



SAPIENZA  
UNIVERSITÀ DI ROMA

# Primary Myelofibrosis

Gene Therapy on MPL gene via CRISPR CjCas9: a possible treatment against PMF

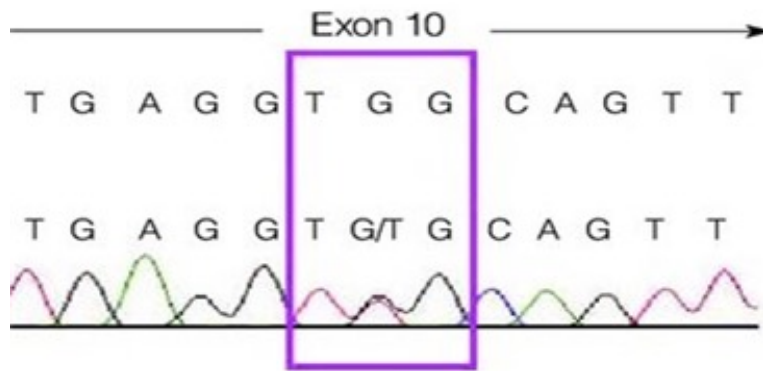
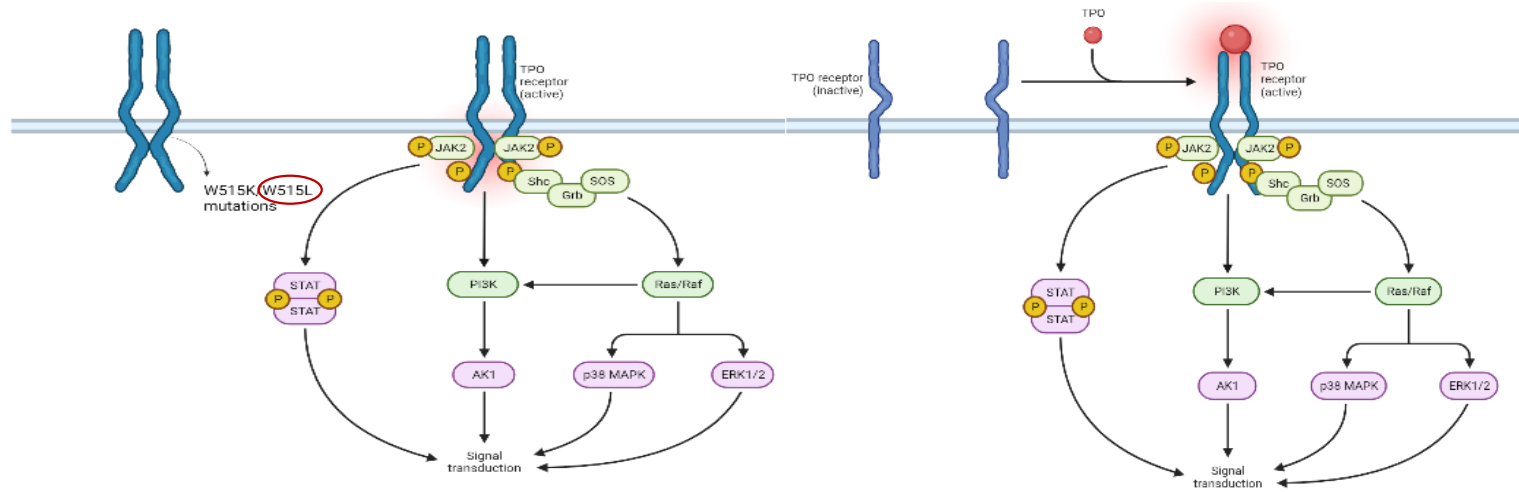
Gene Therapy

Prof.ssa I. Saggio, Dr.ssa M. La Torre, Dr.ssa R. Burla

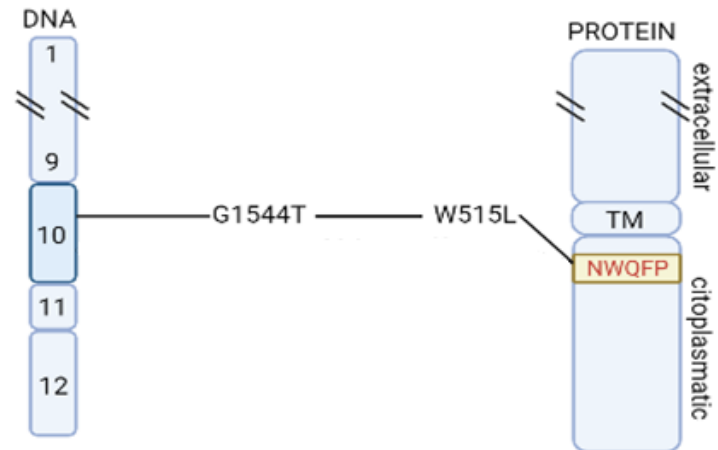
Academic Year 2022/2023

M. Hushi, M. Maio, C. Perini

# PMF: BACKGROUND

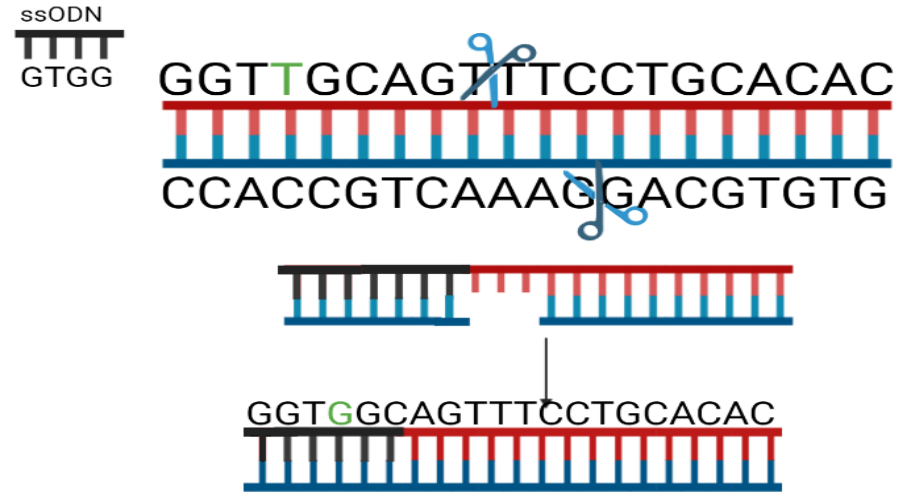
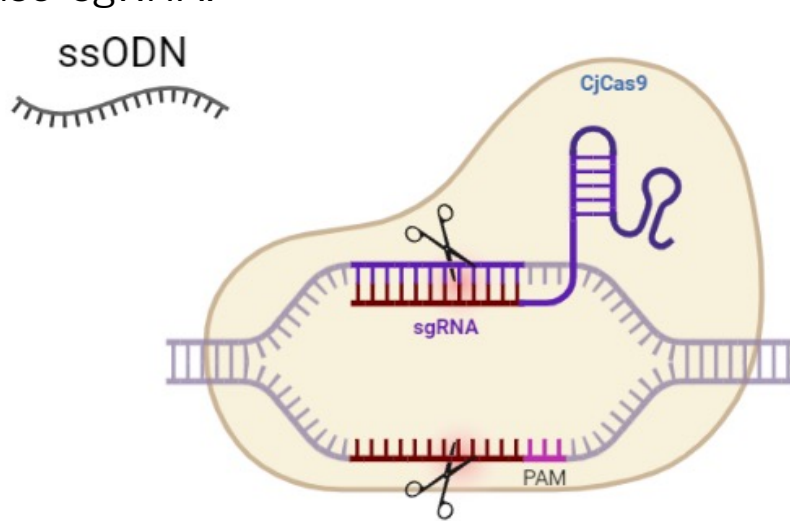


**MPL gene mutation**  
 (Adapted from Kim et al. 2010)



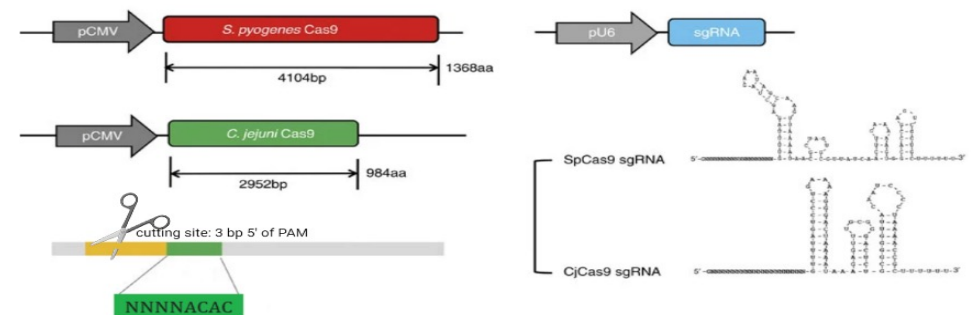
# AIM OF THE PROJECT

Ex vivo therapy to treat Primary Myelofibrosis through the correction of MPL gene sequence with the system CjCas9-sgRNA.

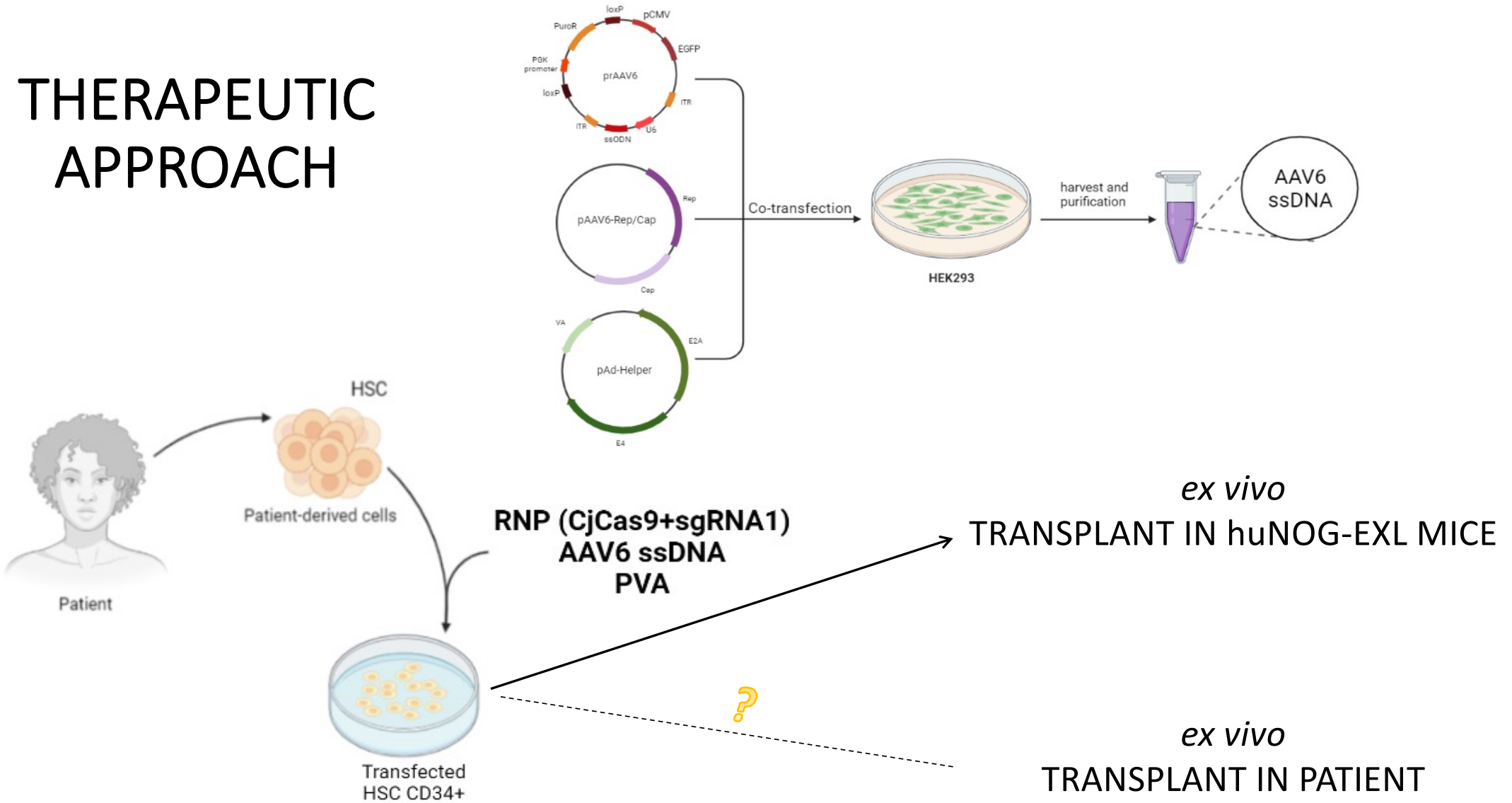


## WHY CRISPR/CJAS9?

	CRISPR/CAS9	CRISPR/CJAS9
Size (base pairs)	4.200 bp	2.952 bp
PAM sequence	NGG	NNNNACAC/NNNNRYAC
sgRNA	20 bp	22 bp



# THERAPEUTIC APPROACH



TARGET SEQUENCE

5' CTGCTGCTGAGGTGGCAGTTTC 3'

sgRNA1

GACGACGACTCCACCGTCAAAG

sgRNA2

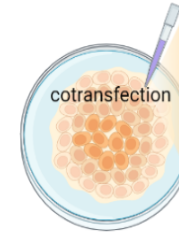
GCTGGGCCTCAGCGCGTCCGG

sgRNA3

GACGGCGCCTCATCCGACAGTG

sgRNA4

GGCAGTTTCCTGCACACTACAG



CjCas9 + sgRNA1

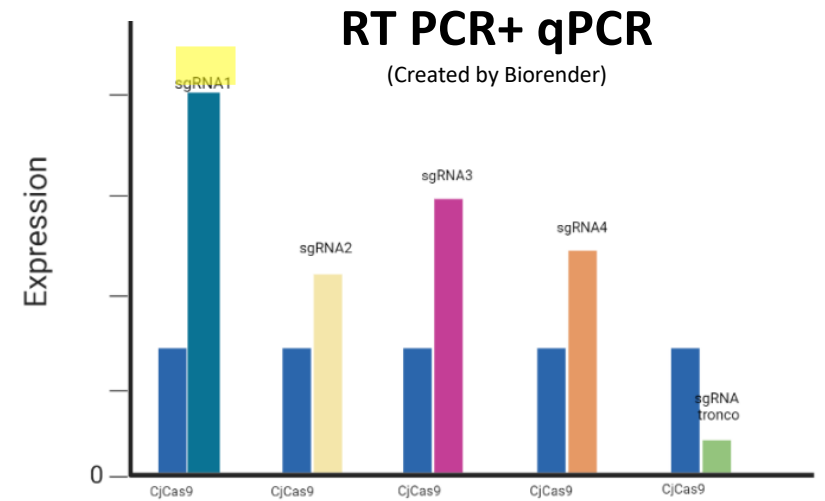
CjCas9 + sgRNA2

CjCas9 + sgRNA3

CjCas9 + sgRNA4

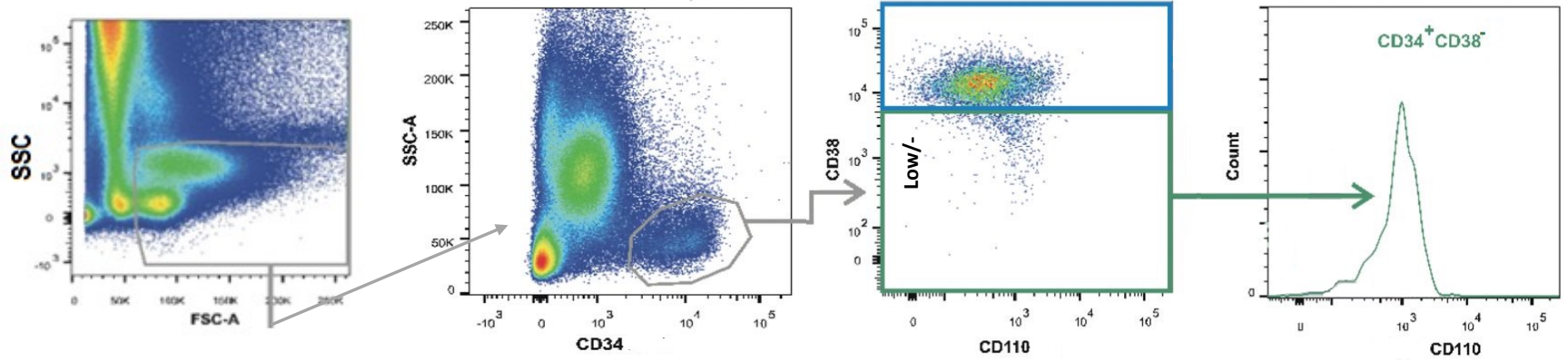
CjCas9 + sgRNA tronco

RT qPCR

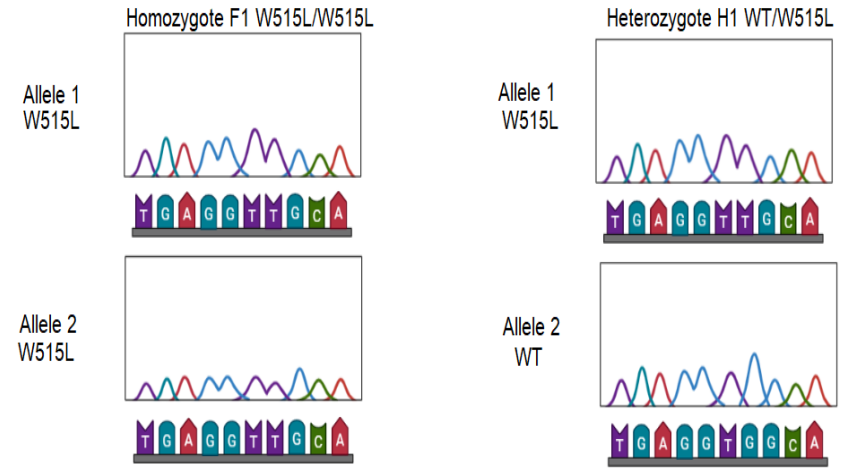
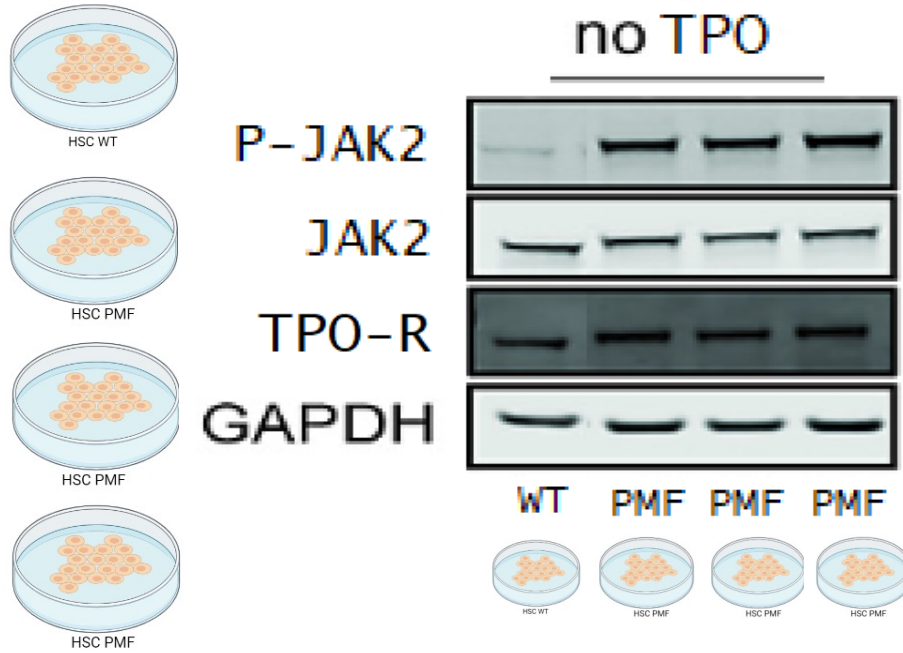


# EXPERIMENTAL PLAN *in vitro*:

**HSC  
CD34+CD110+  
sorting**  
(Adapted from Ballmaier et al. 2015)



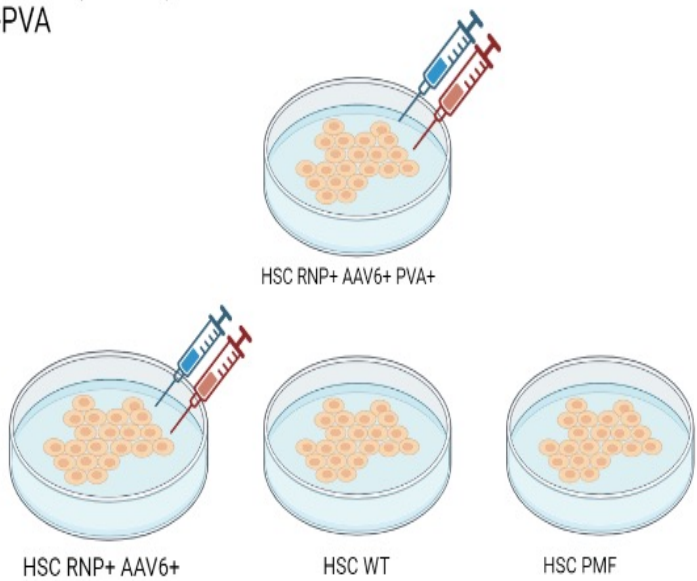
**Western blot**  
(Adapted from Baik et al. 2021)



**NGS**  
(Created by Biorender)

# EXPECTED RESULTS

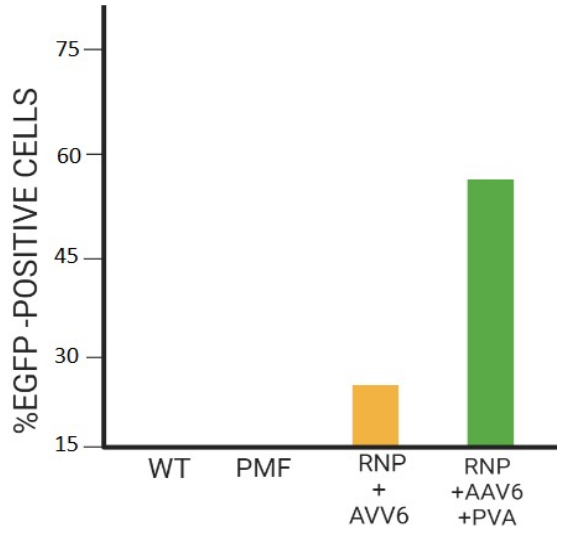
TRANFECTION:  
 -RNP (CjCas9+sgRNA)  
 -AAV6 (ssDNA)  
 -PVA



(Created by Biorender)

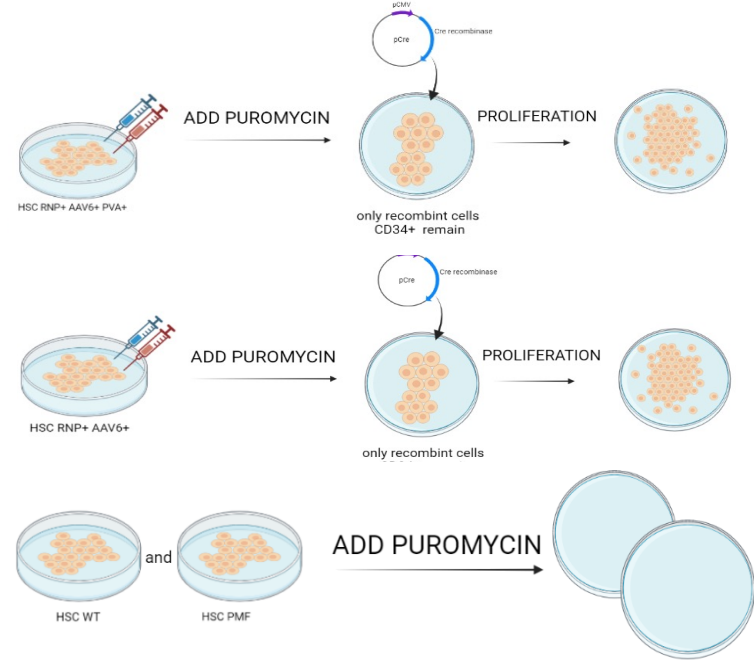
## EGFP Assay

(Created by Biorender)



## Puromycin Antibiotic Assay

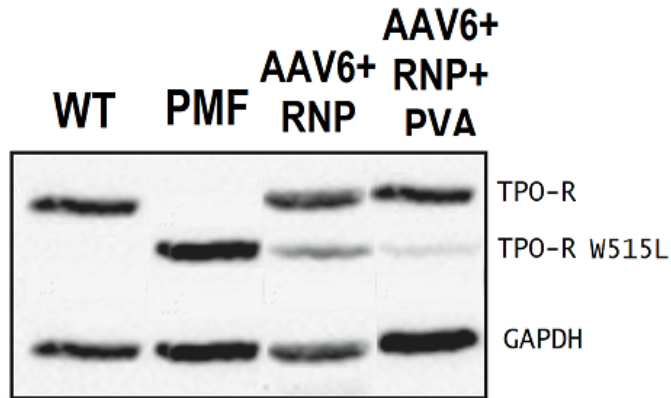
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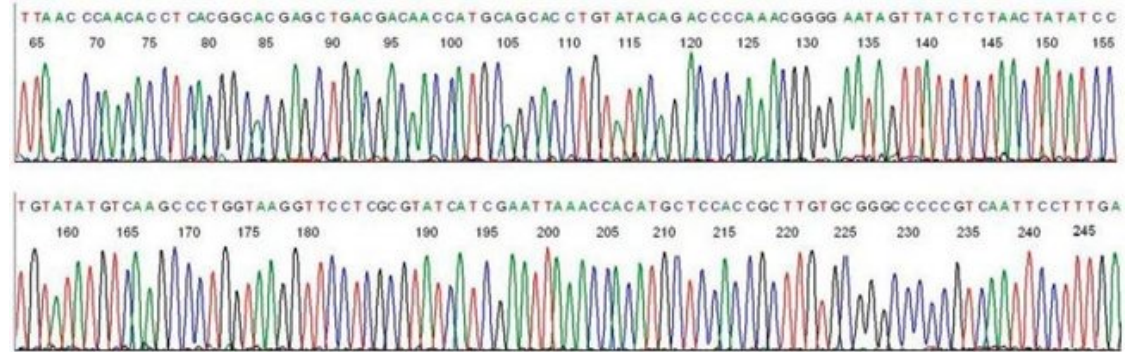
(Created by Biorender)

## Western blotting

(Adapted from ZW et al. 2018)

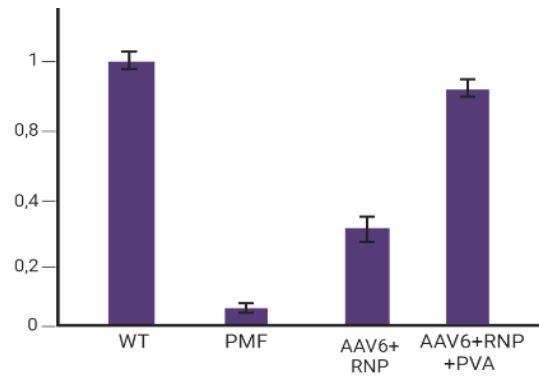
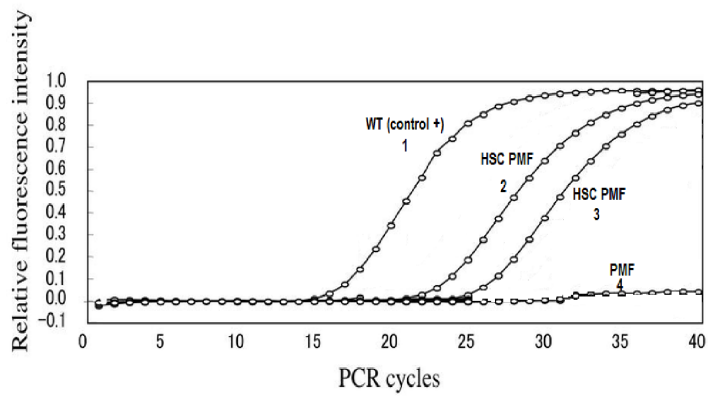


## NGS (whole genome sequence) → off targets detection



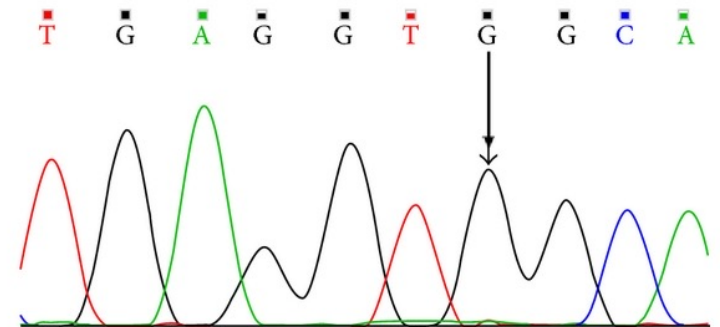
## qPCR

(Adapted from Miyagawa et al. 2008 and Created by Biorender)



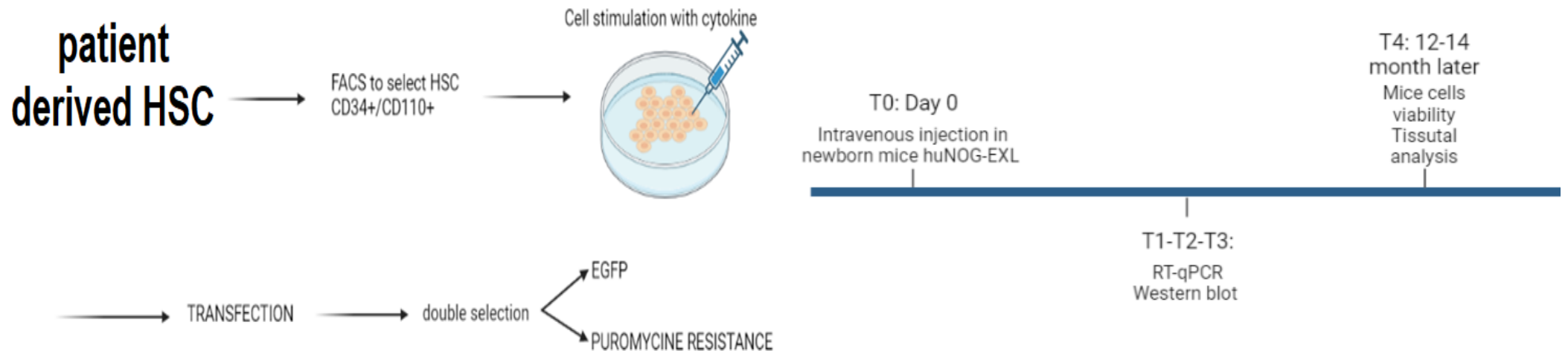
## NGS (target sequence)

(From Wu et al. 2014)

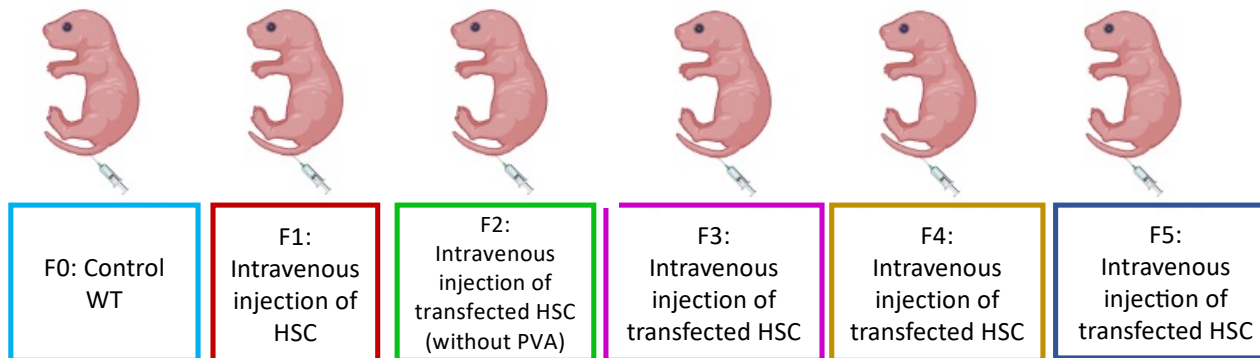




# EXPERIMENTAL PLAN *in vivo*



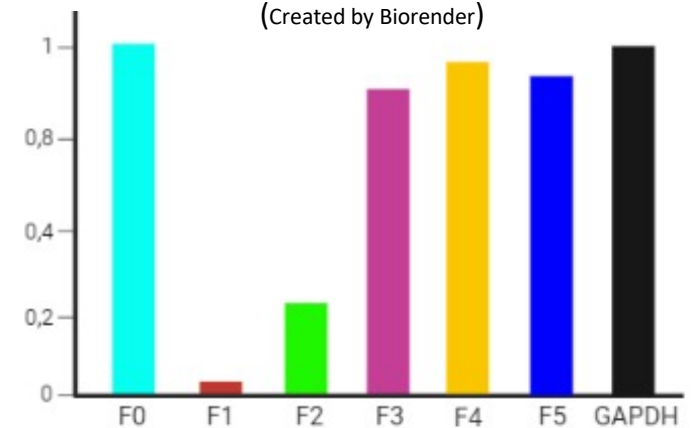
T0



T1-T2-T3

qPCR

(Created by Biorender)



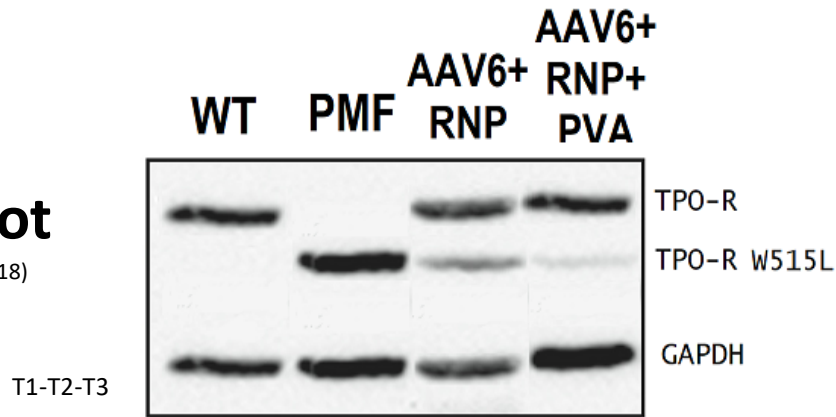
# EXPECTED RESULTS

## Tissutal analysis

(From Pizzi et al. 2015)

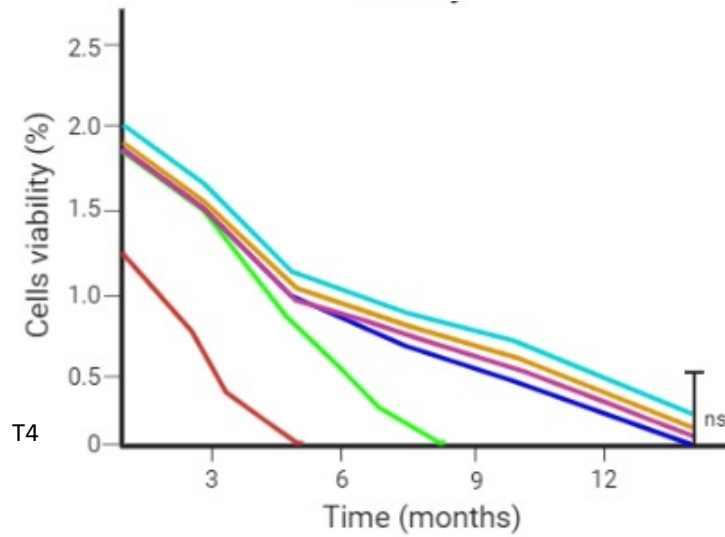
### Western blot

(Adapted from ZW et al. 2018)



### Mice cells viability

(Created by Biorender)



T0 T4

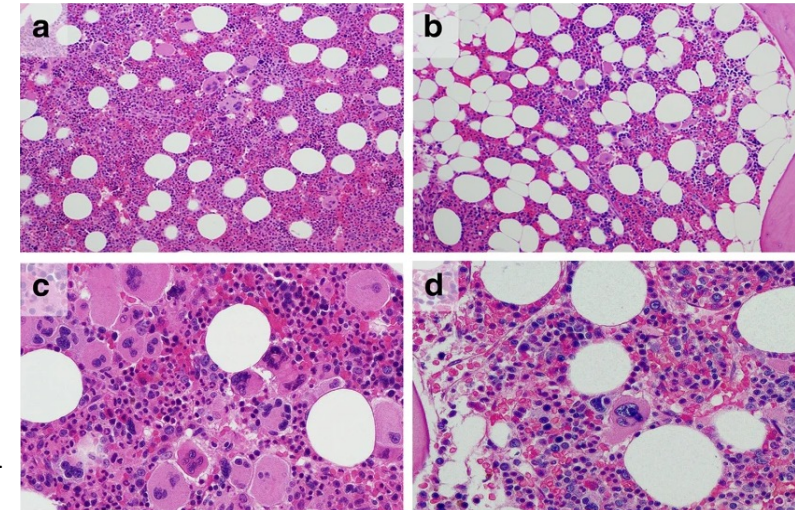


Fig. 1

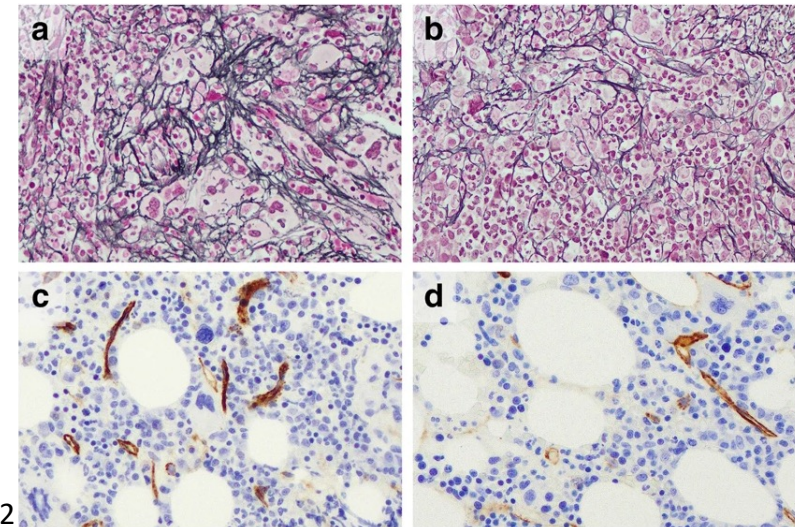


Fig. 2

## CONCLUSIONS

The correction of MPL mutation will restore WT in order to activate TPO-R only in the presence of TPO

In vivo: the post-treatment biopsy shows a significant reduction in the deposition of reticulin and collagen fibers from the engineered mice

## PITFALLS

Limited packaging capacity

Off target activity could negatively affect the experiment

Unstable integration of vector for AAV

## SOLUTIONS

Using fragmented AAV dual vector system

CNV evaluation/more specific sgRNA

Use of lentivirus but random integrations can occur

# MATERIALS AND COSTS (~18 months of research)

Materials	Costs	
Topi NOG-EXL (hGM-CSF/hIL-3 NOG), MALEs 4 to 10- (Taconic)	€344,10 x 5	
Flp-In™-293 Cell Line (Thermo Fisher Scientific)	€2.575,00 x 1mL	
Expi293™ Expression Medium (Thermo Fisher Scientific)	€373 x 1L	
Western Blot Detection Kit (Elabscience)	€200	
StemPro™-34 SFM (1X) (Thermo Fisher Scientific)	€448,00 x 550mL	
Puromycin (InvivoGen)	€381,00 x 500 mg	
Human Hematopoietic Stem Cell Expansion Cytokine Package (Peprotech)	€1,650.00 x 1 pack	
prAAV6 (Packgene)	€1744	
Packaging plasmid AAV rep cap (biolabs)	€600	
Vector plasmid AAV U6 sgRNA Cjcas9 (Addgene)	€3000	
Helper plasmid (Agilent)	€1850	
Control mouse (The Jackson Laboratory)	€35 x 1 ca	
RT-qPCR kit and equipments (Thermo Fisher Scientific)	€1500	
PCR- kit (Thermo Fisher Scientific)	€2000 ca	
Mice stabulation	€10.000	
ACSL1 Polyclonal Antibody (Thermo Fisher Scientific) + cell sorting (ISS)	€428 x 0.1 mL + €768 (€64/hour)	
Disposable plastic (Thermo Fisher scientific)	€5000 (1 year)	<b>Cost per year: €184.272,5</b>
Research team	€150.000 (1 year)	

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