



SAPIENZA  
UNIVERSITÀ DI ROMA

# Treatment of Revesz syndrome through the restoring of the TNF2 gene in HSCs with LV-TNF2wt

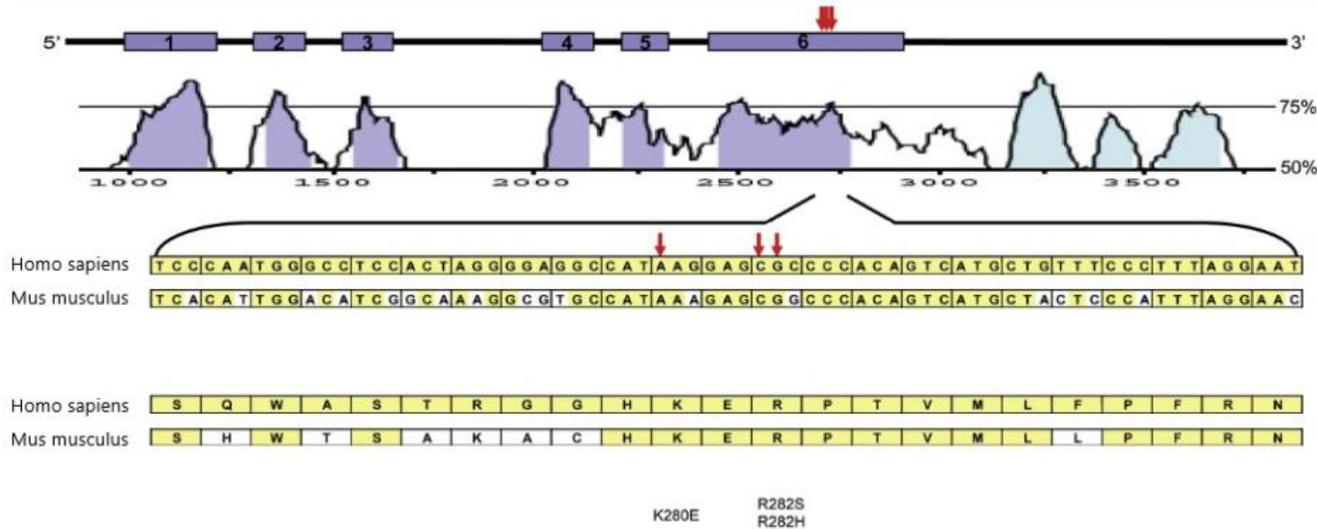
A.A. 2020 - 2021

Corso di Terapia genica e neuroscienze  
Prof.ssa Isabella Saggio  
Tutor Dr.ssa La Torre

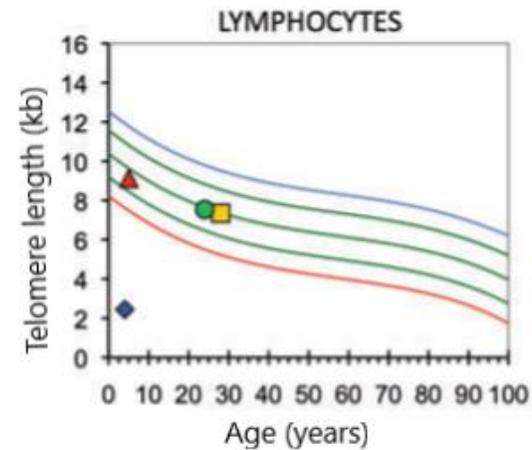
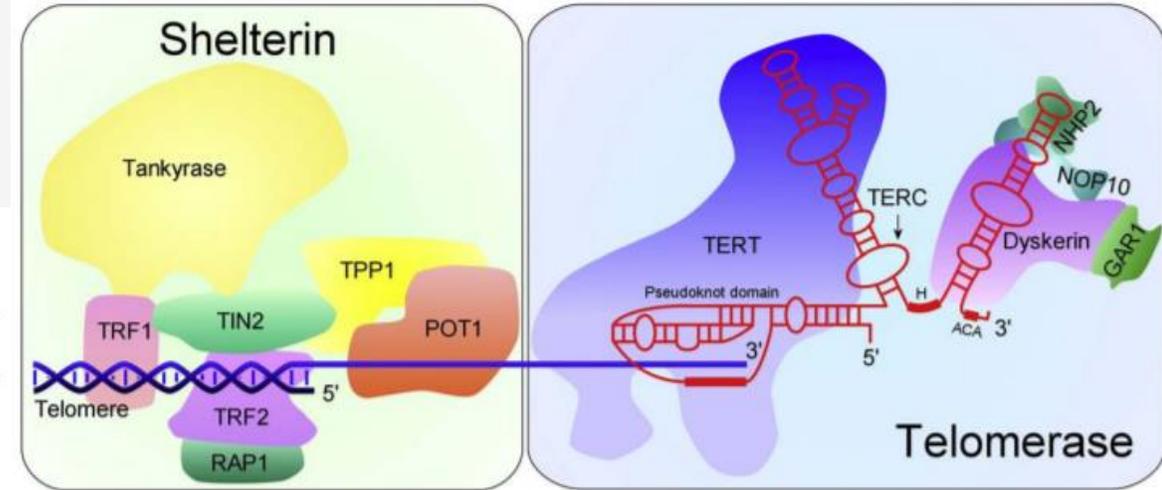
Scognamiglio C. , Barezzi C. , De Cantis L.  
Pezza S. , Caprini E. S. , Di Bella E.

# BACKGROUND

- ➔ It was initially described in 1992 by Revesz et al. in a 6 months old patient.
- ➔ It is caused by heterozygous mutations in TIN2.
- ➔ It is a severe form of a TBD.



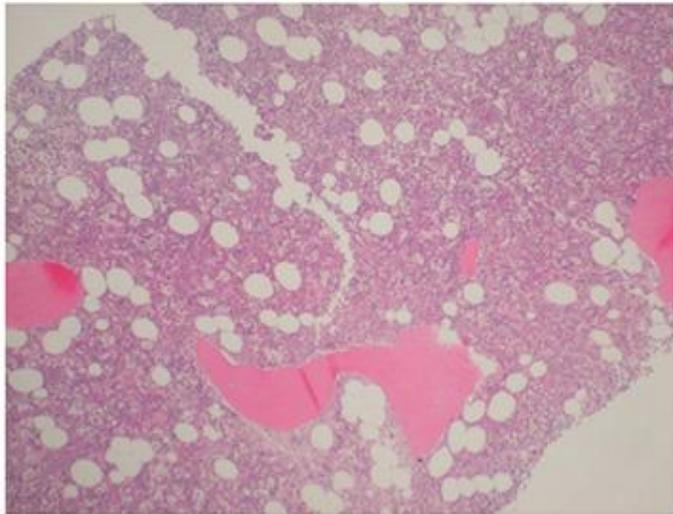
the genomic region of TIN2 consists of 2686 base pairs. The red arrows show the relative positions of the mutations, Ex6p234A/G (K280E), Ex6p240C/A (R282S), and Ex6p241G/A (R282H).



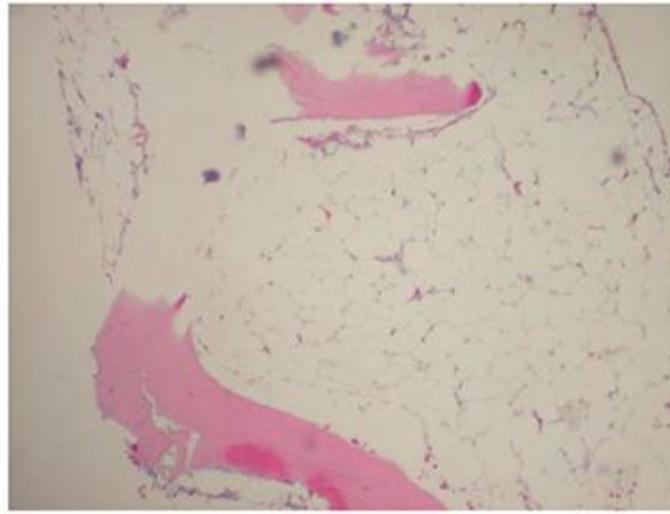
Telomere flow-FISH analysis

# PHENOTYPE OF REVESZ SYNDROME

**Bone marrow failure**, intrauterine growth retardation, fine and sparse hair, reticulate skin pigmentation, bilateral exudative retinopathy, cerebral calcification, cerebellar hypoplasia, and psychomotor retardation

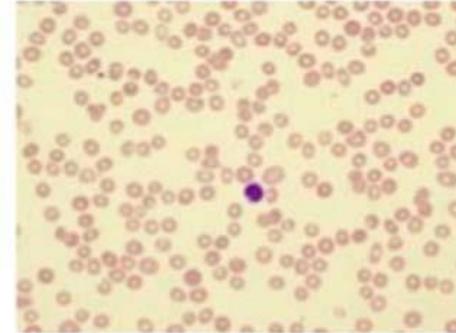


Healthy bone marrow

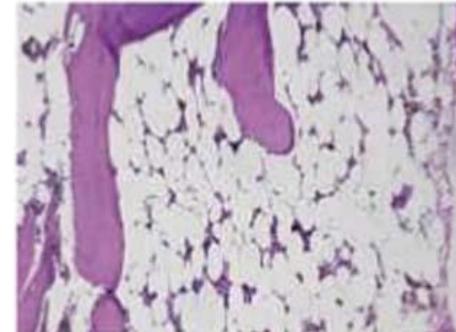
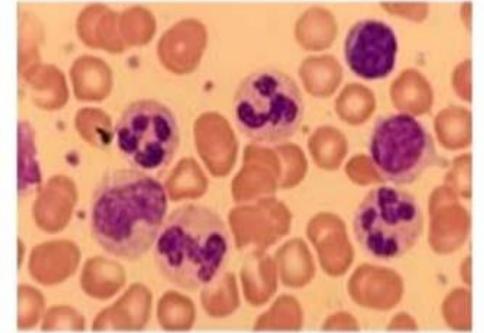


Aplastic anemia

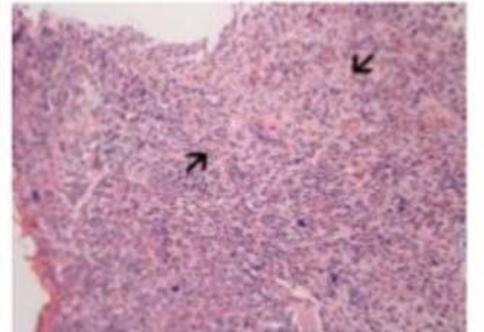
Peripheral blood showing pancytopenia



Normal peripheral blood



Fatty bone marrow in aplastic anemia



Normal cellular bone marrow

# AIMS

WHAT?



**Restore:**  
the function of the protein (TIN2), telomere length and bone marrow function

WHERE?



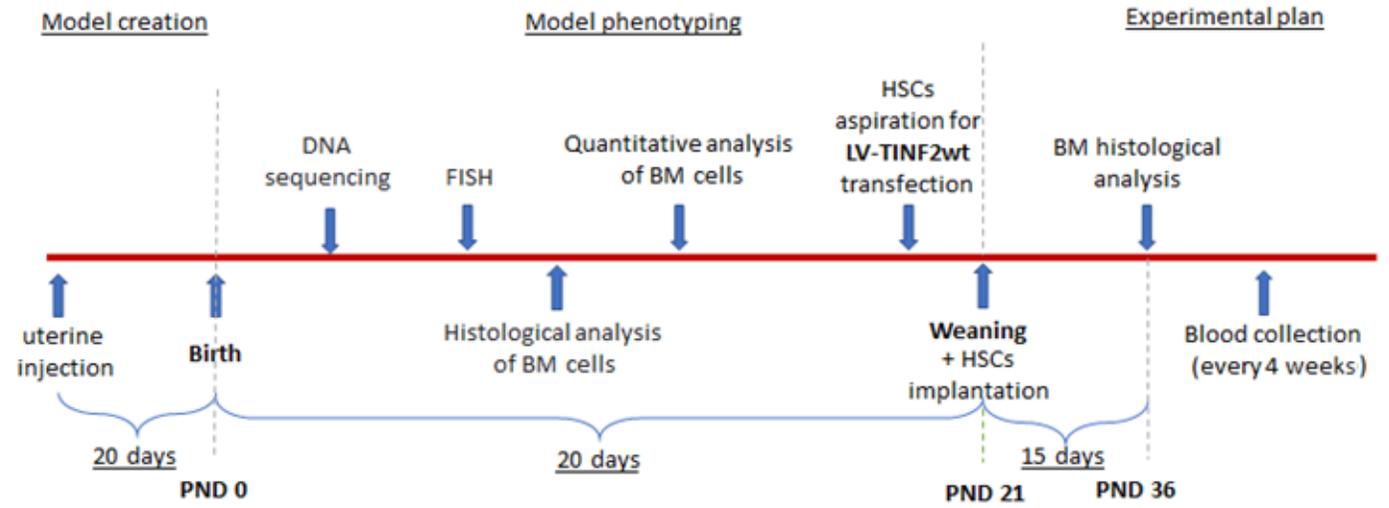
Hematopoietic stem cells

HOW?



Expression of the wild type TINF2 gene with LV-TINF2wt

## EXPERIMENTAL TIMELINE



LEGEND

PND: Post-natal day

LV-TINF2wt: lentiviral vector

BM: Bone marrow

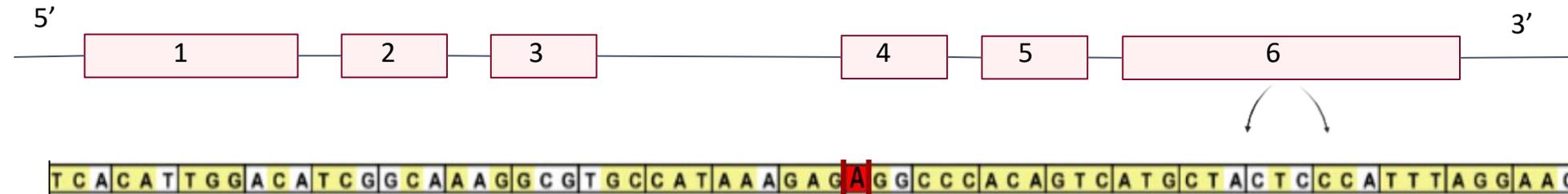
HSCs: hematopoietic stem cells

# EXPERIMENTAL PLAN model creation

## CRISPR/dCas9

sgRNA: - AGACTCCATCTCGGCCTTT - CGAAGGCGGGTCCTCTGAC - GGGATTTTCGCTTTCCCAA

## Donor template:



Mus musculus:  
EXON 6 with  
mutation

Creation of liposome:



Injection into uterus:



# CHARACTERIZATION OF THE MODEL

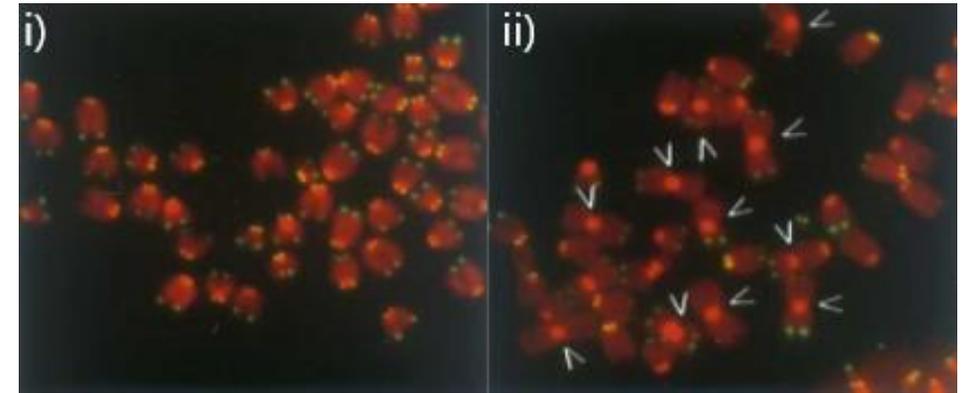
## A Next generation sequencing

AAAGAG**A**GGCCACA

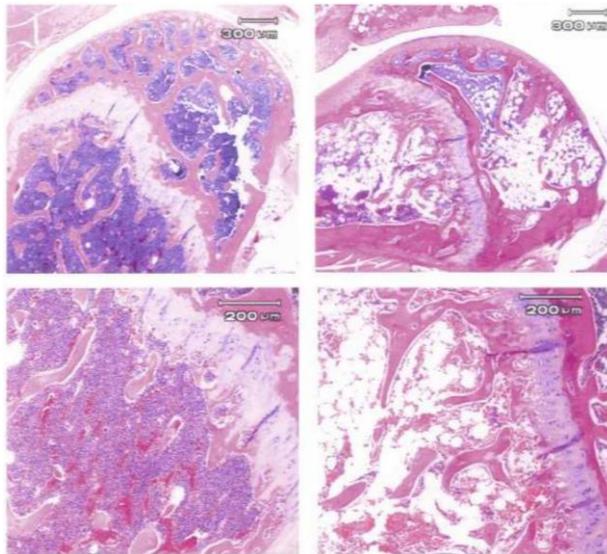
## B Telomere FISH analysis on metaphase chromosome:

i) Wild type

ii)  $TINF2^{-/+}$ : arrows point at chromosome fusions



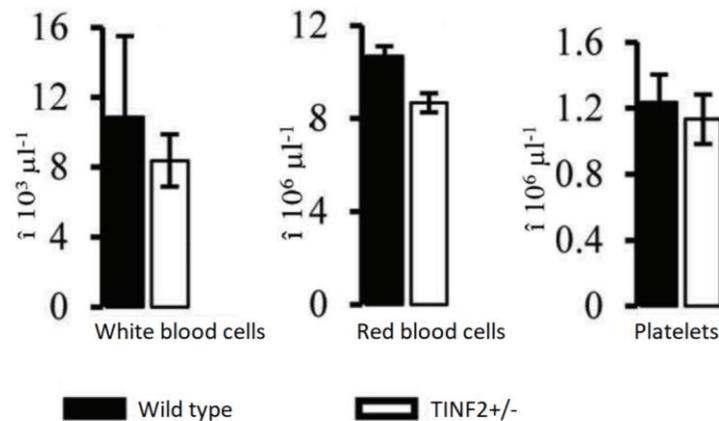
## C Histological analysis of the bone marrow



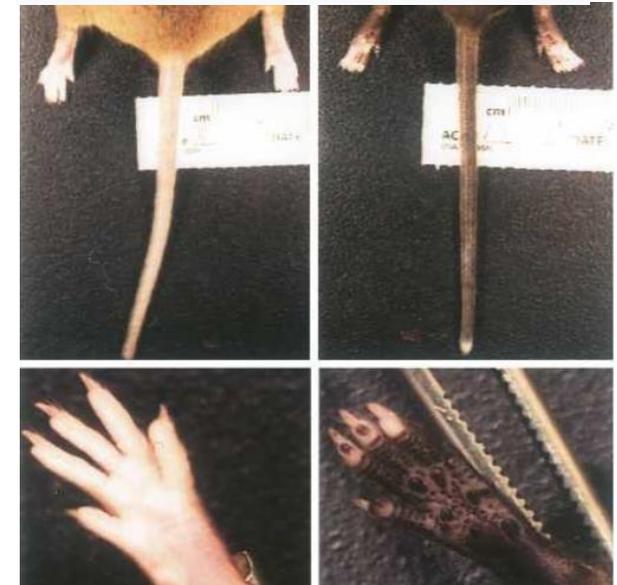
Wild type

$TINF2^{+/-}$

## D Measure of the amount of bone marrow cells



## E Phenotypic characteristics



Wild type

$TINF2^{+/-}$

# CONSTRUCTION OF THE VIRAL VECTOR



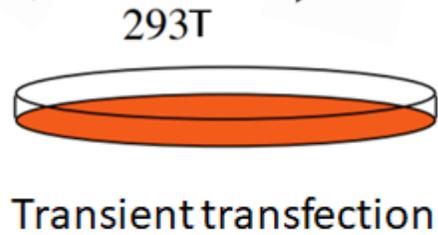
Lentiviral transfer plasmid

pCMV-G: envelope plasmid

pMDLg/pRRE: 3rd generation lentivirus packaging plasmid



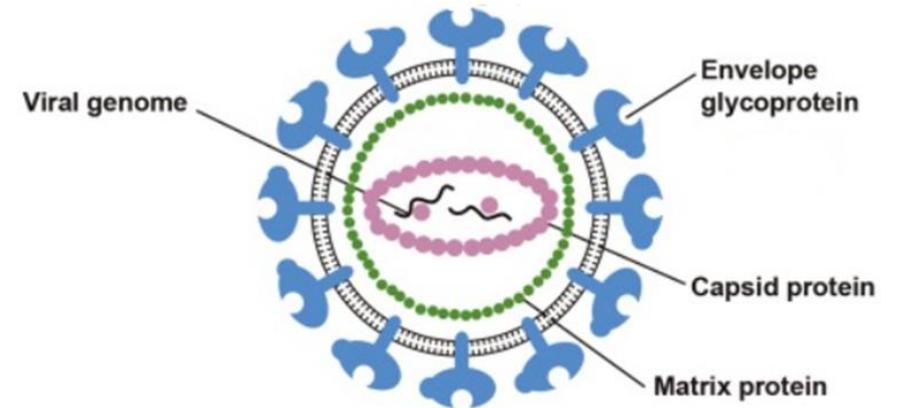
pRSV-Rev: Rev plasmid



Transient transfection

Harvesting after 24 and 48 hours → Ultracentrifugation → Titer (FACS)

Gene of interest: HSV-TK, GFP, TIN2



# EXPERIMENTAL PLAN *ex vivo*

**1**

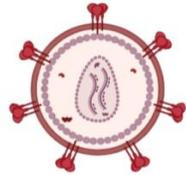
Aspiration of HSC from the bone marrow of model mice

**2**

Infect HSC with LV-TINF2wt

**3**

Positive selection to verify the correct integration



Expansion for 1 month



CD34+ selected cells



VPA, 100 ng / ml di TPO,  
10 ng / ml di SCF,  
fibronectin



HSCs expanded by  
~13,000-fold during  
culture



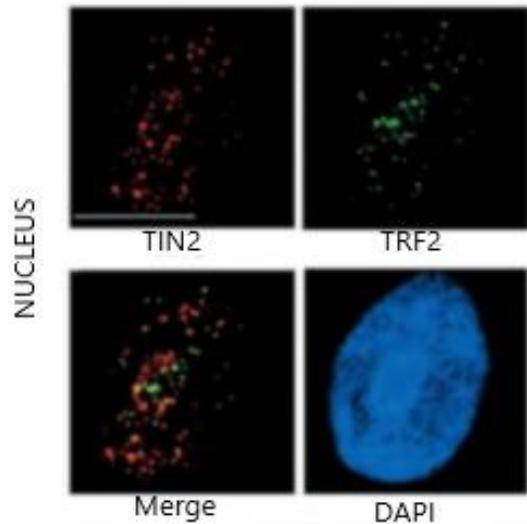
**CONTROL:** infection of a second culture with LV without the TINF2 gene but only with GFP: cells are still proliferant



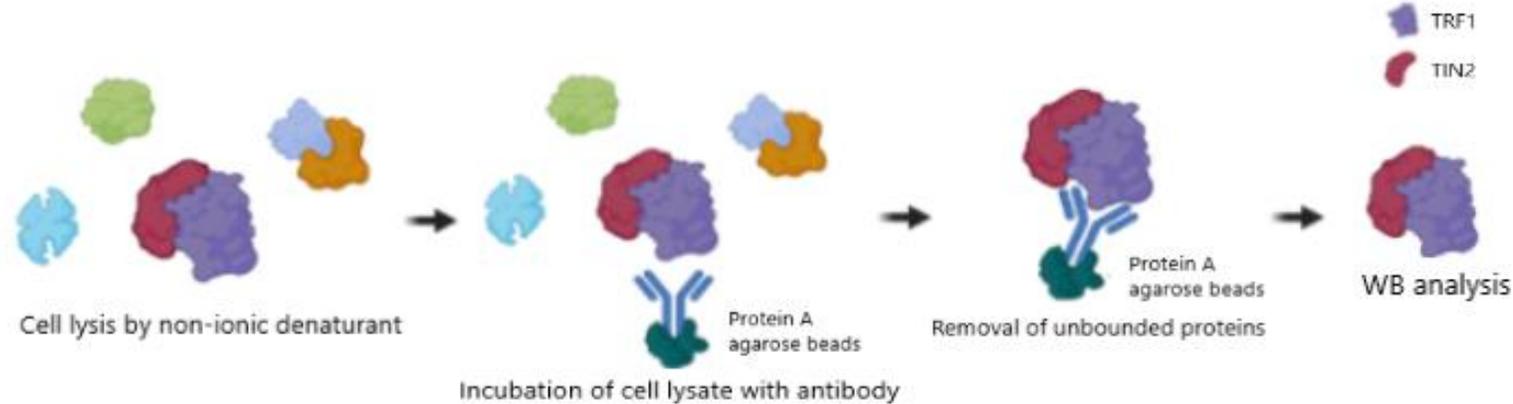
LV-TINF2wt doesn't provoke damage when it is integrated

# EXPERIMENTAL PLAN *in vitro*

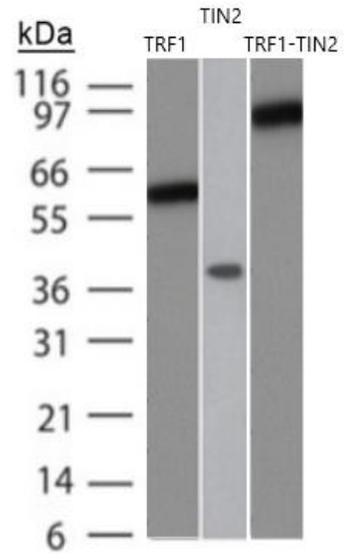
Observation of the co-presence of TIN2 and TRF1 with immunostaining



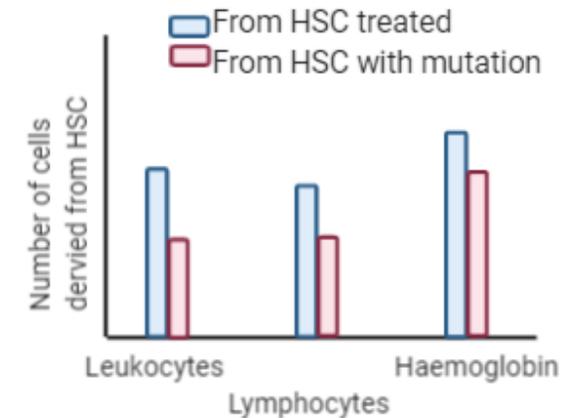
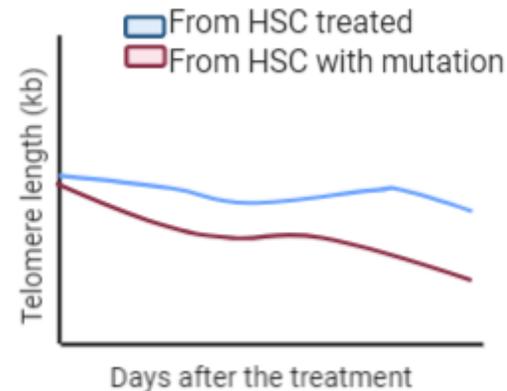
Analysis of the interaction between TIN2 and TRF1 with co-immunoprecipitation



Western Blot



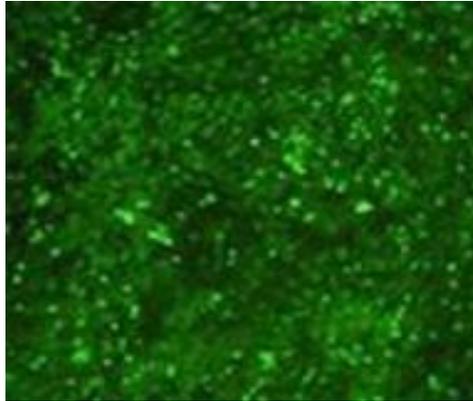
There is an interaction between TRF1 and TIN2 after the treatment and this has an effect on the general health of the cells



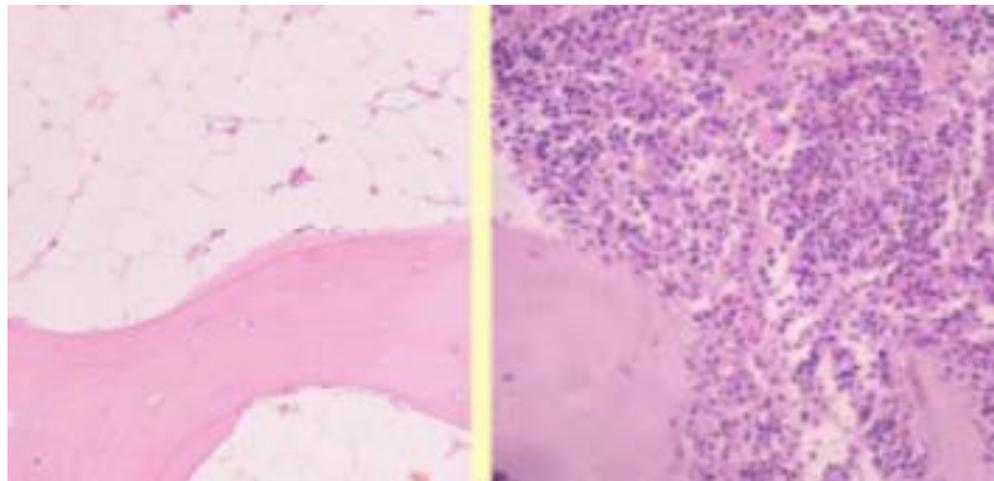
# EXPERIMENTAL PLAN *in vivo*

Intrafemoral injection in the marrow cavity of  $10^4$  cells and observation of the results

Correct integration of GFP + BMMSC



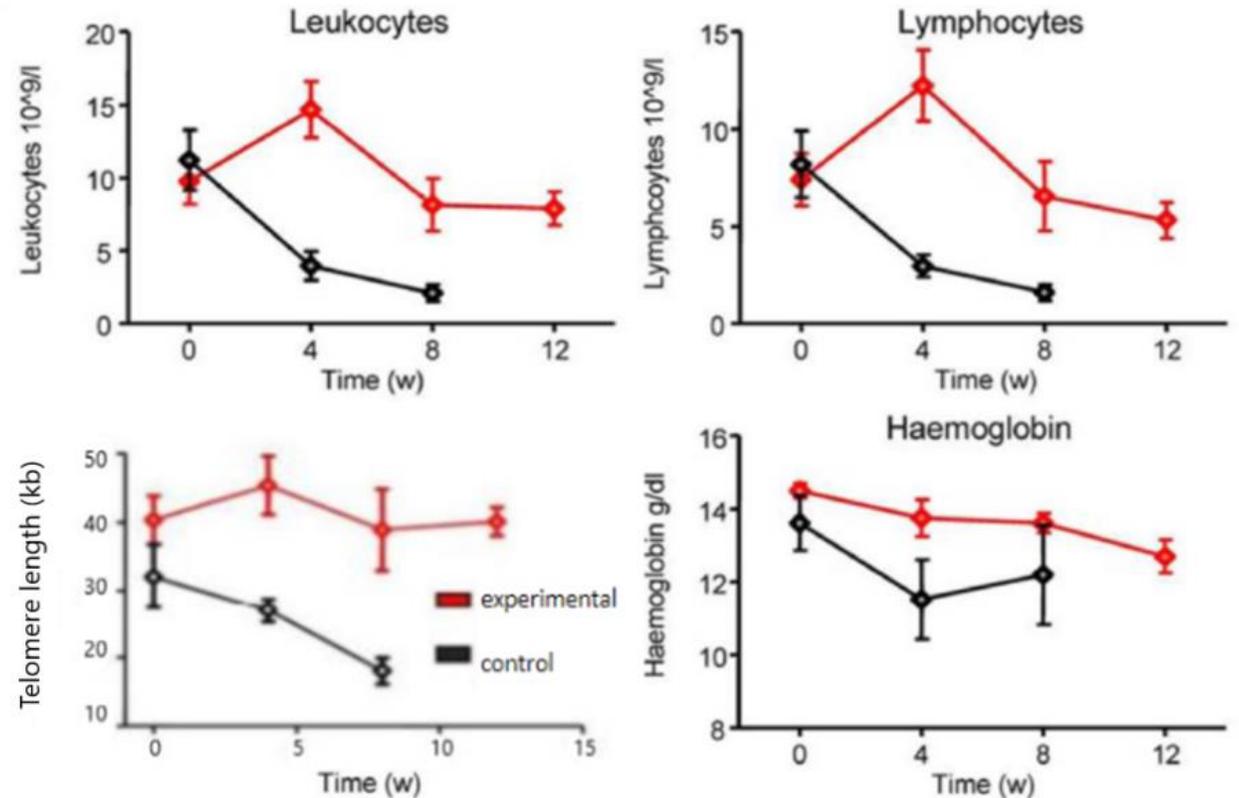
Restoring of the normal histology of BM



TINF2+/-

wt

Concentration of leukocytes, lymphocytes and hemoglobin in relation with time





# PITFALLS AND SOLUTIONS

Development of tumor



Insertion of thymidine kinase gene into the lentiviral vector and somministration of ganciclovir.

Toxicity given by an high quantity of the protein



An alternative therapy that restores the TINF2 gene with the use of a viral vector containing CRISPR/Cas9 and the wild type gene.

# BUDGET



<i>Lentiviral vector</i>	€ 600,00
Liposomes (€100x6)	€ 600,00
12 mice Balb/c (€25x12)	€ 300,00
12 mice C57 BL6 (€25x12)	€ 300,00
Next generation sequencing (€1000x12)	€ 12.000,00
FISH (€1000x12)	€ 12.000,00
Immunostaining and Co-immunoprecipitazione	€ 1.200,00
HSCs culture medium (€200x6)	€ 1.200,00
Animal care starting from (0,50 daily for ten years)	€ 43.200,00
<b>TOTAL</b>	<b>€ 71.400,00</b>

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