SAPIENZA
UNIVERSITÀ DI ROMA

Bone Marrow rescue in Dyskeratosis Congenita patients via autologous transplantation of gene-edited Hematopoietic (HSCs) and Mesenchimal Stem Cells (MSCs)

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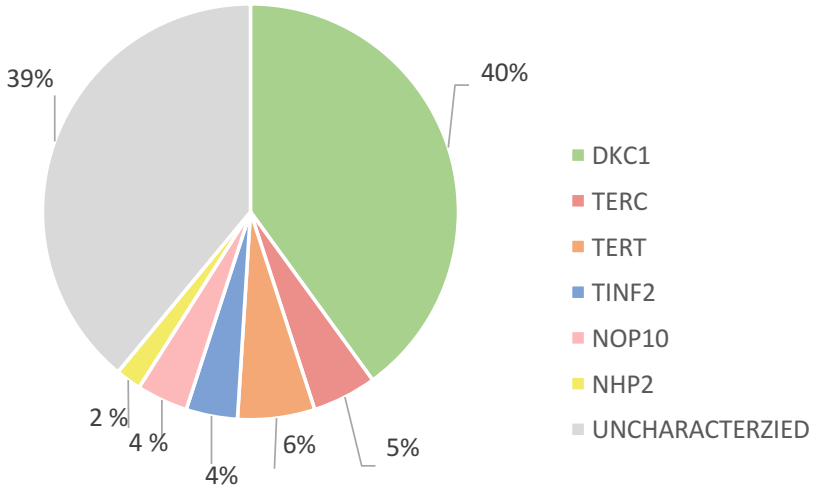
Background



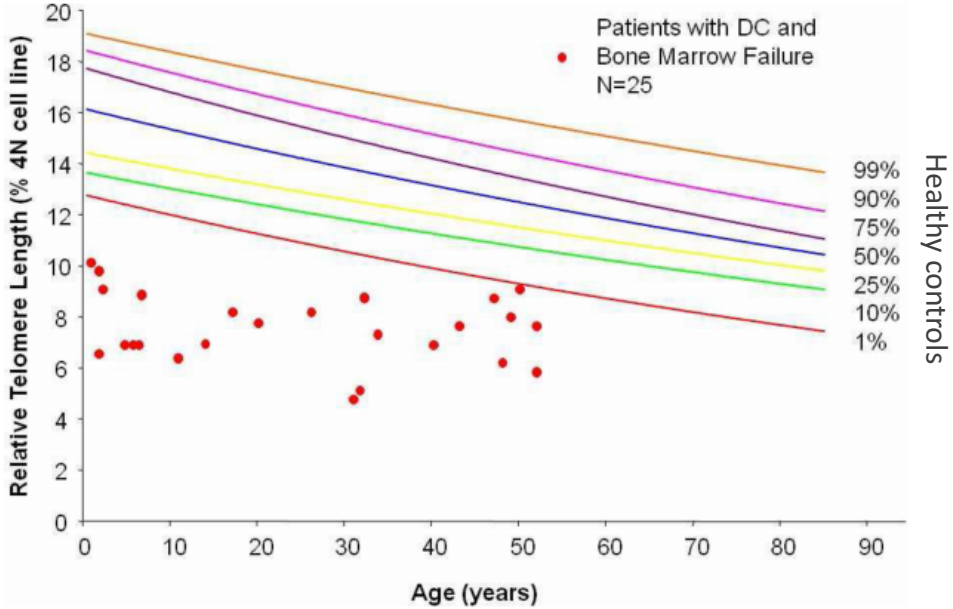
- Classical linkage analysis established the DKC1 gene as the gene responsible for the X-linked Dyskeratosis Congenita (DC).

(Dokal I. et al., Nature Genetics, 1998)

Family	Ethnic Origin	Exon	DNA Change	Protein Change
DCR-004	United Kingdom	11	1058C→T	A353V
DCR-008	United Kingdom	5	115A→G	K39E
DCR-006	Italy	11	1058C→T	A353V
DCR-055	Spain	11	1058C→T	A353V
DCR-009	Italy	10	961C→G	L321V
NIMGS 515	United States	4	196A→G	T66A
DCR-020	France	11	1058C→T	A353V
DCR-021	Austria	11	1050G→A	M350I
DCR-027	India	12	1204G→A	G402R
DCR-029	United States	11	1058C→T	A353V

(Knight S. W. Et al., Am. J. Hum. Genet.)

Background

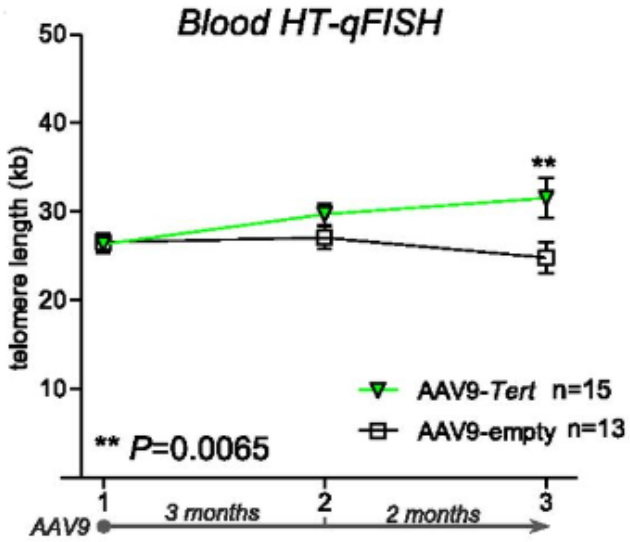


- DC patients have very **short telomeres** in length compared with healthy age-matched controls

(Masona J. Et al., Cancer Genet., 2011)

- **AAV9-Tert treatment** of AA mice rescued mortality due to Aplastic Anemia, concomitant with telomere re-elongation in blood and bone marrow cells.

(Bär C. et al., Blood, 2016)



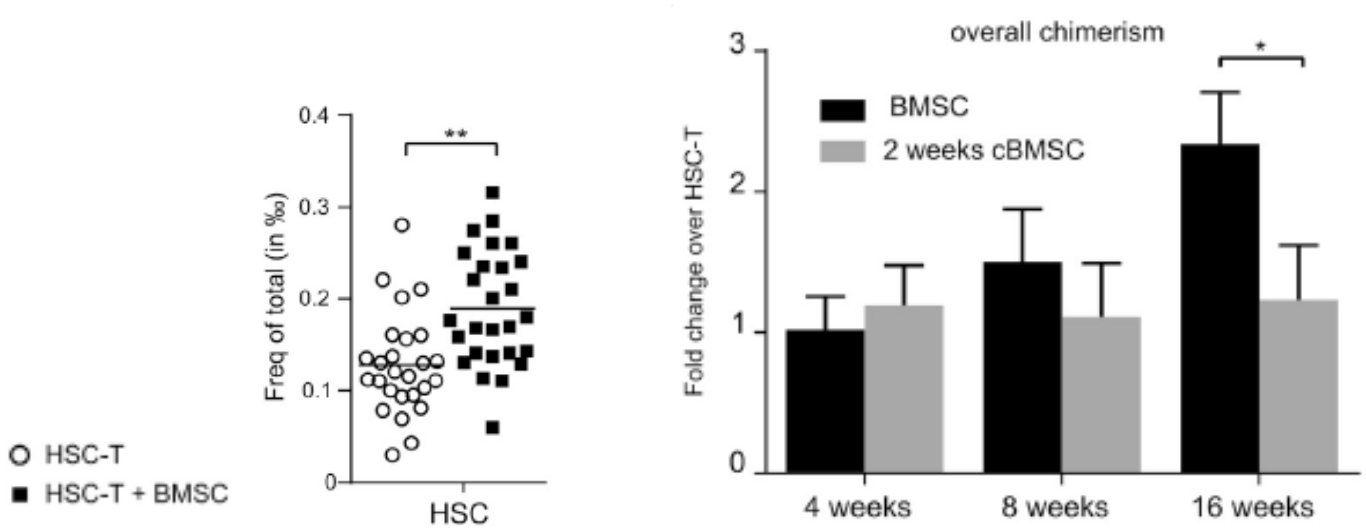
Background

- The only current interventions to treat these diseases involve **organ transplantation**; i.e., BM, liver, and lung

(Stanley S.E. et al., Curr Opin Genet Dev, 2015)

- Long-term (16 weeks) reconstitution assays confirmed expansion of functional HSCs in hosts **co-transplanted** with primary **BMSCs and HSCs** as compared to classical HSC-T alone.

(Abbuehl J.P. et al., Cell Stem Cell, 2017)



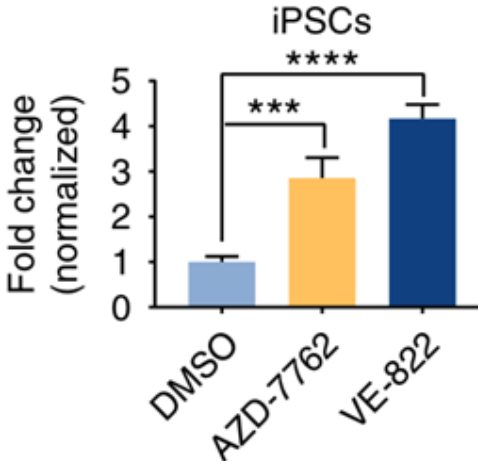
Background- toolkit

	Cas9	**Cpf1**
Components	crRNA, tracrRNA, Cas9	crRNA, Cpf1
Size	spCas9 4.2 kb	3.7 kb
gRNA lenght	100 nt	42 nt
tracrRNA	yes	no
dsDNA cleavage	Blunt end	5’ overhang
PAM sequence	NGG	TTTN

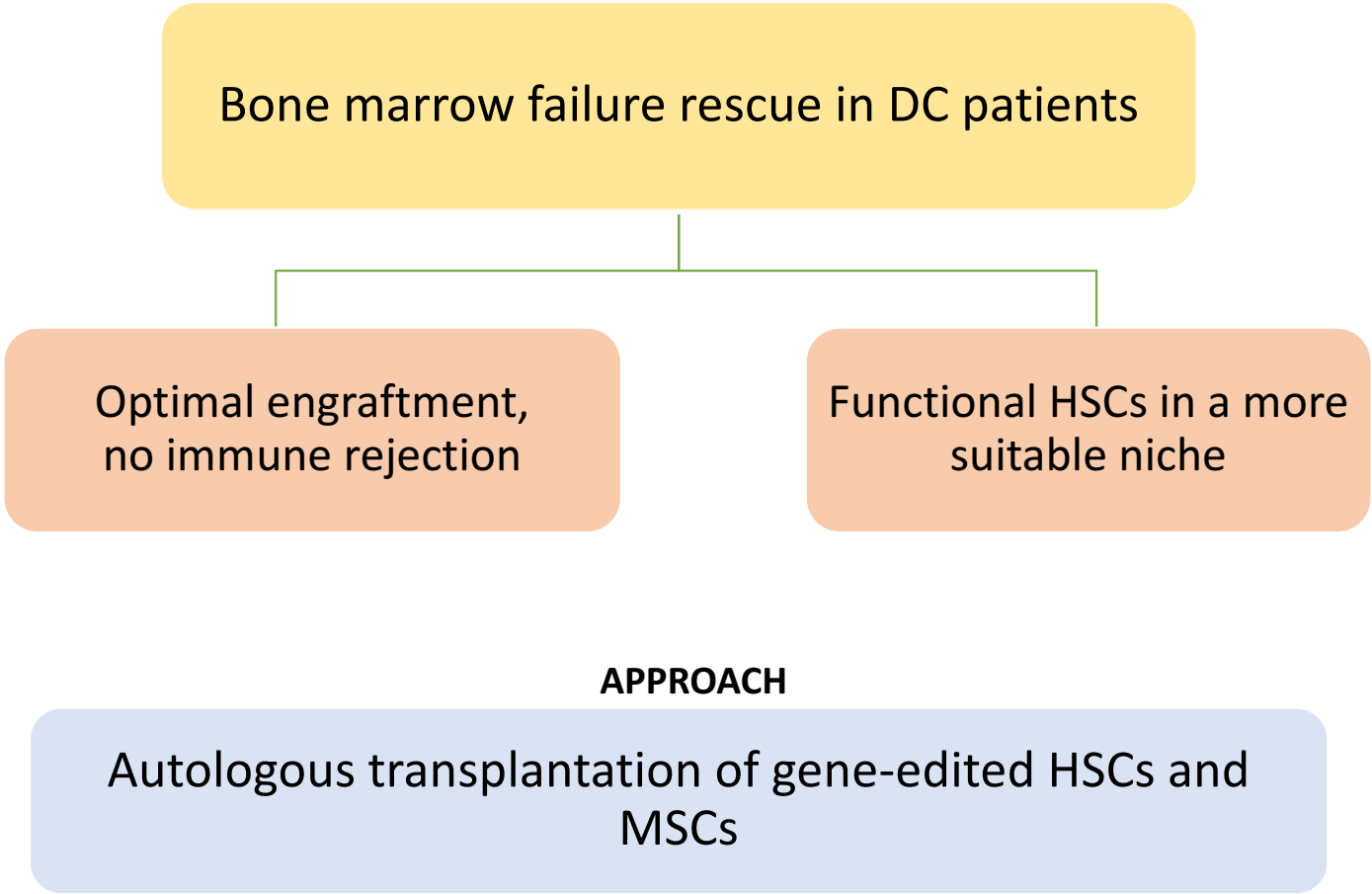
(Zetsche B. et al., Cell, 2015)

- **VE- 822** and **AZD-7762** significantly promoted CRISPR-Cpf1- mediated knock-in in hPSCs by 6-fold.

(Ma X. Et al., Nature Communications, 2018)

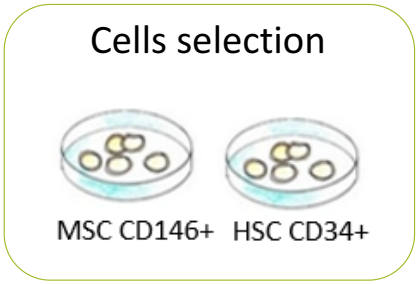


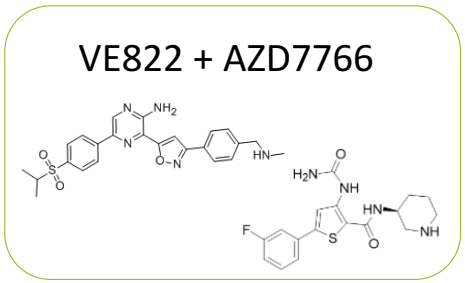
Objectives and strategy

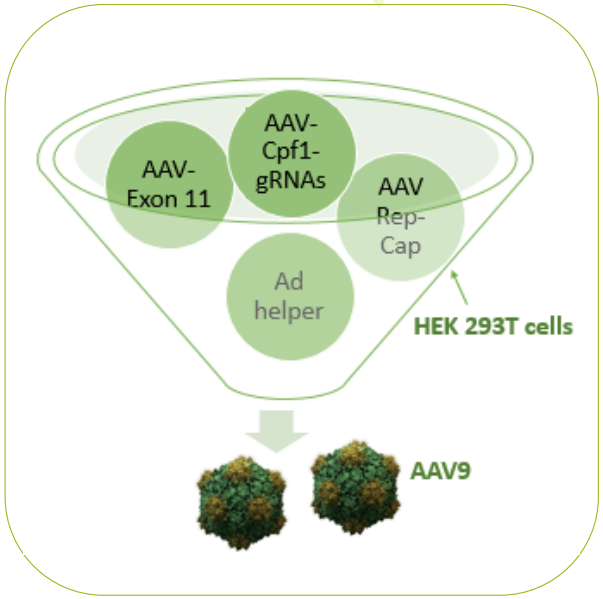


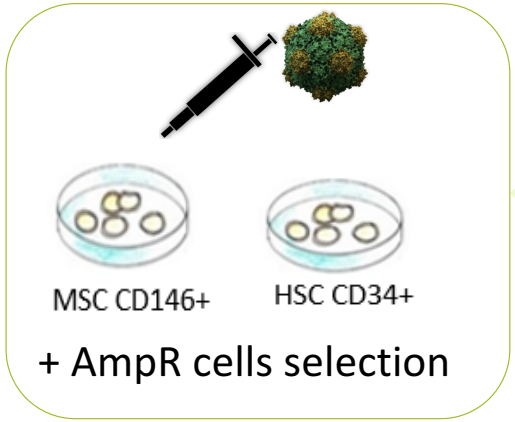
Experimental plan – part one

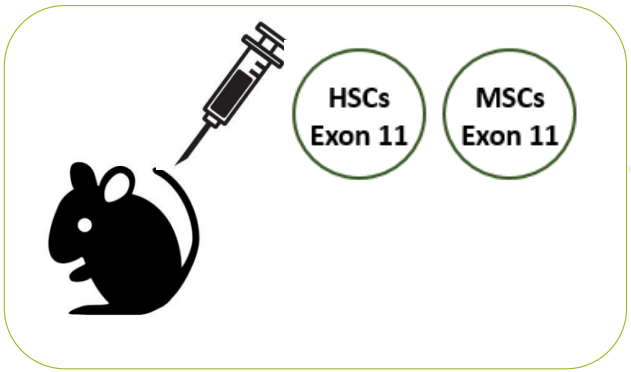
Ex vivo in mice



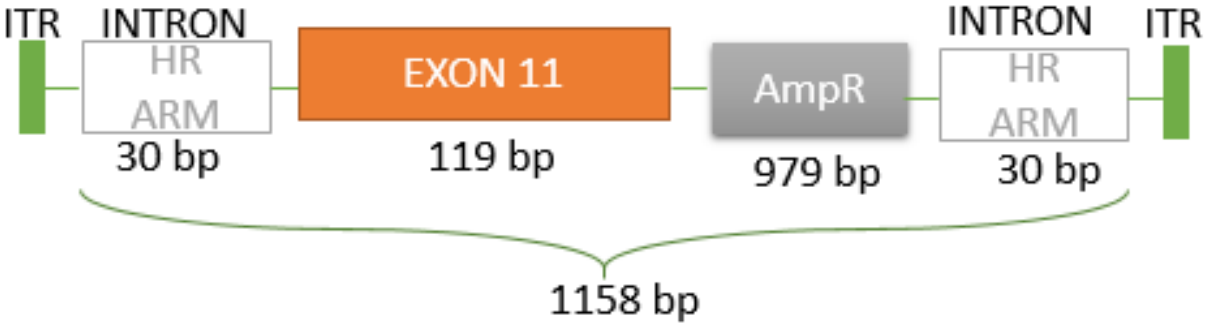




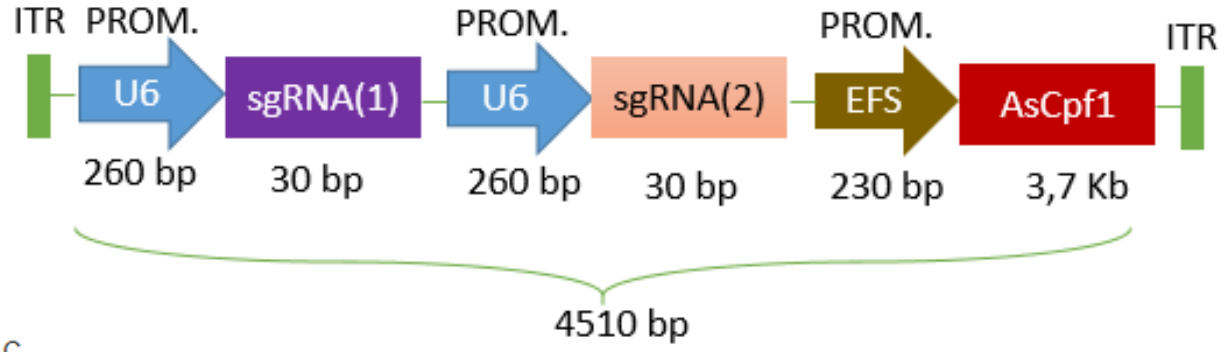




AAV vectors



AAV-9 vector
- non-integrative
- pour immunogenicity
- transduction in both dividing and quiescent cells
- long-term expression



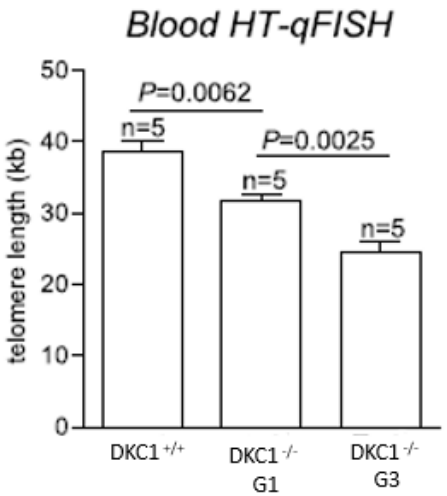
sgRNA(1) TTTTGAGTCGTGTGTAATGTGCGGAGAGTGGTAC

sgRNA(2) GCAGCTAGTGGGCTATAAGTGTCATCCCTGTTTC

(EuPaGDT.com)

Expected results

A



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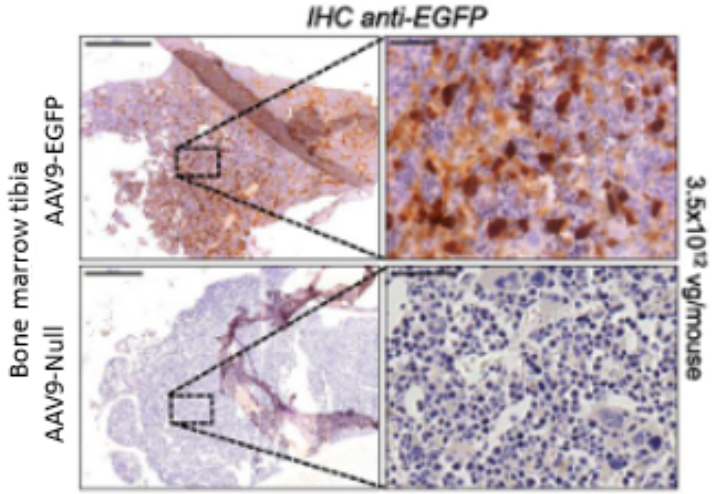
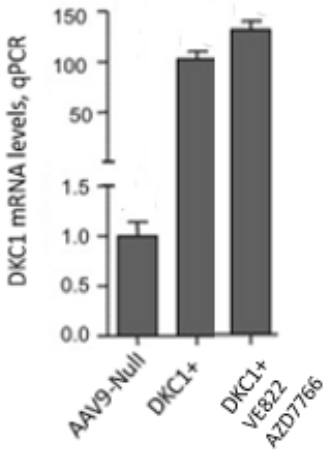
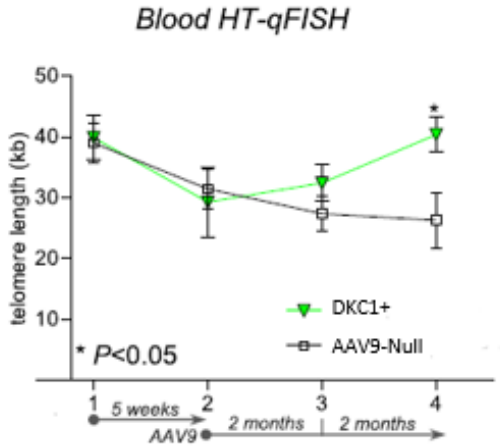


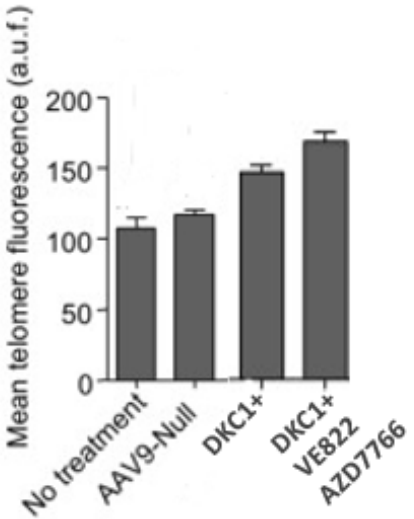
C



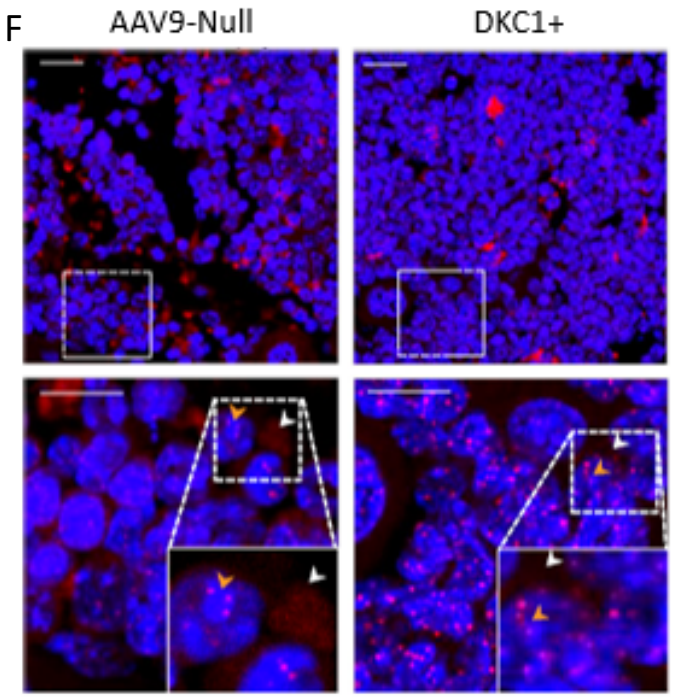
D

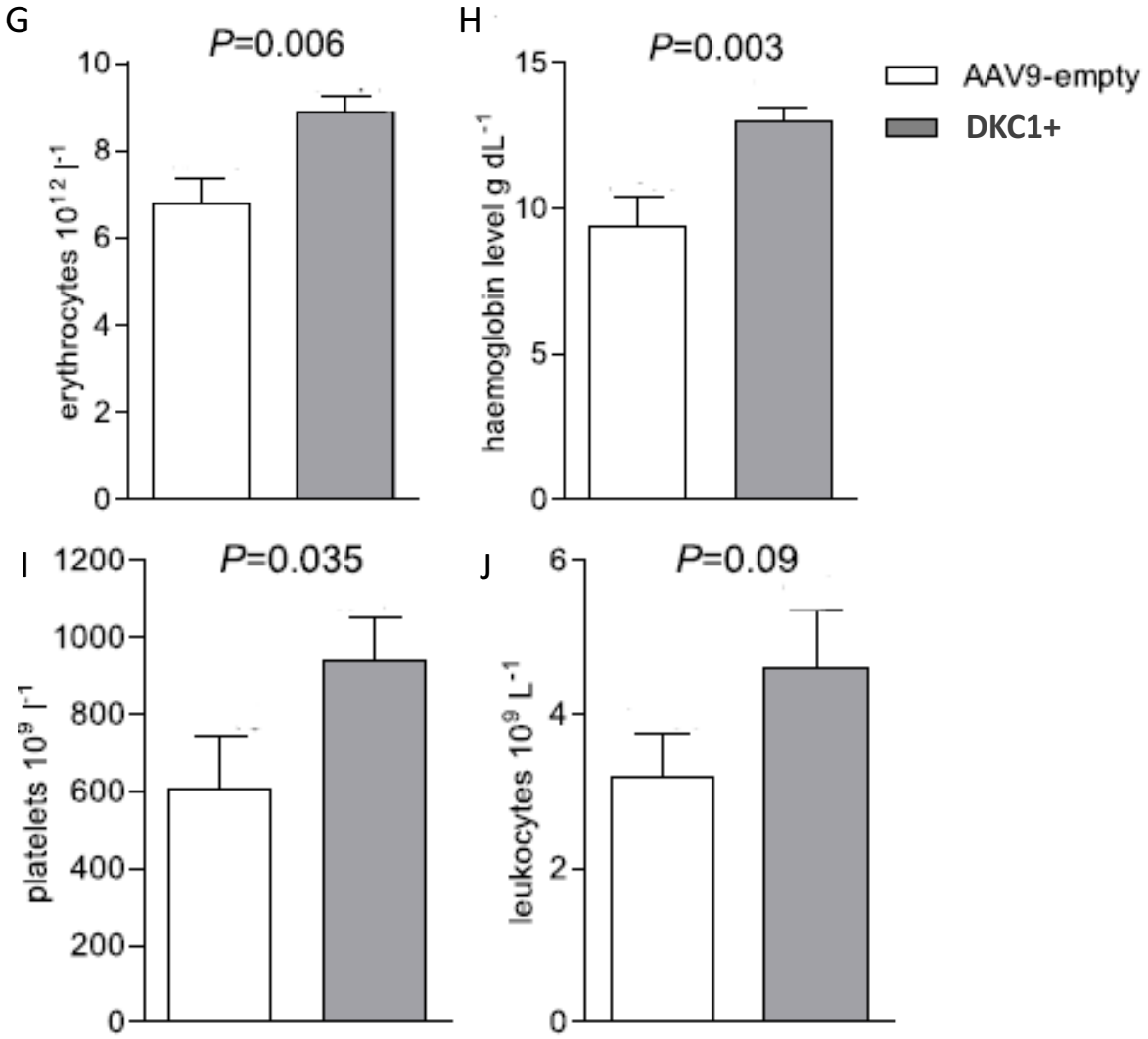


E



Expected results

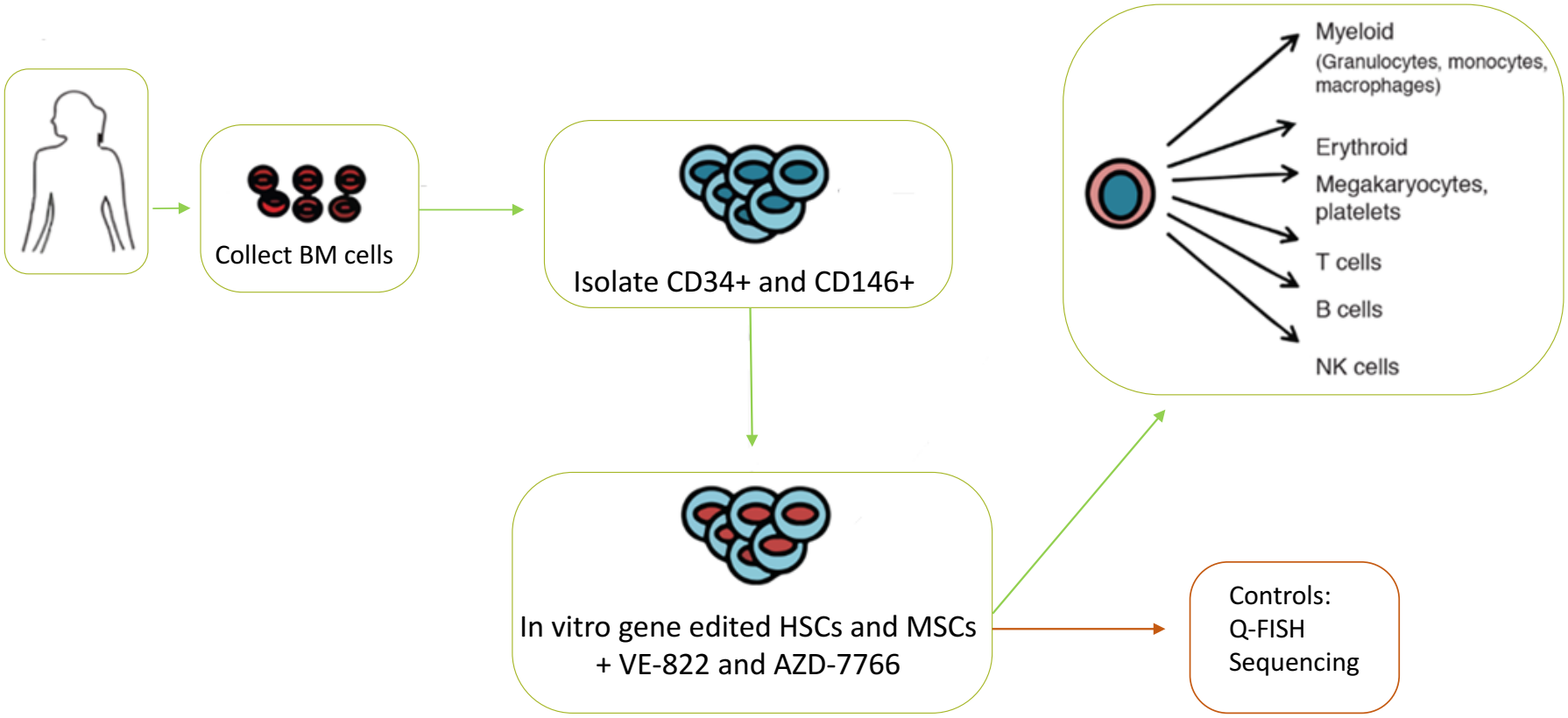




All control experiments to be performed at 4, 12 and 24 weeks

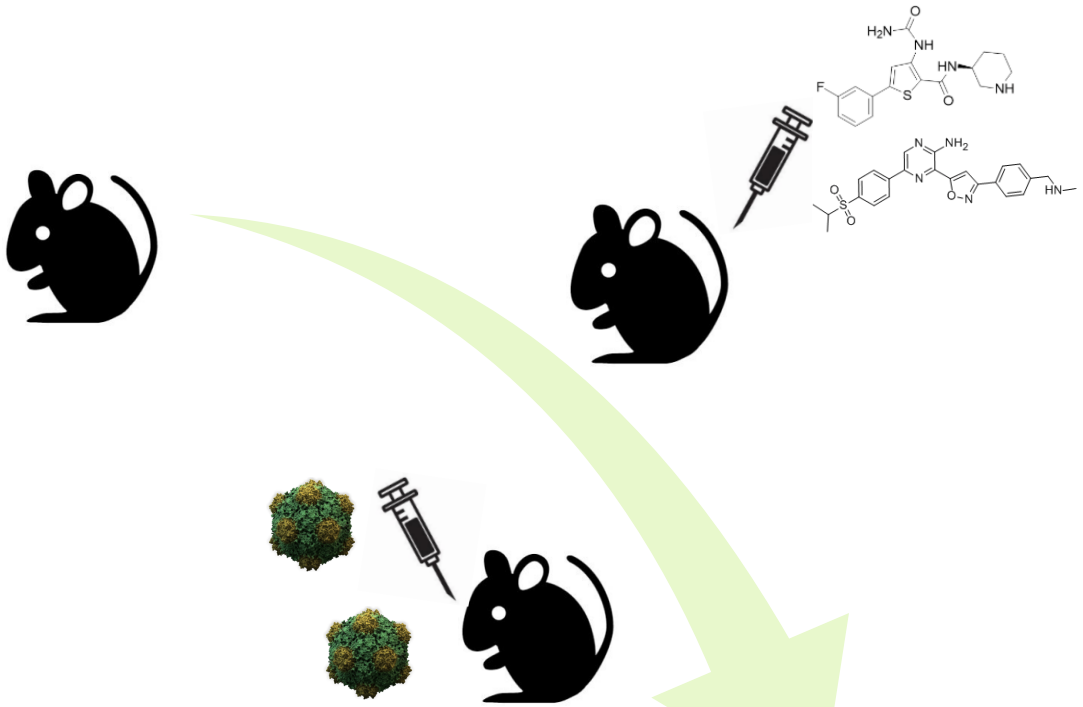
Experimental plan – part two

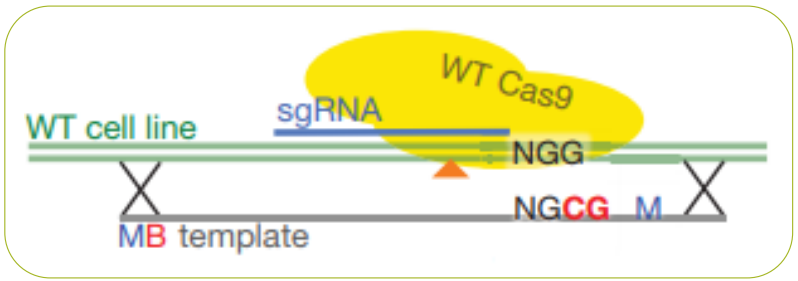
Ex vivo in humans



(Adapted from Jung M. et al., The American Society of Cell &Gene Therapy, 2015)

Experimental plan – part three





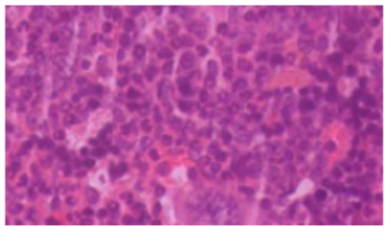
Crispr/Cpf1
attainment check

(Paquet et al., Nature, 2016)

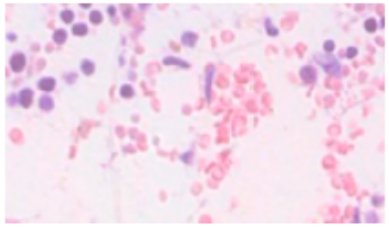
In vivo in mice

Expected results

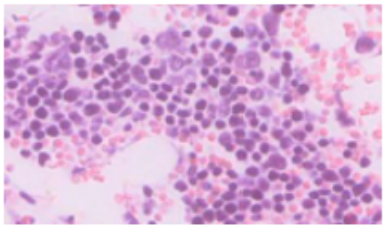
WT mouse



AAV9-Null



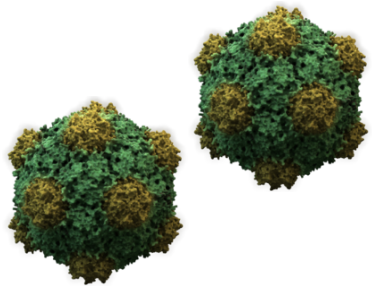
DKC1+



+ Sequencing

Ex-post Evaluation

PITFALLS	SOLUTIONS
Low packaging capacity	Lentiviral delivery
Lower VE-822 and AZD-7766 efficiency than expected	Dosage adjustment



MATERIALS	COSTS	SOURCE
C57BL/6J mouse models	20€ x 40 mice = 800€	thejacksonlaboratory.org
Stabulation	3000€	/
Antibodies	600€	ThermoFisher
VE822 (50 mg) + AZD7766 (50 mg)	240 + 360 € = 600€	MedChemExpress
r-AAV production HEK 293T cells Vector kit Crispr/Cpf1 + EFS promoter sgRNAs + U6 promoter	 557€ 500€ 480€ 110€	 Integrated DNA Technologies // // //
FACS Amp selection kit	 300€	 Cellbiolabsinc.com
HT Q-FISH PBS Acetic acid Methanol DAPI 96-well plates Telomere PNA FISH kits, PNA FISH kit, Cy3	 80€ 60€ 35€ 45€ 900€ 1000€	 Sigmaaldrich.com // // // // Agilent.com
Immunohistochemistry kit	2000€	ThermoFisher
qPCR	300€	LifeTechnologies
Immunofluorescence kit	1500€	ThermoFisher
Blood cells count	300€	Analysis Laboratory
Sequencing	750€	Illumina

Required fundings: € 14.000
Experiment time span: 30 months

References

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