR



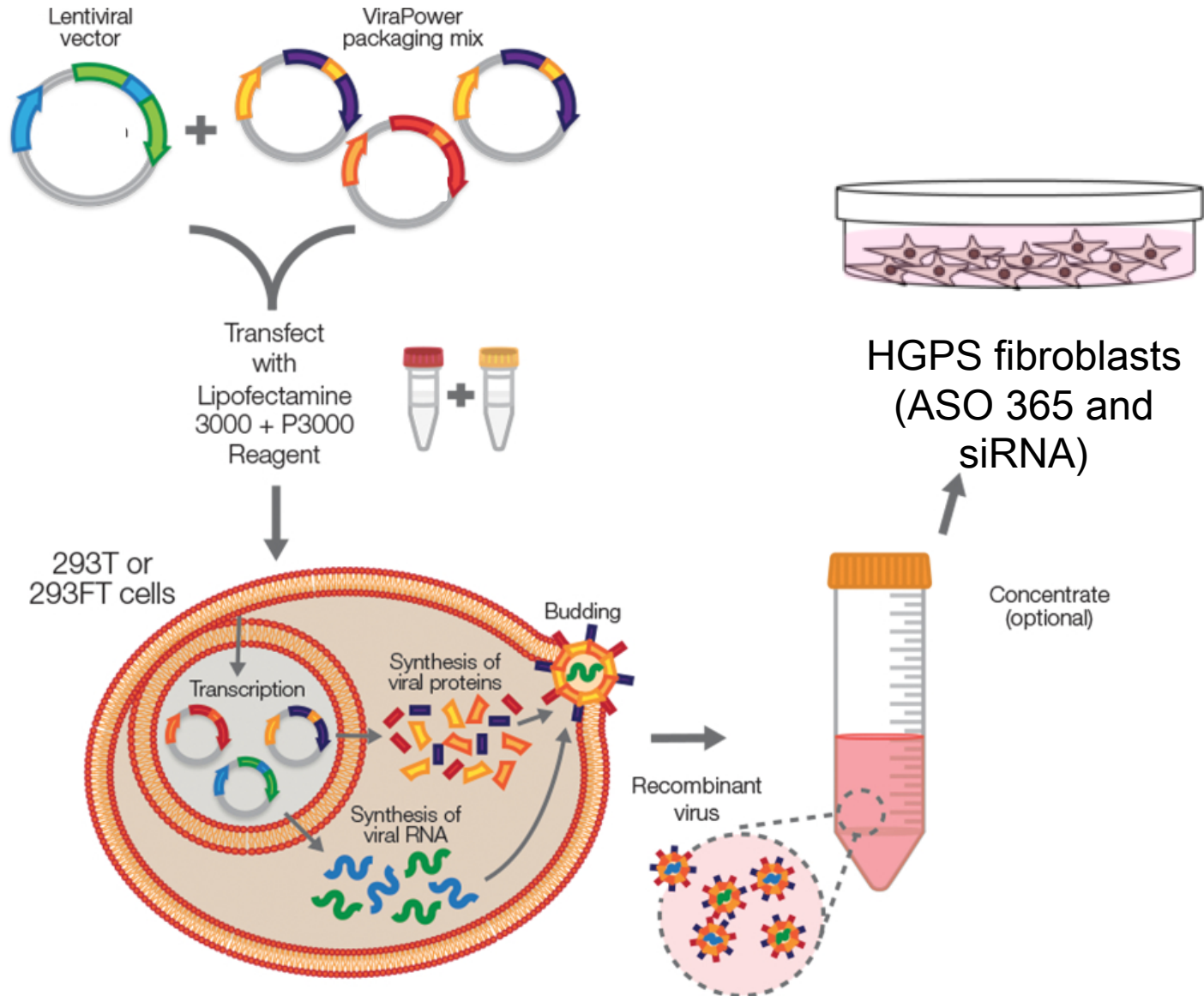
# OUR LENTIVECTOR



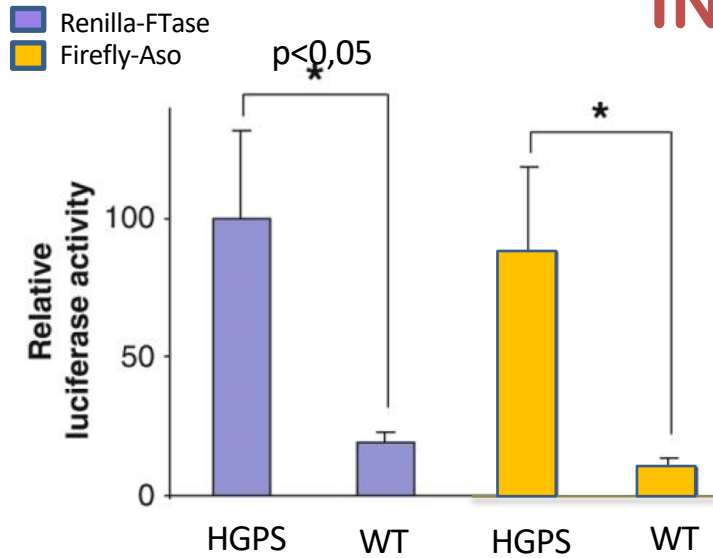
## LV-siRNA



# IN VITRO EXPERIMENTS: planning



# ARE OUR GENE CASSETTES EXPRESSED ONLY IN HGPS CELLS?



- Luciferase assay: correct expression of our genes under GLI-1 promoter only in HGPS cells.

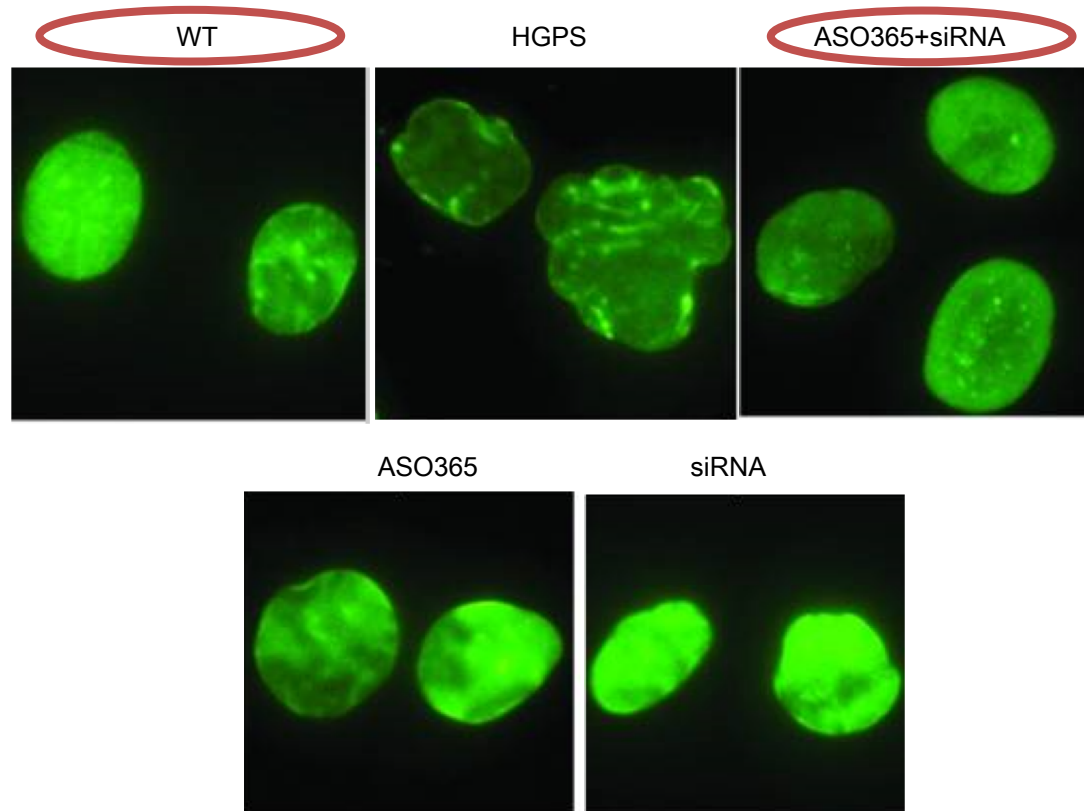


- qPCR: genome integration and the expression in HGPS cells.



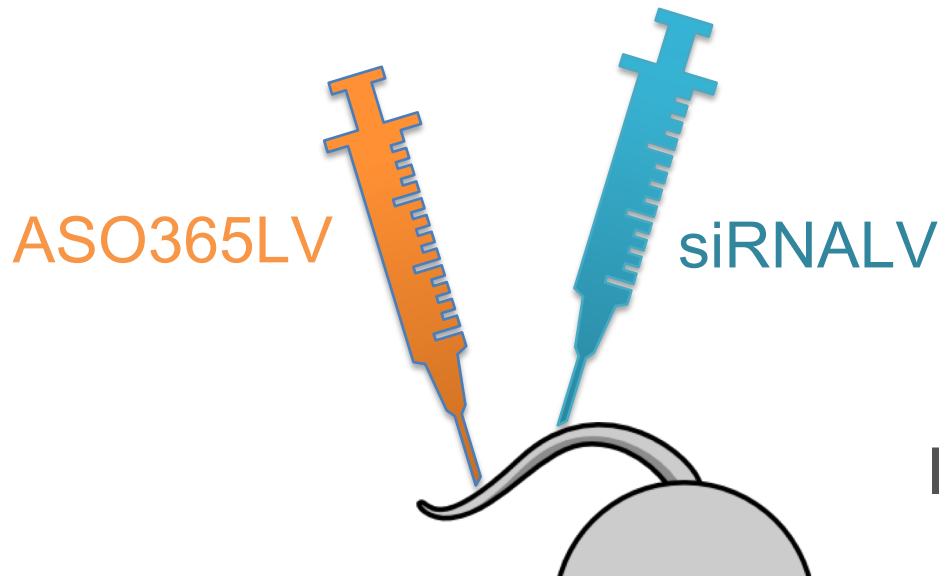


# IS THE NUCLEAR SHAPE RESCUED?



Immunohistochemistry:  
Recover of cellular phenotype in double  
treated HGPS cells

# IN VIVO EXPERIMENTS: planning



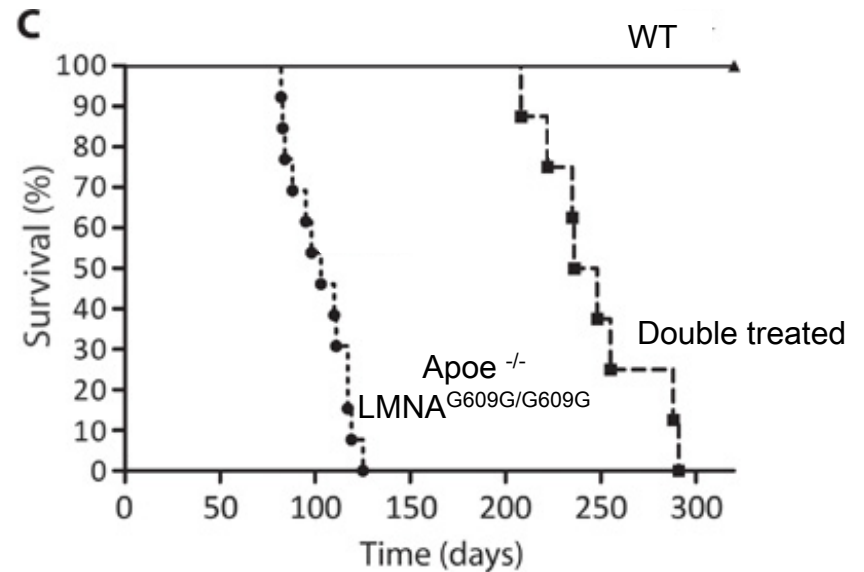
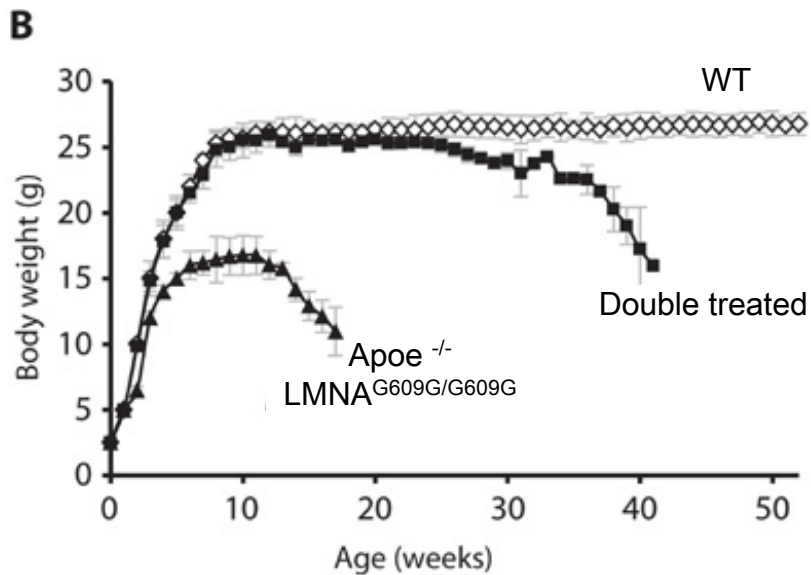
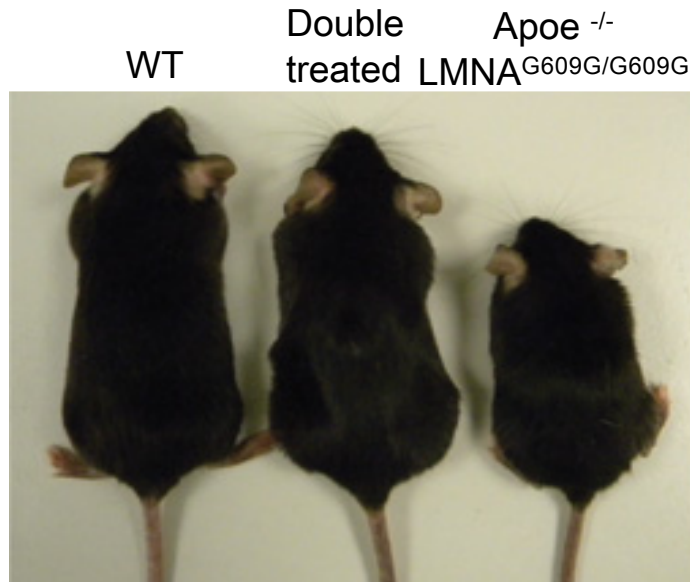
MOUSE MODEL:  $ApoE^{-/-}$   
 $LMNA^{G609G/G609G}$

- Premature aging phenotype
- Medial VSMC loss
- Lipid retention,
- Adventitial fibrosis
- Accelerated atherosclerosis

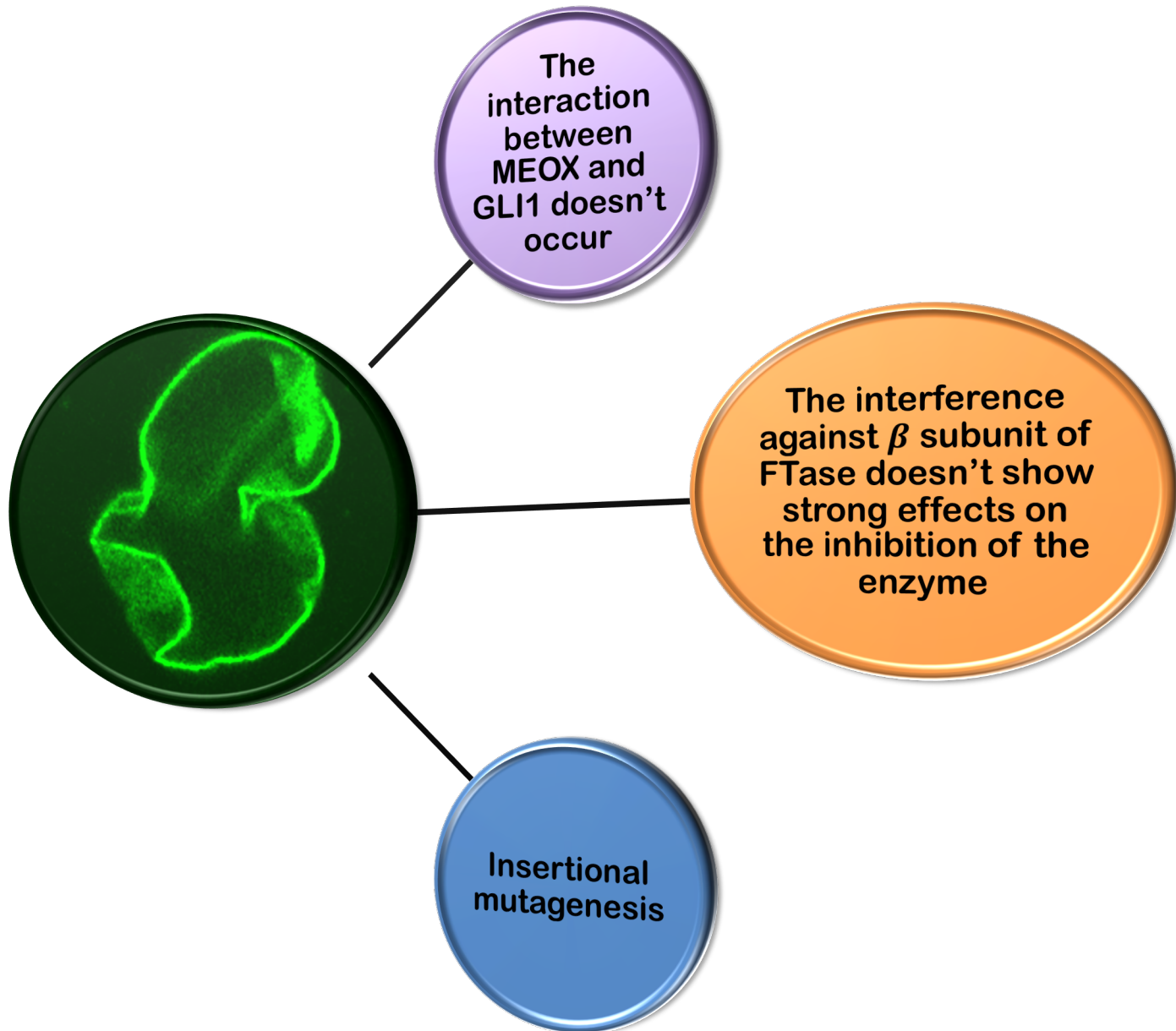
↓

ANALYSIS

# WHAT HAS HAPPENED *IN VIVO*?



# PITFALLS



# SOLUTIONS

**Find another promoter linked to FOXE1 expression**

**Try to induce the cleavage of Ftase subunit  $\alpha$**

**Activating suicide gene (HSV-TK)**



# MATERIALS AND COSTS

- Lentiviral production: 3'500€
- q-PCR and PCR kits: 600€
- WB kit: 2'500€
- Immunofluorescence kit: 1'500€
- Immunohistochemistry kit: 1'250€
- Mouse model (Jackson Labs): 3'373.50€
- Animal Facilities: 30'000€
- Cell Cultures: 1'500€
- Imaging Techniques: 600€
- Laboratory Instruments: 3'000€
- Salary of One Researcher: 36'000€/pp/yy
- Lamin A/C, Progerin MC Antibodies: 1'100€
- ASOs: 50€
- siRNAs: 279€
- Renilla-Firefly Luciferase Dual Assay Kit: 184€

**FINAL COST:** 230'000€ (for 2 years of research)

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