INTERVENTI: Aumento Attività telomerasica

Telomerase gene therapy rescues telomere length, bone marrow aplasia, and survival in mice with aplastic anemia

Christian Bär et al

Blood 2016 127:1770-1779;

- Aplastic anemia is a fatal bone marrow disorder characterized by peripheral pancytopenia and marrow hypoplasia.
- A subgroup of the inherited form is caused by

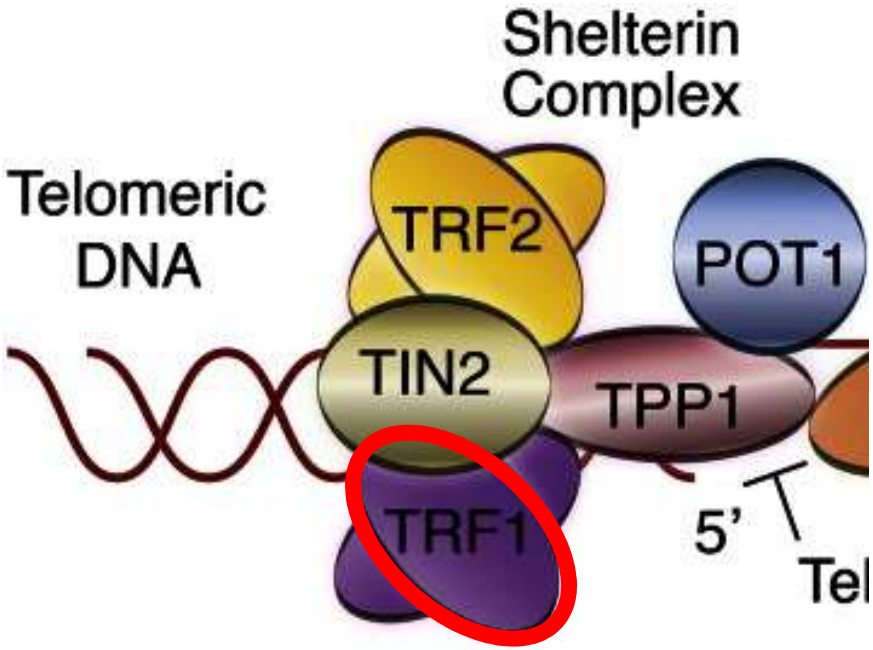
replicative impairment of hematopoietic stem and progenitor cells due to very short telomeres

as a result of mutations in telomerase and other telomere components

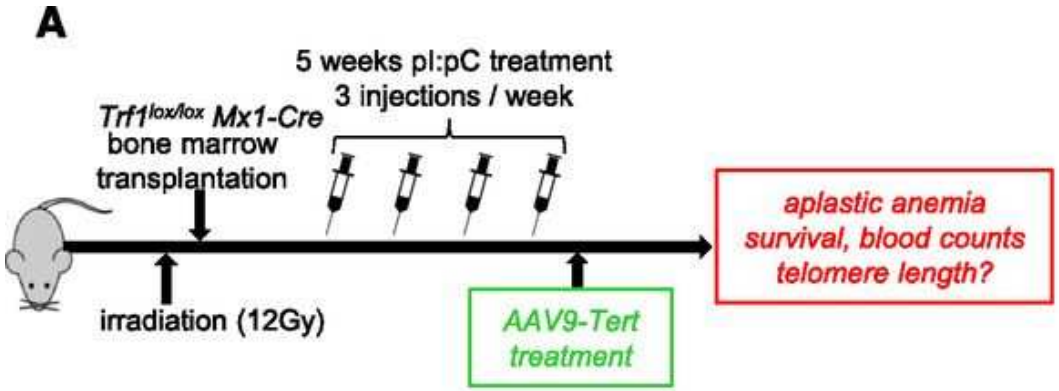
Telomerase gene therapy..

- We test the therapeutic efficacy of telomerase activation by using adeno-associated virus (AAV)9 gene therapy vectors carrying the telomerase *Tert* gene in

mouse models of aplastic anemia due to short telomeres *Trf1-* deficient mice


Shelterin
Complex
Telomeric
DNA
TRF2
POT1
TIN2
TPP1
TRF1
5’
Tel

AAV9-Tert treatment rescues the aplastic anemia phenotype in Trf1lox/lox mice.



- Mice lethally irradiated were transplanted the following day with *Trf1*$^{lox/lox}$*Mx1-Cre* bone marrow.
- *Cre* expression and *Trf1* excision was induced for 5 weeks.
- One week later, mice were injected with AAV9-*Tert* or AAV9-empty particles.

Christian Bär et al. Blood 2016;127:1770-1779

blood

Mice were lethally irradiated and transplanted the following day with *Trf1* *Mx1-Cre* bone marrow. After engraftment, *Cre* expression and *Trf1* ext

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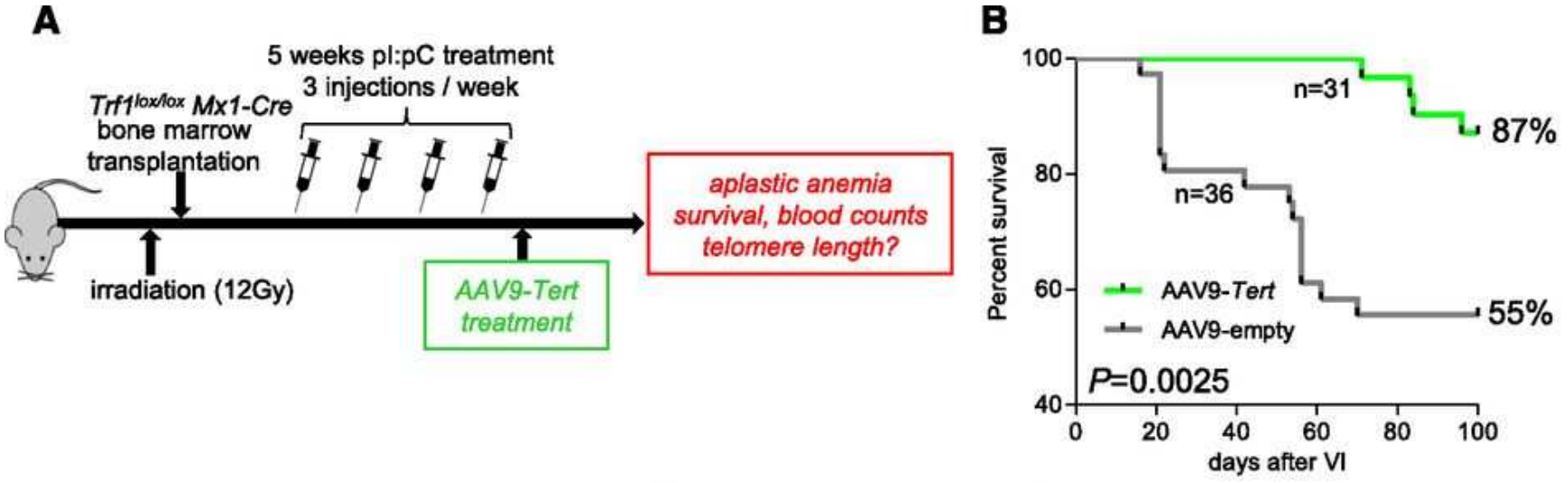
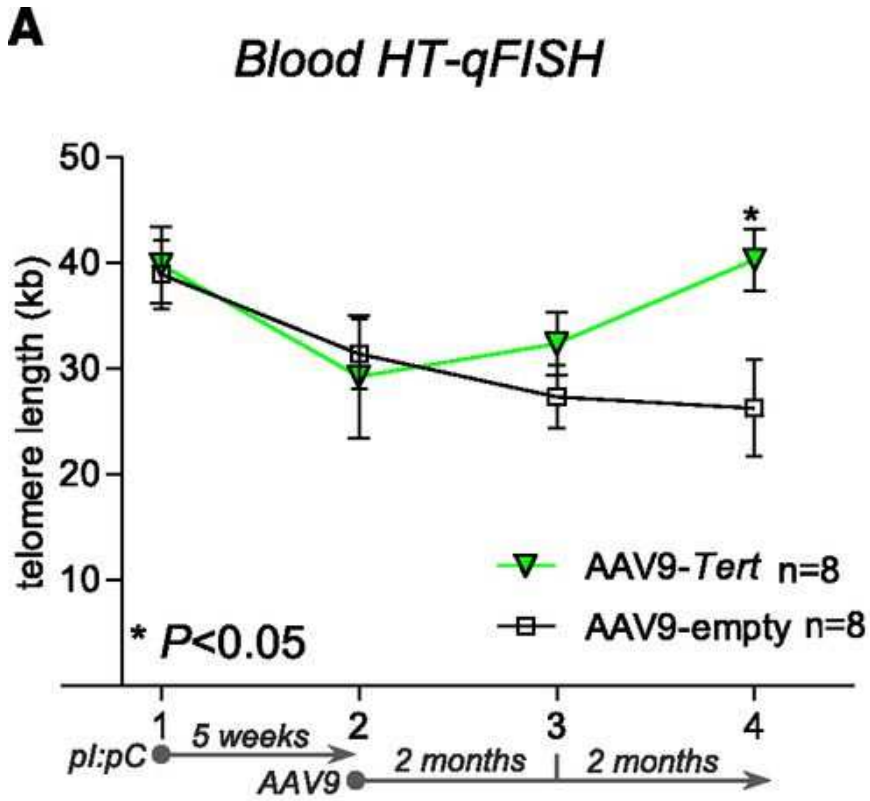


Christian Bär et al. Blood 2016;127:1770-1779

blood™

AAV9-Tert treatment causes telomere elongation in blood and bone marrow.



analysis of telomere length in peripheral blood monocytes

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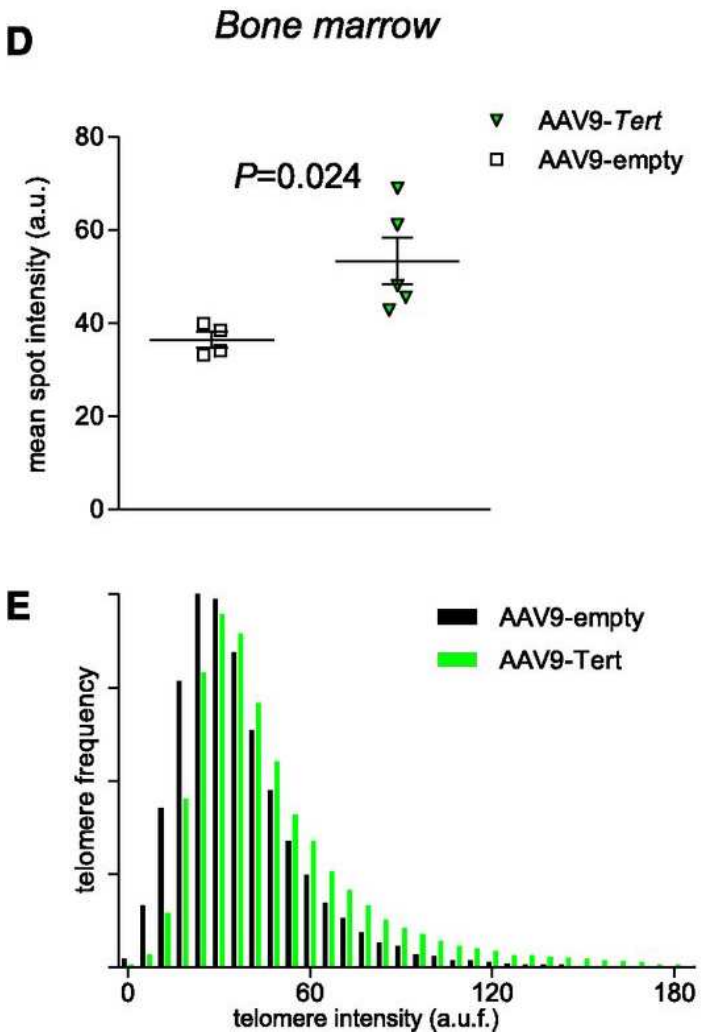
AAV9-Tert treatment causes telomere elongation in blood and bone marrow.

Q-FISH analysis

Relative telomere length in bone marrow

Frequency distribution blot of telomere length



Christian Bär et al. Blood 2016;127:1770-1779

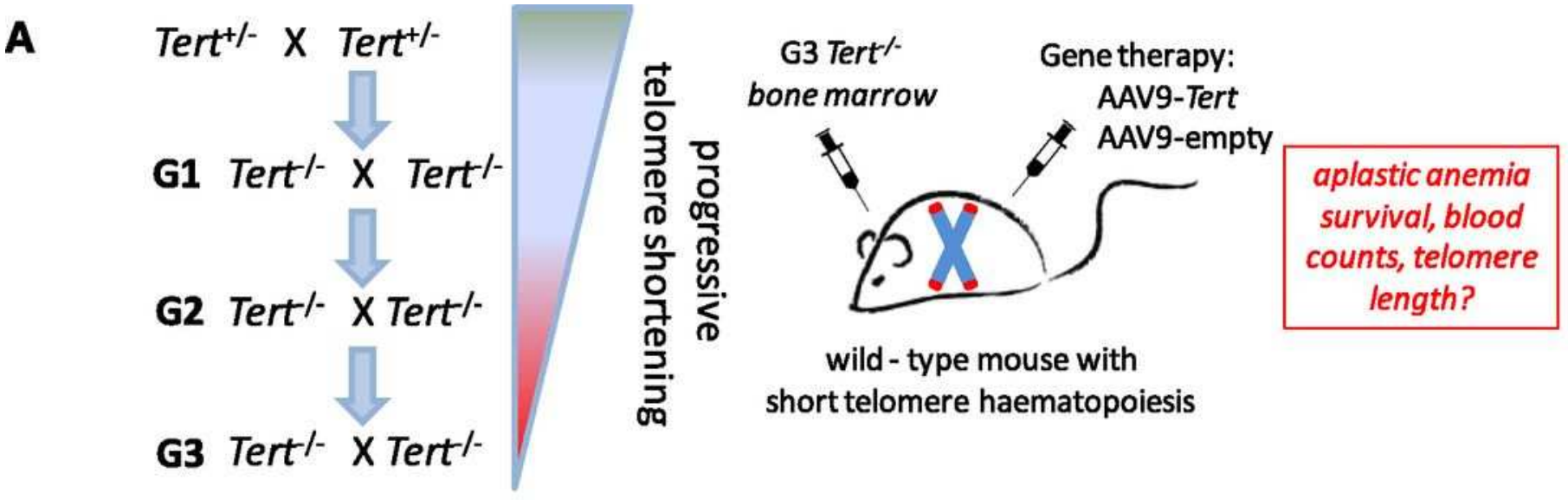
- Aplastic anemia is a fatal bone marrow disorder characterized by peripheral pancytopenia and marrow hypoplasia.
-

- We test the therapeutic efficacy of telomerase activation by using adeno-associated virus (AAV)9 gene therapy vectors carrying the telomerase *Tert* gene in

2nd mouse model of aplastic anemia due to short telomeres

Tert−deficient mice

AAV9-Tert treatment improves blood counts in mice with short telomeres resulting from specific Tert deletion in the bone marrow.



G3 Tert–/– mice with short telomeres were generated by consecutive crosses of Tert-deficient mice

Christian Bär et al. Blood 2016;127:1770-1779

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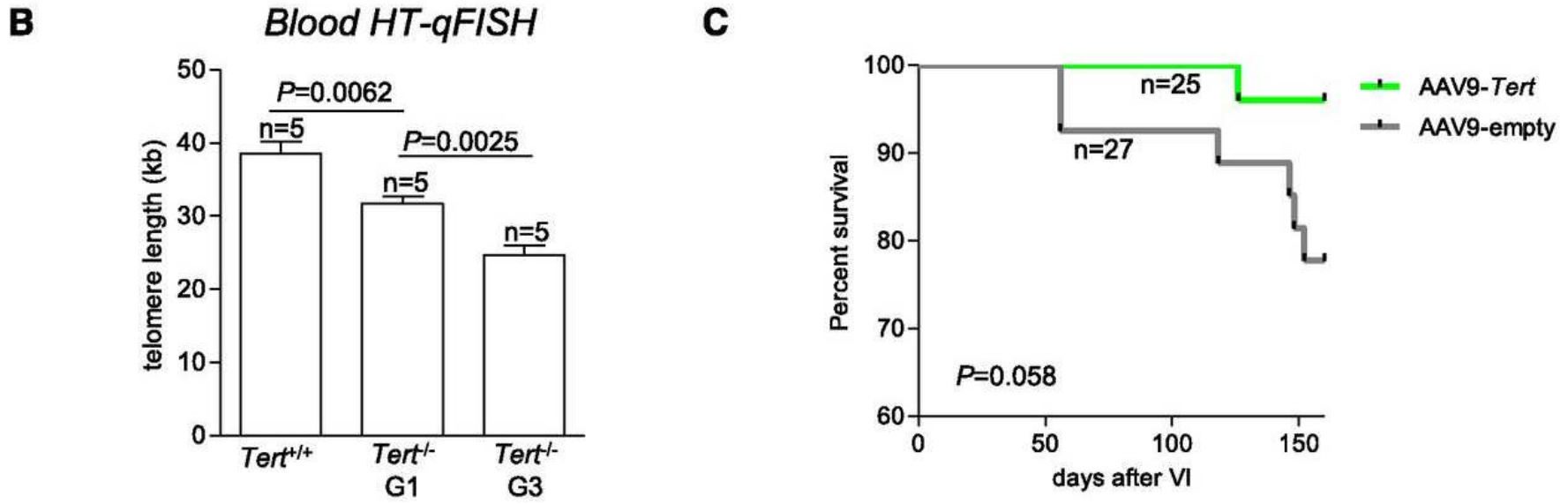

Christian Bär et al. Blood 2016;127:1770-1779

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- A subgroup of the inherited form is caused by replicative impairment of hematopoietic stem and progenitor cells due to very short telomeres as a result of mutations in telomerase and other telomere components.
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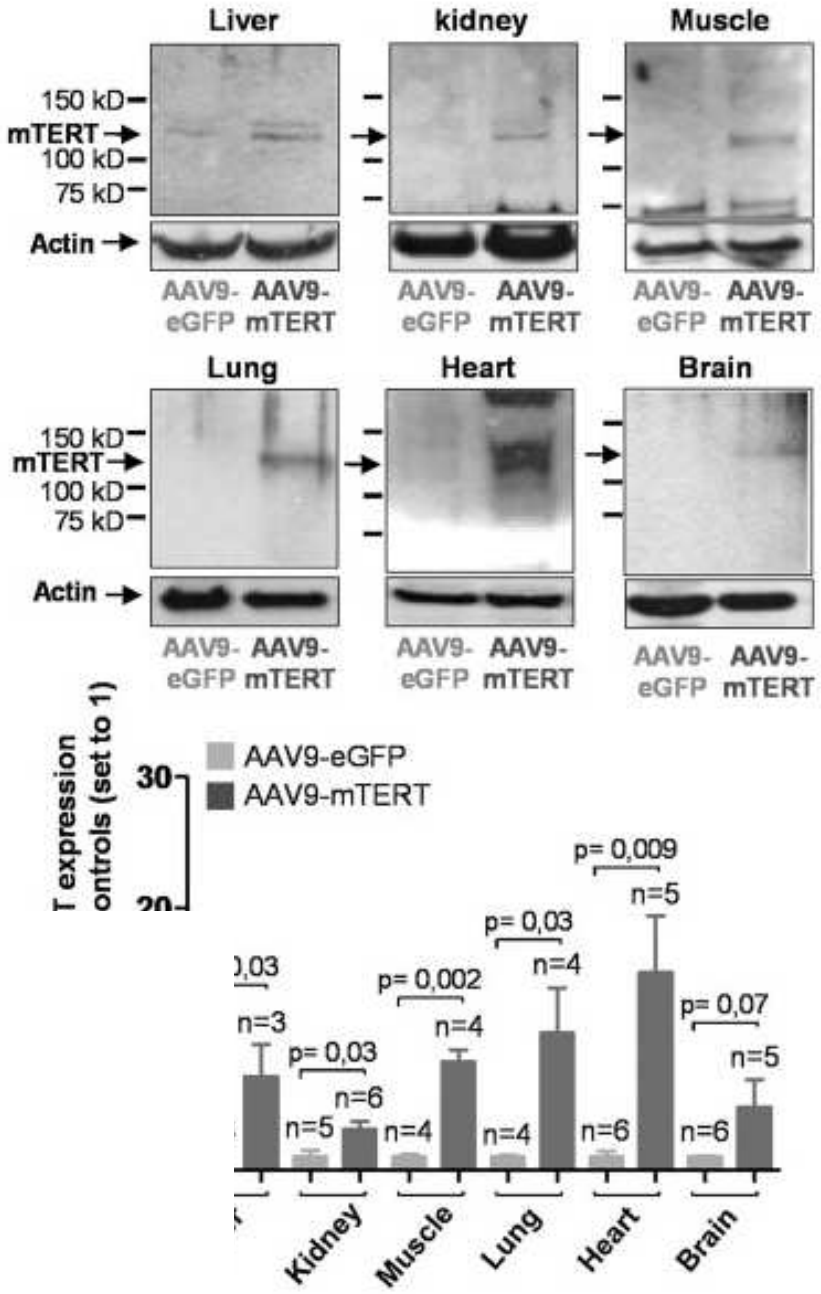
INTERVENTI: Aumento Attività telomerasica

- EMBO Mol Med. 2012

- Telomerase **gene therapy in adult and old mice delays** aging **and increases longevity without increasing cancer.**
- Bernardes de Jesus B, Vera E, Schneeberger K, Tejera AM, Ayuso E, Bosch F, Blasco MA.

- A major goal in aging research is to improve health during aging. In the case of mice, genetic manipulations that shorten or lengthen telomeres result, respectively, in decreased or increased longevity.
- Based on this, we have tested the effects of a telomerase gene therapy in adult (1 year of age) and old (2 years of age) mice.

AAV9-mTERT treated mice
compared to AAV9-eGFP controls



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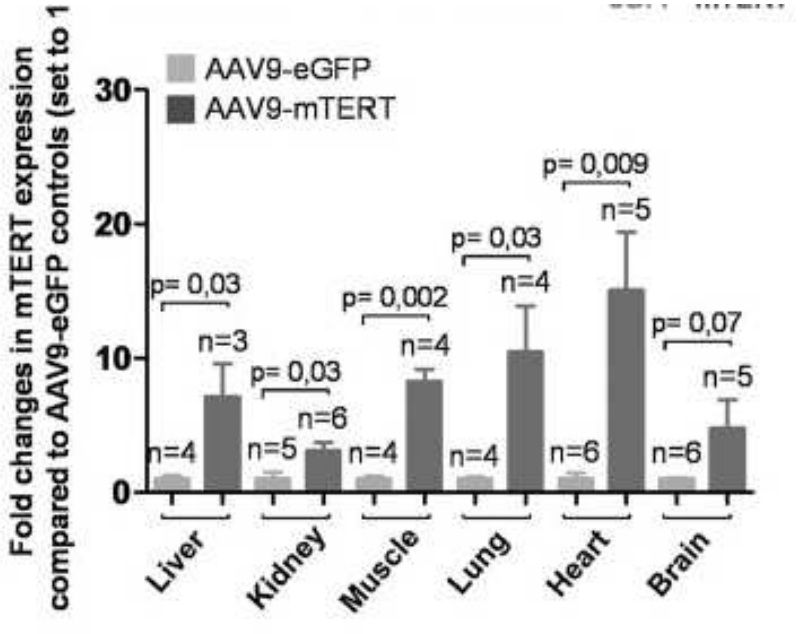
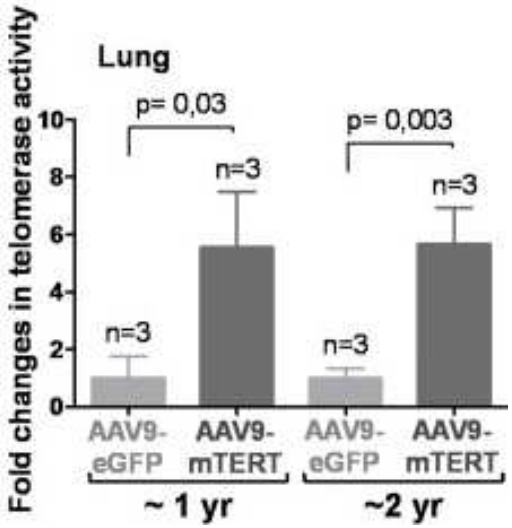


Telomerase activity (measured through
TRAP assay) in several tissues



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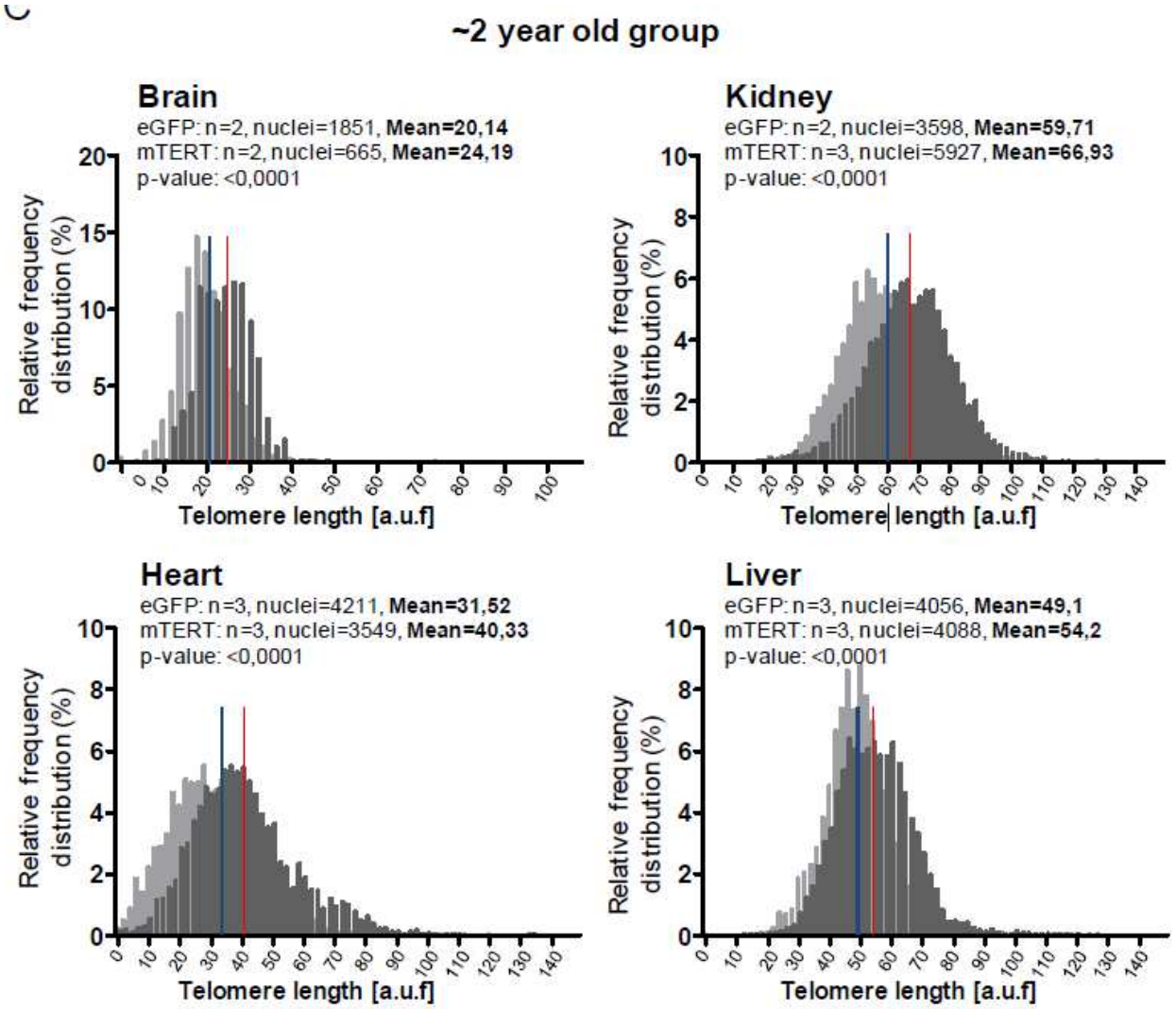
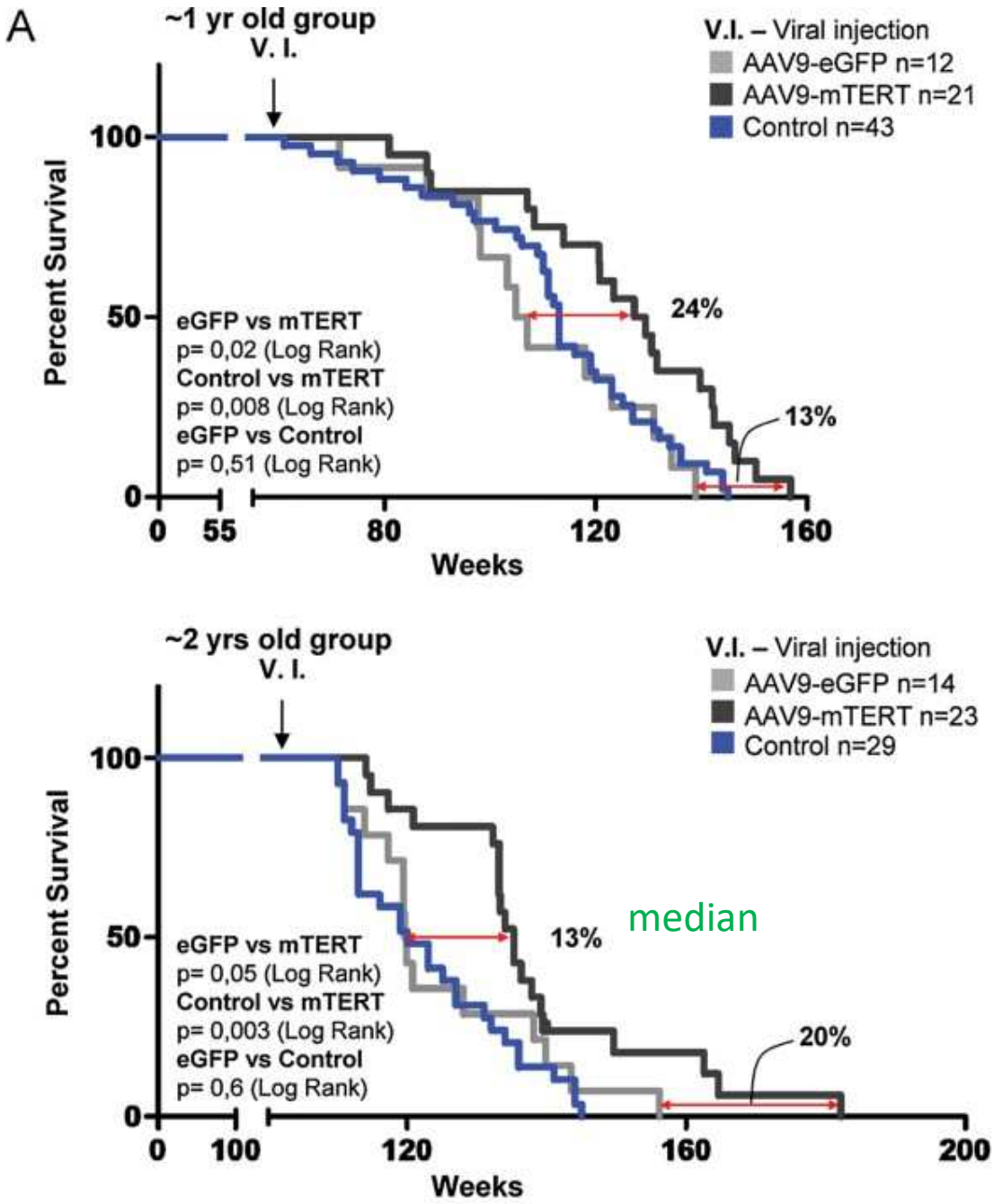
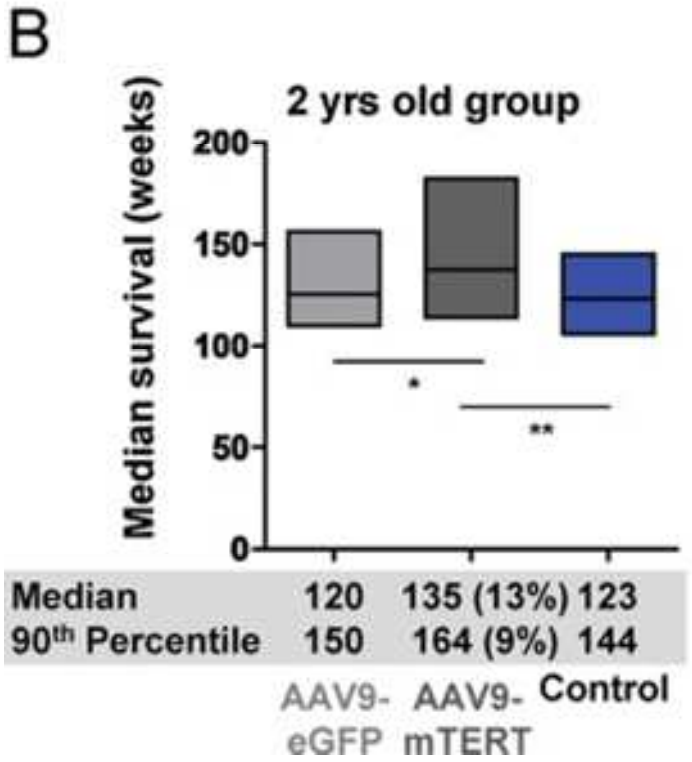

~2 year old group
Brain
eGFP: n=2, nuclei=1851, Mean=20,14
mTERT: n=2, nuclei=665, Mean=24,19
p-value: <0,0001
Relative frequency distribution (%)
Telomere length [a.u.f]
Kidney
eGFP: n=2, nuclei=3598, Mean=59,71
mTERT: n=3, nuclei=5927, Mean=66,93
p-value: <0,0001
Relative frequency distribution (%)
Telomere length [a.u.f]
Heart
eGFP: n=3, nuclei=4211, Mean=31,52
mTERT: n=3, nuclei=3549, Mean=40,33
p-value: <0,0001
Relative frequency distribution (%)
Telomere length [a.u.f]
Liver
eGFP: n=3, nuclei=4056, Mean=49,1
mTERT: n=3, nuclei=4088, Mean=54,2
p-value: <0,0001
Relative frequency distribution (%)
Telomere length [a.u.f]



Increased longevity of AAV9-mTERT treated mice

median

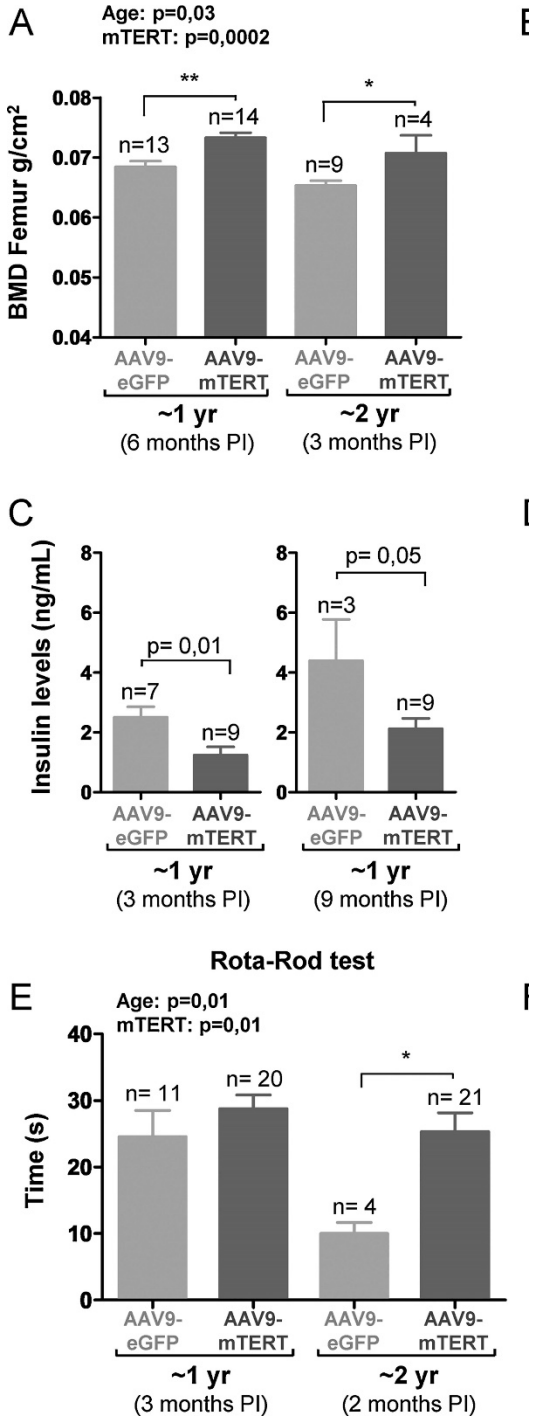
maximum



longevity of AAV9-mTERT treated mice

Delayed aging in AAV9-mTERT treated mice.

Femur bone mineral density



Bruno Bernardes de Jesus et al. EMBO Mol Med. 2012;4:691-704

EMBO Molecular Medicine

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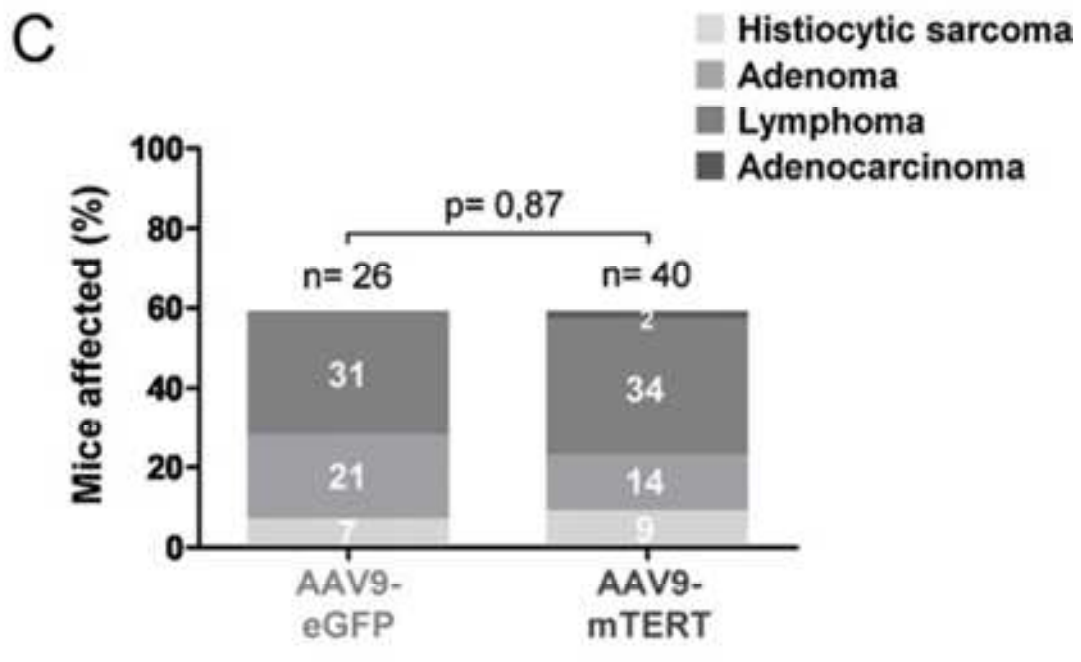
Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer.

- Treatment of 1- and 2-year old mice with an adeno associated virus (AAV) expressing mouse TERT had remarkable beneficial effects on health and fitness, including insulin sensitivity, osteoporosis, neuromuscular coordination and several molecular biomarkers of aging.

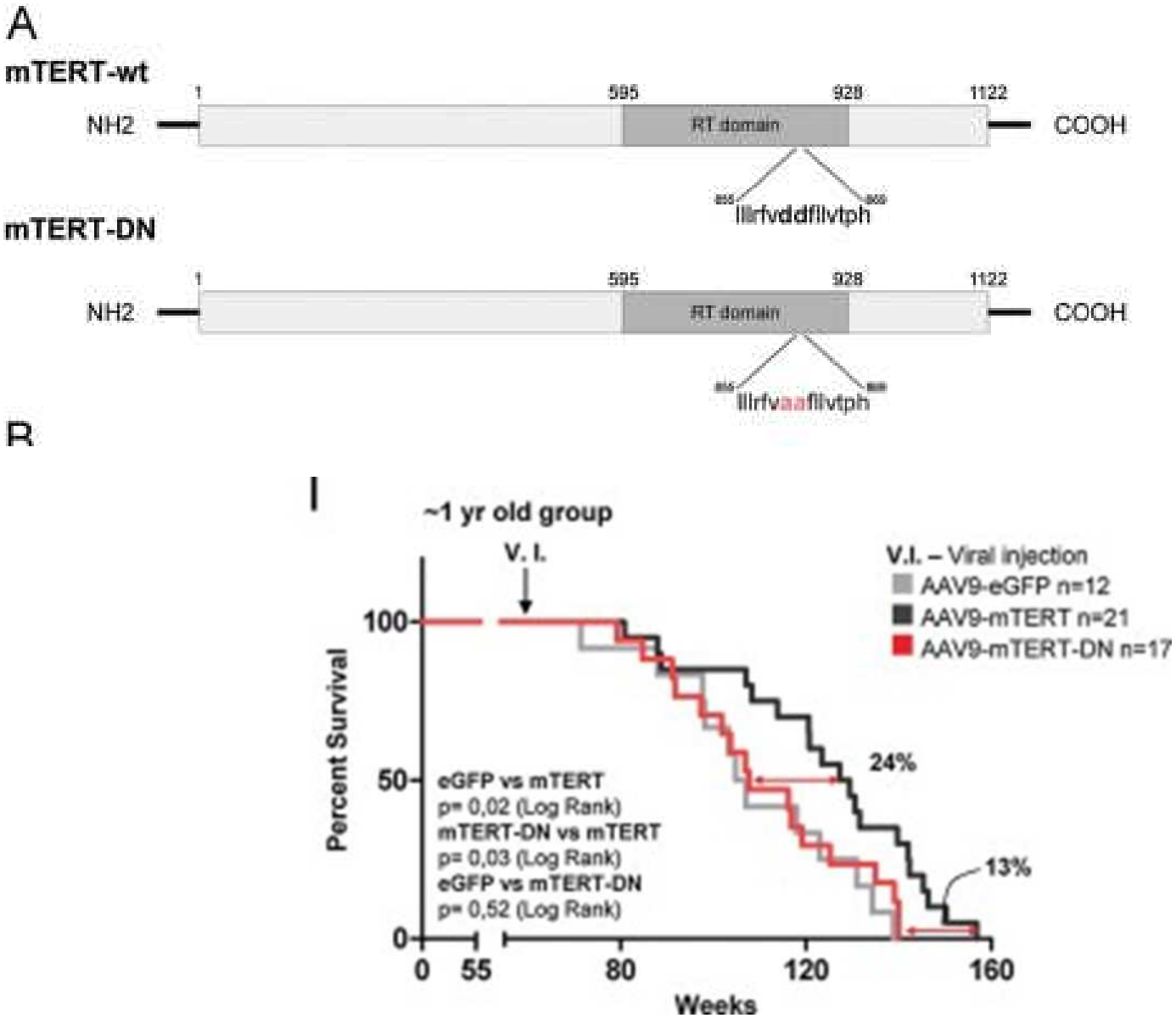
- telomerase-treated mice, both at 1-year and at 2-year of age, had an increase in median lifespan of 24 and 13%, respectively

EMBO Mol Med. 2012;4(8):691-704



Percentage of mice with the indicated tumours at their time of death

telomerase-treated mice did not develop more cancer than their control littermates, suggesting that the known tumorigenic activity of telomerase is severely decreased when expressed in adult or old organisms using AAV vectors.



beneficial effects were not observed with a catalytically inactive TERT, demonstrating that they require telomerase activity.

- Together, these results constitute a proof-of-principle of a role of TERT in delaying physiological aging and extending longevity in normal mice through a telomerase-based treatment, and demonstrate the feasibility of anti-aging gene therapy.

EMBO Mol Med. 2012;4(8):691-704

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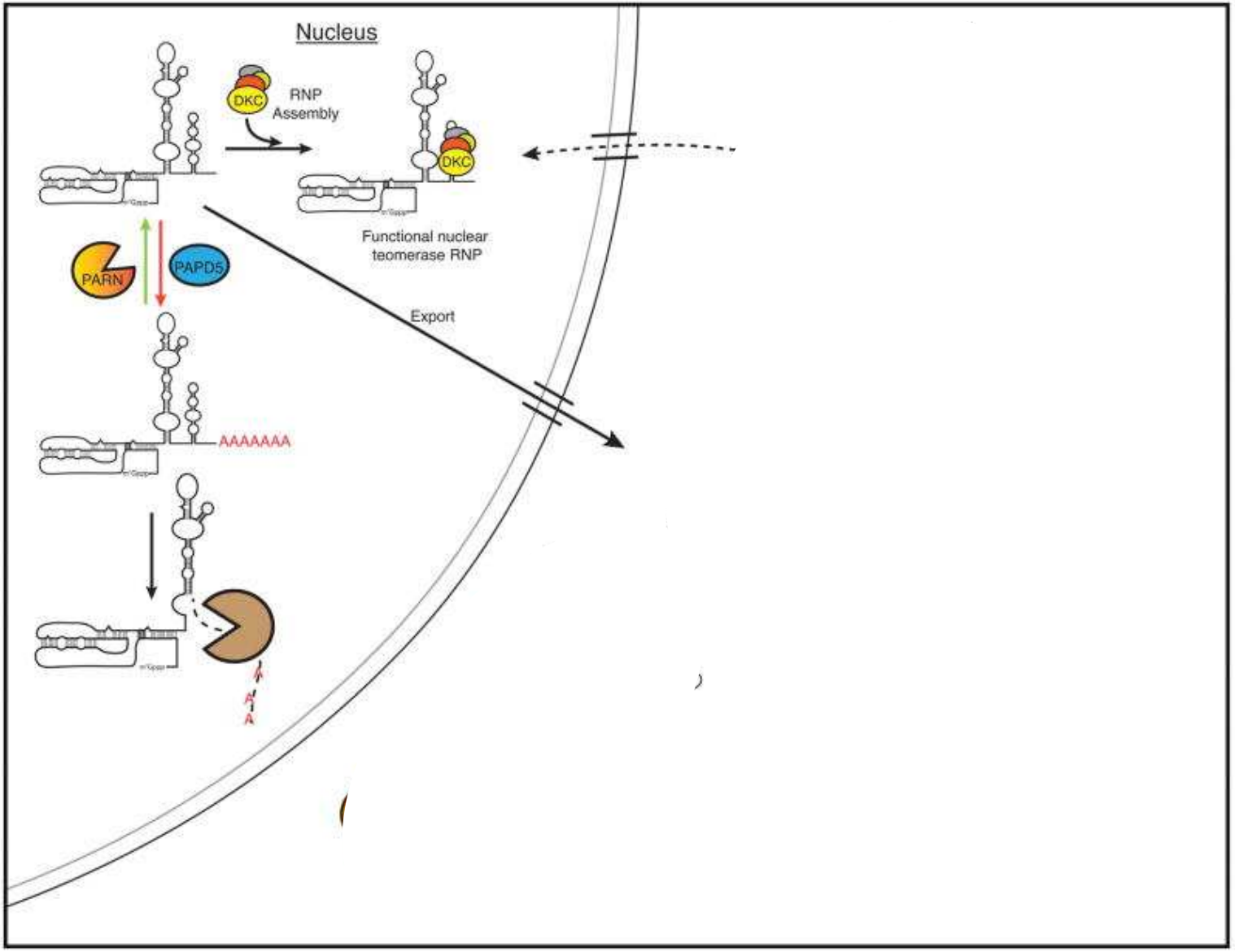
MODELLI DI INTERVENTO BASATI SU RNA STAMPO DELLA TELOMERASI (hTR)

hTR biogenesis



hTR biogenesis

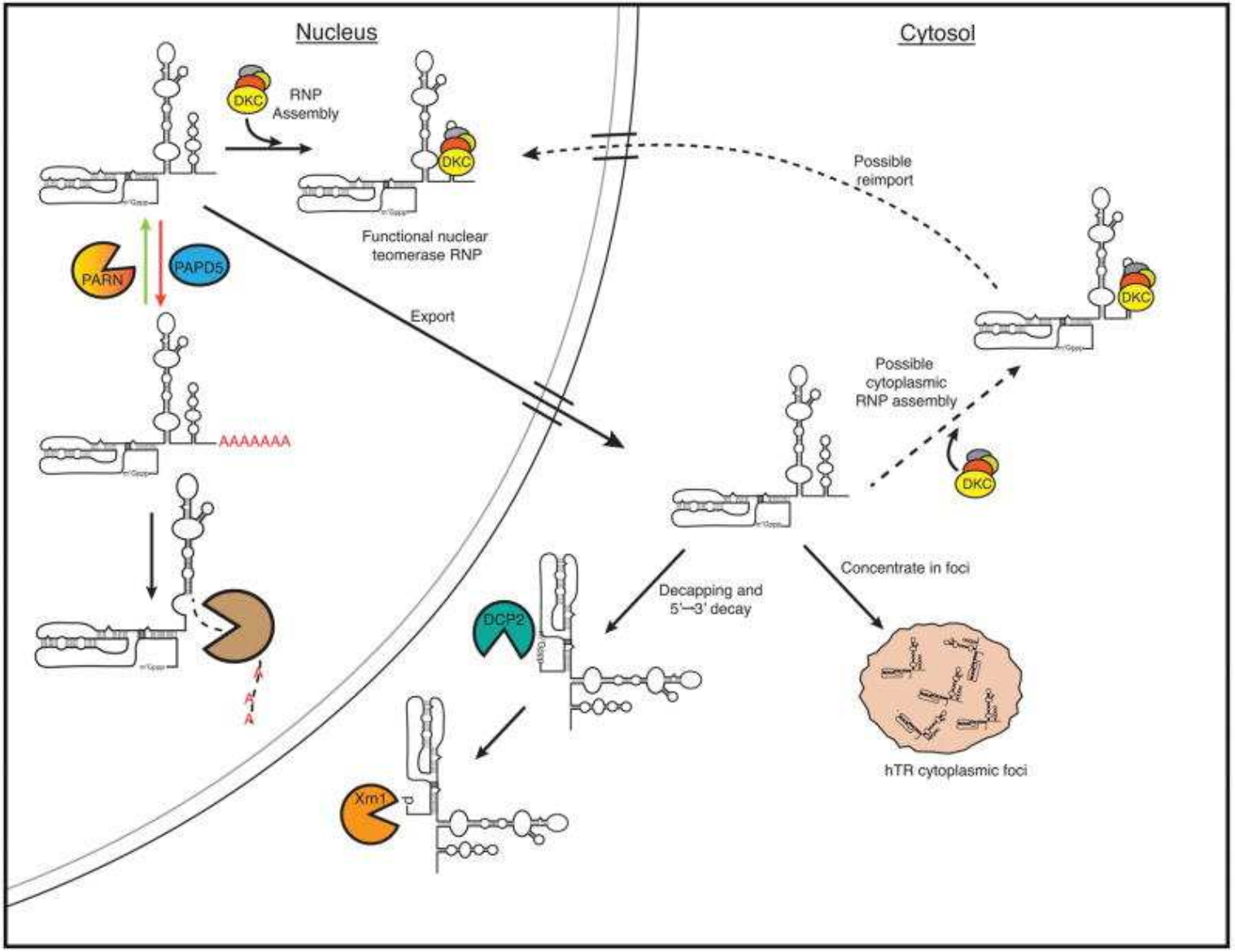
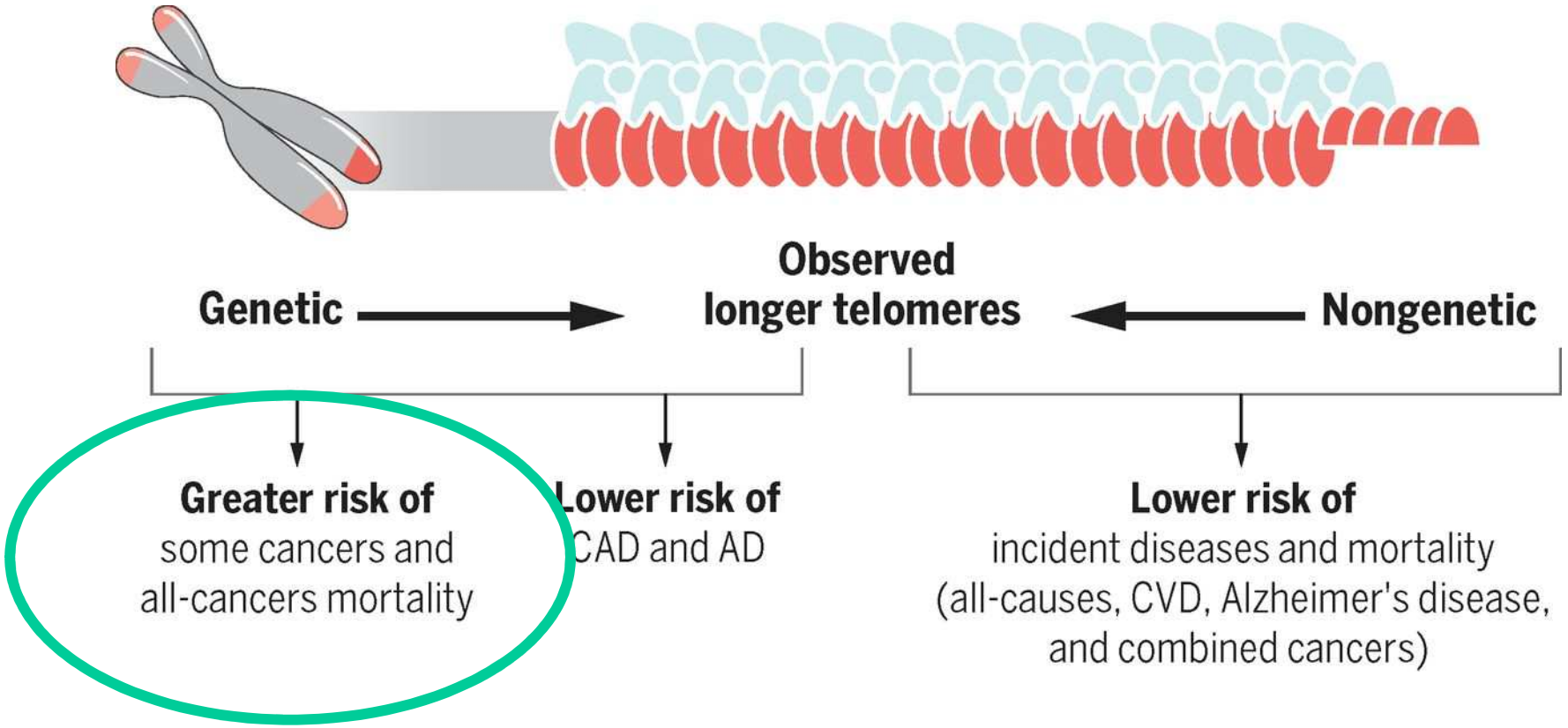
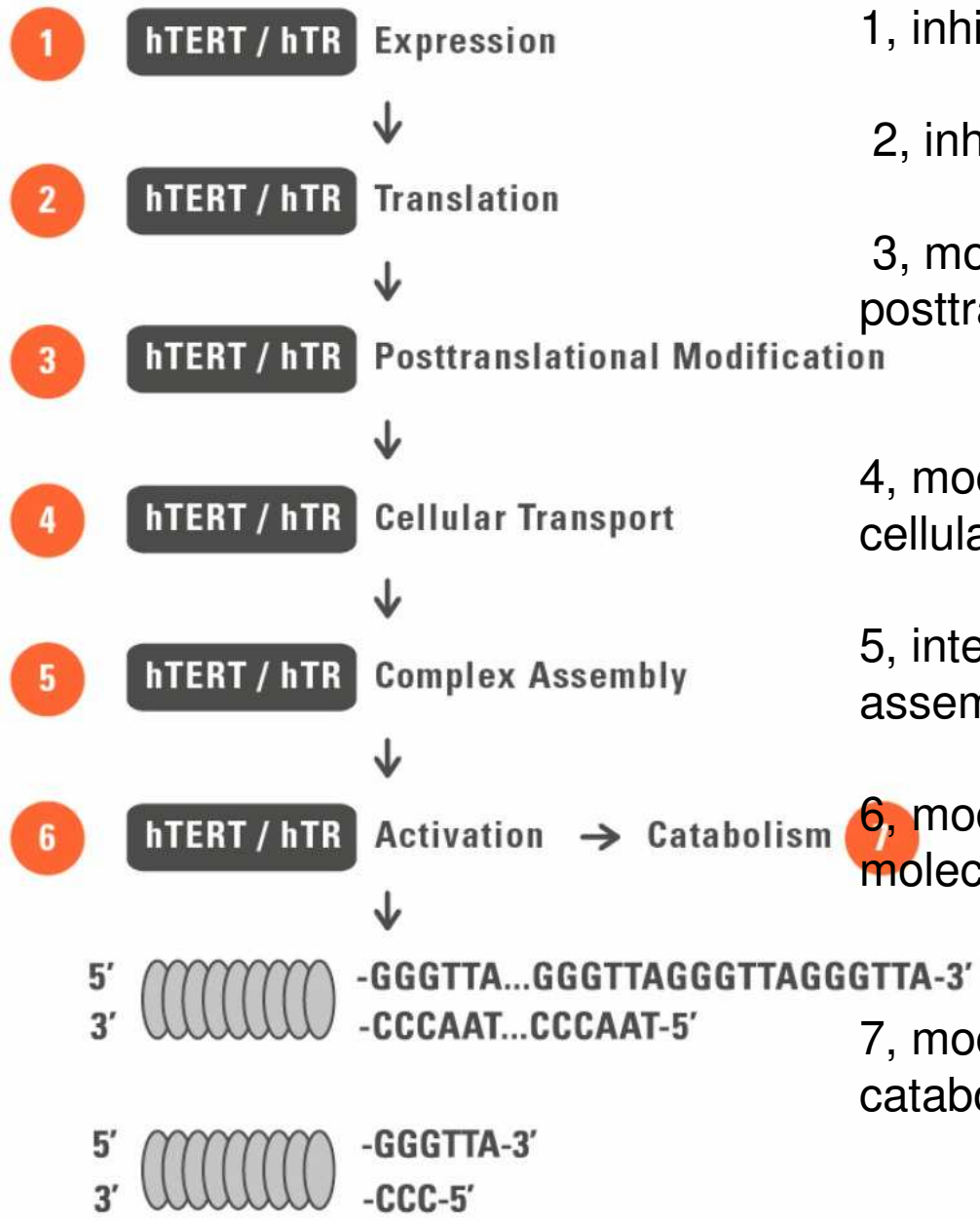


Fig. 3 Different inputs to telomere maintenance have disease-specific consequences.



Elizabeth H. Blackburn et al. Science 2015;350:1193-1198



1, inhibition/activation of gene transcription

2, inhibition/activation of protein synthesis;

3, modulation of activity by posttranslational modifications;

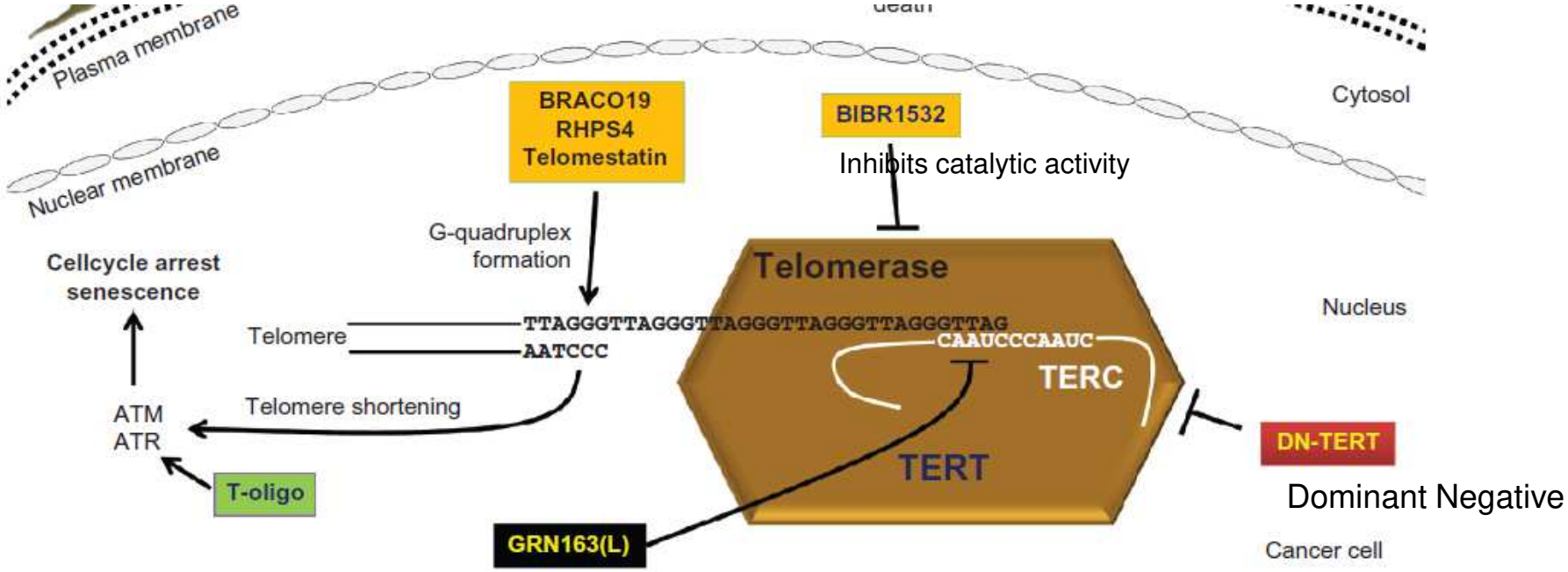
4, modulation of telomerase activity by cellular sequestration;

5, interference with telomerase complex assembly;

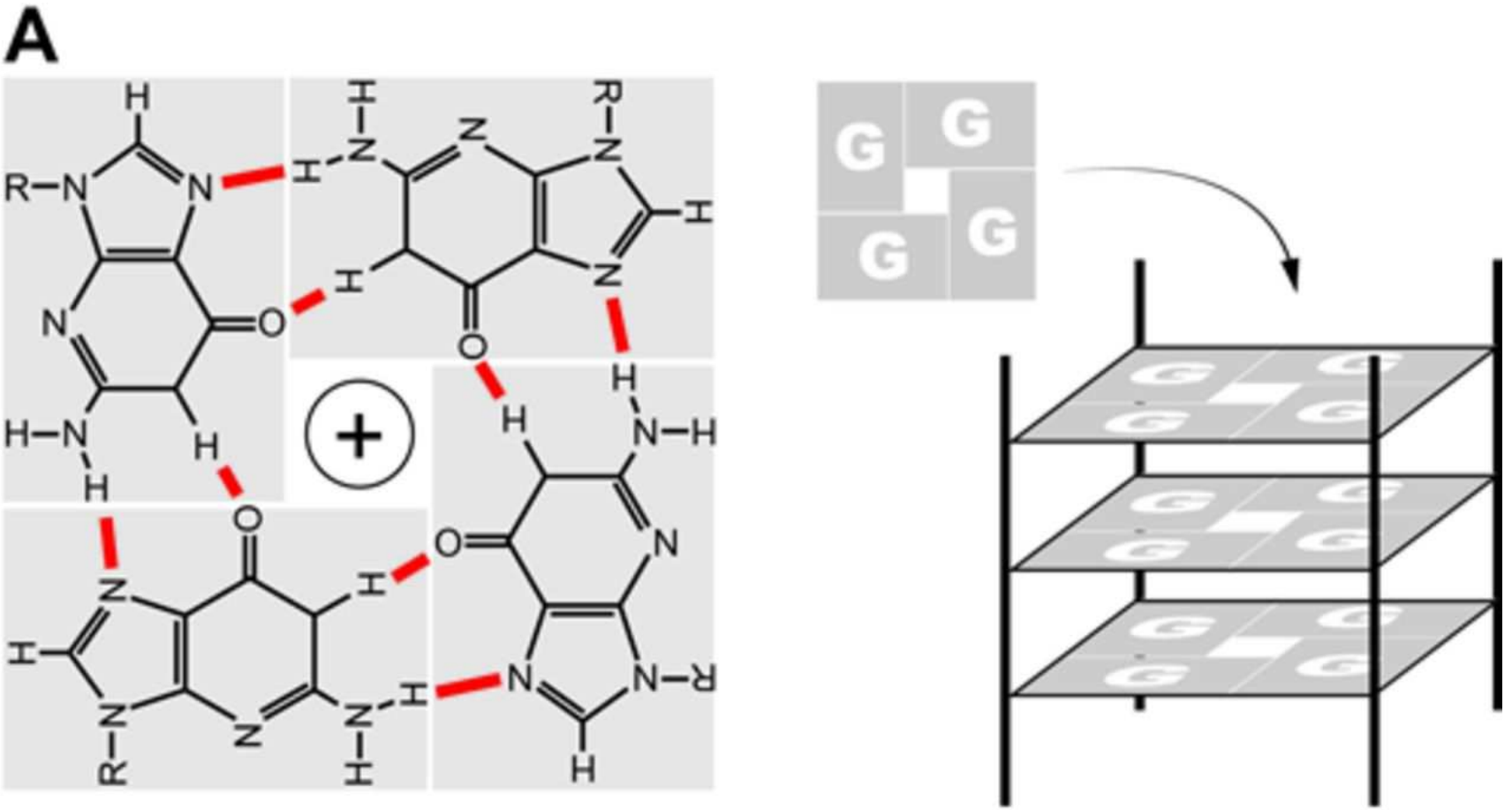
6, modulation of signaling pathways and molecules involved in enzyme activation,

7, modulation of telomerase complex catabolism including vaccine therapy.

BRACO19, RHPS4, and telomestatin promote G-quadruplex formation



Structure of G-quadruplexes.



Daniela Rhodes, and Hans J. Lipps Nucl. Acids Res. 2015;nar.gkv862

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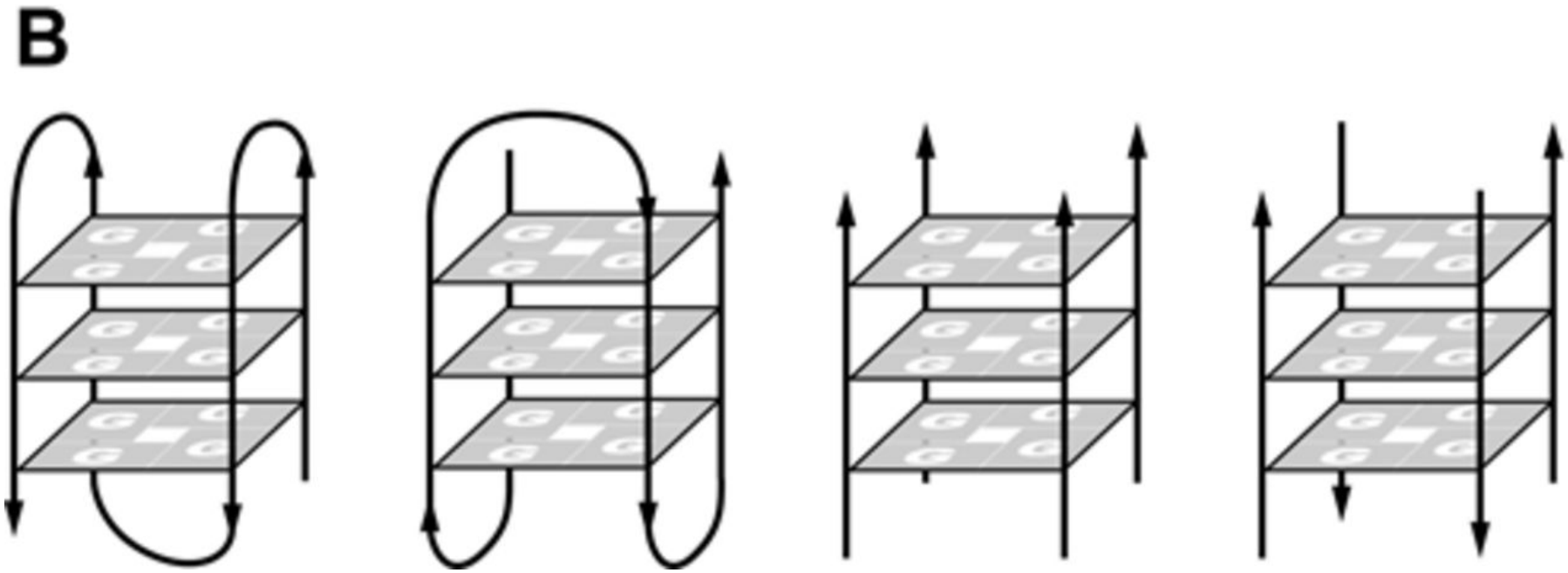

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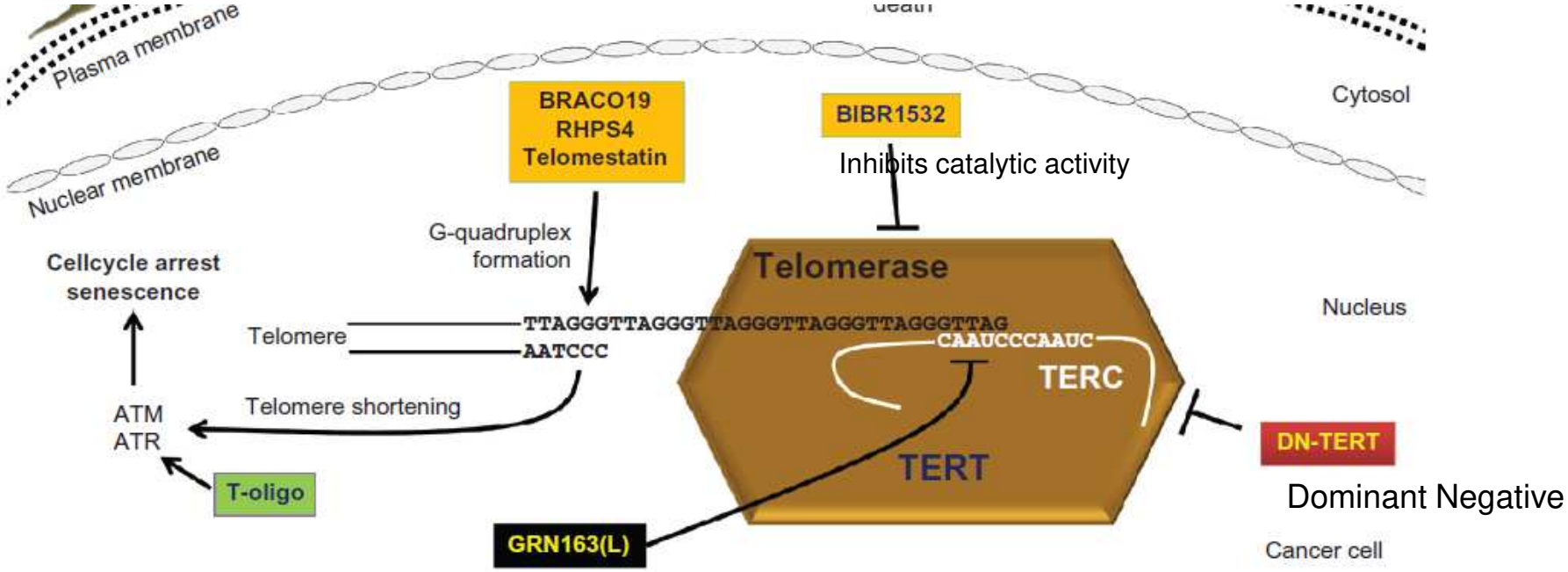
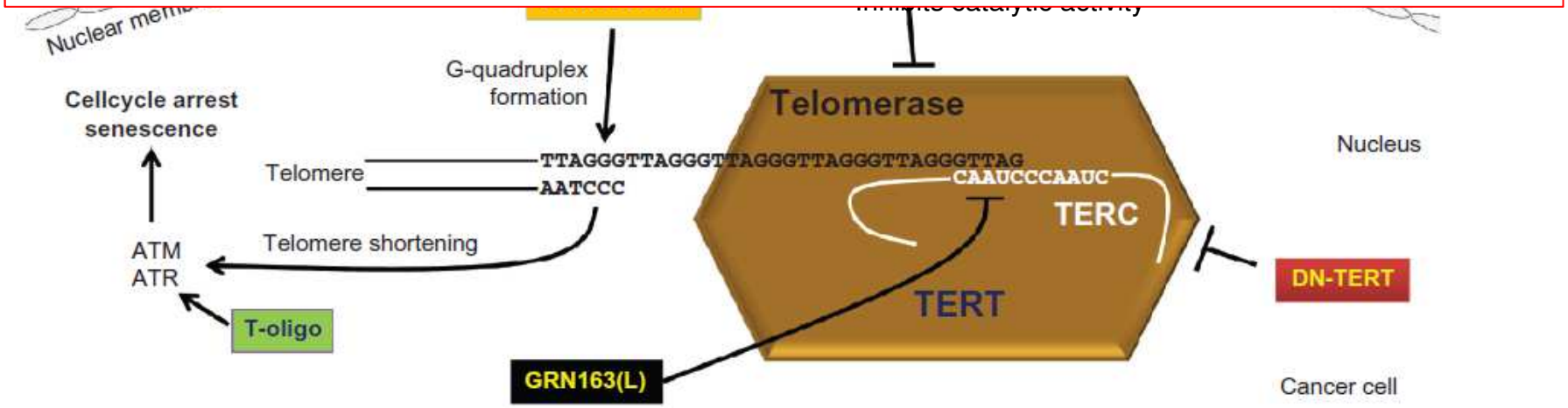


T-oligo mimics dysfunctional telomeres

Vaccination with peptides derived from TERT or introduction of TERT mRNA into dendritic cells activates T and/or B cells, which recognize and eliminate TERT-expressing cancer cell

GV1001 is a 16-amino acid peptide derived from the human telomerase reverse transcriptase (hTERT) protein (616-626; EARPALLTSRLRFIPK), which lies within the reverse transcriptase domain.



Lung Cancer. 2014 Oct;86(1):59-66.

A phase II trial evaluating the clinical and immunologic response of HLA-A2(+) non-small cell lung cancer patients vaccinated with an hTERT cryptic peptide.

Kotsakis A[1],.

Abstract

OBJECTIVES:

The immunological and clinical responses of patients with NSCLC treated, in the context of an expanded action program, with the cryptic hTERT-targeting Vx-001 vaccine are presented.

MATERIALS AND METHODS:

2018 A dose-escalating phase I/II study in patients with pancreatic cancer revealed prolonged survival rates in patients receiving the GV1001 peptide with an immunologic response, compared with those without an immune response

(range, 0.7-39.4) and 19.6 months (range, 0.7-39.4), respectively.

Patients who developed immune response had a numerically higher PFS compared to those who failed to mount any. However, the median survival for the immune-responders was significantly prolonged compared to non-responders (40.0 versus 9.2 months, respectively; p=0.02). Toxicity was <grade 2.

CONCLUSION:

Vx-001 vaccine is well tolerated and induced a TERT-specific immunological response, which was significantly correlated with improved clinical outcome.

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MATERIALS AND METHODS:

Forty-six HLA-A*0201-positive patients with advanced NSCLC and residual (n=27) or progressive (n=19) disease following front-line treatment received two subcutaneous injections of the optimized TERT572Y peptide followed by four injections of the native TERT572 peptide, every 3 weeks.

RESULTS:

Three (7%) patients achieved a partial response and 13 (28%) disease stabilization. The median progression-free survival (PFS) and overall survival (OS) was 3.8 (range, 0.7-99.4) and 19.8 months (range, 0.7-99.4), respectively.

Patients who developed immune response had a numerically higher PFS compared to those who failed to mount any. However, the median survival for the immune-responders was significantly prolonged compared to non-responders (40.0 versus 9.2 months, respectively; p=0.02). Toxicity was <grade 2.

CONCLUSION:

Vx-001 vaccine is well tolerated and induced a TERT-specific immunological response, which was significantly correlated with improved clinical outcome.

Vaccinazione (2)

hTERT-Dendritic Cells were produced from patient-specific leukapheresis, electroporated with an mRNA-encoding hTERT and a lysosomal-targeting sequence, and cryopreserved.

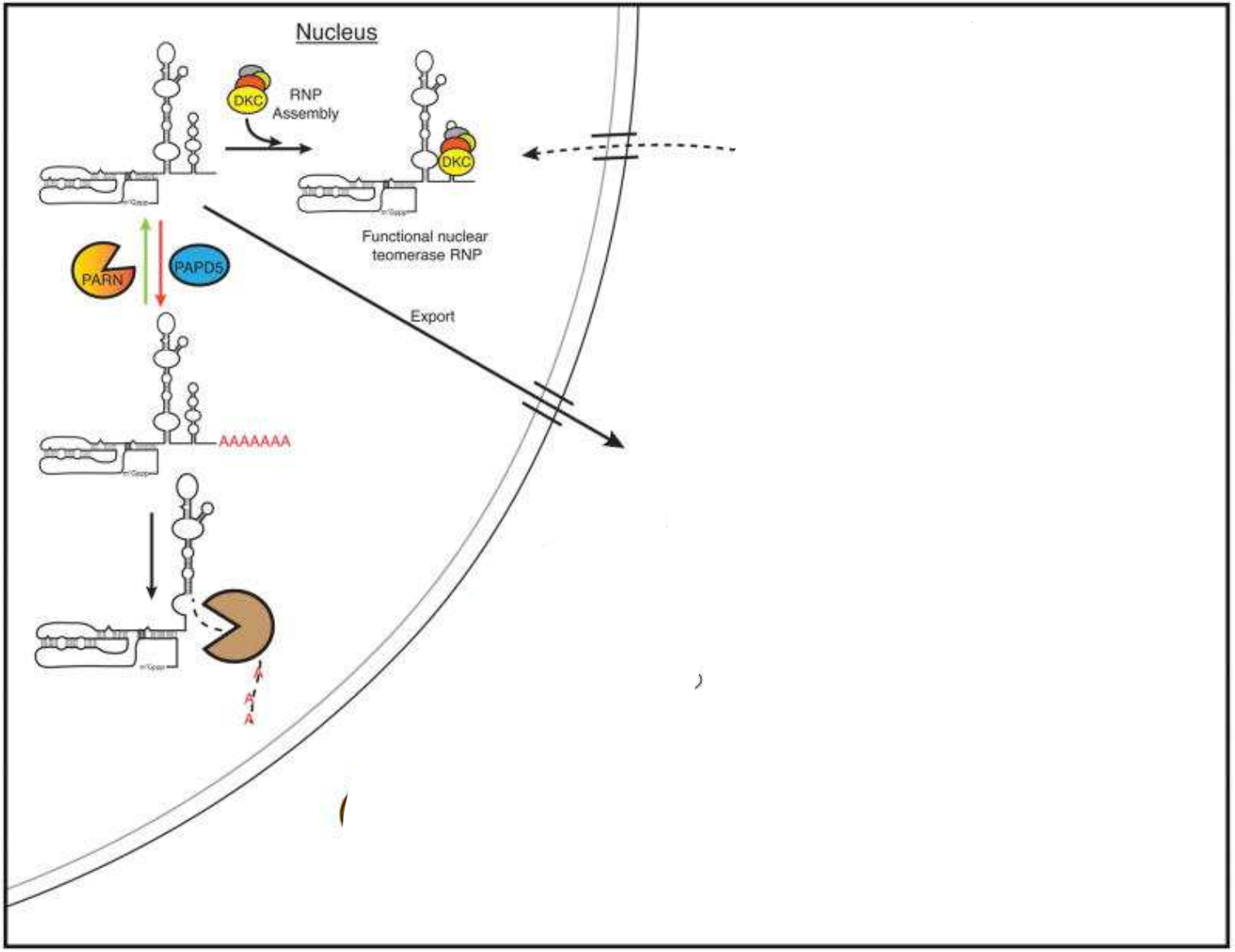
Of the 19 patients receiving hTERT-DCs, 11 patients (58%) developed hTERT-specific T-cell responses

Primarily targeted toward hTERT peptides with predicted low human leukocyte antigen (HLA)-binding affinities.

Cancer. 2017

MODELLI DI INTERVENTO BASATI SU RNA STAMPO DELLA TELOMERASI (hTR)

hTR biogenesis



hTR biogenesis