IMPROVE LIFE EXPECTATION OF PATIENTS AFFECTED BY TELOMEROPATHIES USING CD34+ CELL THERAPY

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Class of Biology of stem cells,
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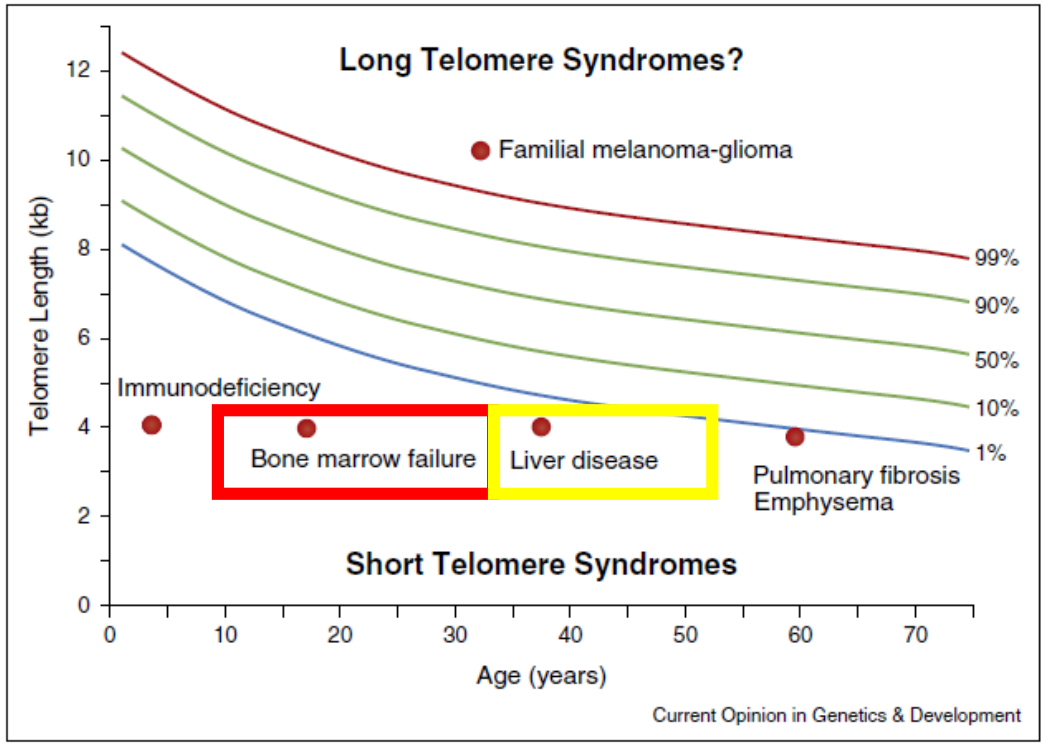
Telomere length and predominant clinical manifestations

Patients with dyskeratosis congenita (DC) suffer from stem cell failure in highly proliferative tissues. **DKC1** gene is the gene responsible for the X-linked Dyskeratosis Congenita.

Table 1 Telomere erosion and human disease

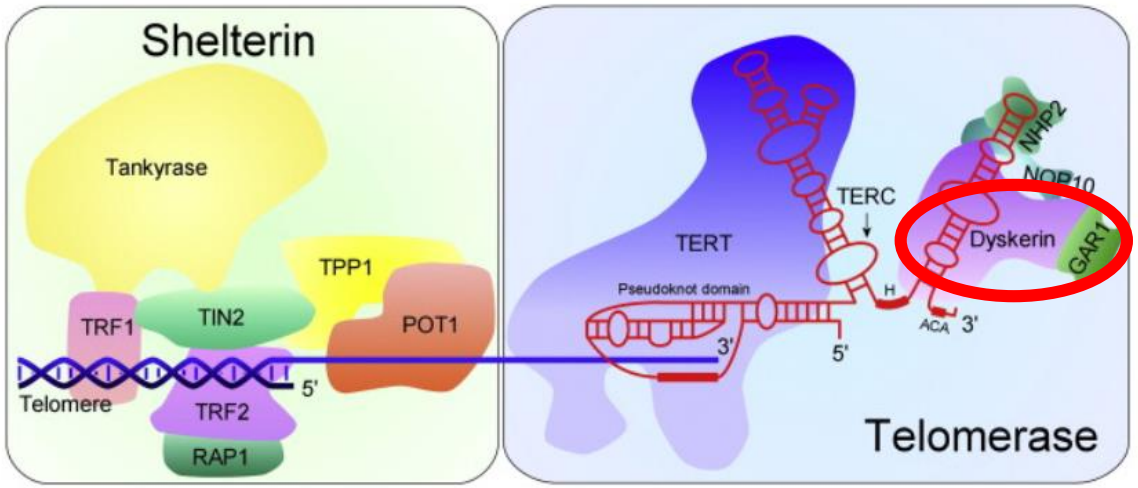
	Telomerase mutations as genetic determinants	Telomerase mutations as genetic risk factors
Characteristics	High penetrance Childhood onset disease Congenital clinical manifestations	Low penetrance Adult onset disease Single or multiple organs
Disease	Dyskeratosis congenita	Aplastic anemia Lung fibrosis Liver cirrhosis Telomere syndromes

Carulli et al., 2014

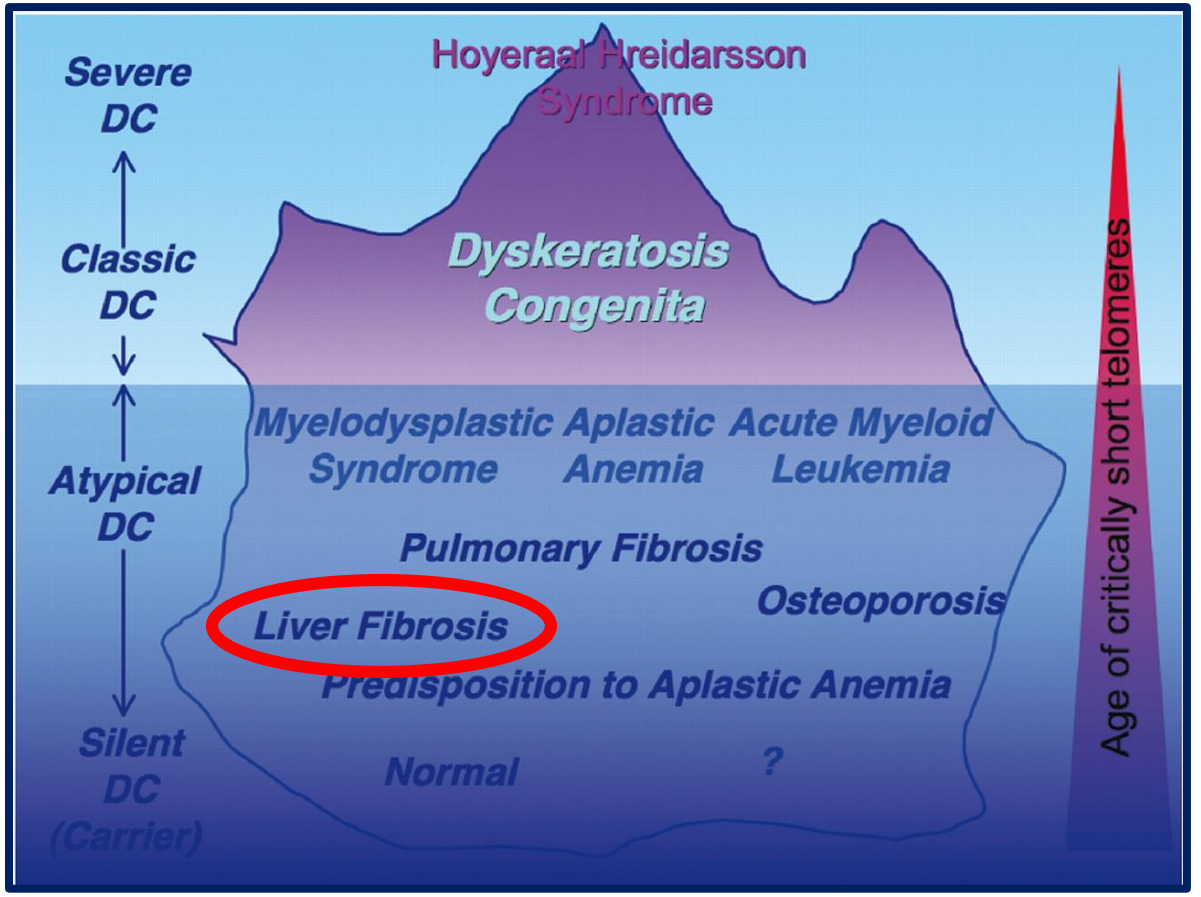


Armanios et al., 2015

Kirwan and Dokal, 2009

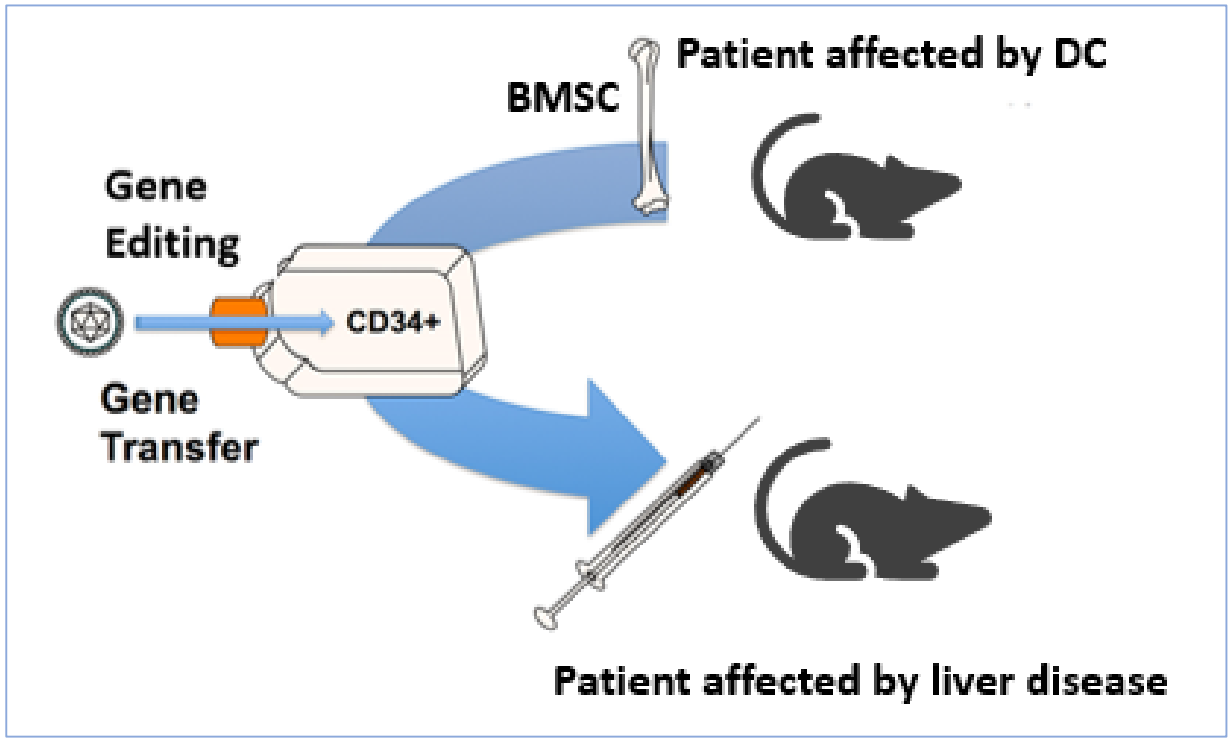


The **dysckerin complex** is a protein encoded by the gene DKC1. This cause a selective defect in the translation of a subgroup of internal ribosome entry site (IRES)–containing cellular mRNAs.



M. Bessler, Hong-Yan Du, Baiwei Gu, P. J.Mason, 2007

AIMs of the gene therapy



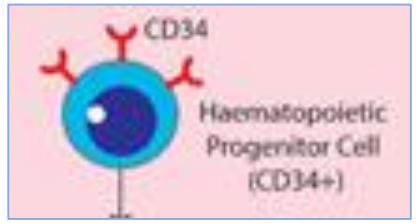
Reduce disease damage

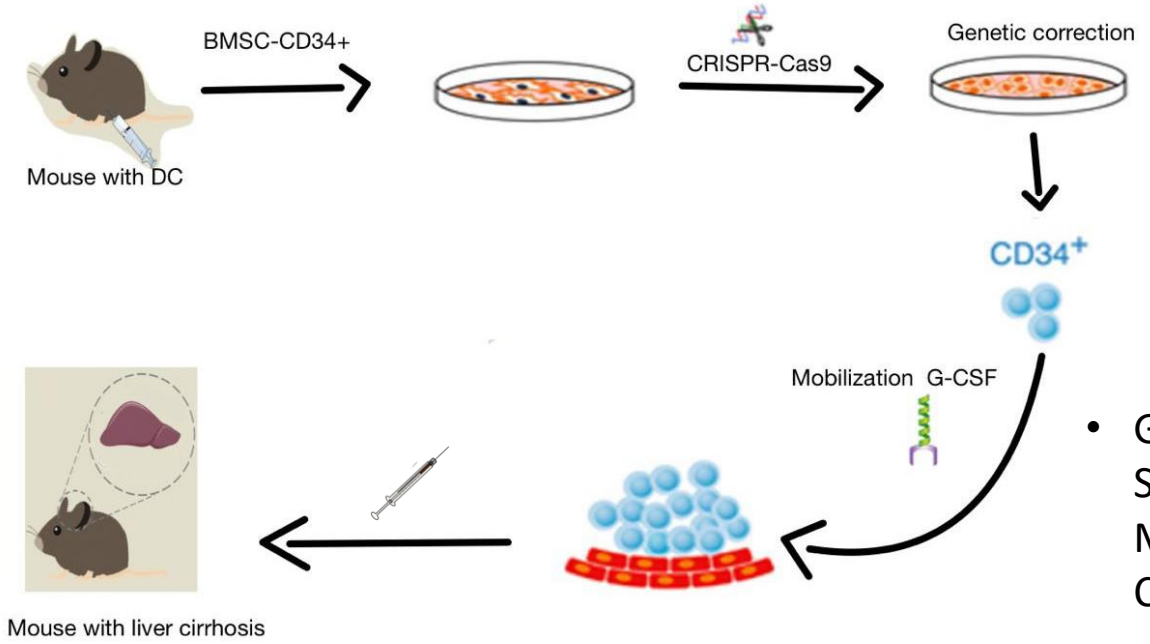
Attenuate impact of telomeropathies

Improve life expectations

The short telomere phenotype in children and young adults represents more severe disease. Bone marrow failure is its most common first manifestation, and **stem cell transplantation** alleviates this condition pointing to a stem cell-autonomous defect in this compartment.

COMBINED THERAPY





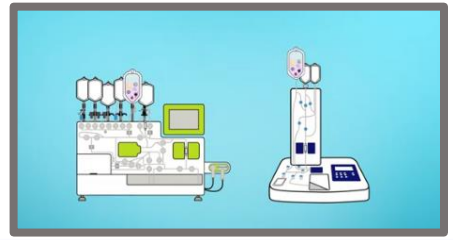
FIRST THERAPY

- ISOLATION OF BMSC – CD34+ from a sample of bone marrow of DC patients
- GENE EDITING with CRISPR *in vitro* to correct **DKC1** mutation

SECOND THERAPY

- GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) TO MOBILIZE HEMATOPOIETIC STEM CELLS (BMSC-CD34+) *in vitro*
- TRANSPLANT *in vivo* OF CD34+ IN PATIENT AFFECTED BY LIVER DISEASE

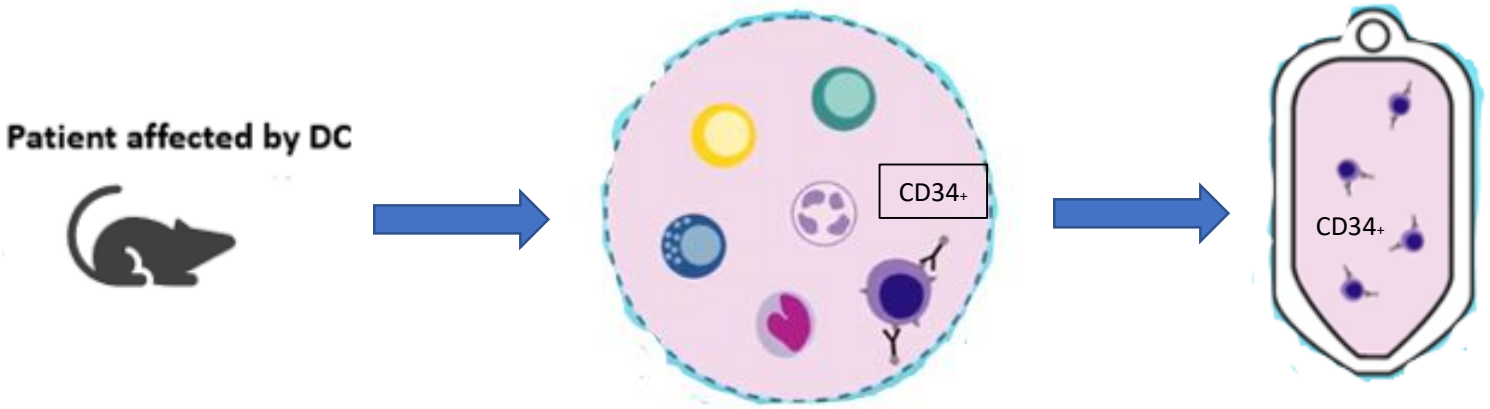
ISOLATION OF CD34+

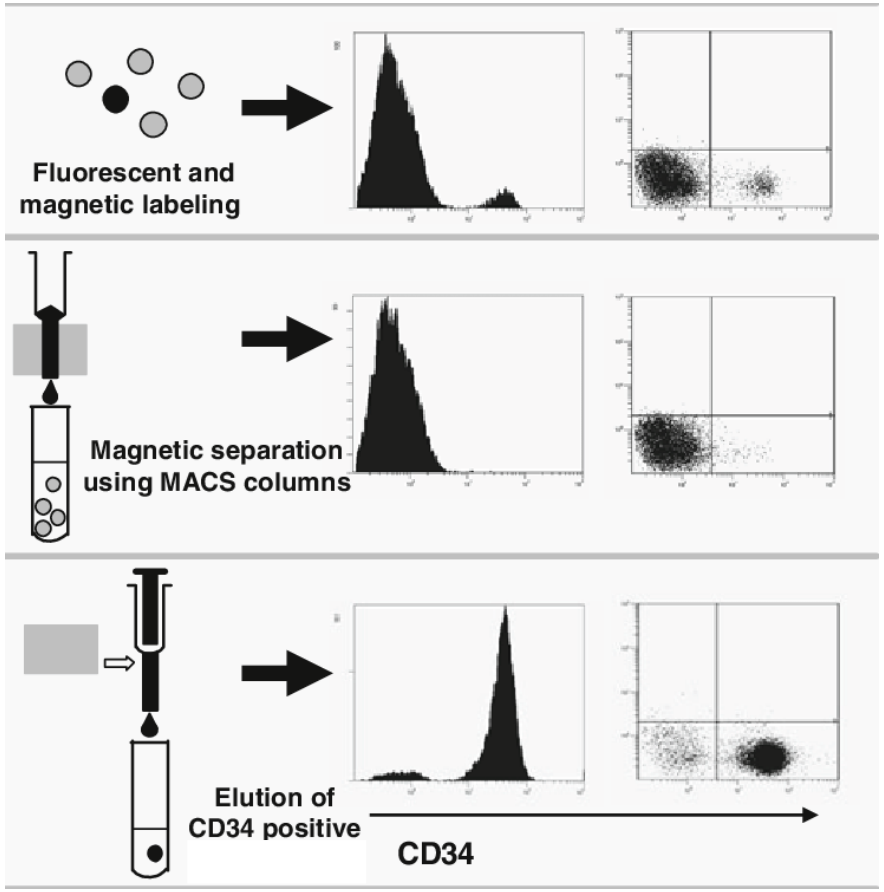


- BONE MARROW SAMPLE FOR THE ISOLATION OF CD34+ FRACTION with **CliniMACS® System**

BM blood cell = 10.000/µL. → **CD34+** → $> 2 \times 10^6/1$

BMCs were sampled for cell counts and immunophenotyping by flow cytometry prior to processing.

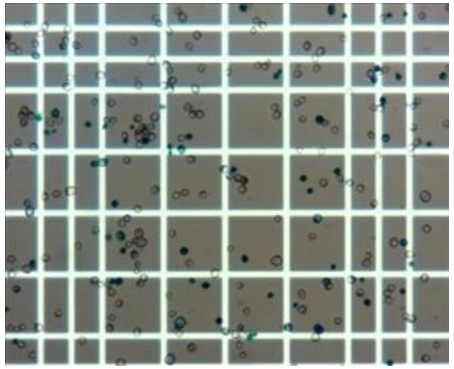


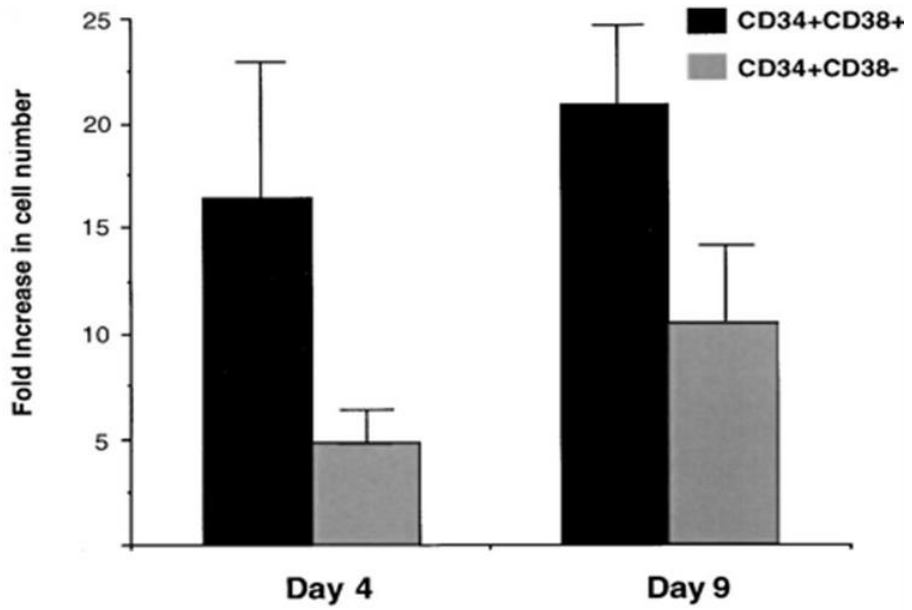


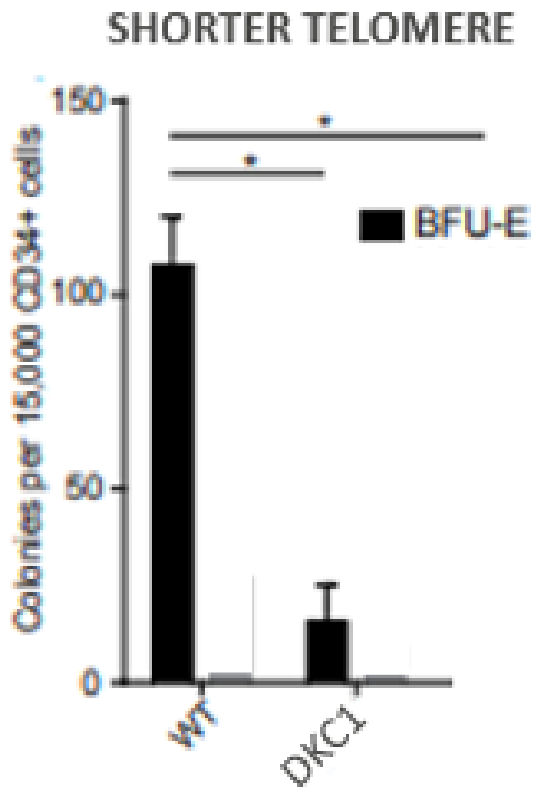
COLONIES OF CD34+

The percentage of CD34+ and viability were determined by flow cytometry analysis, used to calculate total CD34+ cells number.

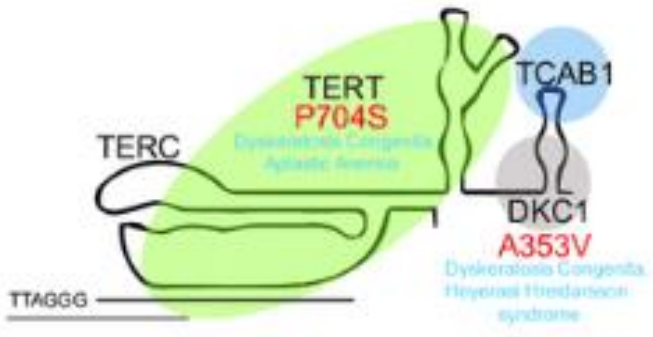
CD34+ cell enumeration

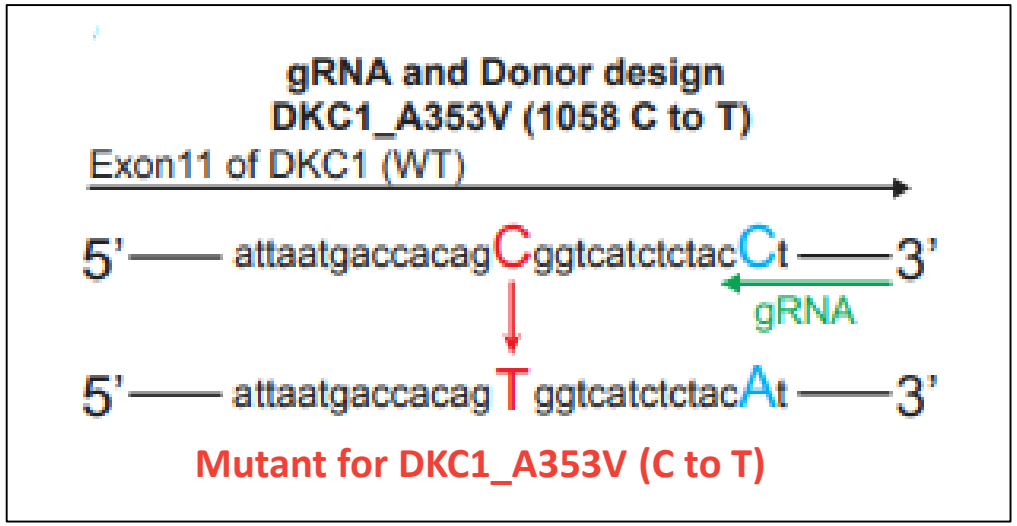


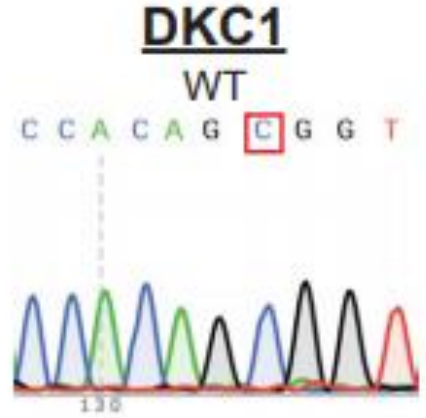




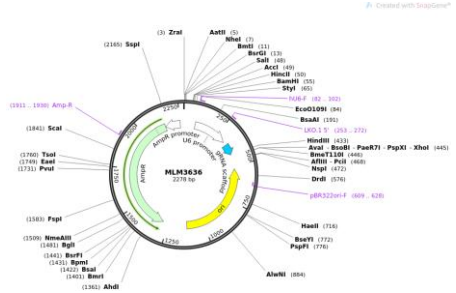
CRISPR-Cas9 to correct DCK1 mutation





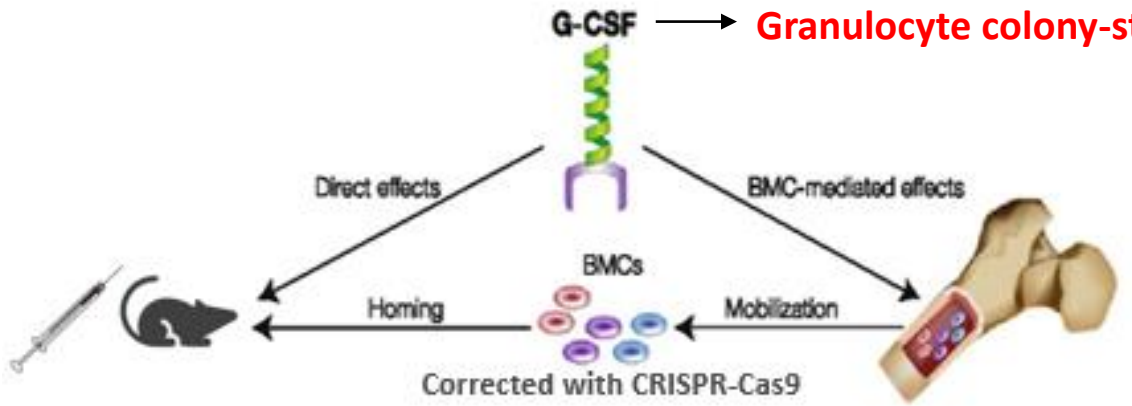


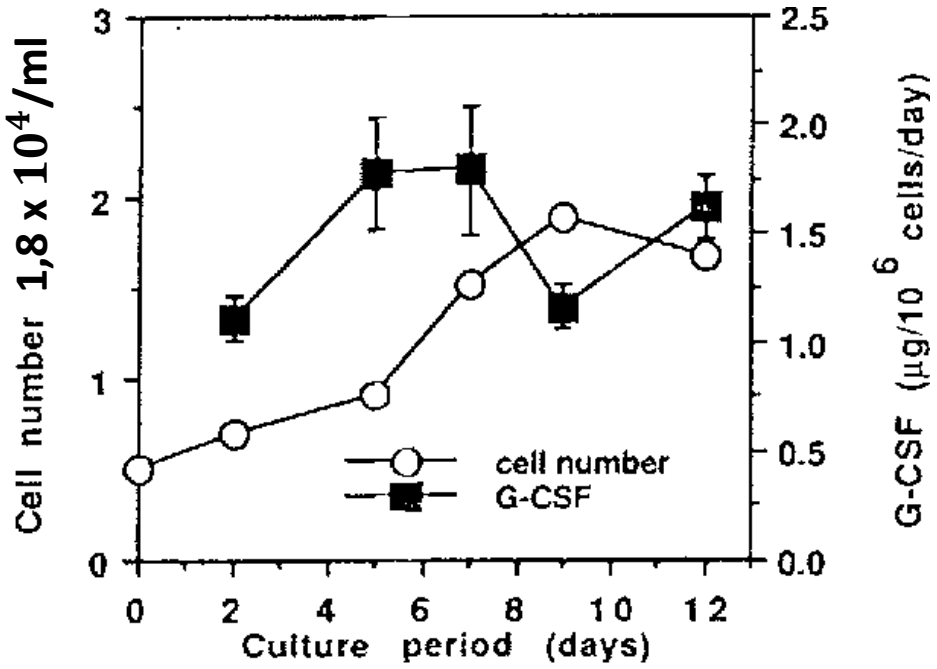
CRISPR gRNAs were inserted into the **MLM3636 plasmid** and cotransfected with a plasmid carrying Cas9.

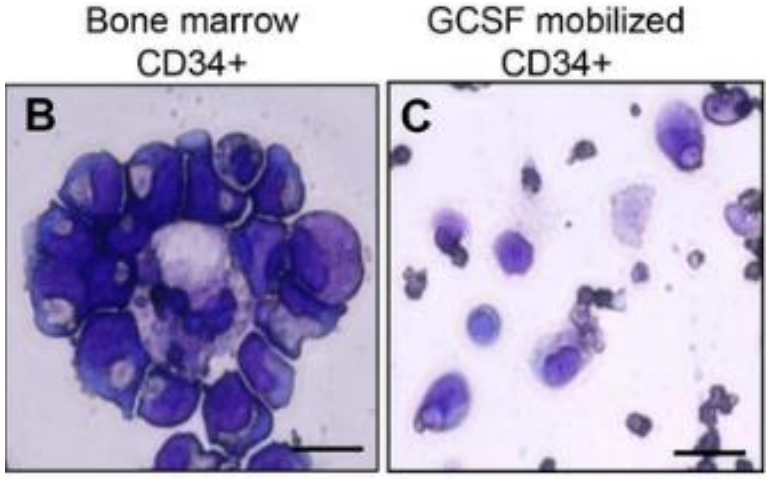


Sequencing traces confirming genome modification →

CD34+ cultured in G-CSF



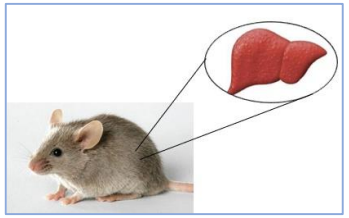




Methylthioninium chloride

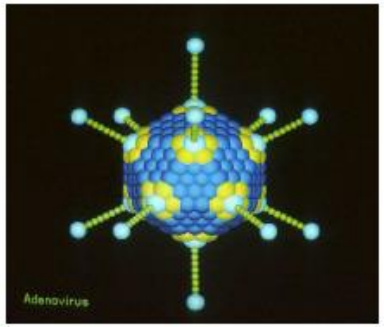
For 12 days, cells were cultured in **G-CSF**, washed and placed on superfrost slides, to stimulate the survival and the proliferation.

TRANSPLANT



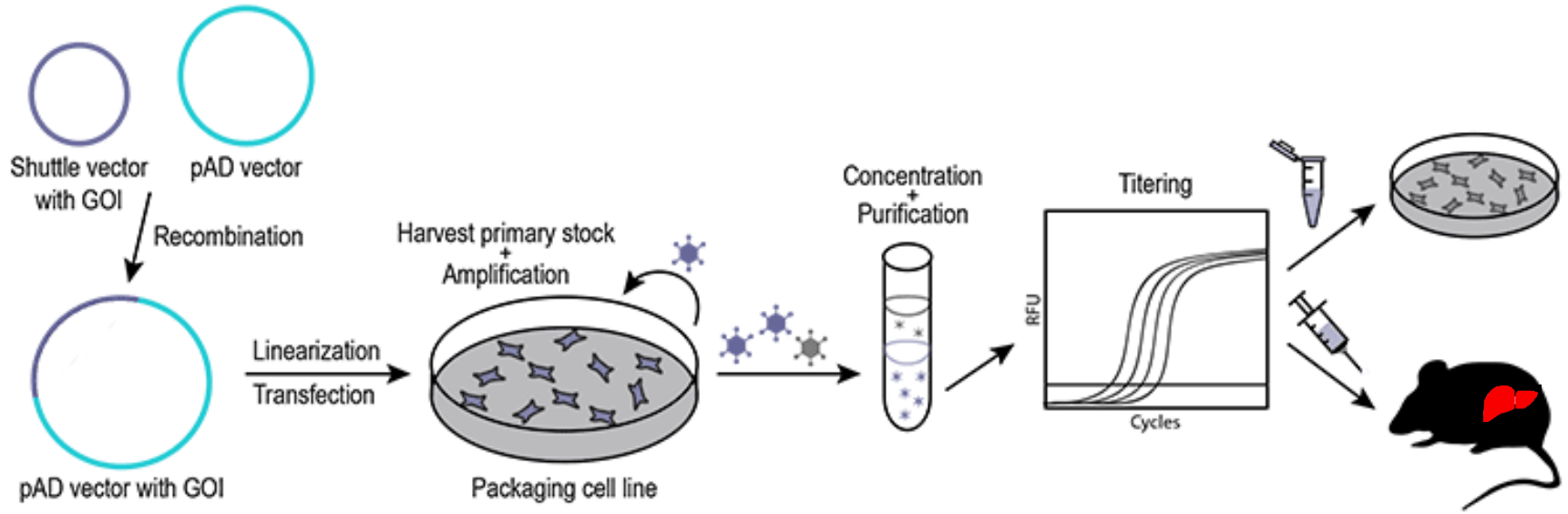
$2 \times 10^5/mL$

Cellular fraction containing CD34+ cells genetically modified with a **RECOMBINANT pAD ADENOVIRAL VECTOR** containing the DNA sequence that encodes for DKC1 .



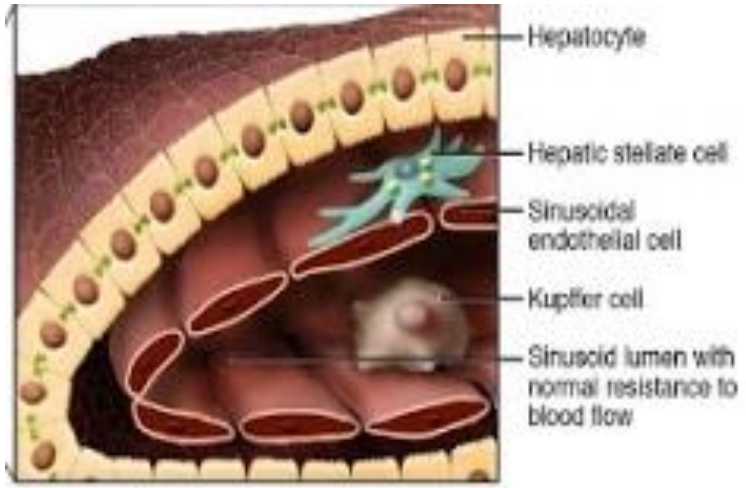
ADVANTAGES:

- Large packaging capacity (~7.5 kb)
- High levels of expression that can often be observed within 24 hours
- Does not integrate into host genome
- Infects most cell types with nearly 100% efficiency

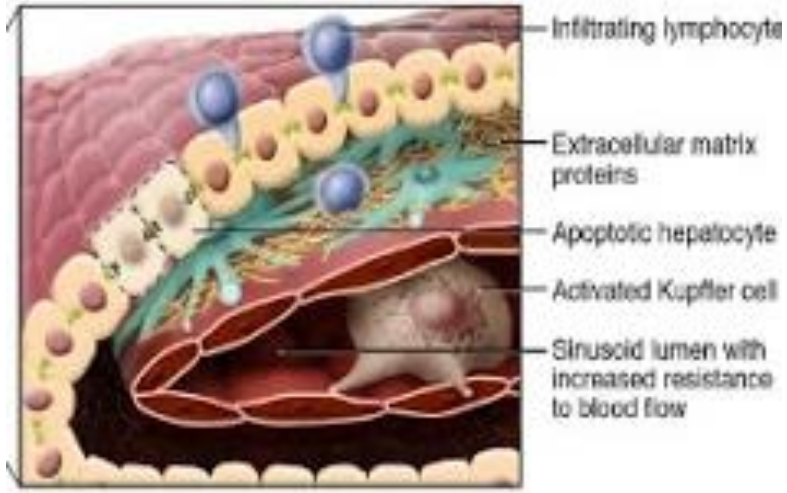


RESULTS

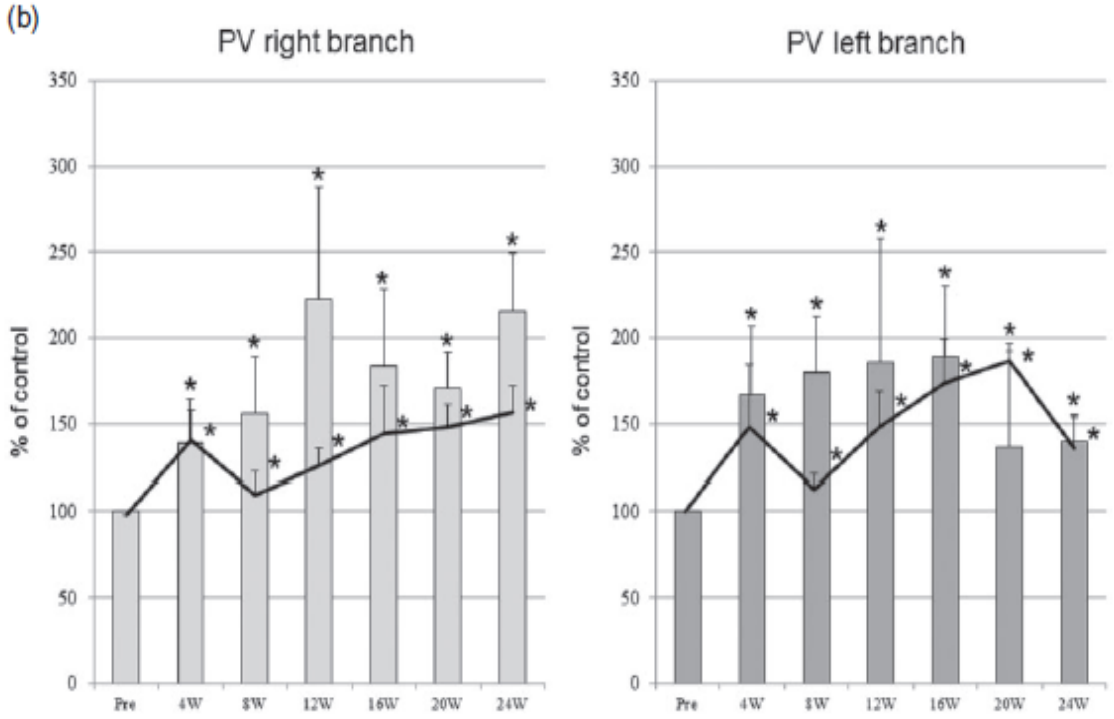
NORMAL LIVER



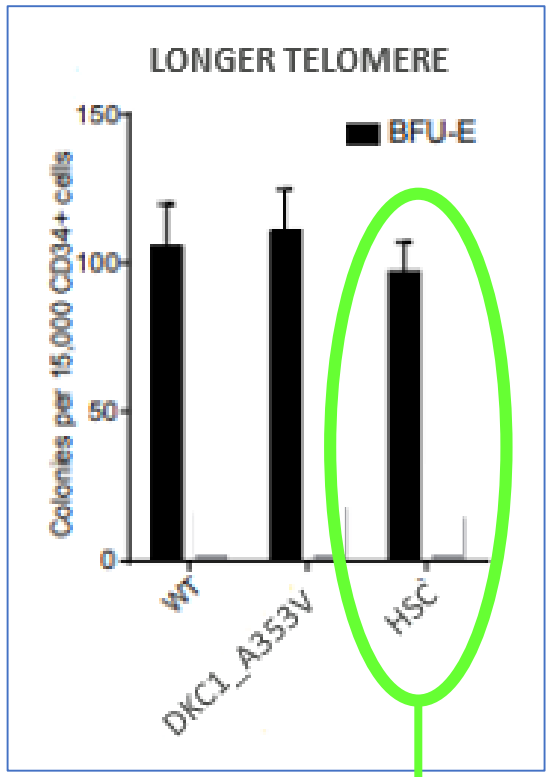
LIVER WITH FIBROSIS

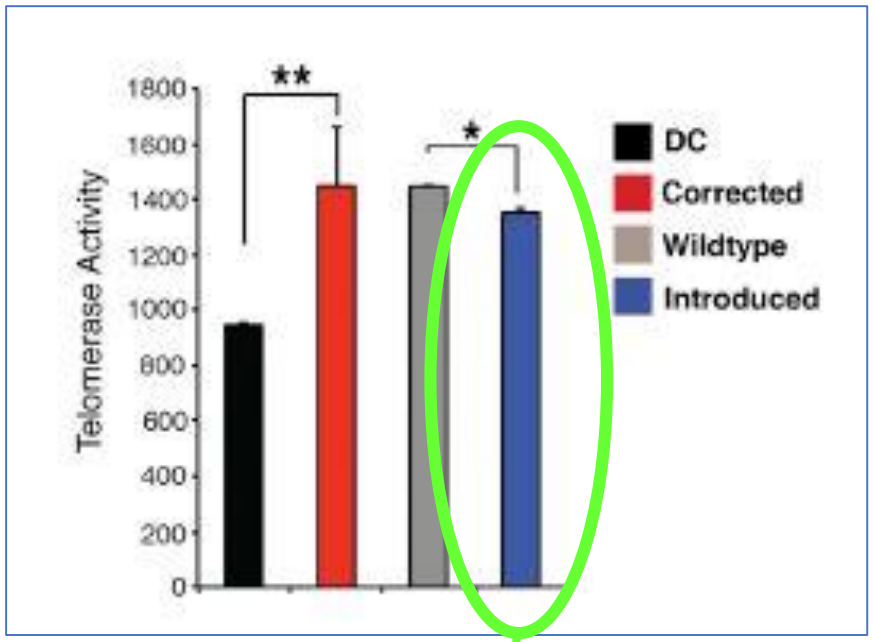


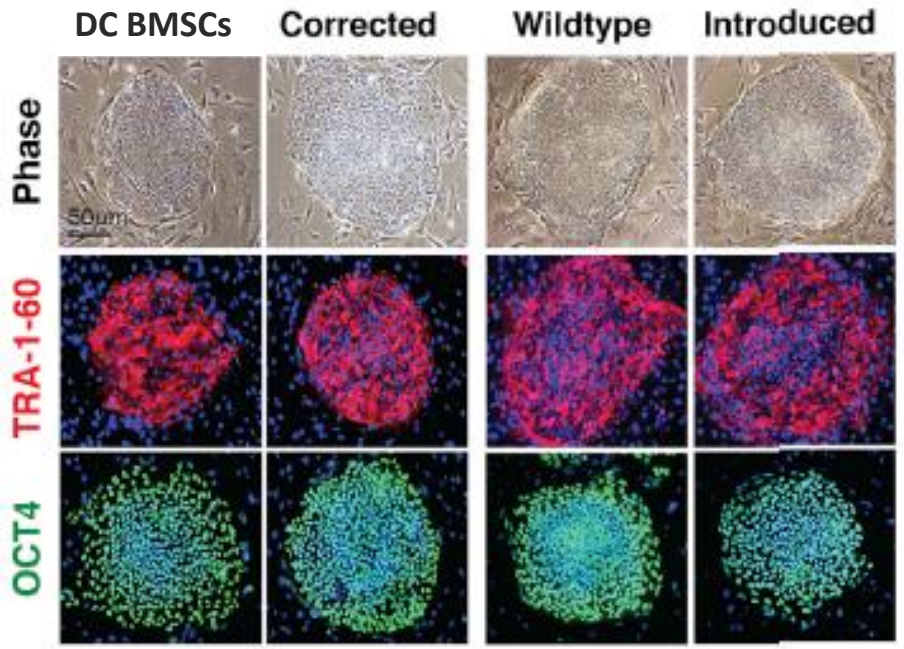
Improvement of liver function after CD34+ cell transplantation based on the analysis of **portal blood flow and velocity in both branches of the portal vein.**



Telomere length quantified by Telomere Repeat Fragment Analysis (TRF).

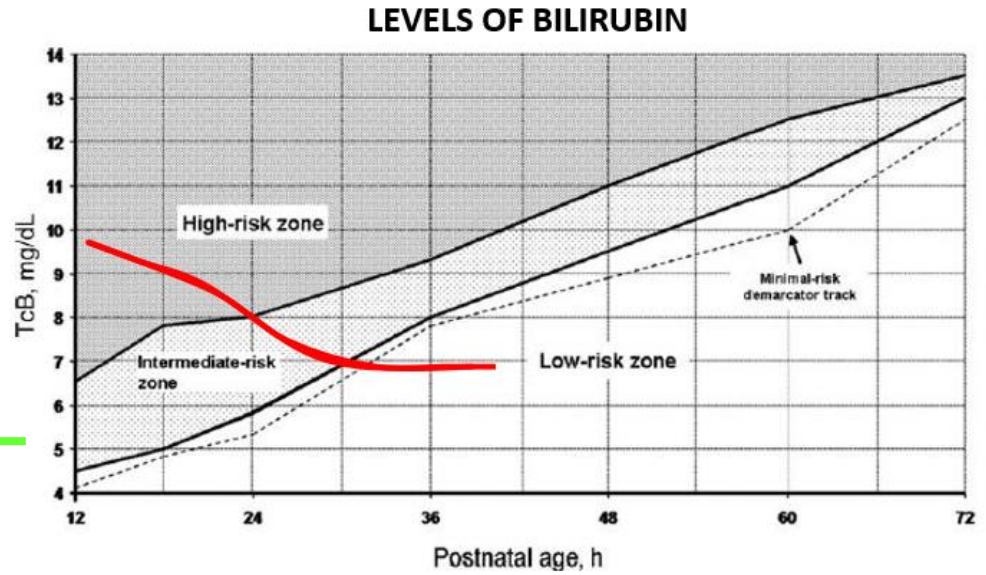






HEPATHIC STEM CELLS resulted to have longer telomere without the DKC1 mutation.

BILIRUBIN LEVEL has been restored in the low risk zone



Materials & budget

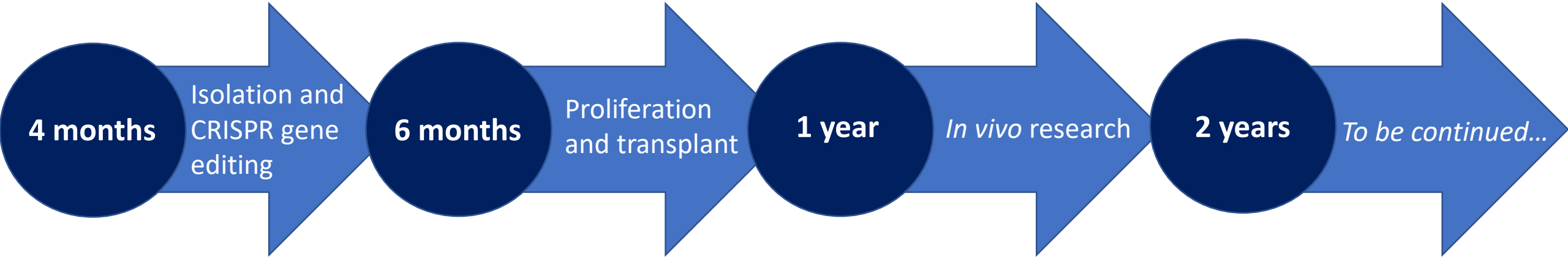
DKC1 mouse models	20€ x 10 models = 200,00 €
Liver cirrhosis mouse + WT mouse models	20€ x 30 models = 600,00 €
CliniMACS® System TS 500 for Research Use	1.650,00 €
CRISPR-Cas9 Mutation Detection Kit	160,00 €
G-CSF Recombinant Protein	500,00 €
pAD Adenoviral vector	1.200,00 €
Additional costs (results analysis, markers,…)	500,00 €
Salary of researchers	3.500,00 €
TOT.	8.310,00 €

Pitfalls

- May trigger a substantial immune response *in vivo*
- Transient expression
- Cloning can be challenging due to large genome size
- Risk of hepathic tumor

Solutions

- Correction of liver disease mutation linked to telomeropathies



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THANKS FOR YOUR ATTENTION!