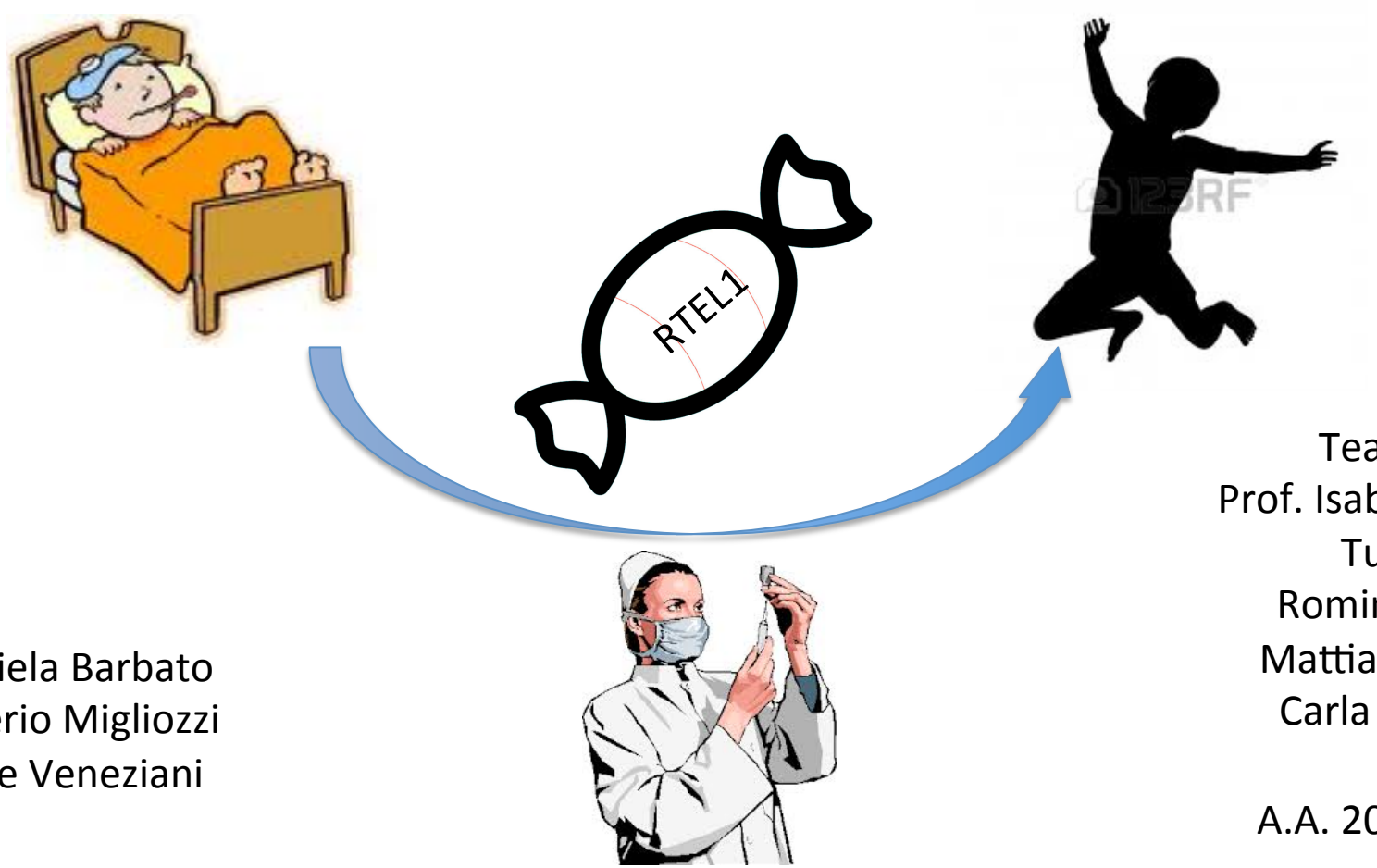
Gene Therapy for Dyskeratosis Congenita (DKC) and Hoyeraal-Hreidarsson Syndrome (HHS) by the rescue of RTEL1 activity



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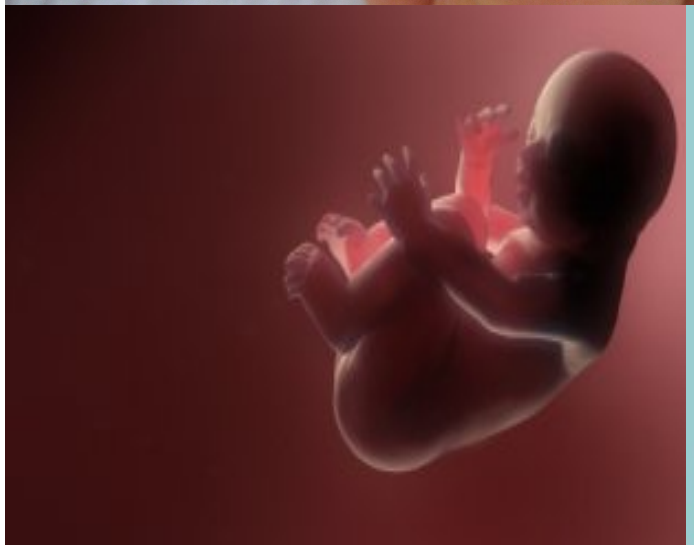
Background

Dyskeratosis Congenita (DKC)

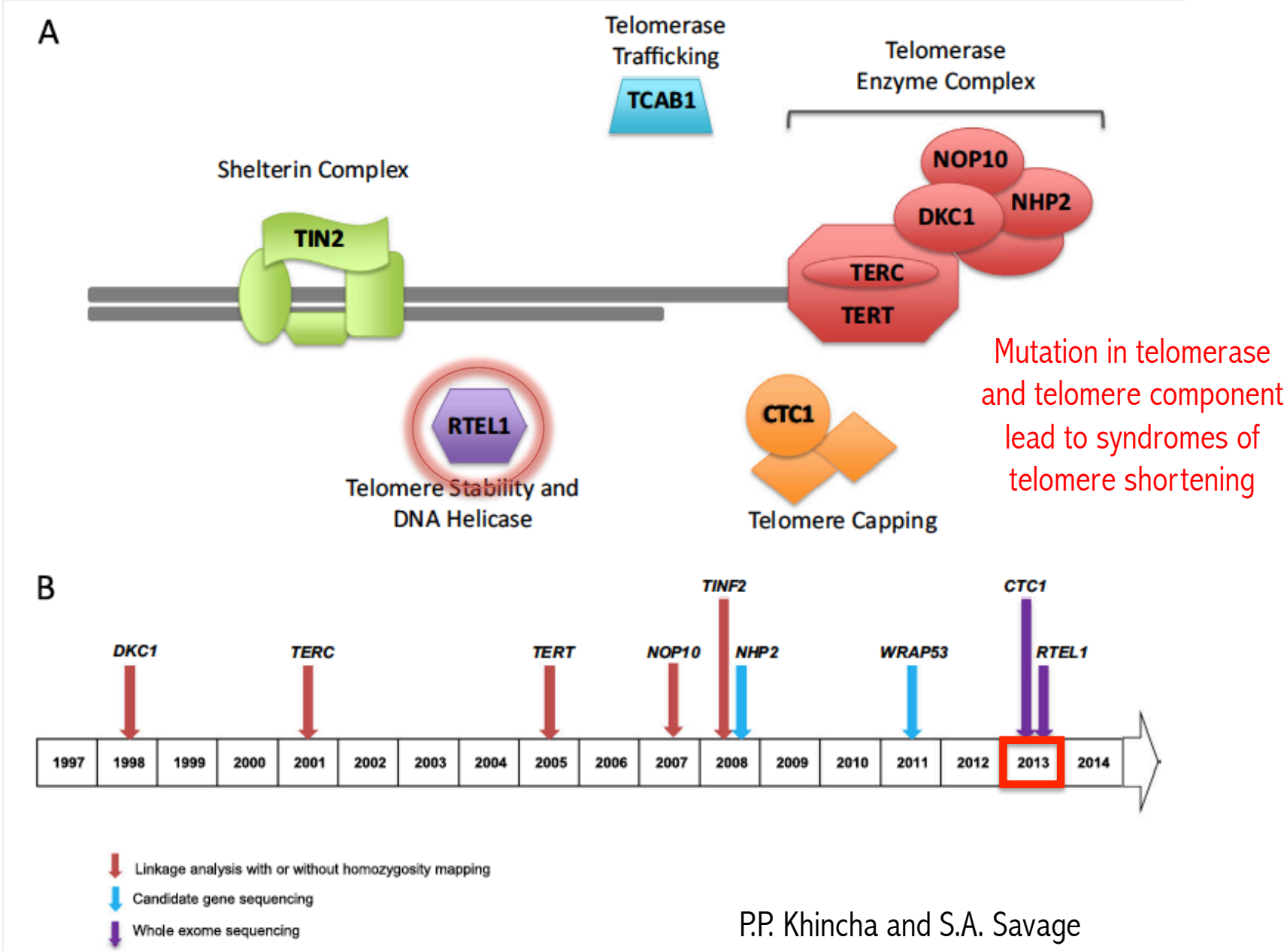
Hoyeraal-Hreidarsson Syndrome (HHS)

- Abnormal skin pigmentation
- Nail dystrophy
- Oral Leukoplakia



- Bone Marrow failure
- Cerebellar Hypoplasia
- Intrautrine grow retardation

Which genes are involved in these diseases?

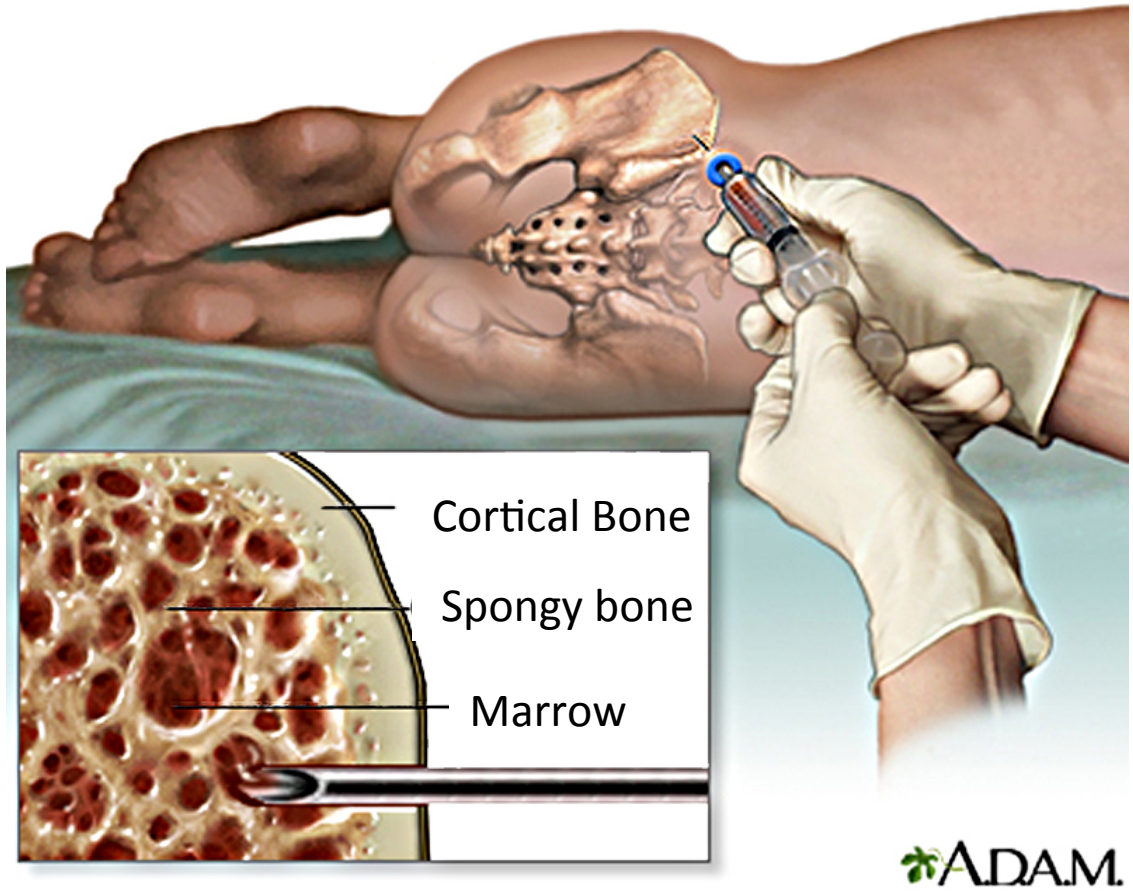


Mutation in telomerase and telomere component lead to syndromes of telomere shortening

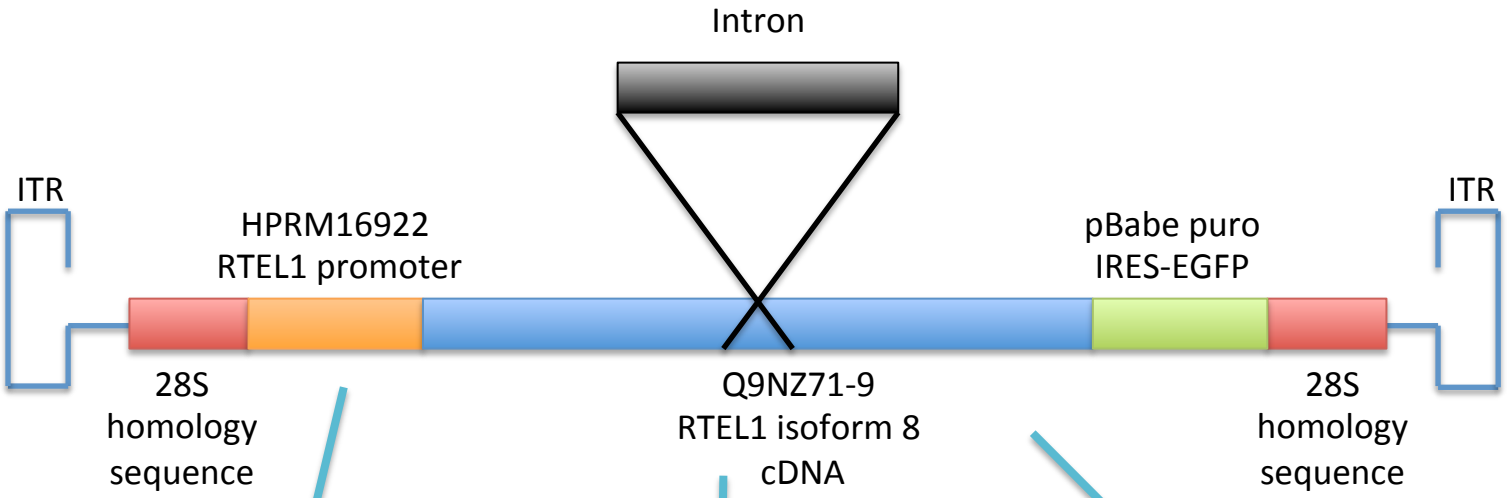
P.P. Khincha and S.A. Savage

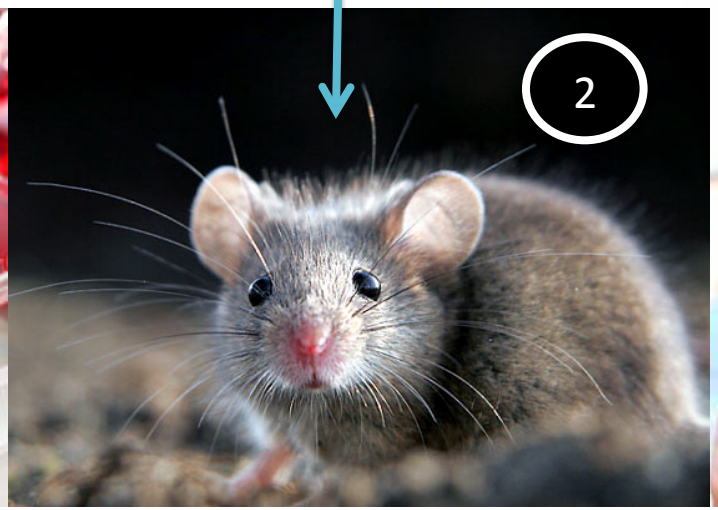
Therapy

Bone marrow failure (BMF) is the most important aspect of this disease. Our main goal is to do a gene therapy on hematopoietic stem cell (HSC) CD34+ to rescue the function of RTEL1, in BM, with the use of a recombinant Adeno-associated vector (rAAV).



Experimental plan





Experimental plan

In vitro:

HSC CD34+ from patients, trasduced with rAAV

- LAM-PCR to evaluate the insertion of rAAV
- Quantitative RT-PCR to analyze the level of expression of the inserted gene (number of copies)
- Q-FISH to evaluate the rescue of the telomere length
- Immunofluorescence CD34+/GFP+
- Chip to demonstrate that the rescue of the telomere length is due to an increased activity of RTEL1 (injection with rAAV)
- FACS analysis of CD34+/GFP+ cells → sorting and amplifications

Ex vivo:

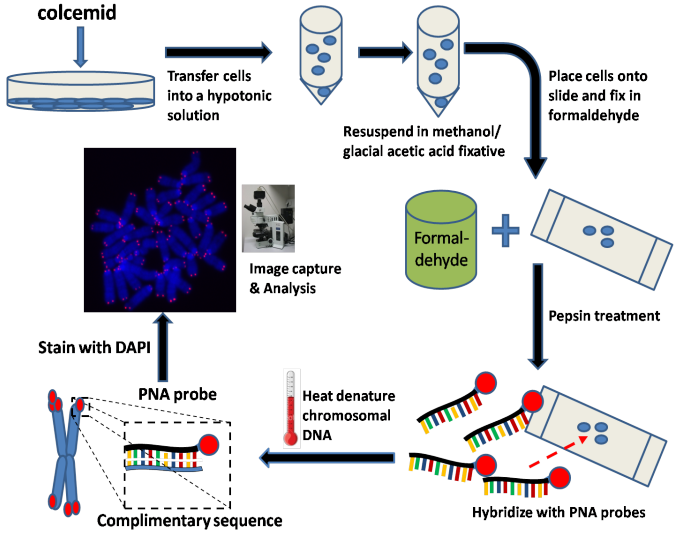
(mouse cell after intravenous injection of our rAAV)

- Northern blot to evaluate the presence of RTEL1mRNA
- Western blot to analyze the protein presence
- Q-FISH to study the telomeres length
- Southern blot to recognize the specific recombinant sequence
- FACS to evaluate the repopulation of bone marrow using specific marker

All the experiments will be done on Wild-Type, unthreated CD34+ and trasduced CD34+

In vitro experiments:

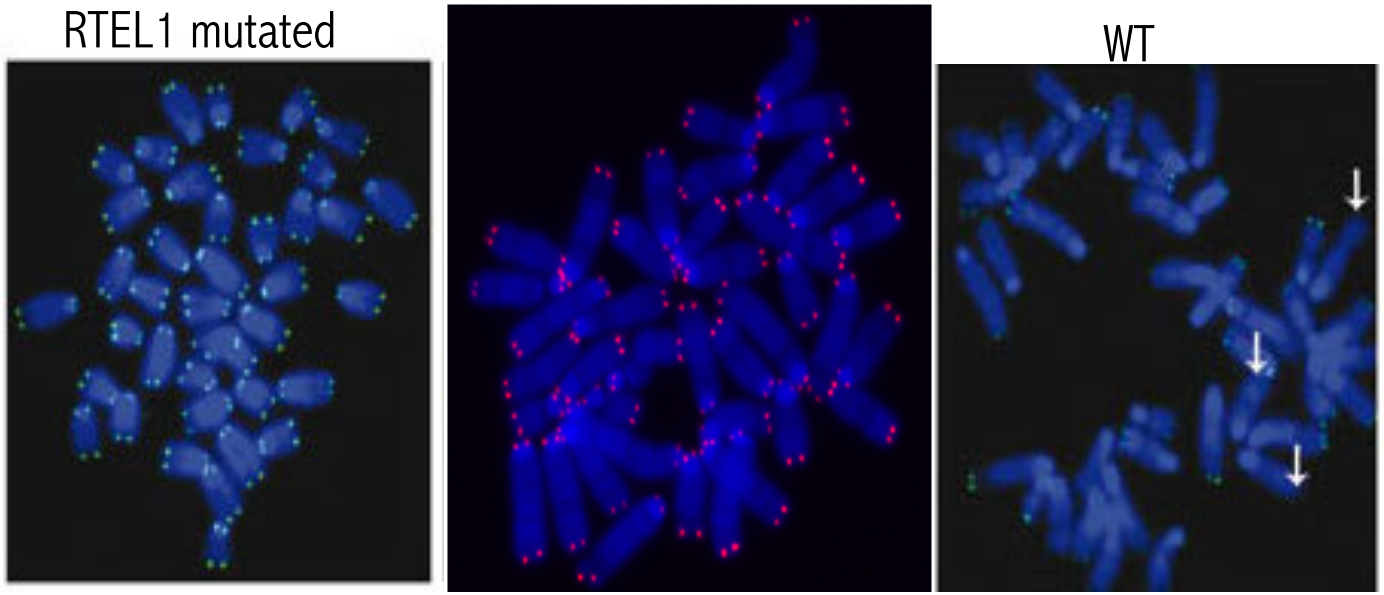
Q-FISH to evaluate the rescue of the telomere length



RTEL1 mutated

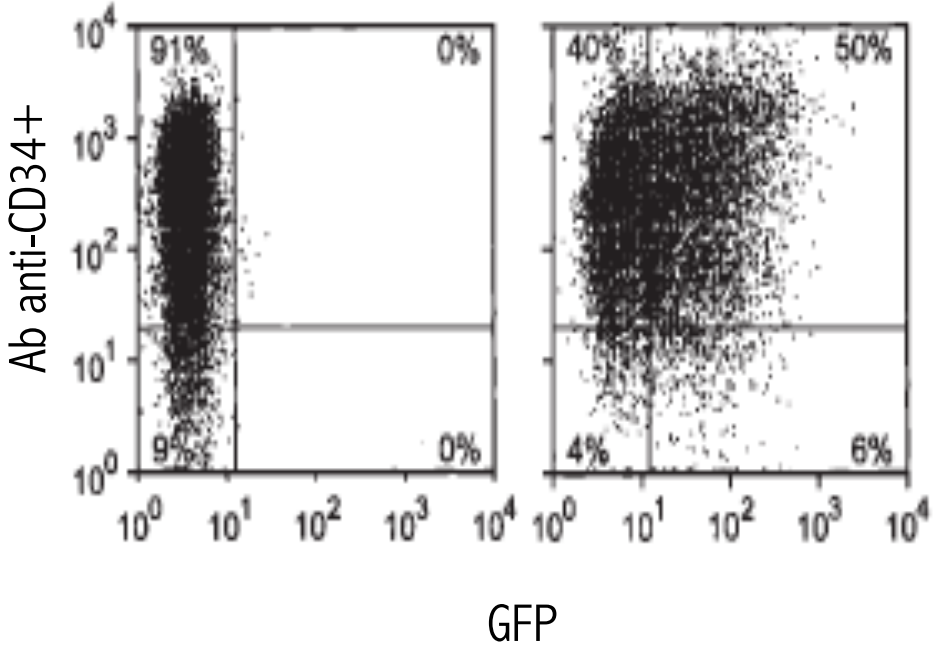
Treated with rAAV

WT



In Vitro Experiments

FACS analysis of CD34+ and sorting of positive population

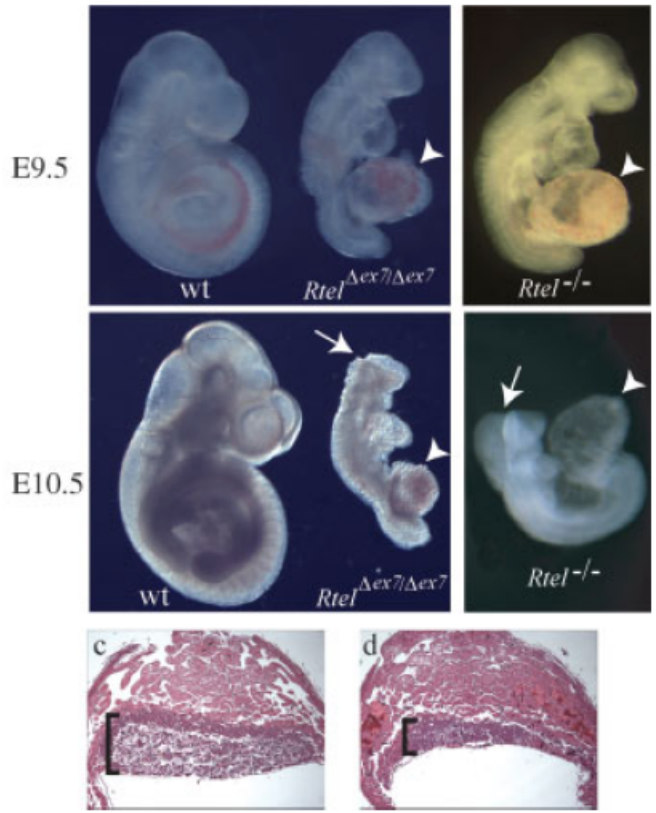


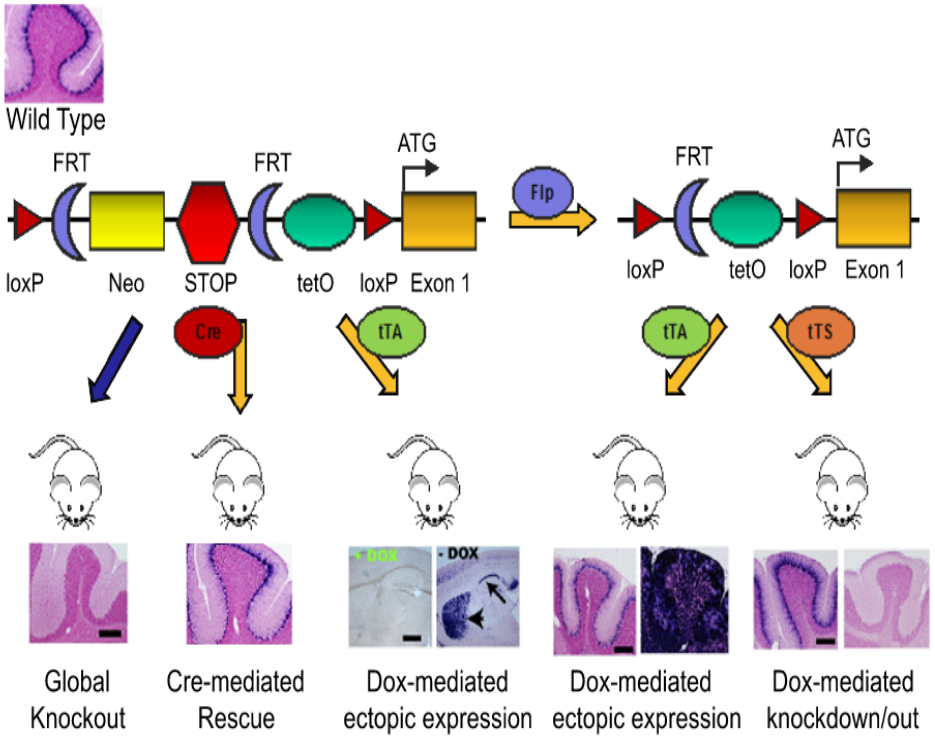
Cytofluorimetric representation of wild-type CD34+ cells and trasduced CD34+ (trasduced with rAAV). GFP is our selection marker; It points out the population of trasduced cells

A.C. Nathwani et al., Efficient gene transfer into human cord blood CD34+ cells and the CD34+CD38- subset using higly purified recombinant adeno-associated viral vector preparations that are free of helper virus and wild-type AAV

Inducible mouse model for RTEL1

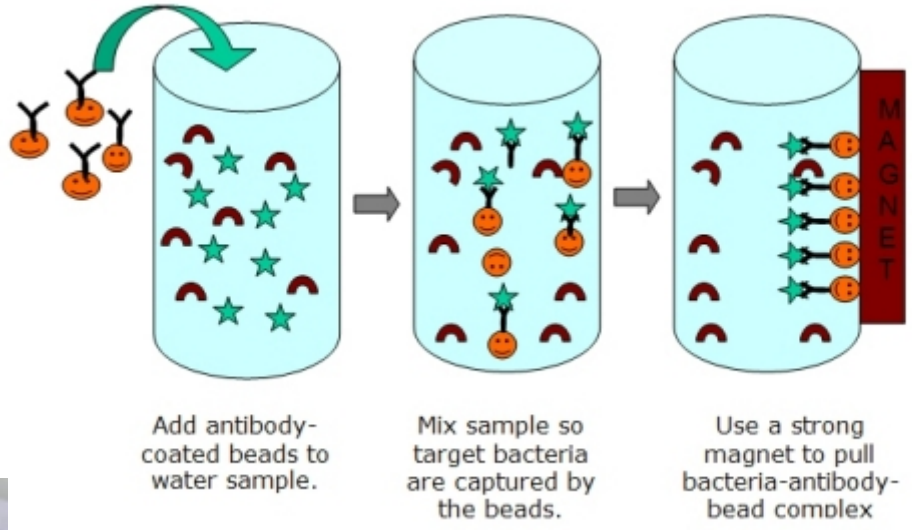
- Use a mouse model that presents two sequences *loxp* near the exon 7 with the Cre gene under the control of a TetOn promoter





Wu et al. Establishment of Conditional Knockout Alleles for the Gene Encoding the Regulator of Telomere Length (RTEL)

1. Giving 1.650 mg/kg of doxiciclin
2. Extraction of BM from some mice



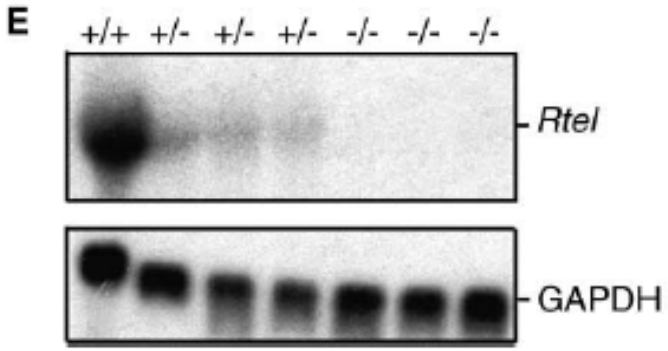
3. Isolation of murine HSC using immunomagnetic sorting

4. Trasduction of rAAV in mHSCs overnight at 37°C
5. After trasduction, cells were suspended in PBS

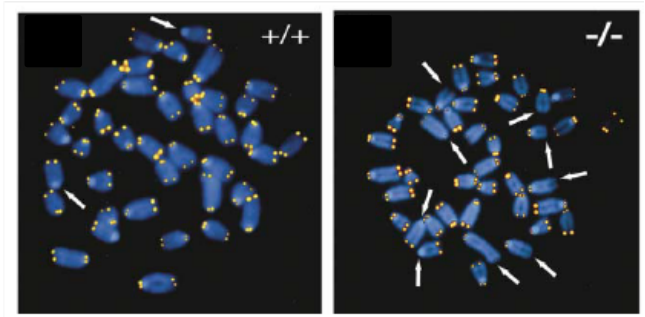
Received mice are lethally irradiated with 950 cGy before receiving transplantation.

Mice are transplated with 10*10^6 cells

8-14 days after transplantation we killed mice and analyzed the cells with:

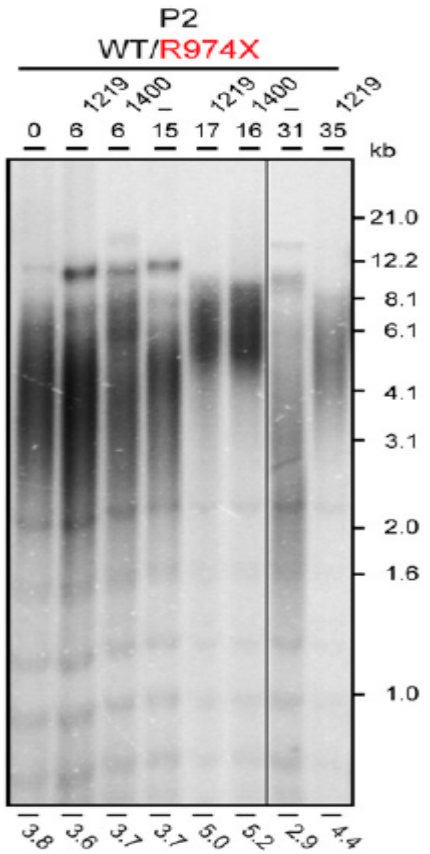


Northern blot analysis of Rtel mRNA in mouse



Defective Differentiation and Telomere Phenotype of Rtel$^{-/-}$

H. Ding et al., Regulation of Murine Telomere Length by Rtel: An Essential Gene Encoding a Helicase-like Protein



Z. Deng et al., Inherited mutations in the helicase RTEL1 cause telomere dysfunction and Hoyeraal–Hreidarsson syndrome

Future perspectives

IF

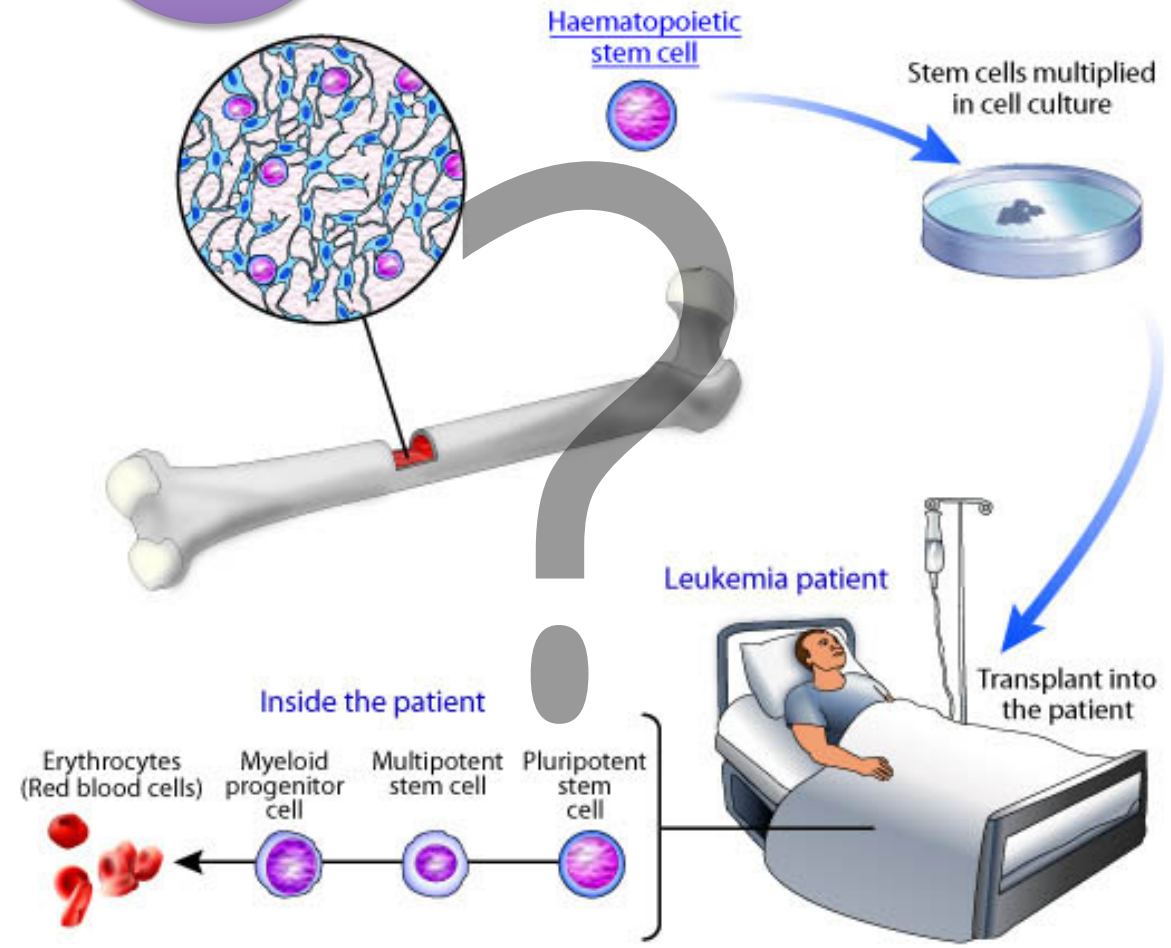


Illustration by Cell Imaging Core of the Center for Reproductive Sciences.

Pitfalls and solutions

Even if they have some very desirable characteristics, like they're not immunogenic and we can drive the expression in specific tissue by using different capsides, our main problem remains the low package capacity of the AAV, respect of other vector, that force us to use two vector to gain the full cassette. However we can use another type of vector, like the **Lentivirus**, that are a subclass of Retrovirus, which have a major package capacity (8-10 Kb)than the AAV and are able to integrate in the genome, but the site of integration is unpredictable, which can pose a problem because the integrated cassette can disturb the function of cellular genes and lead to activation of oncogenes promoting the development of cancer. However, studies have shown that lentiviral vectors have a low tendency to integrate in places that potentially cause cancer. In particular, one study found that lentiviral vectors did not cause either an increase in tumor incidence or an earlier onset of tumors in a mouse strain with a much higher incidence of tumors.

Another possible strategy involved the use of the innovative non viral vector called **Ormosil** (*organically modified silica or silicate)* wich can be engineered to deliver a specifical gene to a specific tissue.

Materials	Price
Anti-CD34 ab (EP373Y) (ABCAM)	390€
iScript Advanced cDNA Synthesis Kit for RT-qPCR (BIO-RAD)	371€
FISH Tag™ RNA Red Kit, with Alexa Fluor® 594 dye (LifeTechnology)	585€
Immunoprecipitation Kit (Life Technology)	350€
NorthenMax kit (Life Technology)	224€
Western Blot kit (Life Technology)	218€
Southern Blot (Life Technology)	381€
Anti CD90 / Thy1 (MRC OX-7) (ABCAM)	420€
Inducible mouse model (Ingenius Targeting Laboratory)	20000€ (2 mice)
Two complementary vectors for Trans-splicing	5.500€

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