Treatment of Revesz syndrome through the restoring of the TINF2 gene in HSCs with LV-TINF2wt
It was initially described in 1992 by Revesz et al. in a 6 months old patient.
It is caused by heterozygous mutations in TINF2.
It is a severe form of a TBD.

The genomic region of TINF2 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).
**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

*Shimamura A., Expert’s Corner, 2017*
AIMS

**WHAT?**
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
**EXPERIMENTAL PLAN** model creation

**CRISPR/dCas9**

**sgRNA:**
- AGACTCCATCTCGGCCTTT
- CGAAGGCGGGTCCTCTGAC
- GGGATTTTCGCTTTCCAA

**Donor template:**

Mus musculus: EXON 6 with mutation

**Creation of liposome:**

**Injection into uterus:**
CHARACTERIZATION OF THE MODEL

A Next generation sequencing

B Telomere FISH analysis on metaphase chromosome:
   i) Wild type
   ii) TINF2-/-: arrows point at chromosome fusions

C Histological analysis of the bone marrow

D Measure of the amount of bone marrow cells

E Phenotypic characteristics

Hockemeyer D. et al., Genes & Dev., 2008 June
CONSTRUCTION OF THE VIRAL VECTOR

Gene of interest: HSV-TK, GFP, TIN2

Harvesting after 24 and 48 hours

Ultracentrifugation → Titer (FACS)
**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

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

Chunyi Hu et al., Cell Res., 2017
EXPERIMENTAL PLAN *in vivo*

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

Correct integration of GFP + BMMSC

Concentration of leukocytes, lymphocytes and hemoglobin in relation with time

Restoring of the normal histology of BM

Beier et al., Blood, 2012
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.
Lentiviral vector € 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
TOTAL € 71.400,00
REFERENCES

Engineered telomere degradation models dyskeratosis congenita.

Long-term ex vivo hematopoietic stem cell expansion affords nonconditioned transplantation.


Novel mutation of the TINF2 gene resulting in severe phenotypic Revesz syndrome.

TINF2, a Component of the Shelterin Telomere Protection Complex, Is Mutated in Dyskeratosis Congenita.

Three novel truncating TINF2 mutations causing severe dyskeratosis congenita in early childhood.

Production of Lentiviral Vectors for Transducing Cells from the Central Nervous System.

Necroptosis in spontaneously-mutated hematopoietic cells induces autoimmune bone marrow failure in mice.