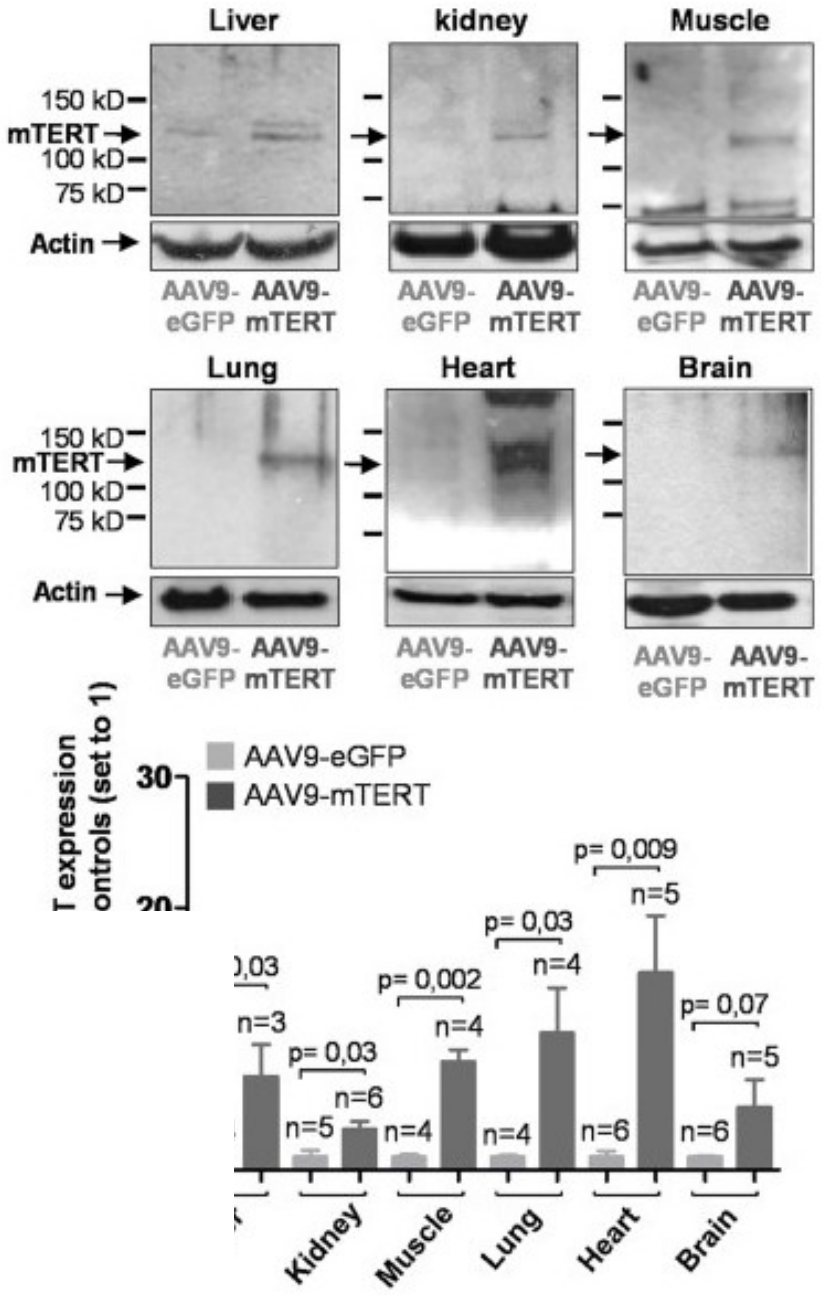
INTERVENTI: Aumento Attività telomerasica

- 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



AAV9-mTERT treated mice
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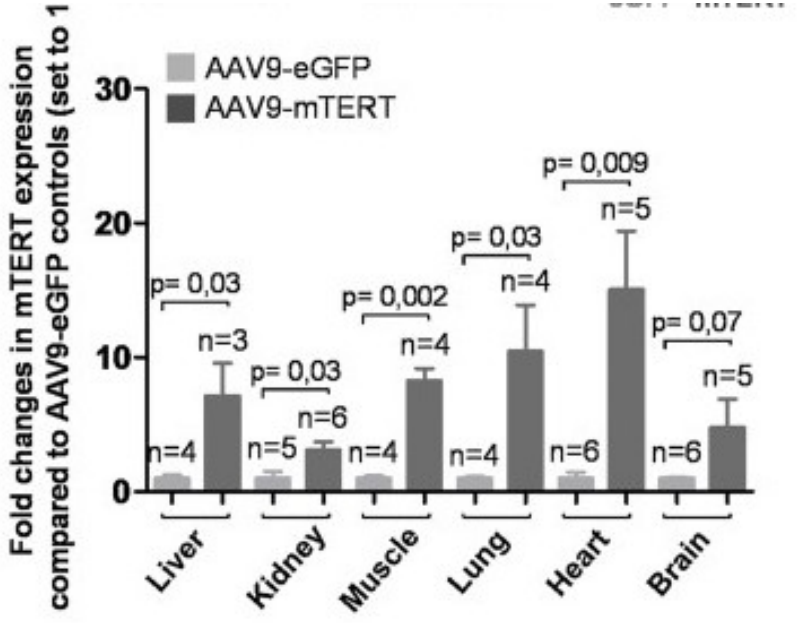
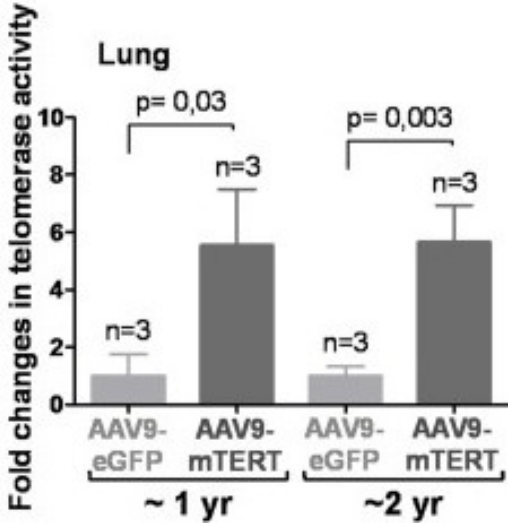


Telomerase activity (measured through TRAP assay) in several tissues from AAV9-eGFP or AAV9-mTERT mice



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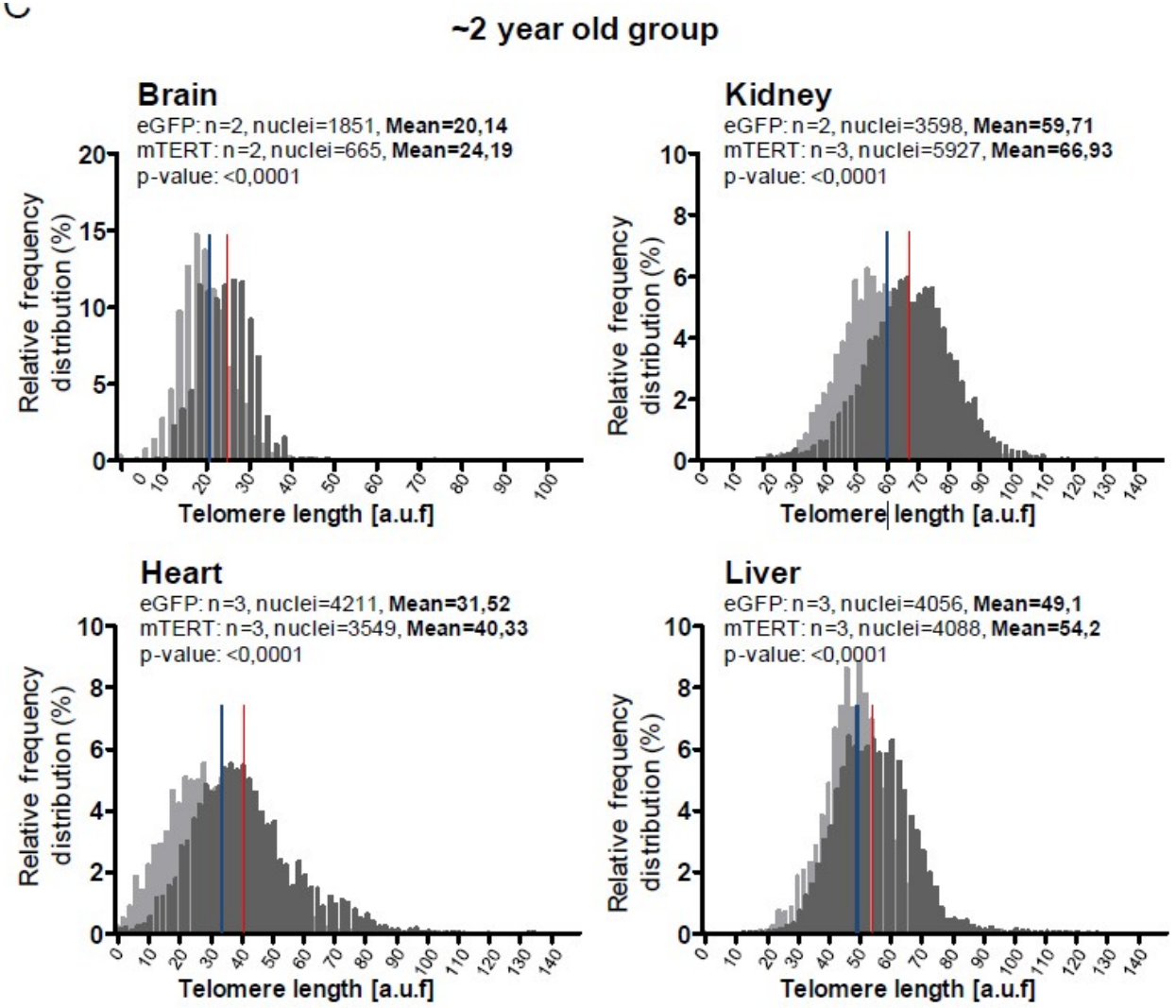
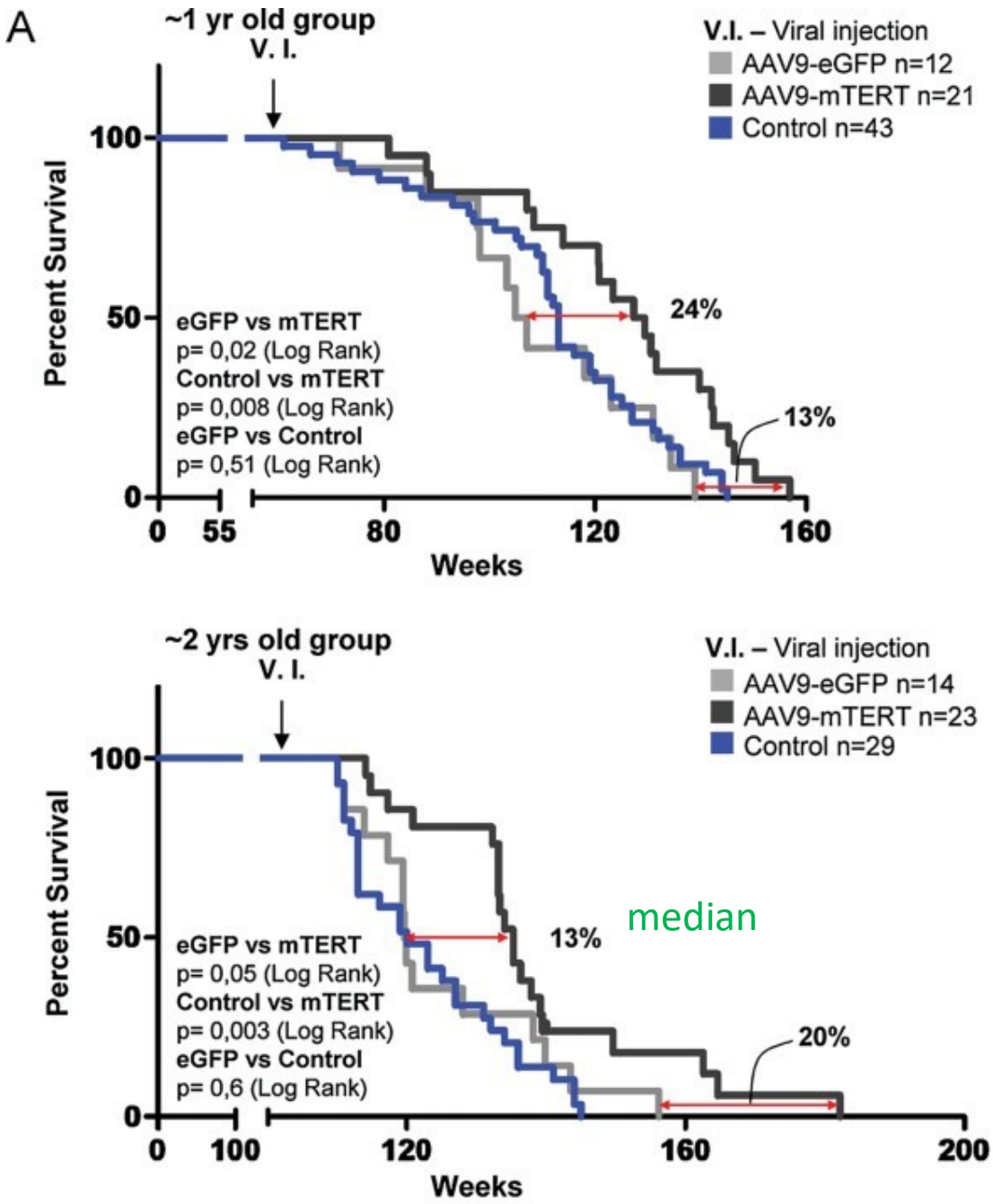
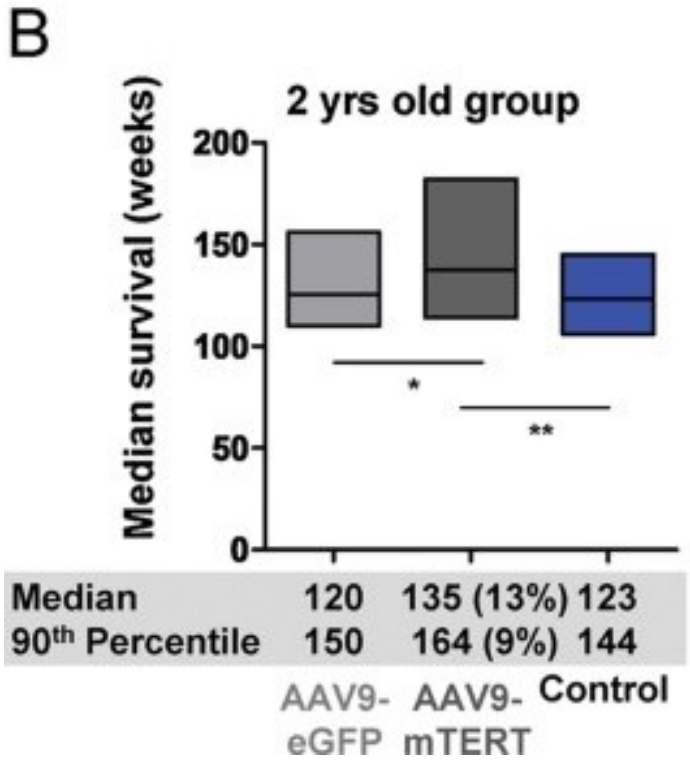

~2 year old group
Brain
eGFP: n=2, nuclei=1851, Mean=20,14
mTERT: n=2, nuclei=665, Mean=24,19
p-value: <0,0001
Relative frequency distribution (%)
Telomere length [a.u.f]
Kidney
eGFP: n=2, nuclei=3598, Mean=59,71
mTERT: n=3, nuclei=5927, Mean=66,93
p-value: <0,0001
Relative frequency distribution (%)
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

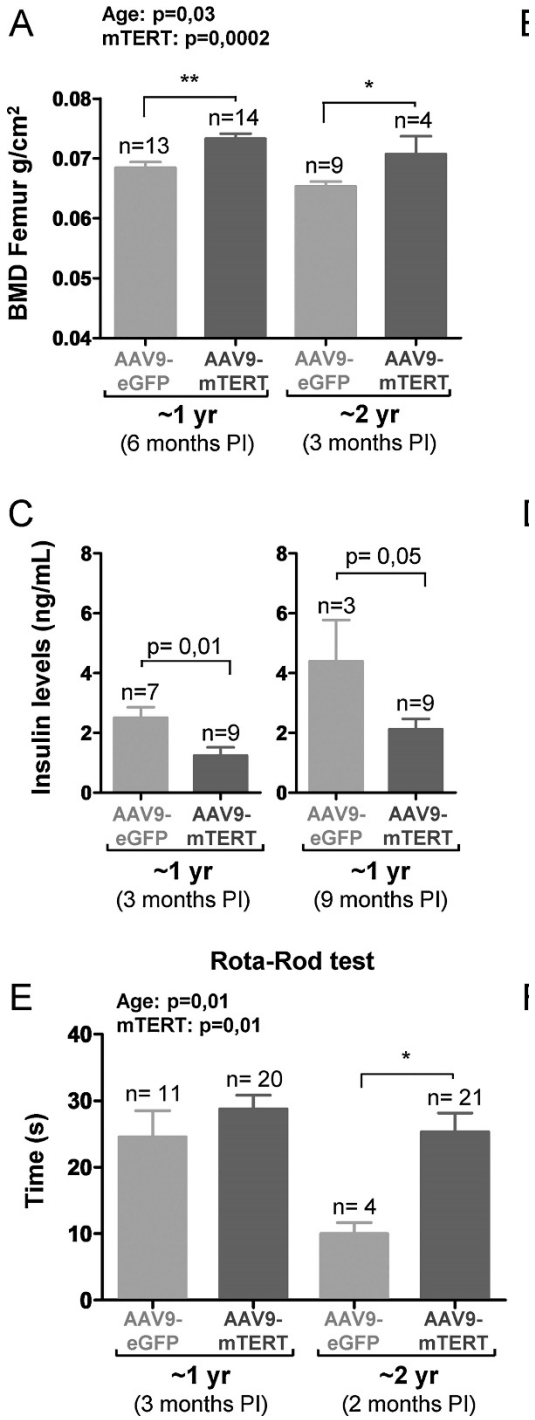
maximum



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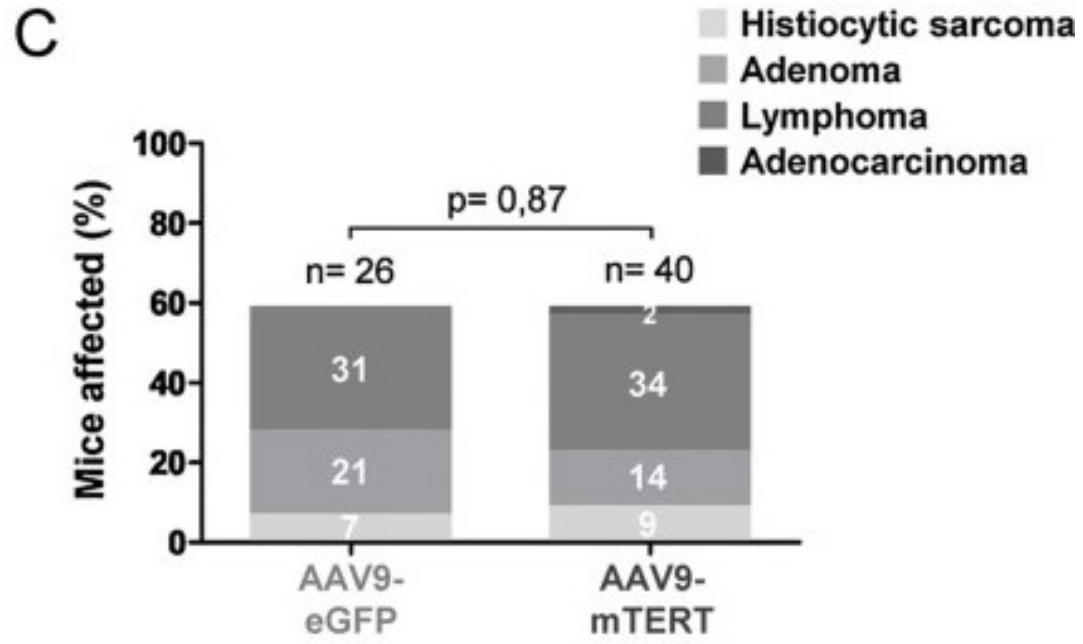
Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer.

- 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.

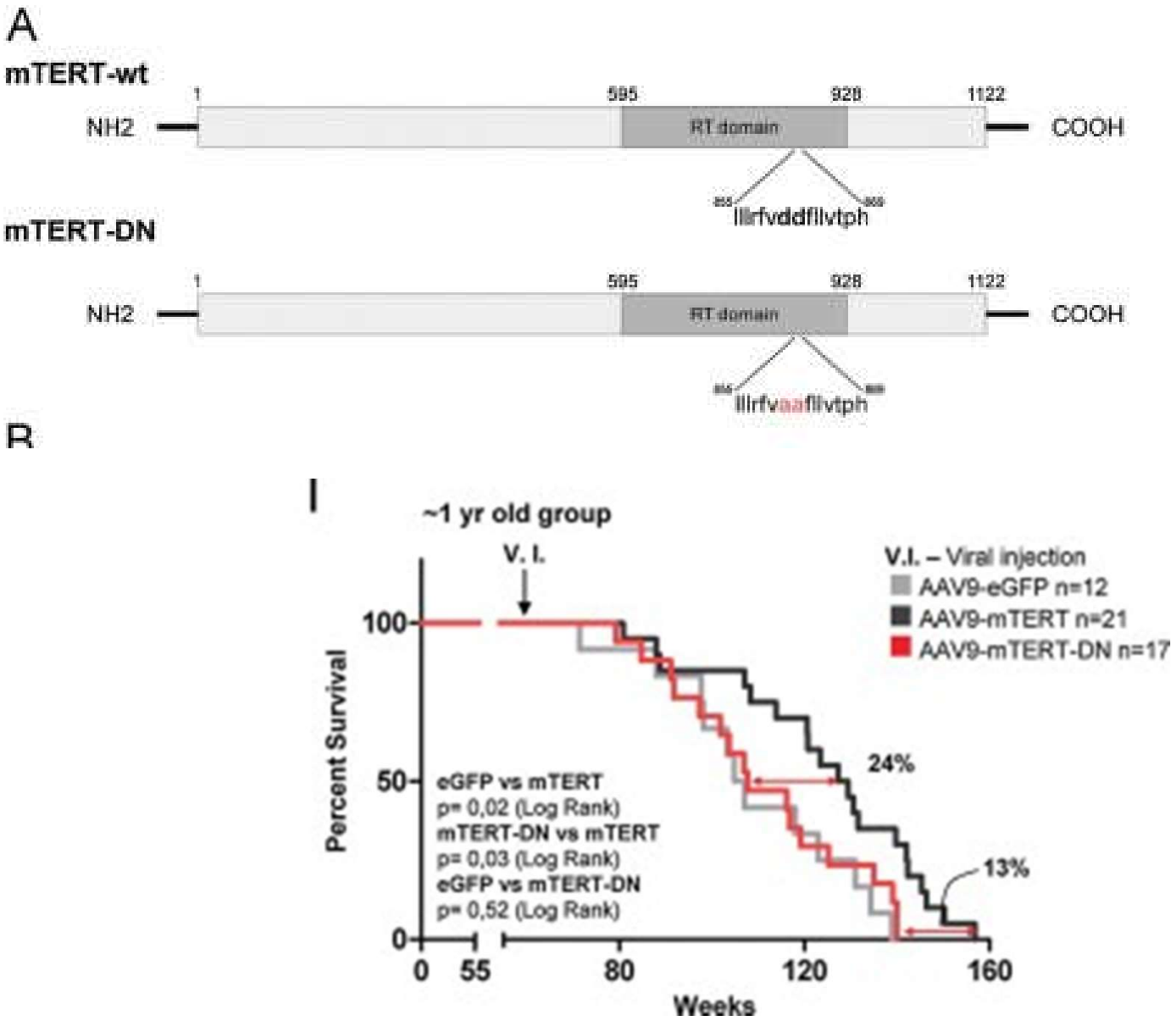
- telomerase-treated mice, both at 1-year and at 2-year of age, had an increase in median lifespan of 24 and 13%, respectively

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.



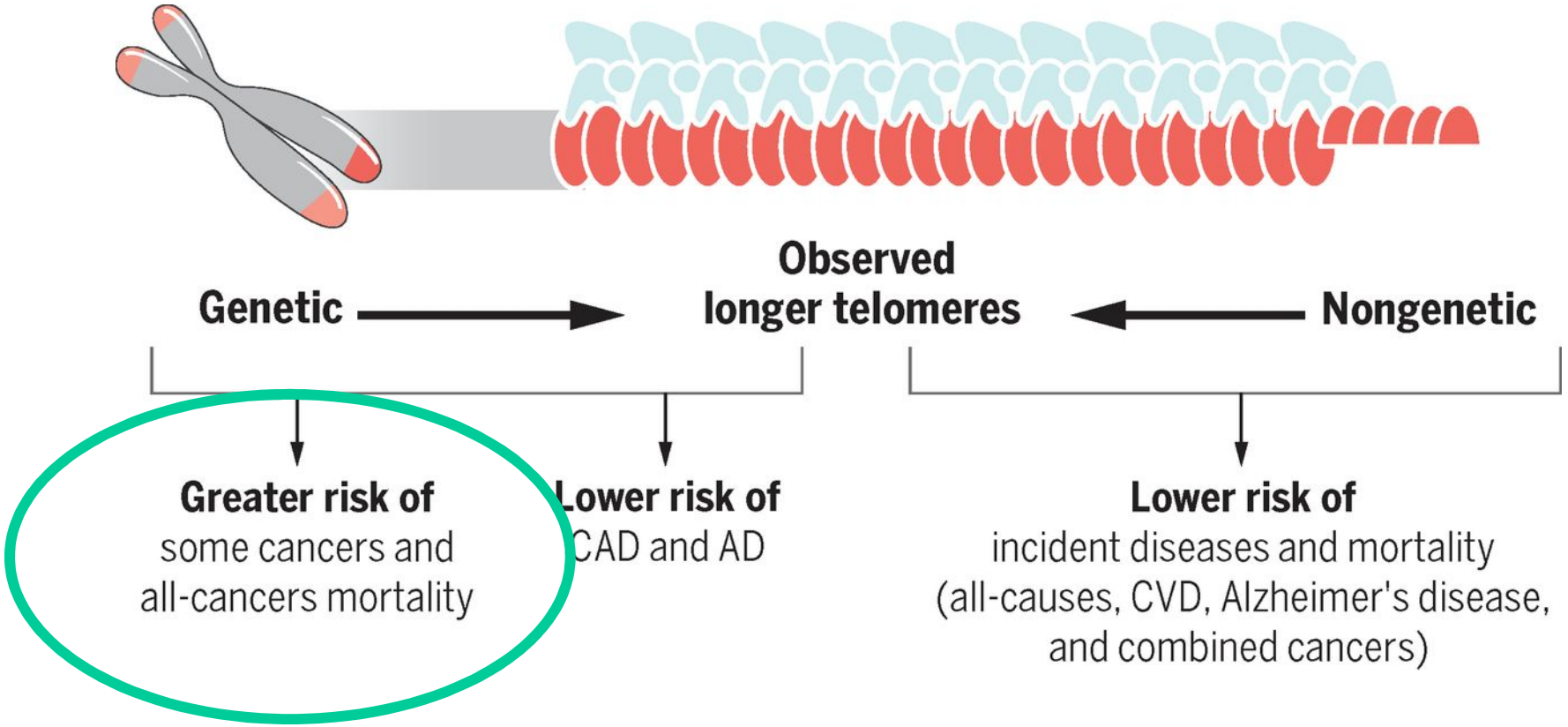
beneficial effects were not observed with a catalytically inactive TERT, demonstrating that they require telomerase activity.

- 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.

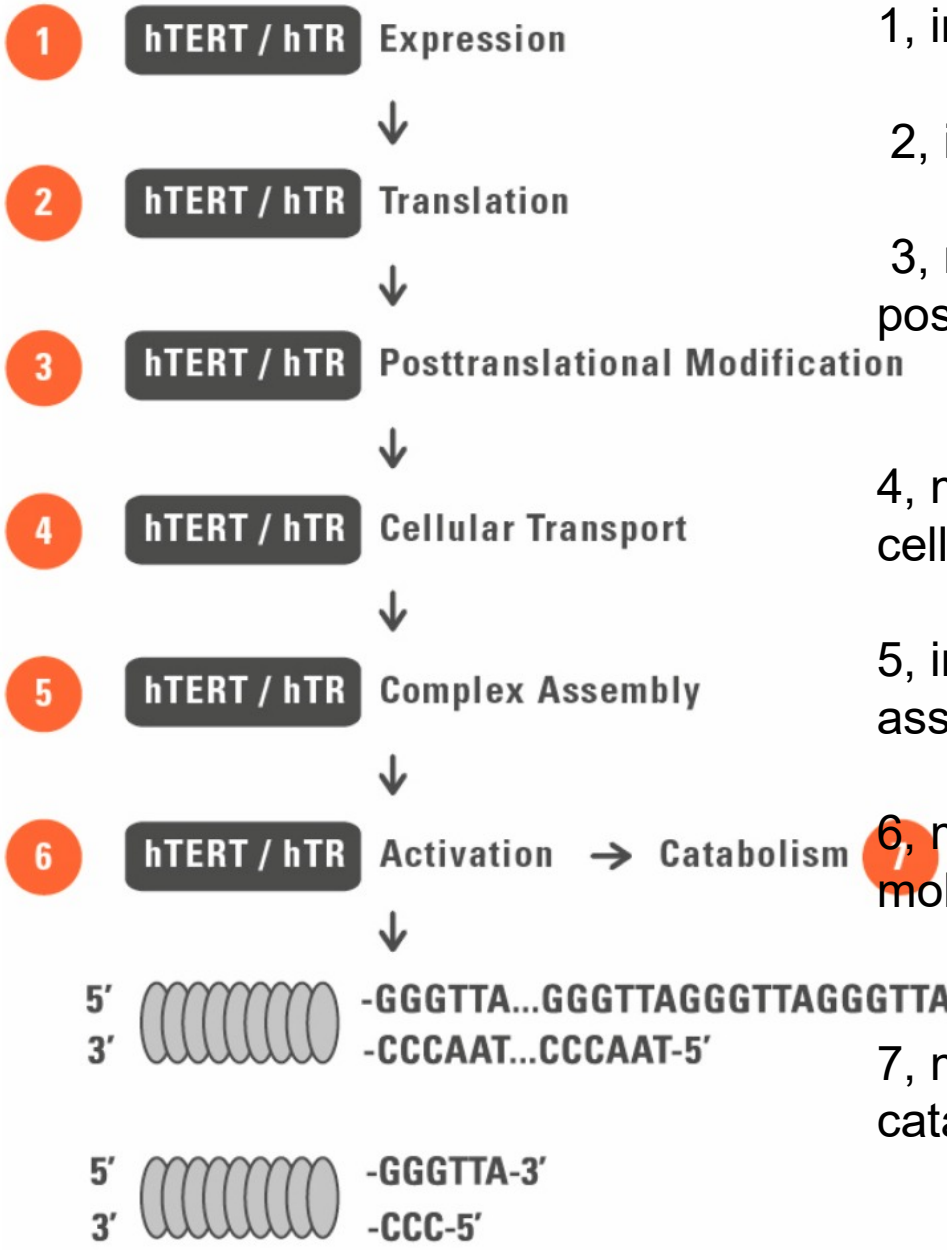
EMBO Mol Med. 2012;4(8):691-704

Strategie anti tumorali basate sulla inibizione della telomerasi

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;

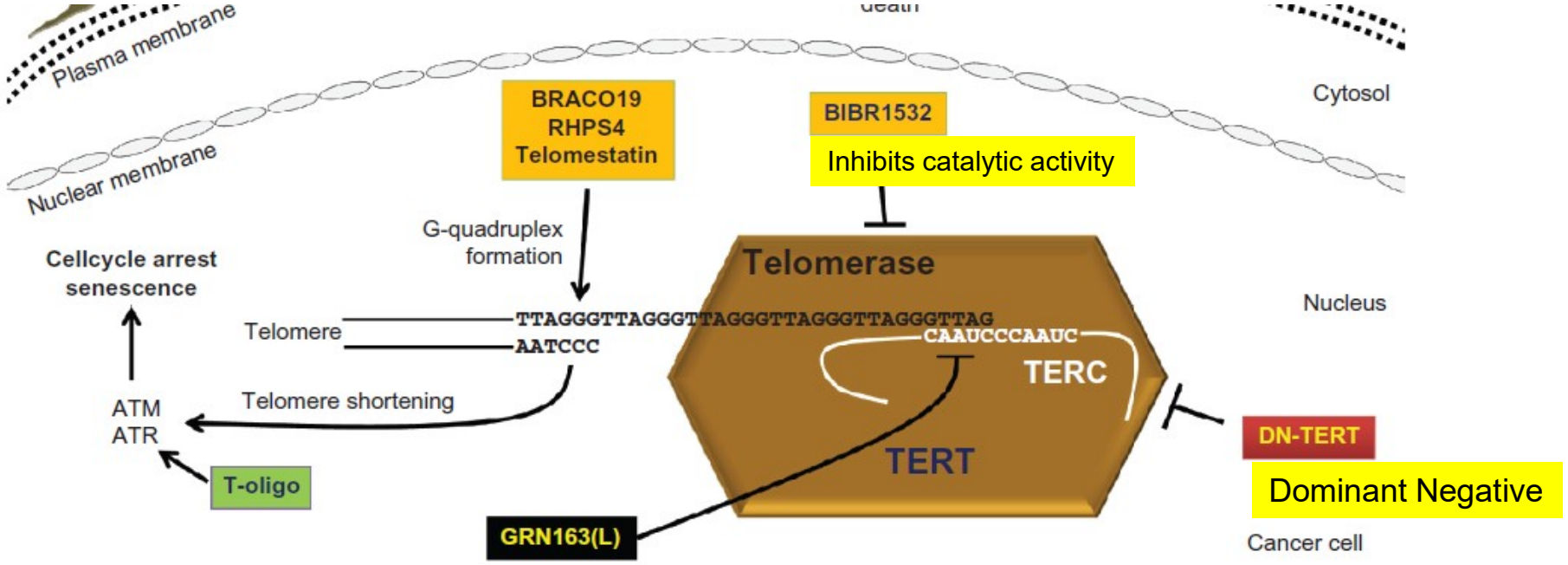
4, modulation of telomerase activity by cellular sequestration;

5, interference with telomerase complex assembly;

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

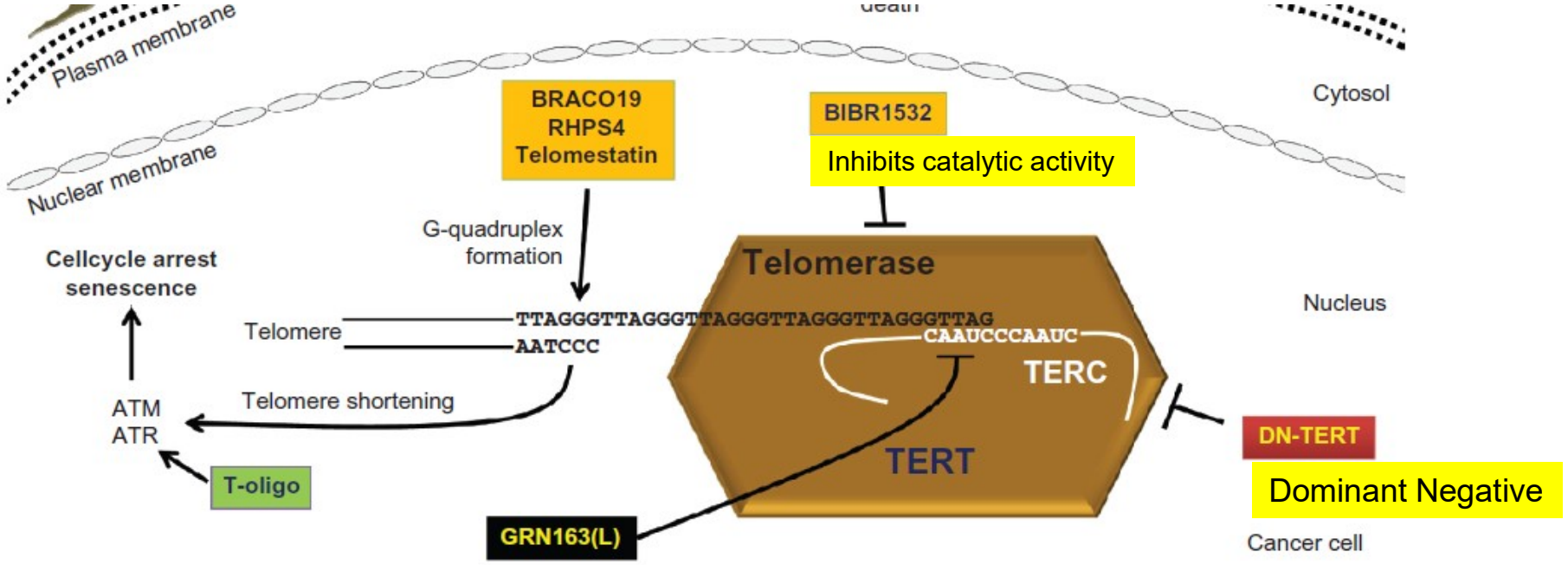
7, modulation of telomerase complex catabolism including vaccine therapy.

GRN163(L), DN-TERT, and BIBR1532 directly inhibit telomerase



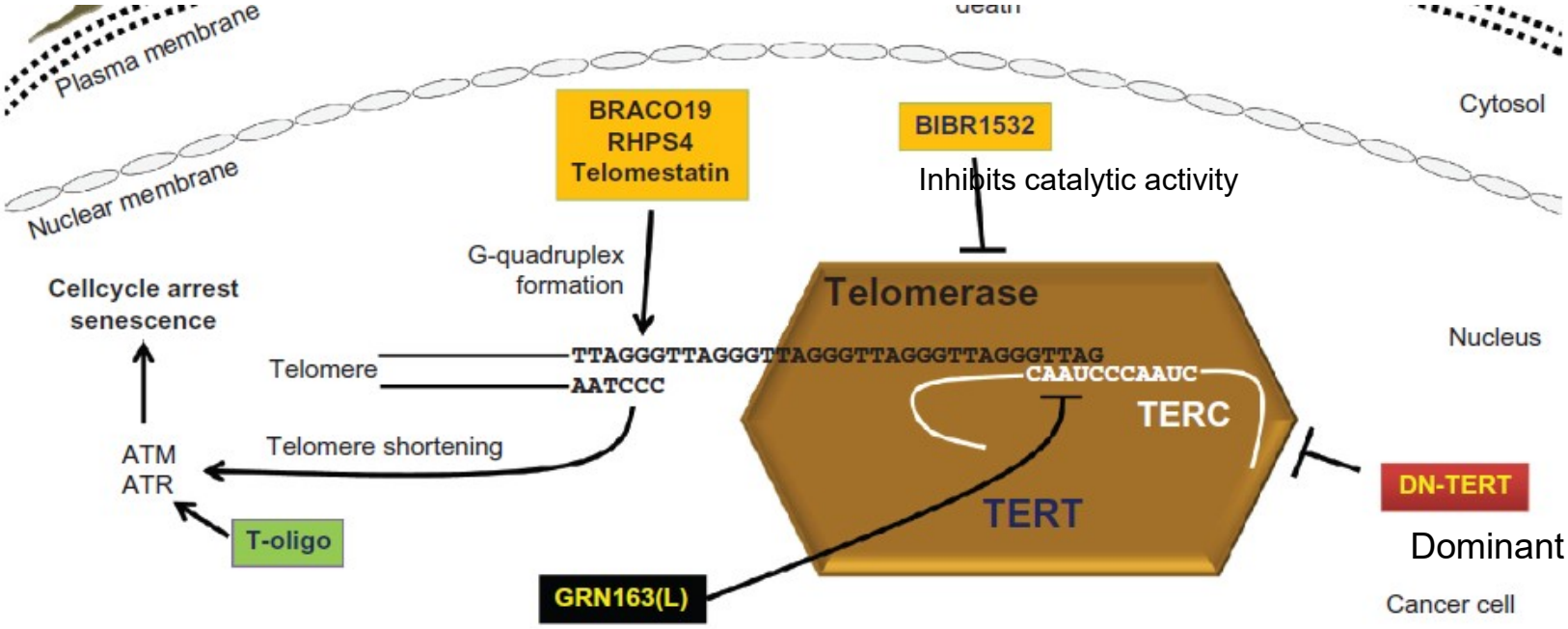
GRN163(L) directly inhibit telomerase

Imetelstat (GRN163L) is a 5’ palmitoylated 13-mer thiophosphoramidate oligonucleotide, composed of the sequence 5’-TAGGGTTAGACAA-3’,
complementary to the template region of human telomerase RNA component (hTR)
It is a highly specific and potent competitive inhibitor of telomeric repeat addition.
Imetelstat are provided by Geron Corporation, California, USA.



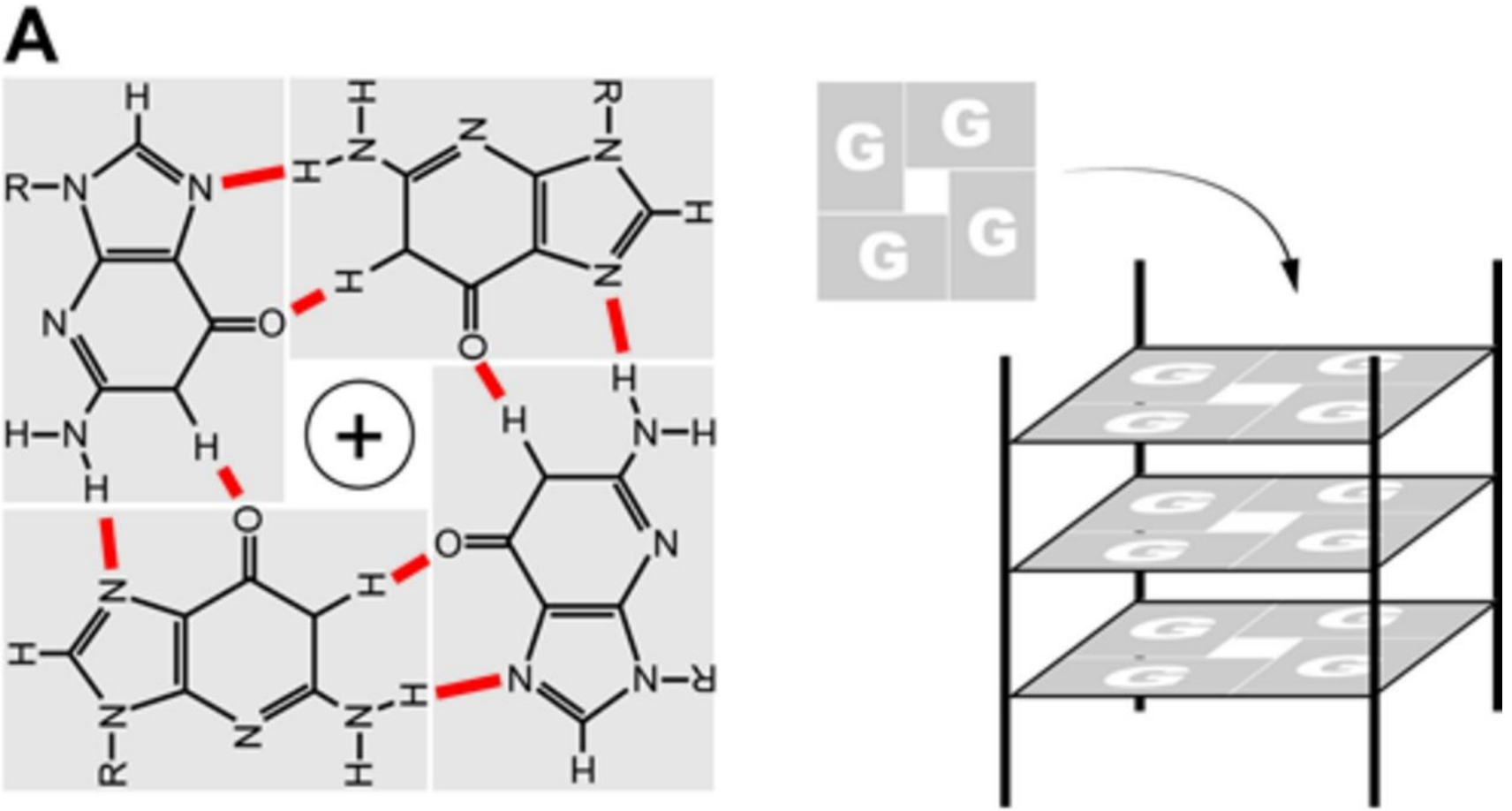
BRACO19, RHPS4, and telomestatin promote G-quadruplex formation

T-oligo mimics dysfunctional telomeres



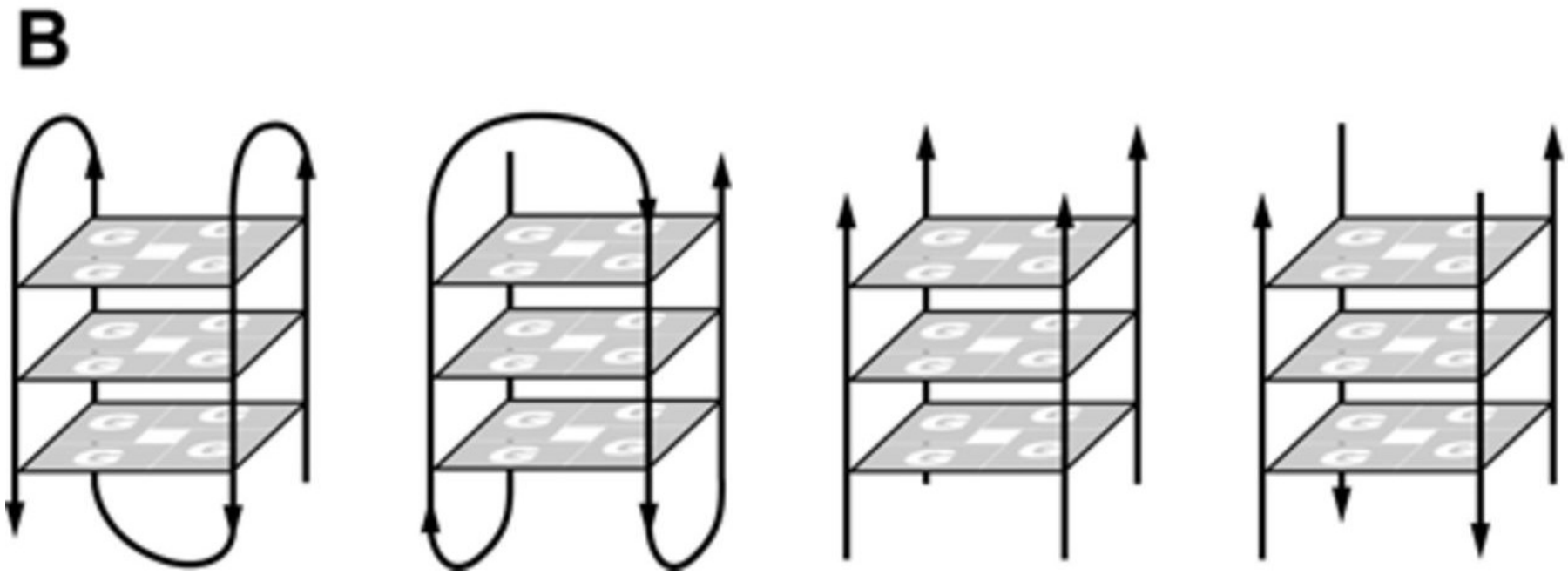
13-mer modified oligonucleotide, binds terC

Structure of G-quadruplexes.



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

Structure of G-quadruplexes.



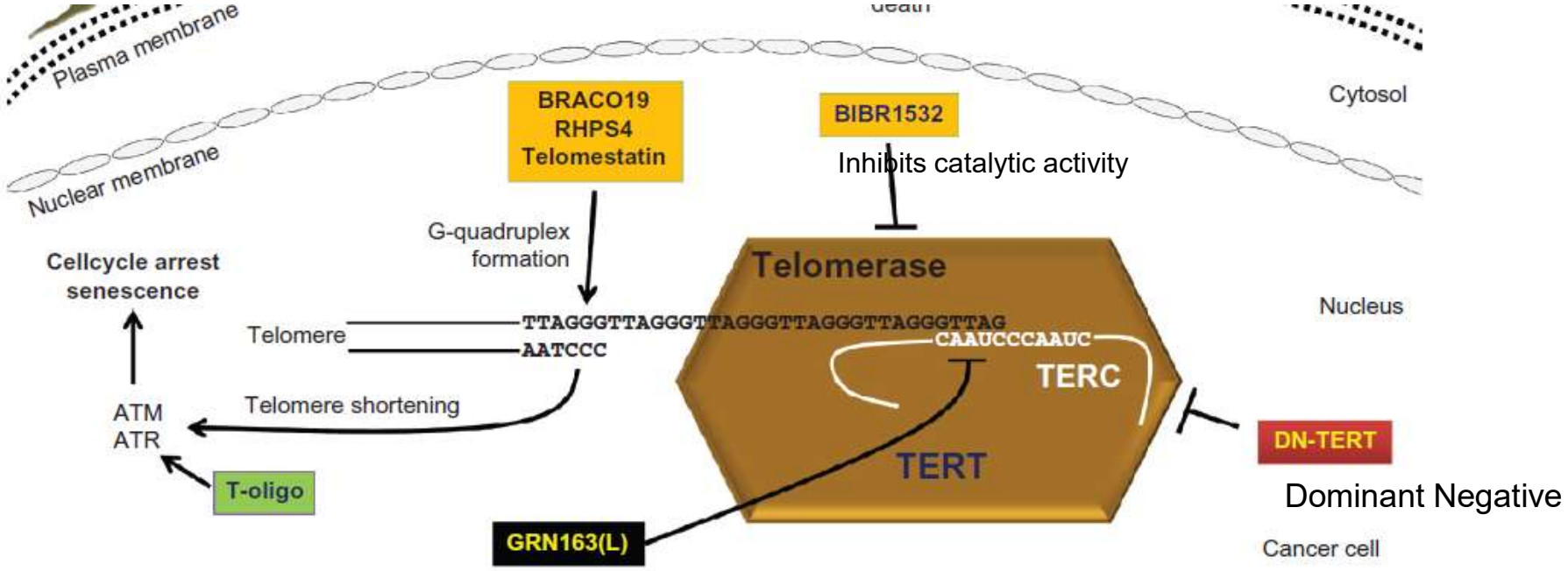
Daniela Rhodes, and Hans J. Lipps Nucl. Acids Res. 2015;nar.gkv862

GRN163(L), DN-TERT, and BIBR1532 directly inhibit telomerase

BRACO19, RHPS4, and telomestatin promote G-quadruplex formation

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



13-mer modified oligonucleotide, binds terC

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:

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:

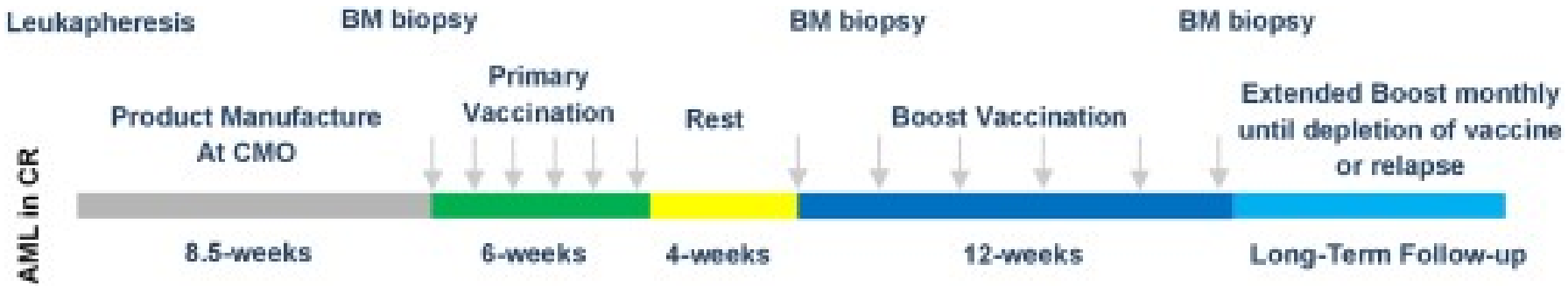
Three (7%) patients achieved a partial response and 13 (28%) disease stabilization. The median progression-free survival (PFS) and overall survival (OS) was 3.8 (range, 0.7-99.4) and 19.8 months (range, 0.7-99.4), respectively.

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.

Immune responses and long-term disease recurrence status after telomerase-based dendritic cell immunotherapy in patients with acute myeloid leukemia

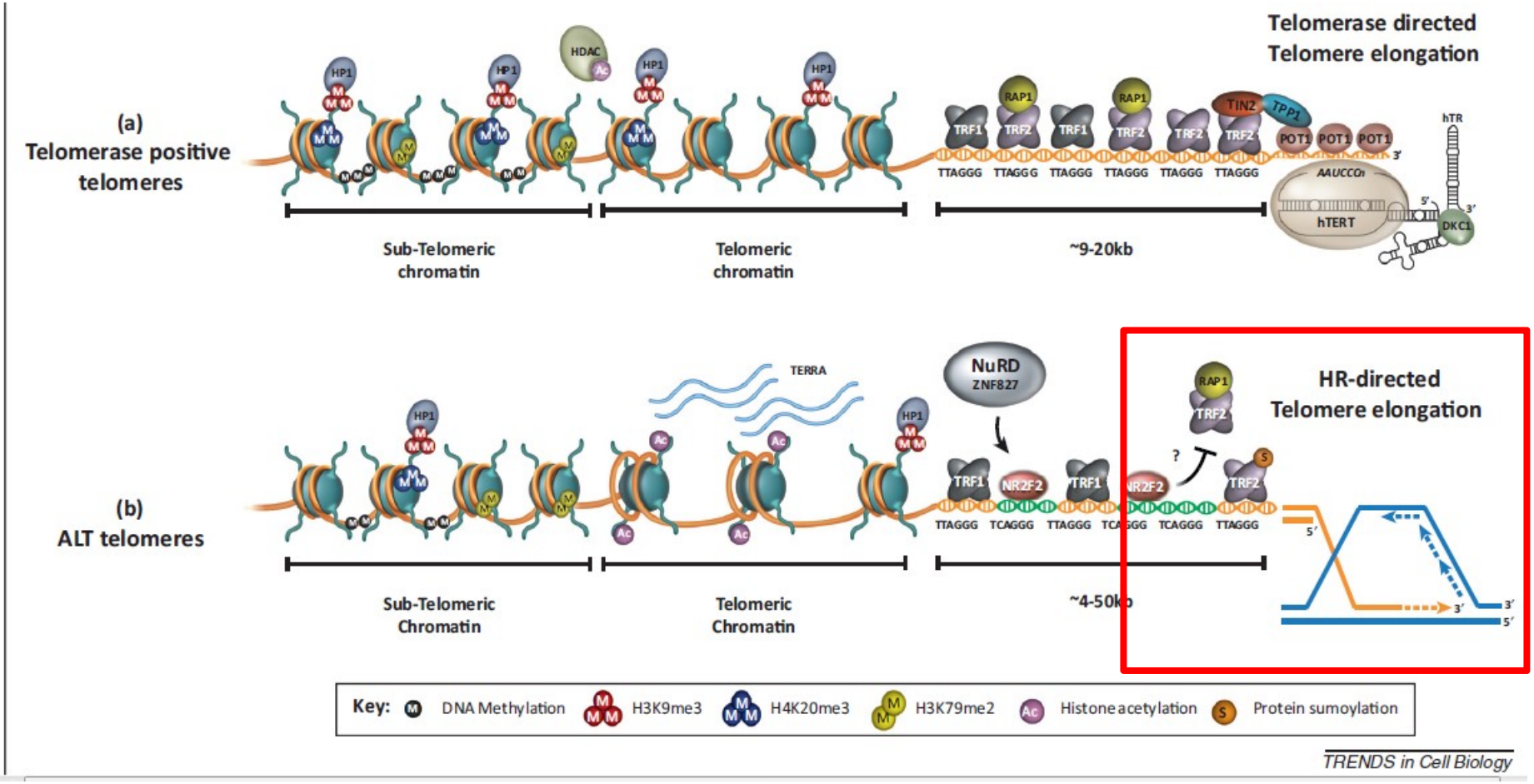


human telomerase reverse transcriptase (hTERT)-expressing autologous dendritic cells (hTERT-DCs)

With a median follow-up of 52 months, 58% of patients were free of disease recurrence at the time of their last follow-up visit; 57% of the patients who were aged ≥60 years (4 of 7 patients) also were found to be free of disease recurrence at a median follow-up of 54 months

Cancer
Volume 123, Issue 16, pages 3061-3072, 14 APR 2017 DOI: 10.1002/cncr.30696
http://onlinelibrary.wiley.com/doi/10.1002/cncr.30696/full#cncr30696-fig-0001

Alternative lengthening of telomeres (ALT)


(a)
Telomerase positive telomeres
HDAC
HP1
Telomerase directed
Telomere elongation
RAP1
TRF1
TRF2
TIN2
TPP1
POT1
hTR
TTAGGG
AAUCCCn
hTERT
DKC1
Sub-Telomeric chromatin
Telomeric chromatin
~9-20kb
(b)
ALT telomeres
TERRA
NuRD
ZNF827
NR2F2
HR-directed
Telomere elongation
TCAGGG
Sub-Telomeric Chromatin
Telomeric Chromatin
~4-50kb
Key:
DNA Methylation
H3K9me3
H4K20me3
H3K79me2
Histone acetylation
Protein sumoylation
TRENDS in Cell Biology

ALT telomeres are distinct in that they contain variant C-type TCAGGG repeats and sumoylated TRF2.

These features of ALT telomeres may lead to displacement of TRF2

A small but significant number of cancers do so via the exchange of telomeric DNA between chromosomes by alternative lengthening of telomeres - ALT

Alternative lengthening of telomeres (ALT) telomeres are considerably longer (4–50 kb)

These provide a platform for the binding of proteins such as the nucleosome remodeling complex.
ALT telomeres display reduced levels of H3K9me3.
As a result, they may contain elevated levels of histone acetylation as suggested by the reduced compaction of nucleosomal arrays.

Sub-telomeric chromatin also displays reduced heterochromatic marks and altered DNA methylation patterns.
The **more open chromatin configuration of ALT telomeres may promote homologous recombination** directed telomere elongation and greater transcription of the telomeric non-coding RNA (TERRA).