SAPIENZA
UNIVERSITÀ DI ROMA

A Leap Back in Time: Lentiviral Vector-Mediated Expression of WRN Gene in Werner Syndrome

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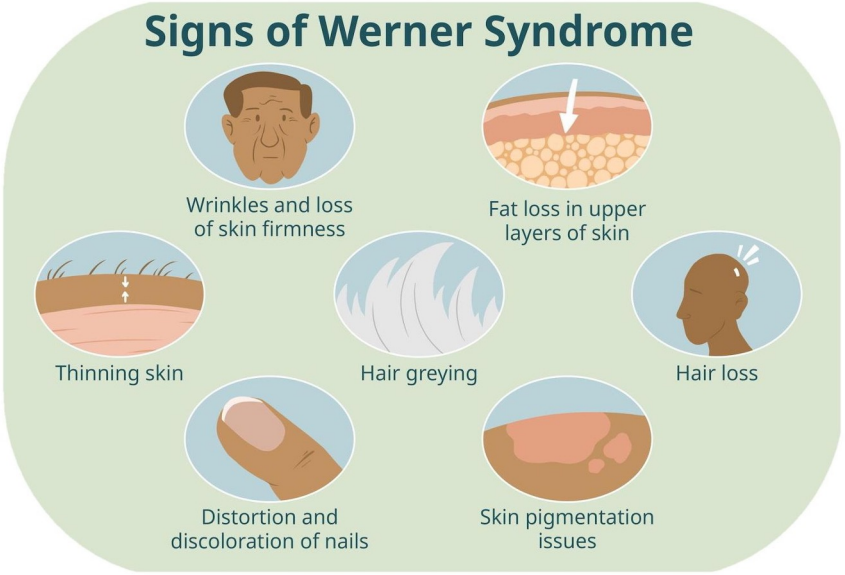
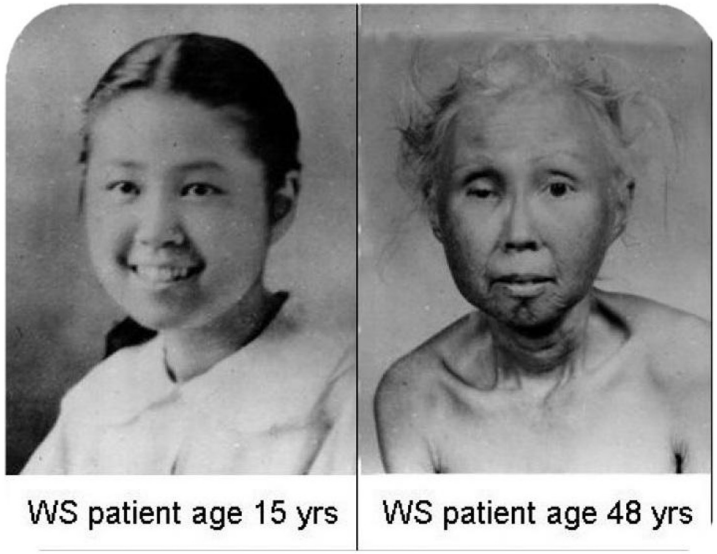
Winter School 2023-24

Background of Werner Syndrome

Rare autosomal recessive genetic disorder.

Loss of function in the WRN gene.





RecQ3: DNA helicase with a 3'→5' exonuclease activity.

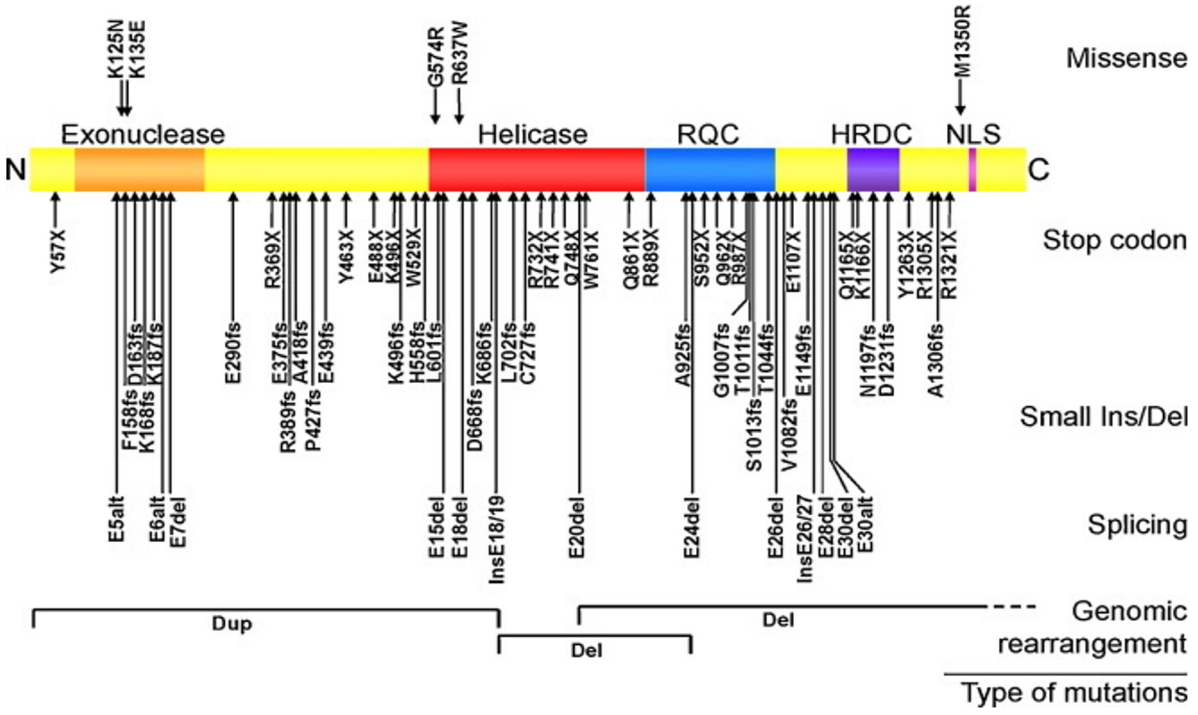
I. genome instability
II. DNA repair, replication, transcription and telomere maintenance
III. age-related diseases

Aim

Providing a functional WRN gene with a lenvi-viral vector as a potential treatment for Werner Syndrome

Why can't we correct the mutation?

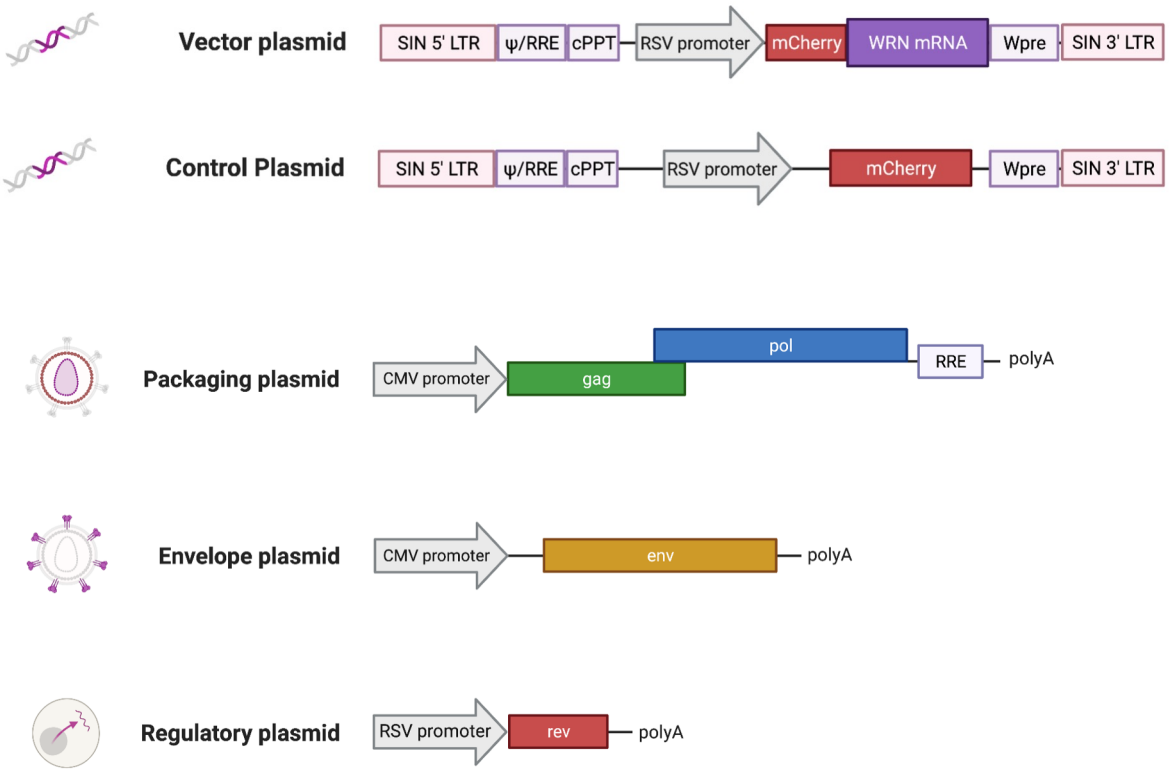
More than 80 different homozygous or compound heterozygous mutations in WRN gene



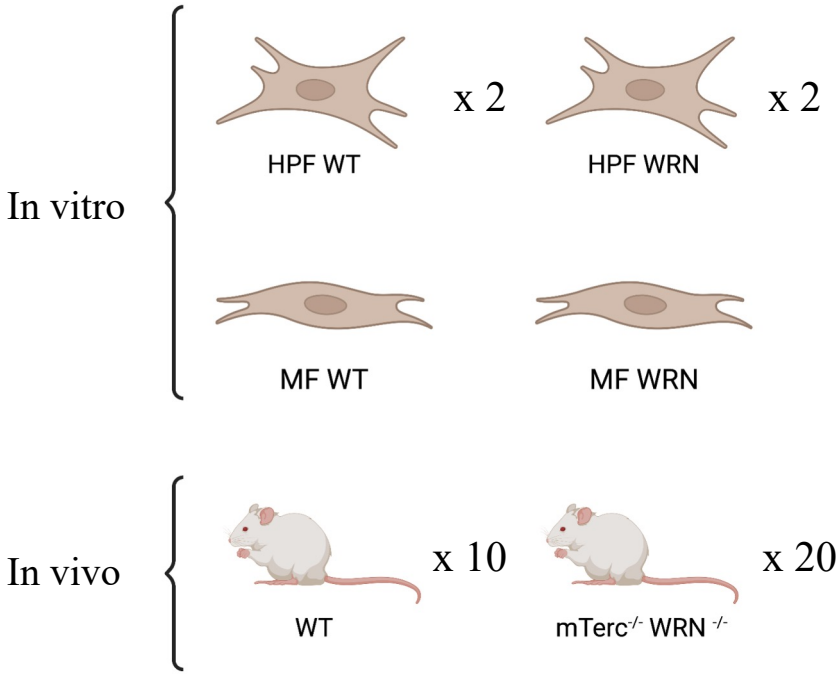
Oshima, 2017

Materials

Third-Generation Lentiviral Vector System



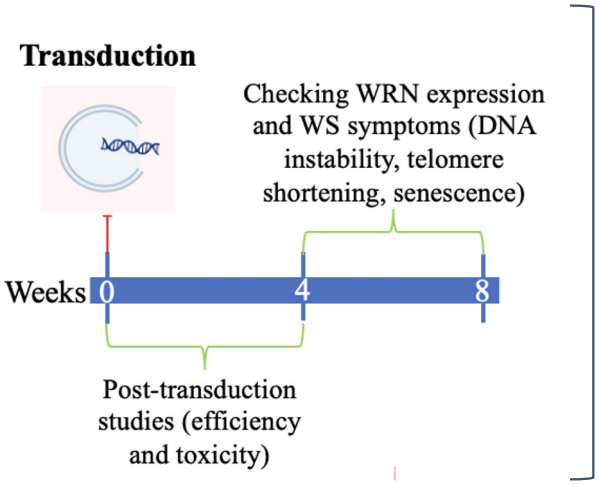
Model System



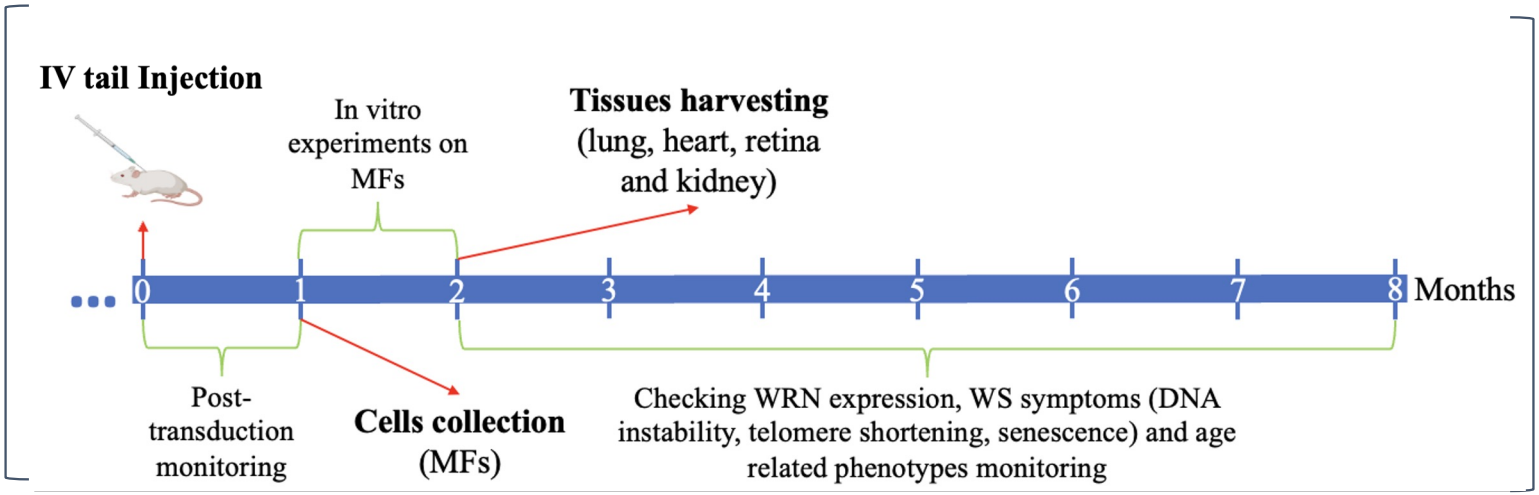
WRN-/- mice do not show WS phenotypes, due to their longer telomeres compared to humans. Instead, mTerc-/- WRN-/- mice shows premature aging phenotypes and heart failure.

Timeline

in vitro



in vivo



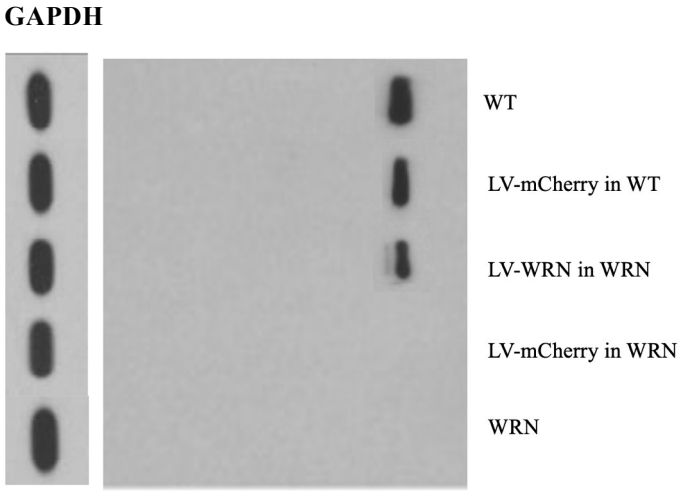
Mice

- 10 WT
  - 5 WT
  - 5 LV-mCherry
- 20 WRN
  - 5 WRN
  - 5 LV-mCherry
  - 10 LV-WRN
    - 5 sacrificed at 2 months
    - 5 sacrificed at 8 months

In vitro

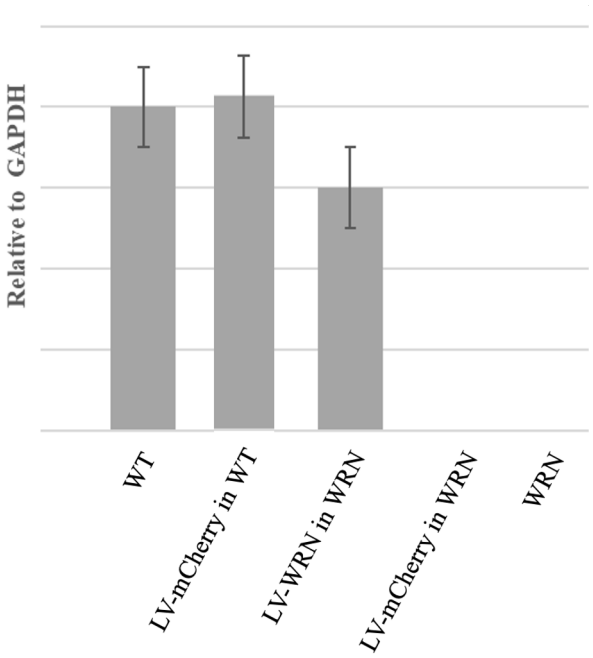
A. Is the transduction successful?

1) WB
2) rt PCR
3) IF



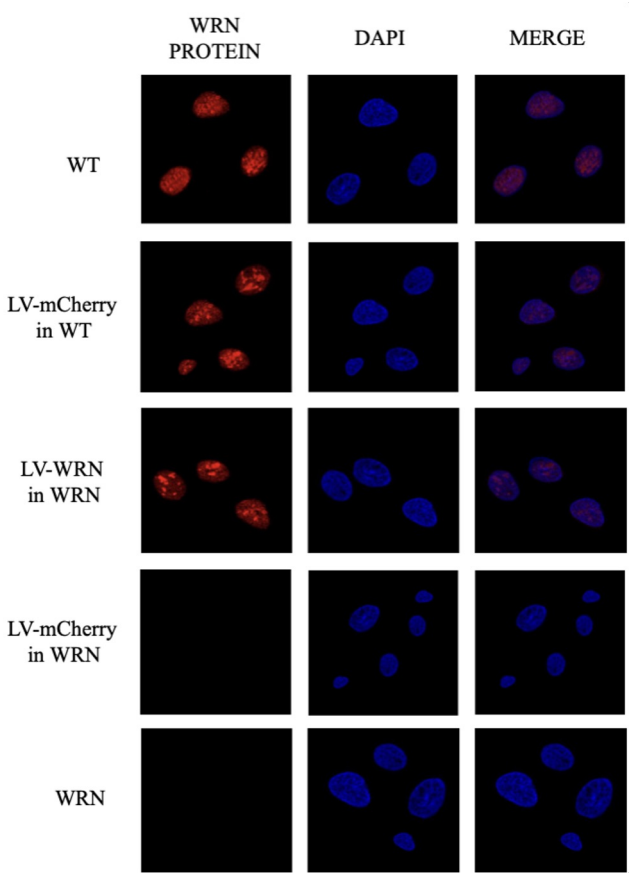
Adapted from Opresko et al, 2002.

A.1)



Adapted from Liu, 2014.

A.2)



Adapted from: Confocal imaging of U-2 OS cells using DHX9/RNA Helicase A Rabbit mAb. Abclonal.

A.3)

In vitro

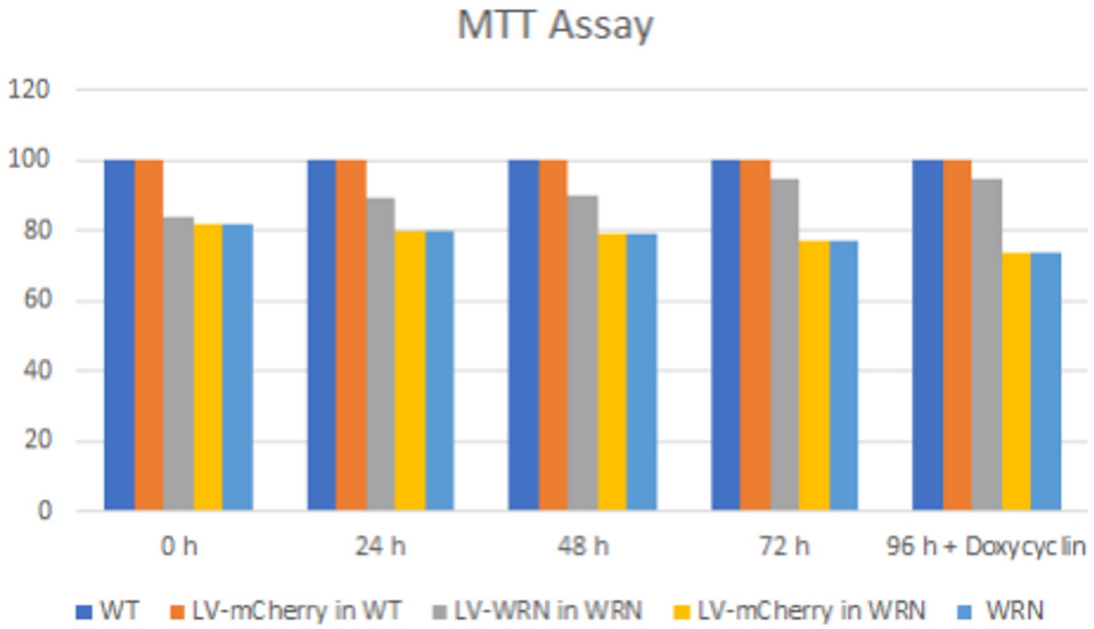
B. Is the treatment cytotoxic?

MTT assay

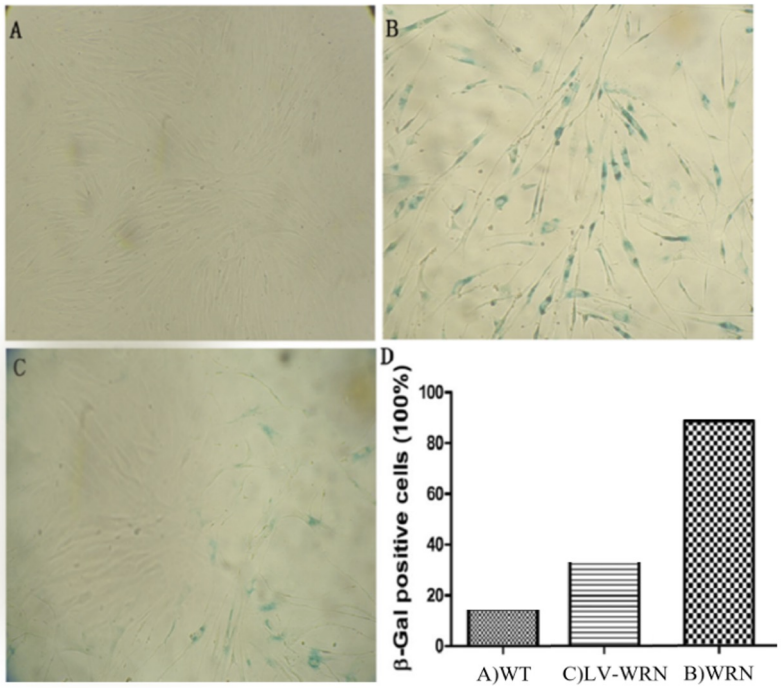
C. Does our vector delay the senescence outcame?

Senescence-associated β-galactosidase (SA-β-gal) Staining

B)



C)



Adapted from Niu et al, 2014

In vitro

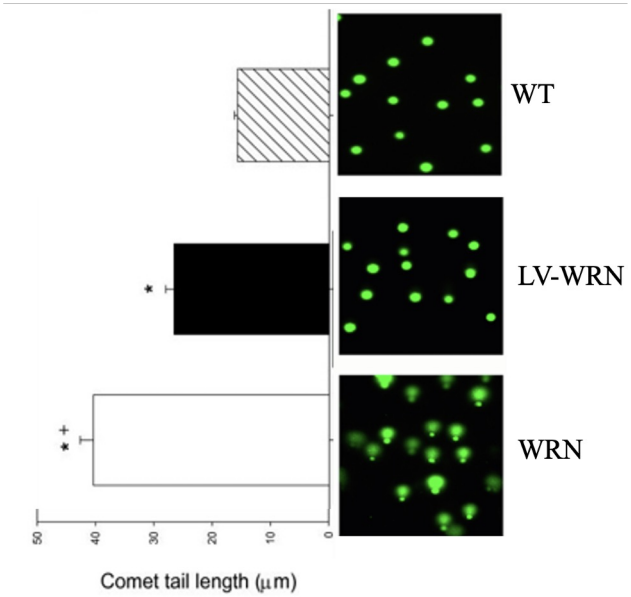
D. Telomere length:

1) TIF
2) TRF
3) qFISH

E. DNA instability:

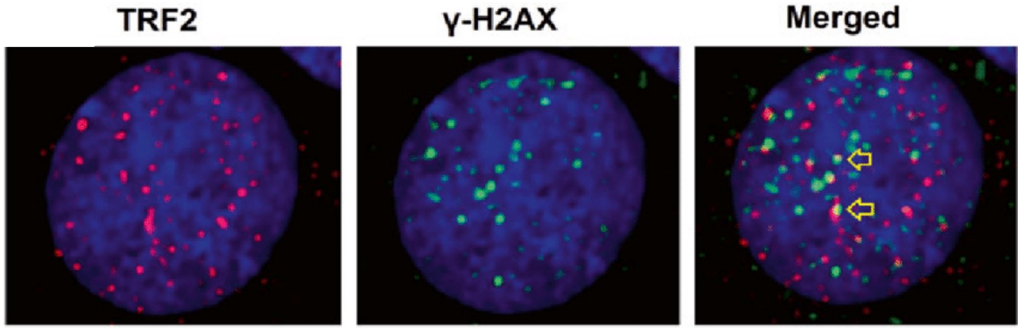
Comet Assay

E)



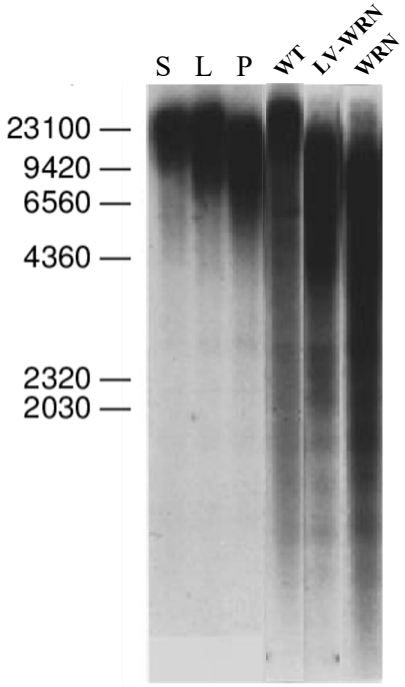
Adapted from Guo et al, 2017.

D.1)



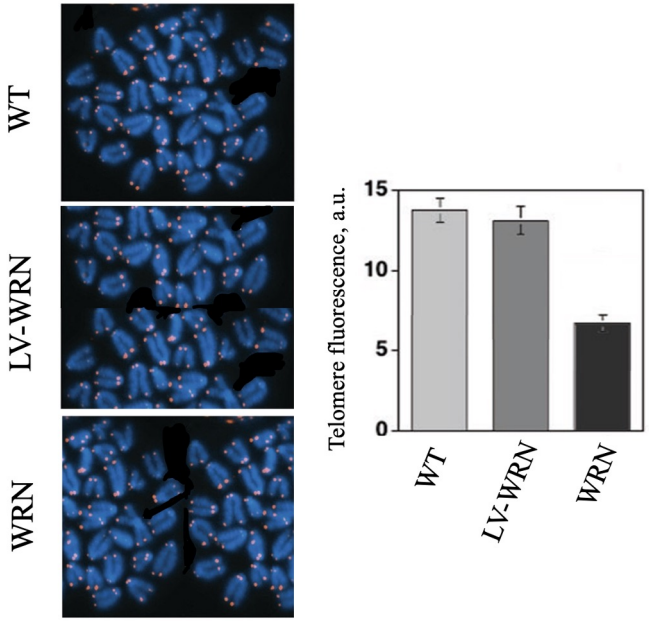
Adapted from Kroustallaki,2015

D.2)



Adapted from Tahara et al, 2017.

D.3)



Adapted from Du et al, 2004.

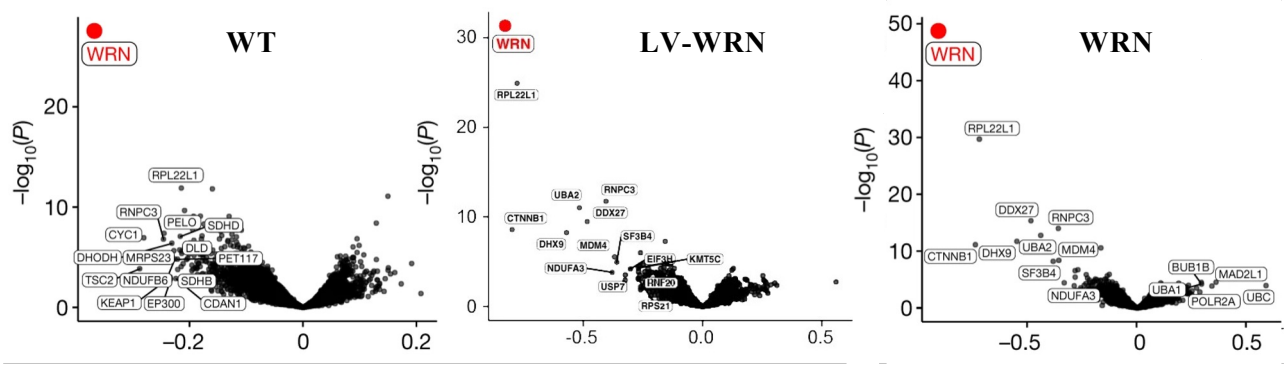
In vitro

F. Monitoring the gene expression:

RNA-seq:

1) Volcano plot
2) Mutation Rate
3) Gene Ontology

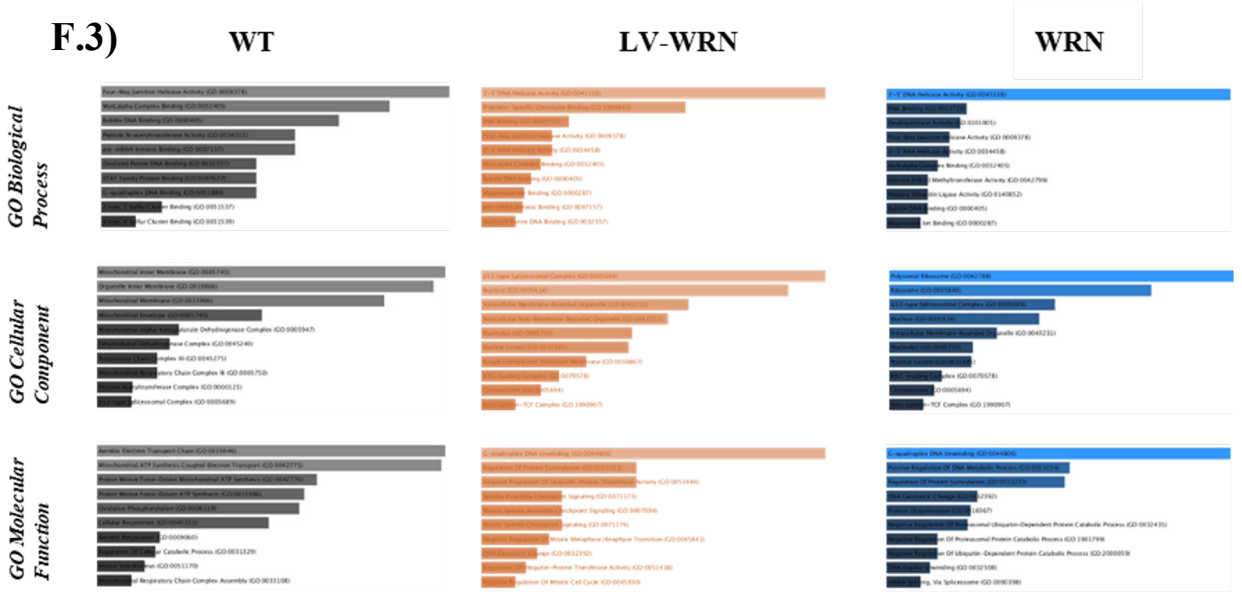
F.1)



Adapted from Chan et al, 2019.

F.2)

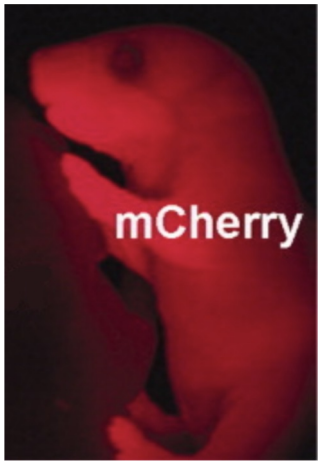
F.3)



By EnrichR.

In vivo

Does the vector affect all the organs?

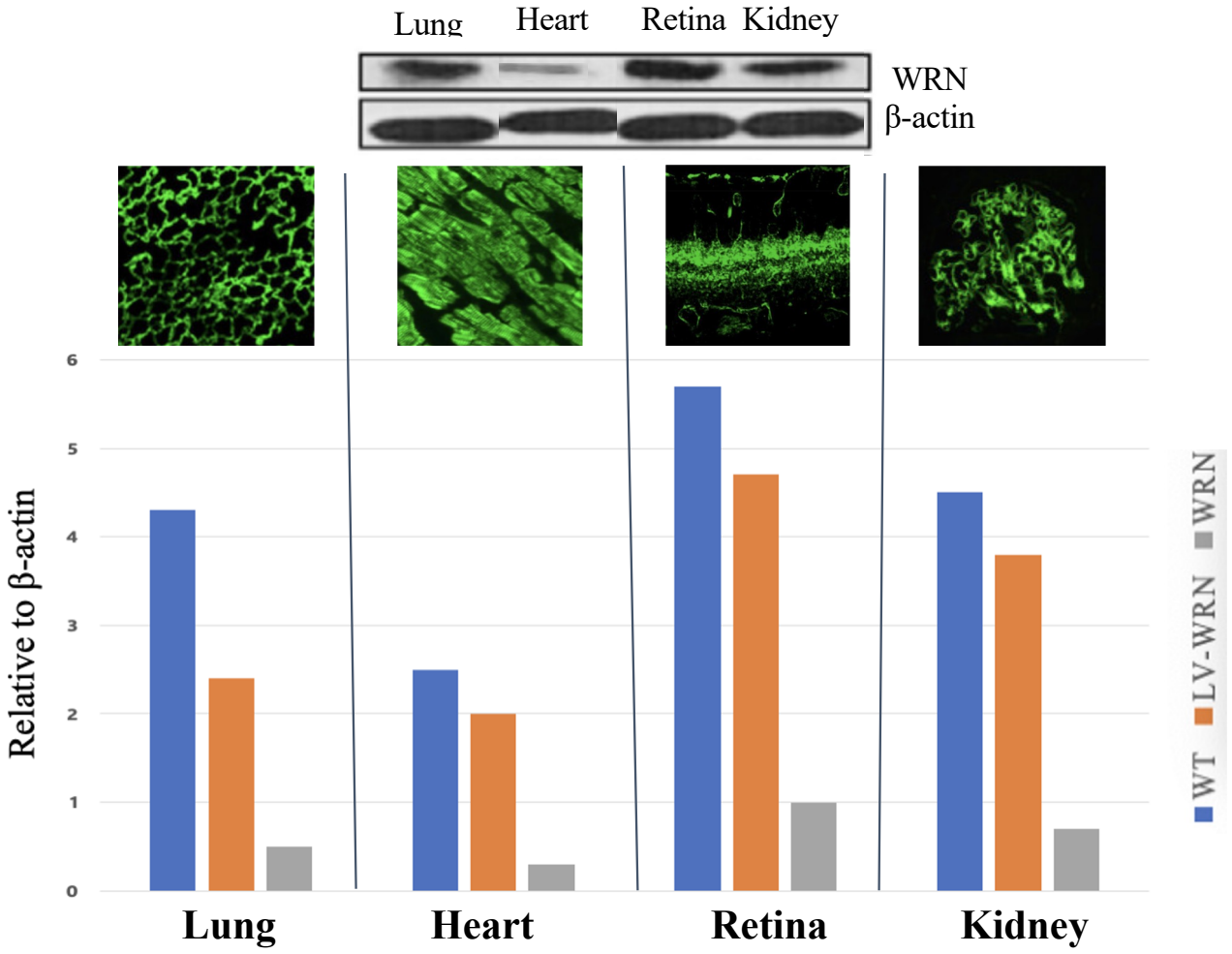


Adapted from Fink et al, 2010.

Why do we choose the heart as a representative tissue?

Comparing WRN expression in LUNG, Heart, Retina and Kidney by WB, IF and rt PCR

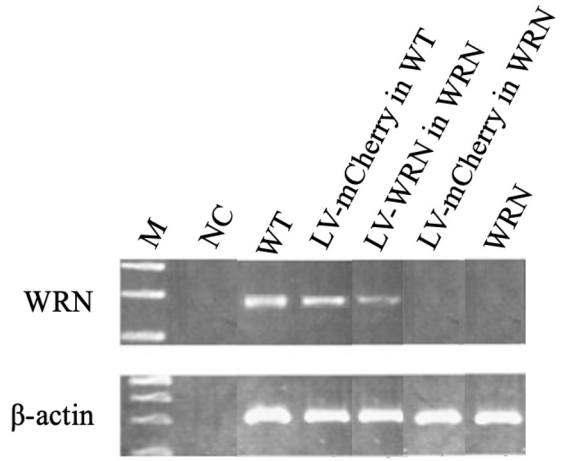
Adapted from Liu et al, 2014.



In vivo
(cardiomyocytes)

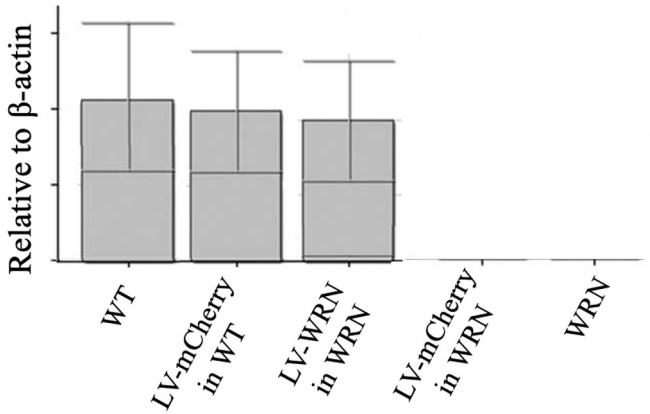
A. Is the transduction successful?

1) WB
2) rt PCR
3) IF



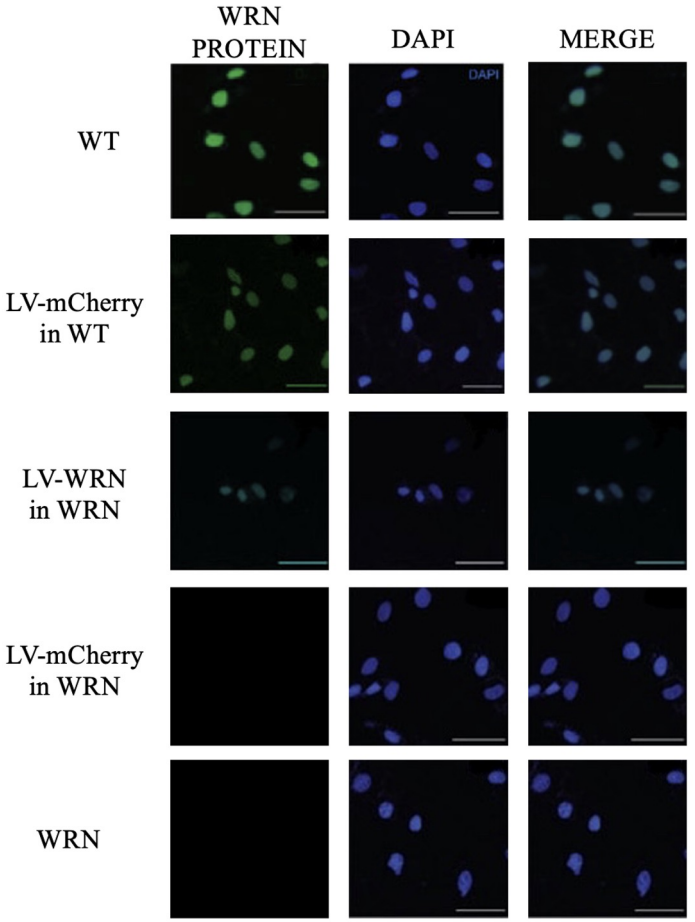
Adapted from Masuda et al, 2012.

A.1)



Adapted from Liu, 2014.

A.2)



Adapted from Ryskalin et al, 2022.

A.3)

In vivo
(cardiomyocytes)

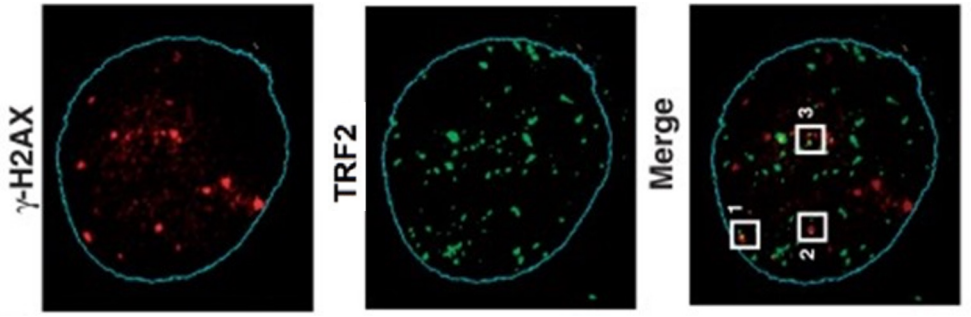
B. Do we see changes in the telomere length?

1) TIF
2) TRF
3) qFISH

C. Does the therapy decrease the DNA damage level?

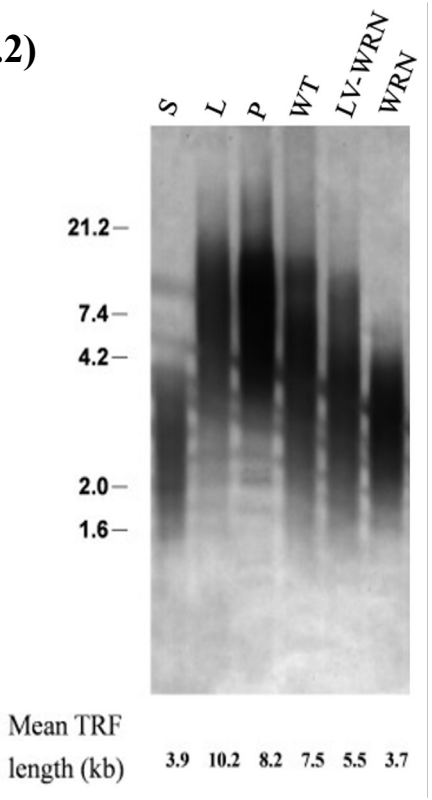
Comet assay

B.1)



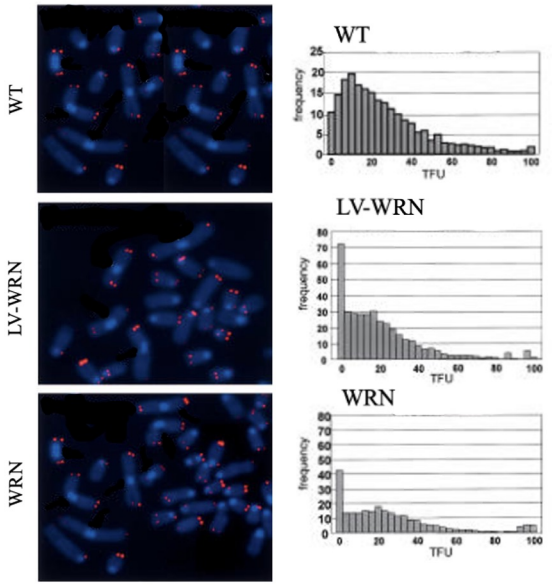
Adapted from Jullien et al, 2012.

B.2)



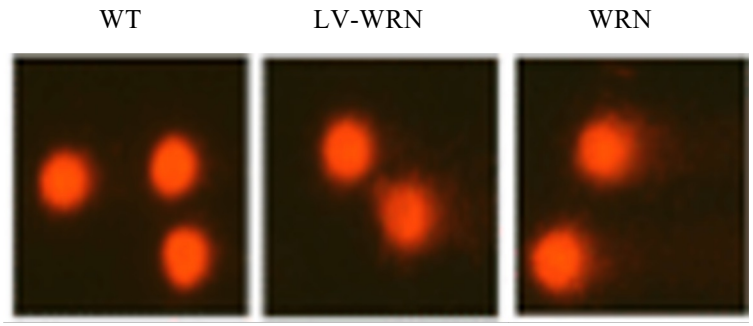
Adapted from Zhu et al, 2018.

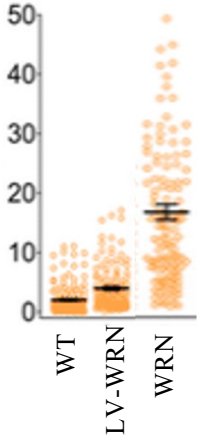
B.3)



Adapted from Laud et al, 20.

C)





Adapted from Marabitti et al, 2020.

In vivo

D. Do we see phenotypic changes?

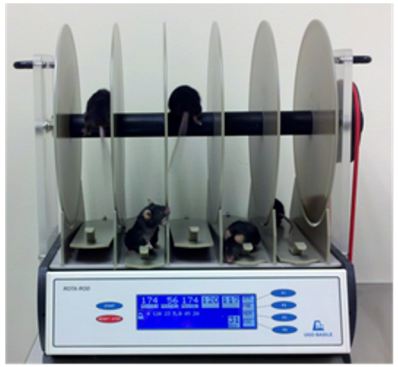
E. Do we see any changes in behaviour?

Rotarod assay

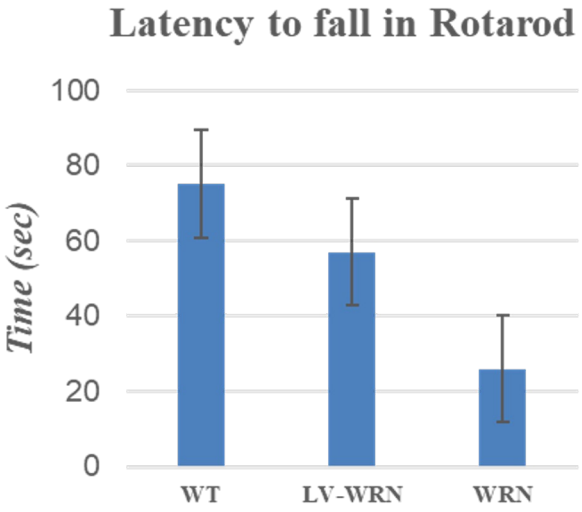
D)

	WT	LV-WRN	WRN
Glucose tolerance	-	-	++ at 4 months
Insulin resistance	-	-	+ at 4 months
Hair regrowth	-	-	+ at 4 months
Subcutaneous adipose	-	-	++ at 4 months
Gonad mass	-	-	+ at 4 months
Voiding spot assay	-	-	+ at 4 months
Urinary Albumin Creatinine Ratio	-	+	+++ from 4 to 8 months
Bodyweight	-	-	++ at 8 months
Lordokyphosis	-	-	+ at 8 months
Osteoporosis	-	-	+ at 8 months
Cataract	-	-	++ at 8 months
Hair graying	-	-	++ at 8 months
Alopecia	-	-	+ at 8 months
Muscle mass	-	-	+ at 8 months
Spleen mass	-	+	+++ at 8 months

Adapted from Chang et al, 2005.



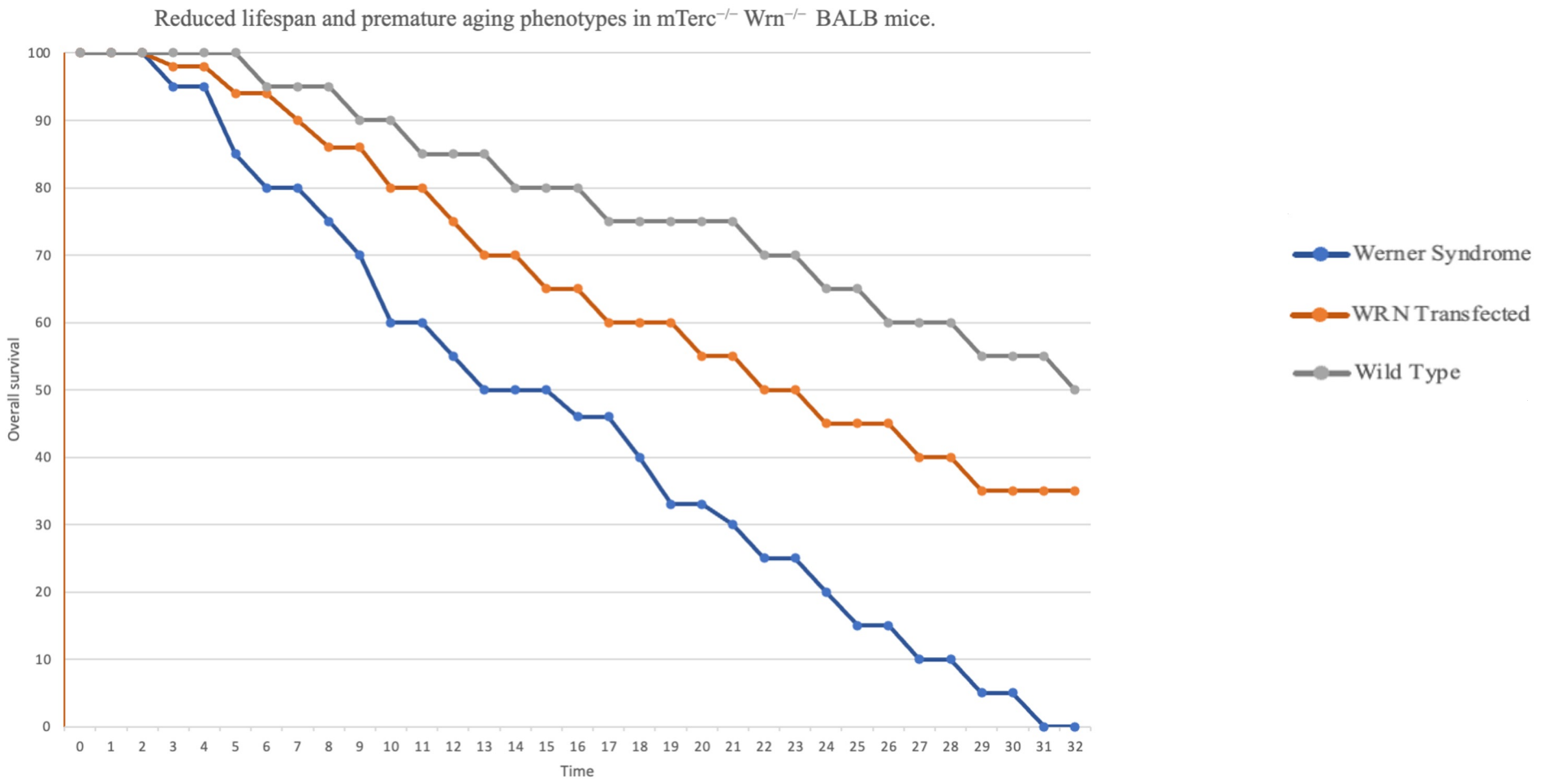
E)



Adapted from Halder et al, 2017.

In vivo

Kaplan Meier curve



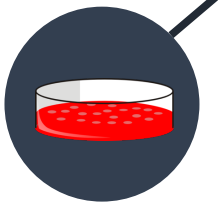
Budget

Materials: 37K

Immunohistochemistry: 1.4K
Pulsed Field Electrophoresis: 25K
TRF: 1K
Fluorescence in situ hybridization: 3K
RT PCR: 1.2K
Western Blot: 1.1K
FISH: 3K
qPCR: 1.3K

Workers: 75K

Postdoc (x1): 30K each
PhD (x3): 15K each



Total:
~200K / year
for 2 years

Cell lines: 1K

HPFs: 1K
MFs: extracted from mice

Mouse models: 80K

Pitfalls and Solutions

Werner patients develop multiple, rare cancers which is the most common cause of death, and has no treatment at the moment.

Additional diagnosis tests for cancer such as Cytogenetic analysis.

Translatability between human patients and animal models.

Testing our therapy on other model animals, trying to use higher level species, such as rabbits, dogs and primates.

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Thank you for your attention.