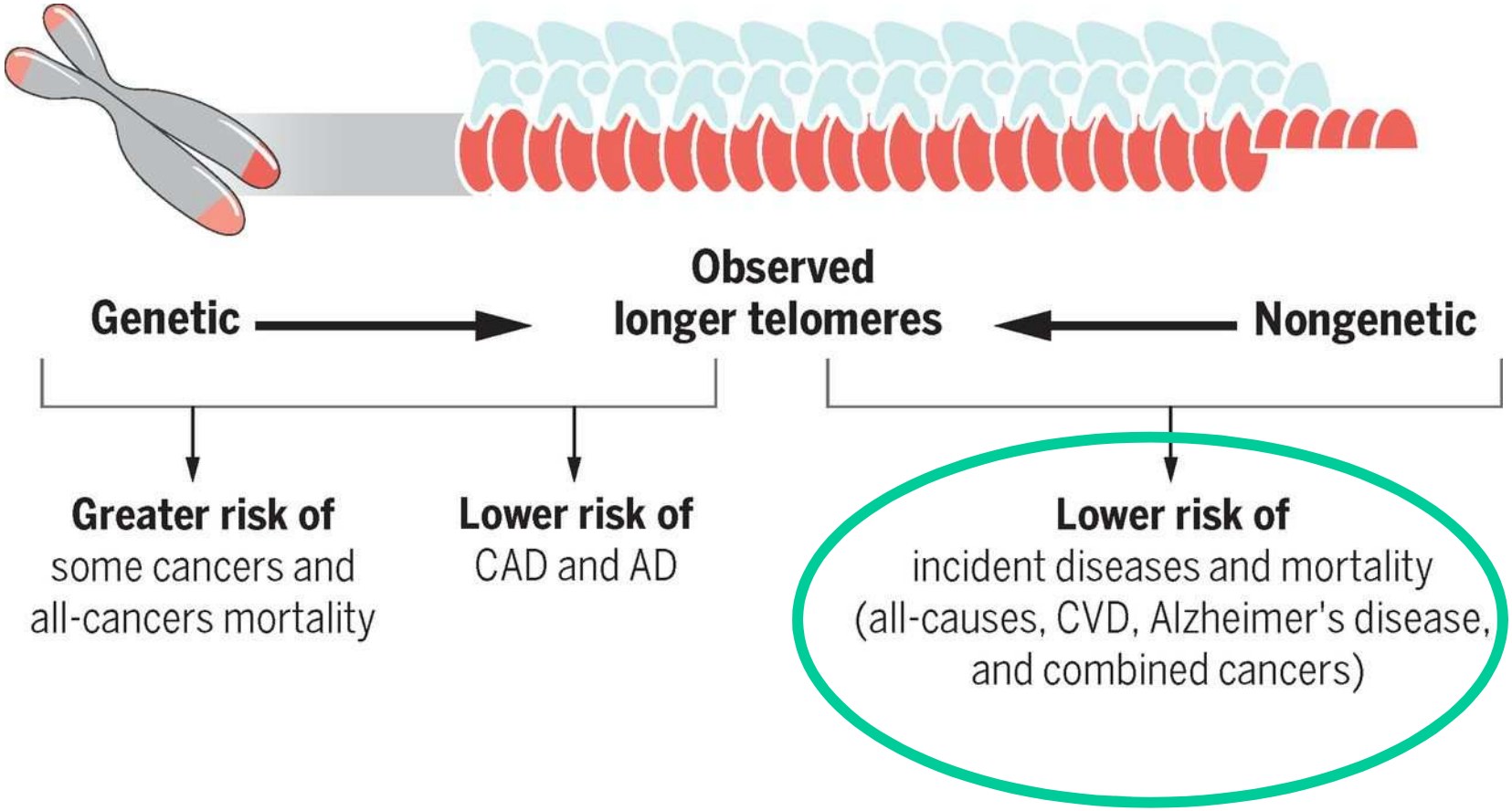
Fig. 3 Different inputs to telomere maintenance have disease-specific consequences.



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

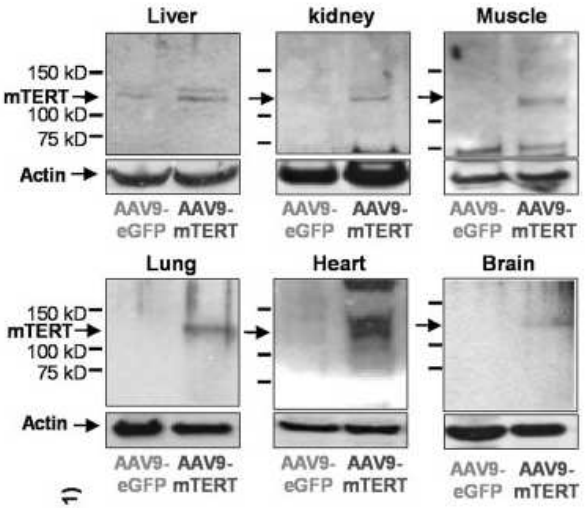
INTERVENTI: Aumento Attività telomerasica

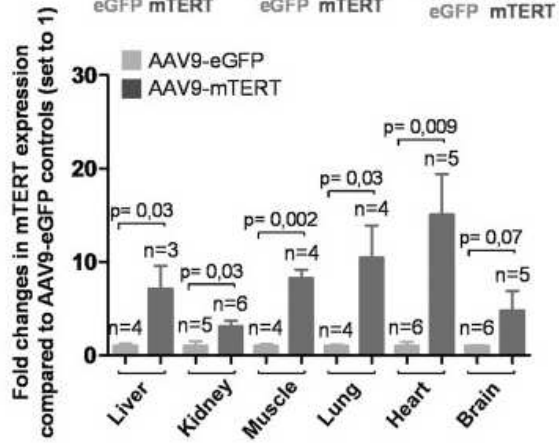
- EMBO Mol Med. 2012

- 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.

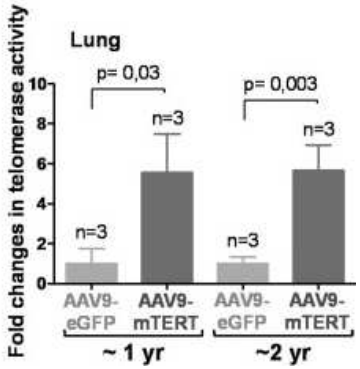
- A major goal in aging research is to improve health during aging. In the case of mice, genetic manipulations that shorten or lengthen telomeres result, respectively, in decreased or increased longevity. Based on this, we have tested the effects of a telomerase gene therapy in adult (1 year of age)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

AAV9-mTERT treated mice
compared to AAV9-eGFP controls





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



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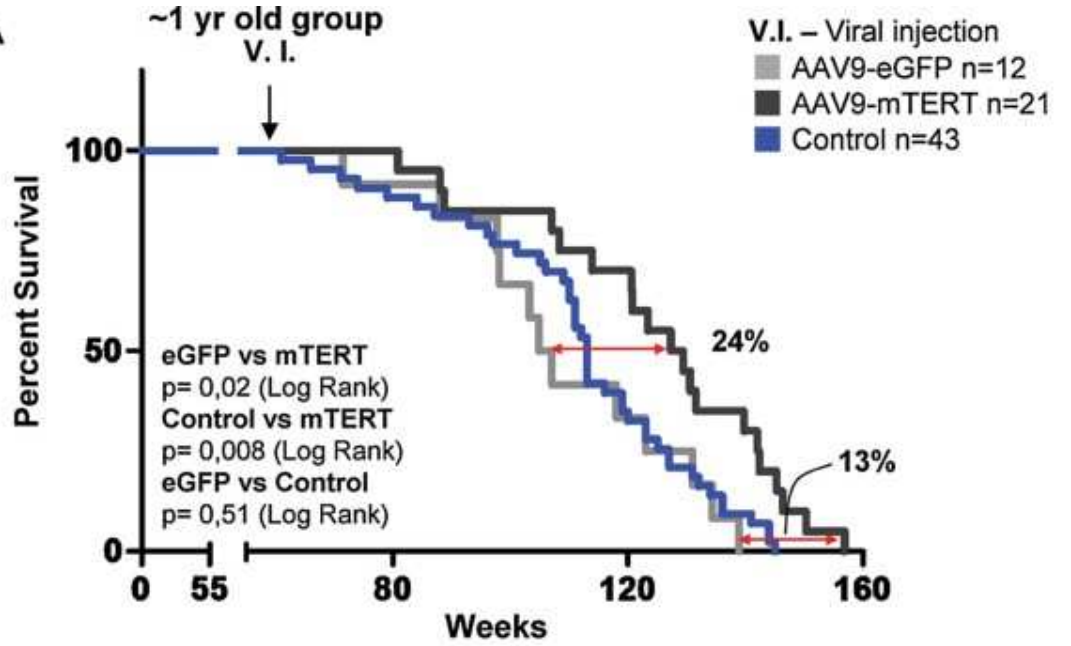
Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer.

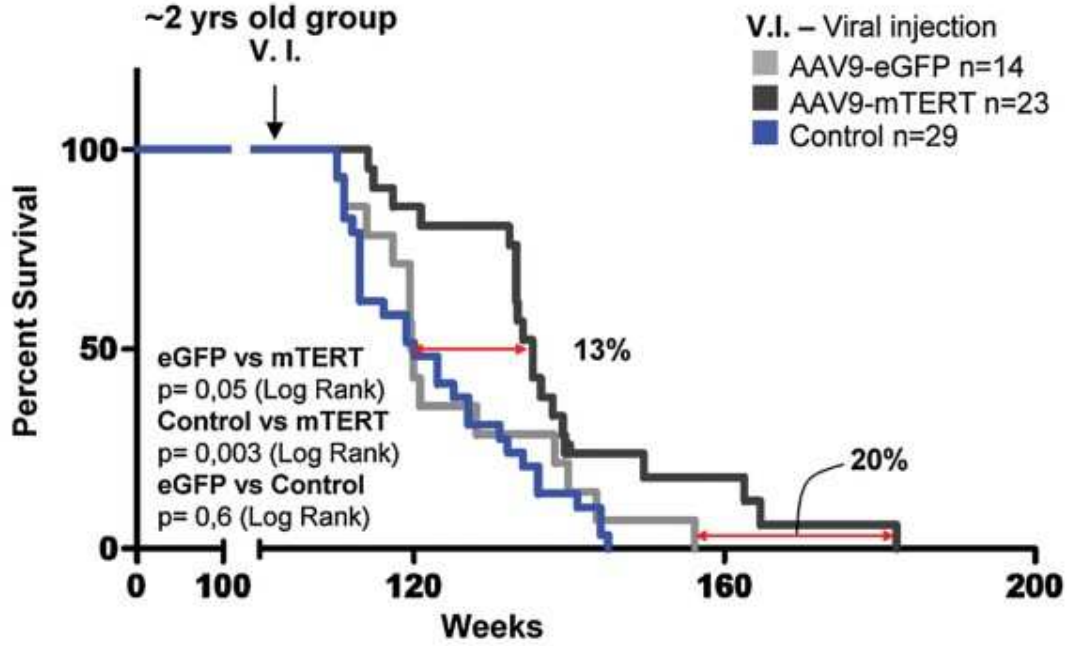
- Treatment of 1- and 2-year old mice with an adeno associated virus (AAV) expressing mouse TERT had remarkable beneficial effects on health and fitness, including insulin sensitivity, osteoporosis, neuromuscular coordination and several molecular biomarkers of aging.

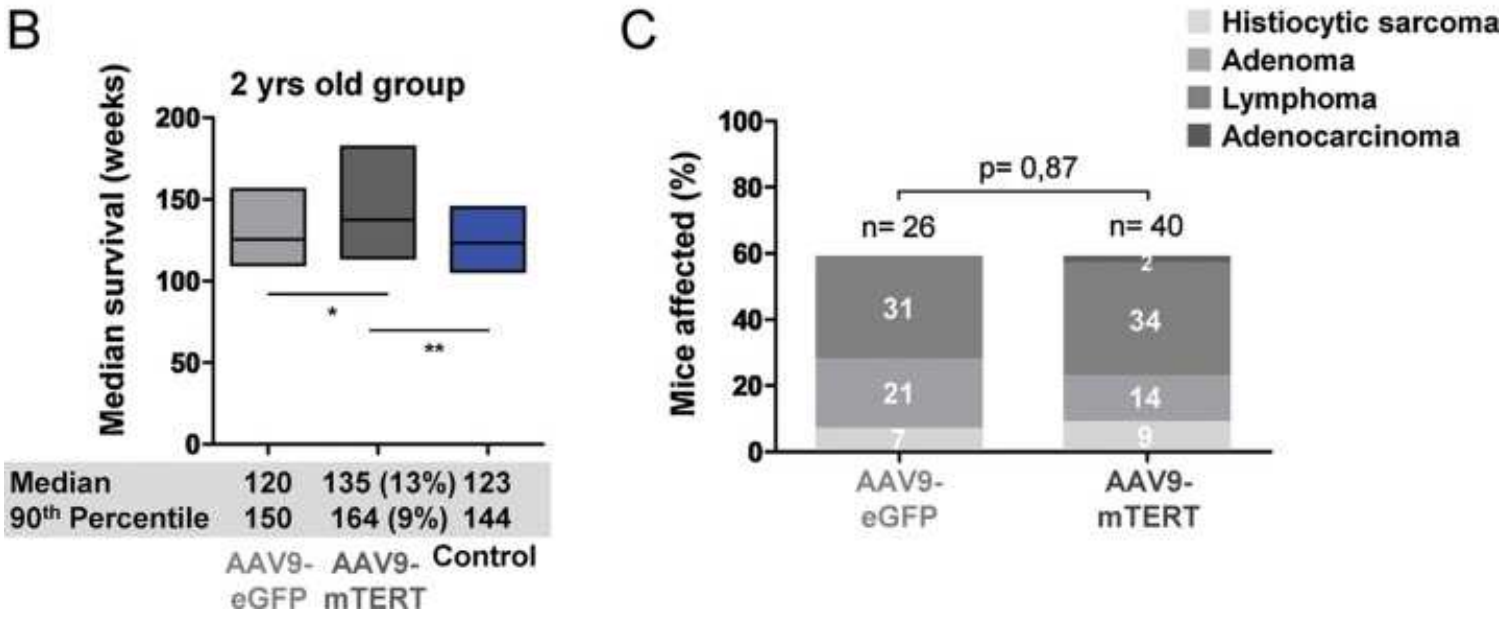
- telomerase-treated mice, both at 1-year and at 2-year of age, had an increase in median lifespan of 24 and 13%, respectively

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

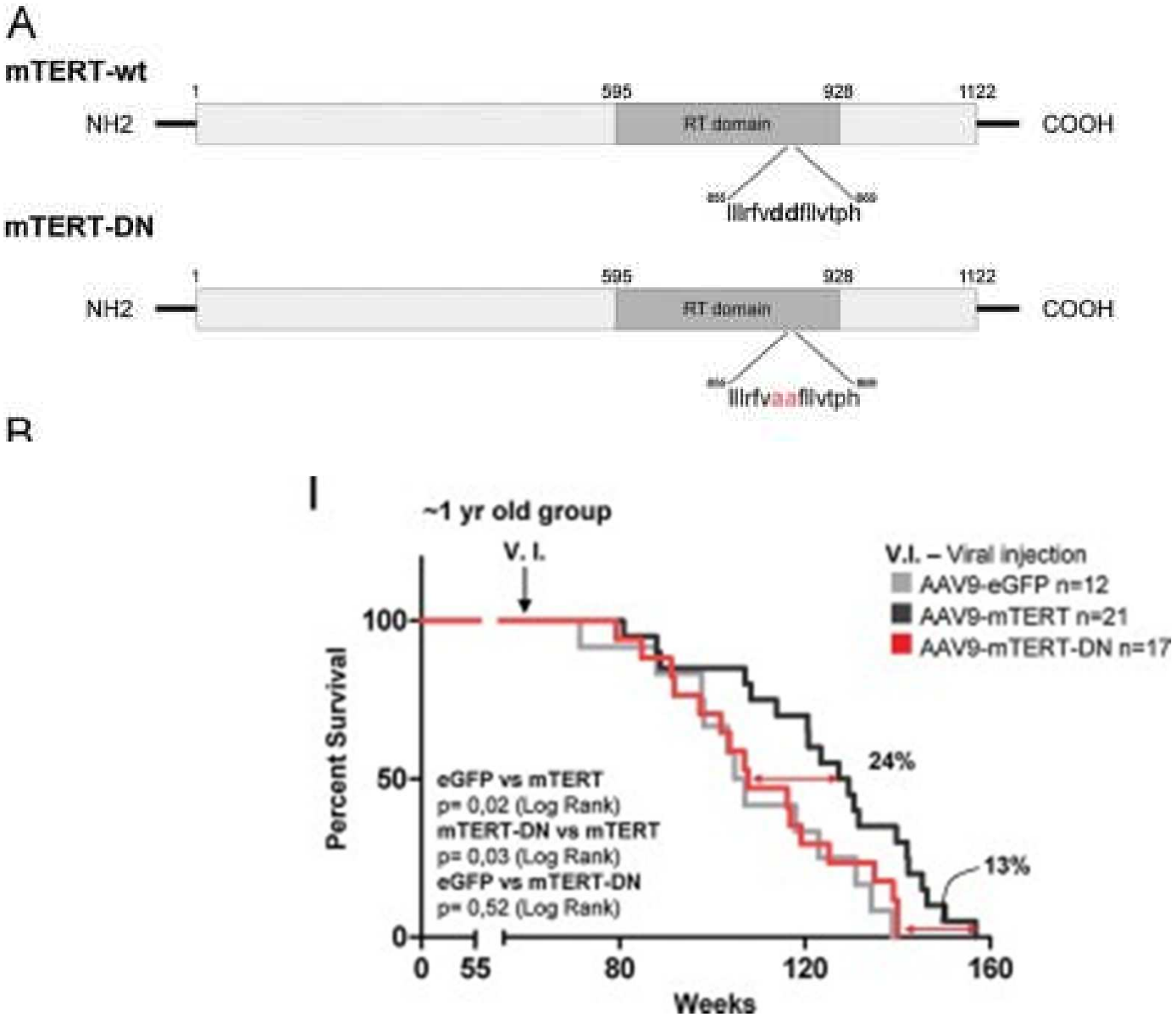
A


~1 yr old group
V. I.
V.I. – Viral injection
AAV9-eGFP n=12
AAV9-mTERT n=21
Control n=43
Percent Survival
100
50
0
eGFP vs mTERT
p= 0,02 (Log Rank)
Control vs mTERT
p= 0,008 (Log Rank)
eGFP vs Control
p= 0,51 (Log Rank)
24%
13%
0
55
80
120
160
Weeks


~2 yrs old group
V. I.
V.I. – Viral injection
AAV9-eGFP n=14
AAV9-mTERT n=23
Control n=29
Percent Survival
100
50
0
eGFP vs mTERT
p= 0,05 (Log Rank)
Control vs mTERT
p= 0,003 (Log Rank)
eGFP vs Control
p= 0,6 (Log Rank)
13%
20%
0
100
120
160
200
Weeks



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

Rejuvenation Res. 2016 Salvador L[1],

A Natural Product Telomerase Activator Lengthens Telomeres in Humans: A Randomized, Double Blind, and Placebo Controlled Study.

Abstract

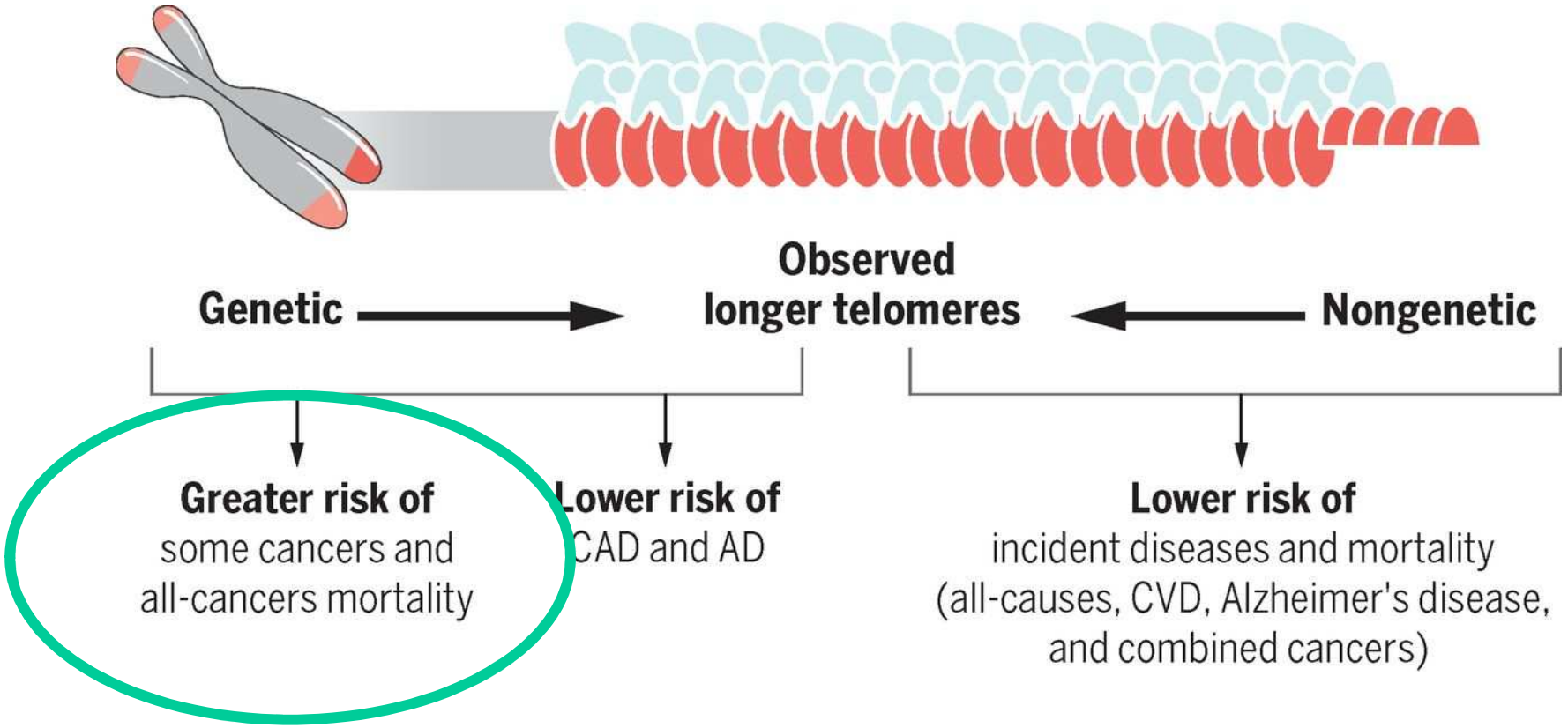
TA-65 is a dietary supplement based on an improved formulation of a small molecule telomerase activator that was discovered in a systematic screening of natural product extracts from traditional Chinese medicines.

This study summarizes the findings on telomere length (TL) changes from a randomized, double blind, placebo controlled study of TA-65 over a 1 year period.

The study was conducted on 117 relatively healthy cytomegalovirus-positive subjects aged 53-87 years old. Subjects taking the low dose of TA-65 (250 U) significantly increased TL over the 12 months period (530 ± 180 bp; $p = 0.005$), whereas subjects in the placebo group significantly lost TL (290 ± 100 bp; $p = 0.01$).

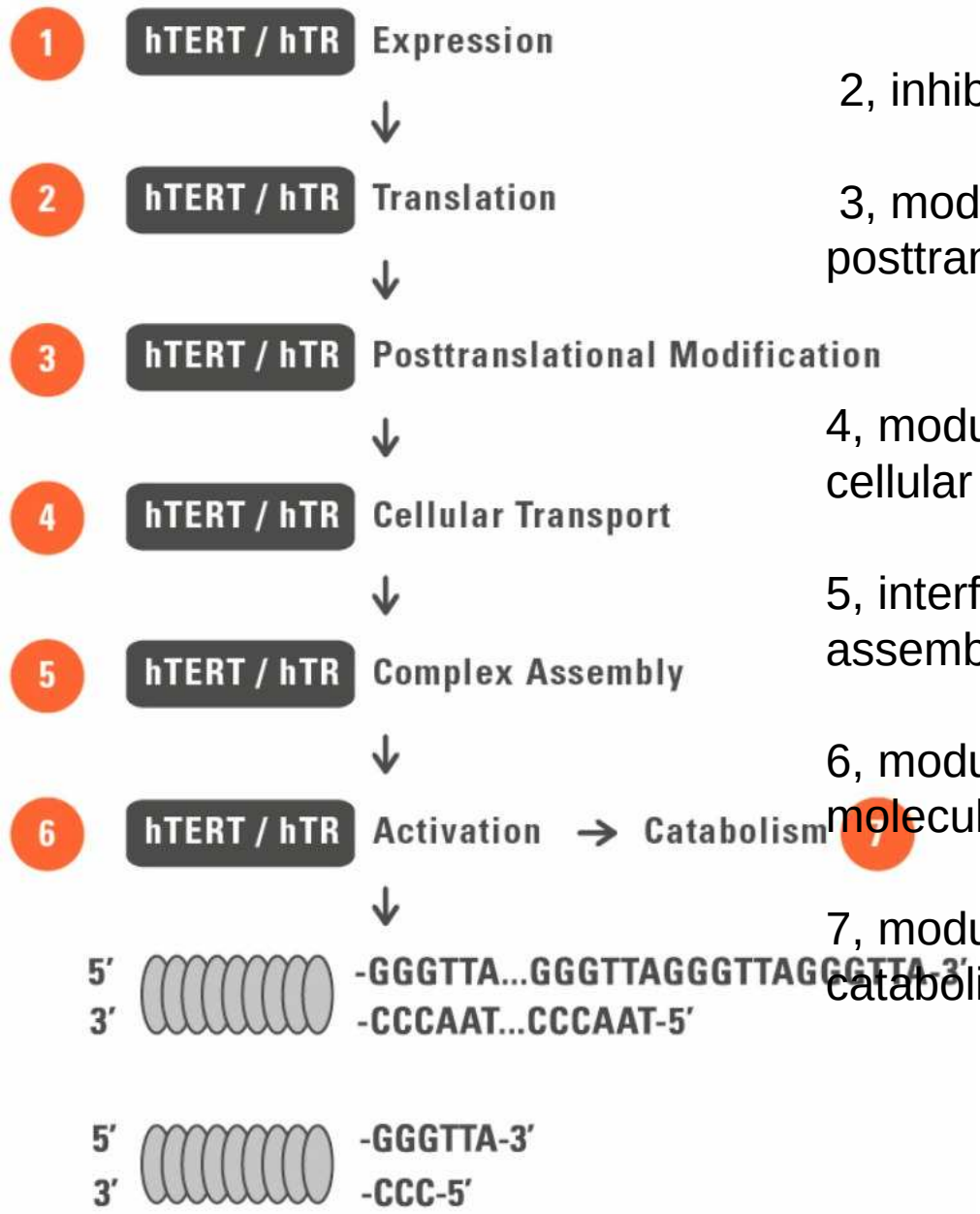
The findings suggest that TA-65 can lengthen telomeres in a statistically and possibly clinically significant manner.

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



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

Strategie anti tumorali basate sulla inibizione della telomerasi



1, inhibition/activation of gene transcription

2, inhibition/activation of protein synthesis;

3, modulation of activity by posttranslational modifications;

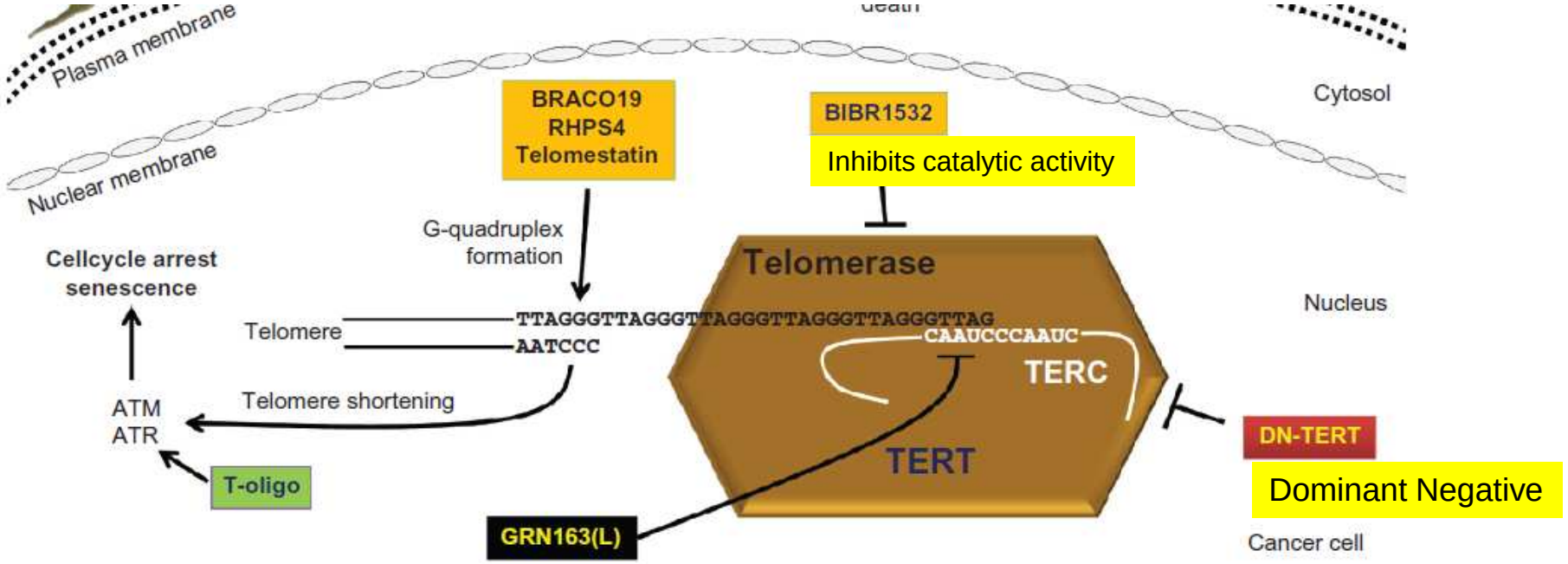
4, modulation of telomerase activity by cellular sequestration;

5, interference with telomerase complex assembly;

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

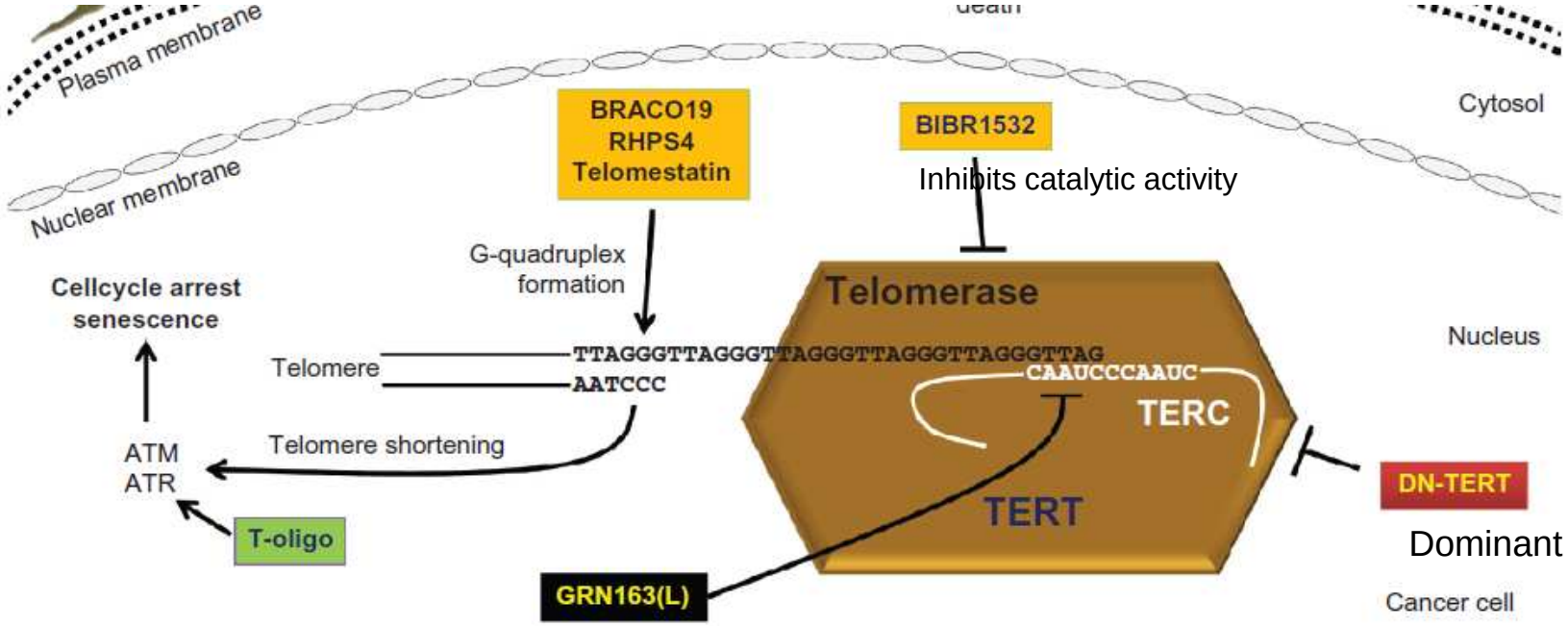
7, modulation of telomerase complex catabolism including vaccine therapy.

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



BRACO19, RHPS4, and telomestatin promote G-quadruplex formation

T-oligo mimics dysfunctional telomeres



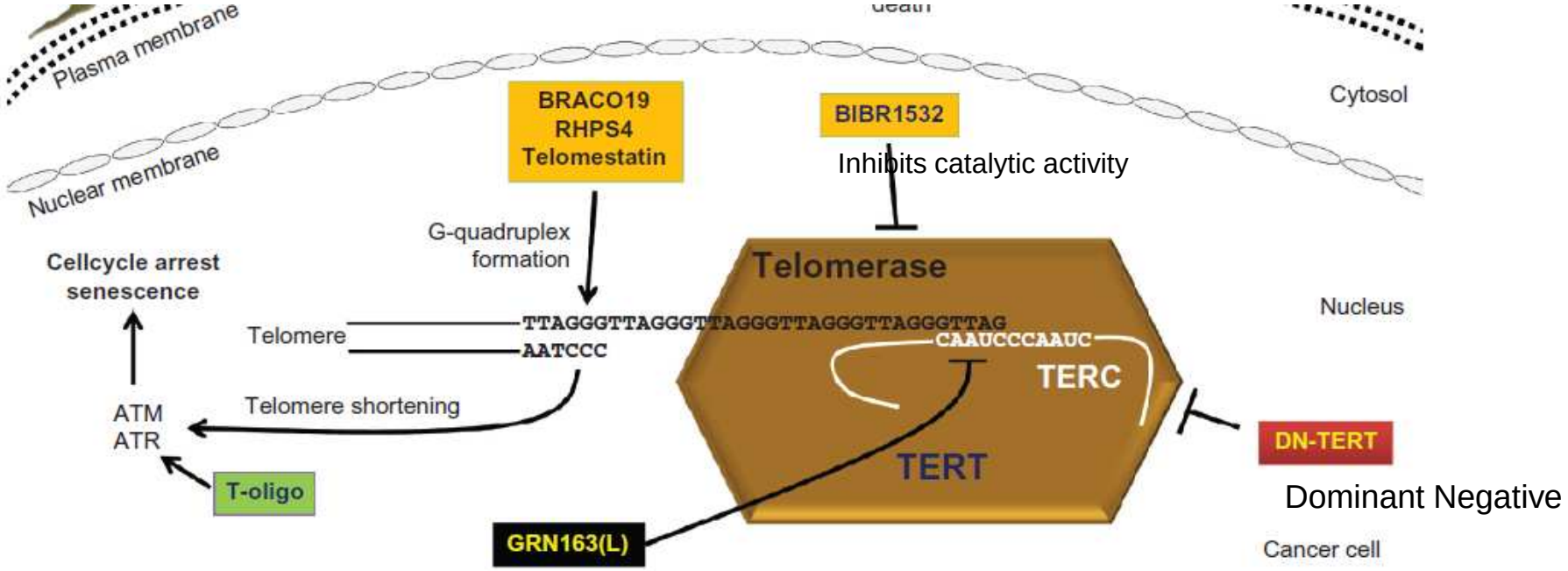
13-mer modified oligonucleotide, binds terC

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:

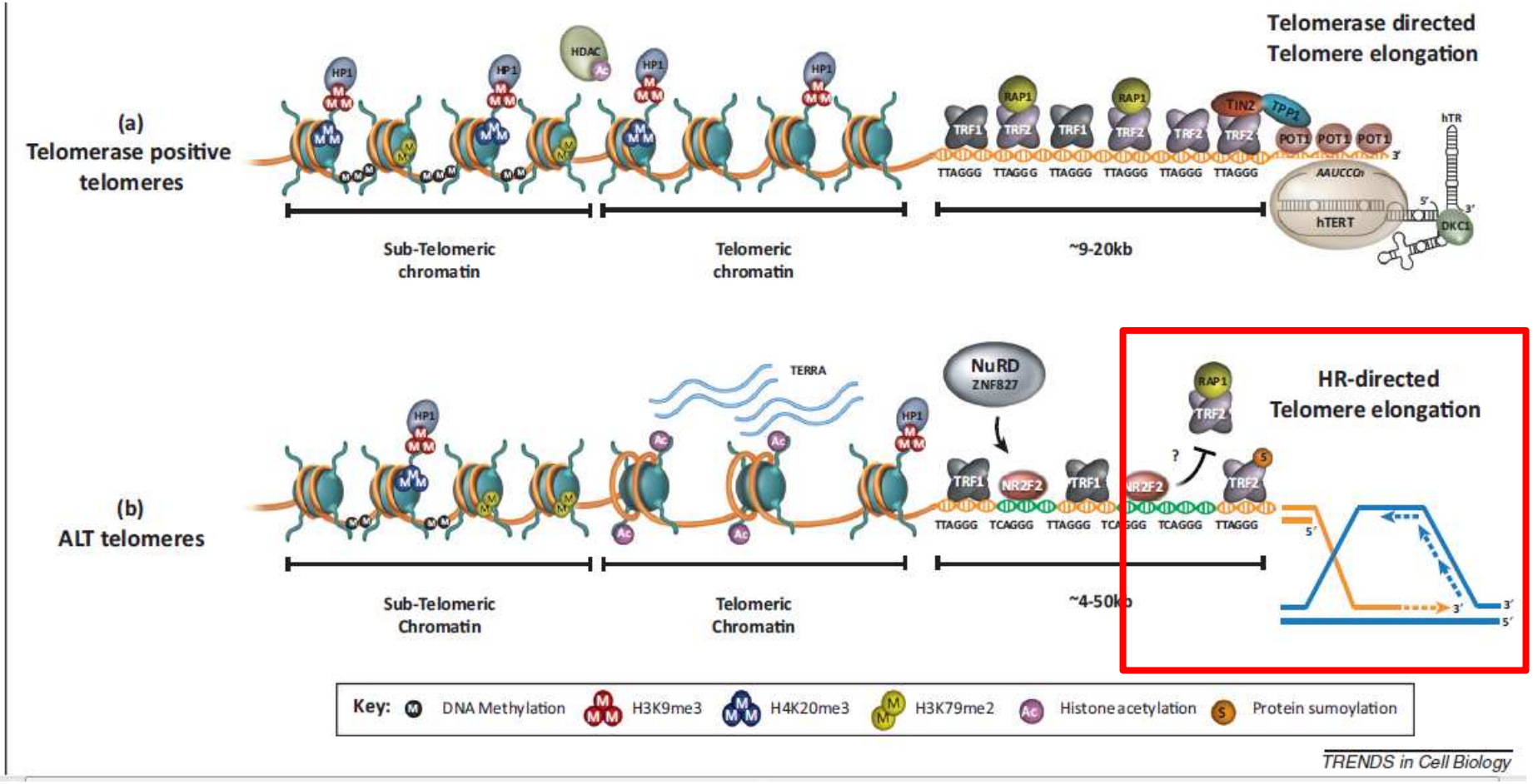
Three (7%) patients achieved a partial response and 13 (28%) disease stabilization. The median progression-free survival (PFS) and overall survival (OS) was 3.8 (range, 0.7-99.4) and 19.8 months (range, 0.7-99.4), respectively.

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.

Alternative lengthening of telomeres (ALT)


(a)
Telomerase positive telomeres
Telomerase directed Telomere elongation
HDAC
HP1
TRF1
TRF2
RAP1
TIN2
TPP1
POT1
hTR
hTERT
DKC1
TTAGGG
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.

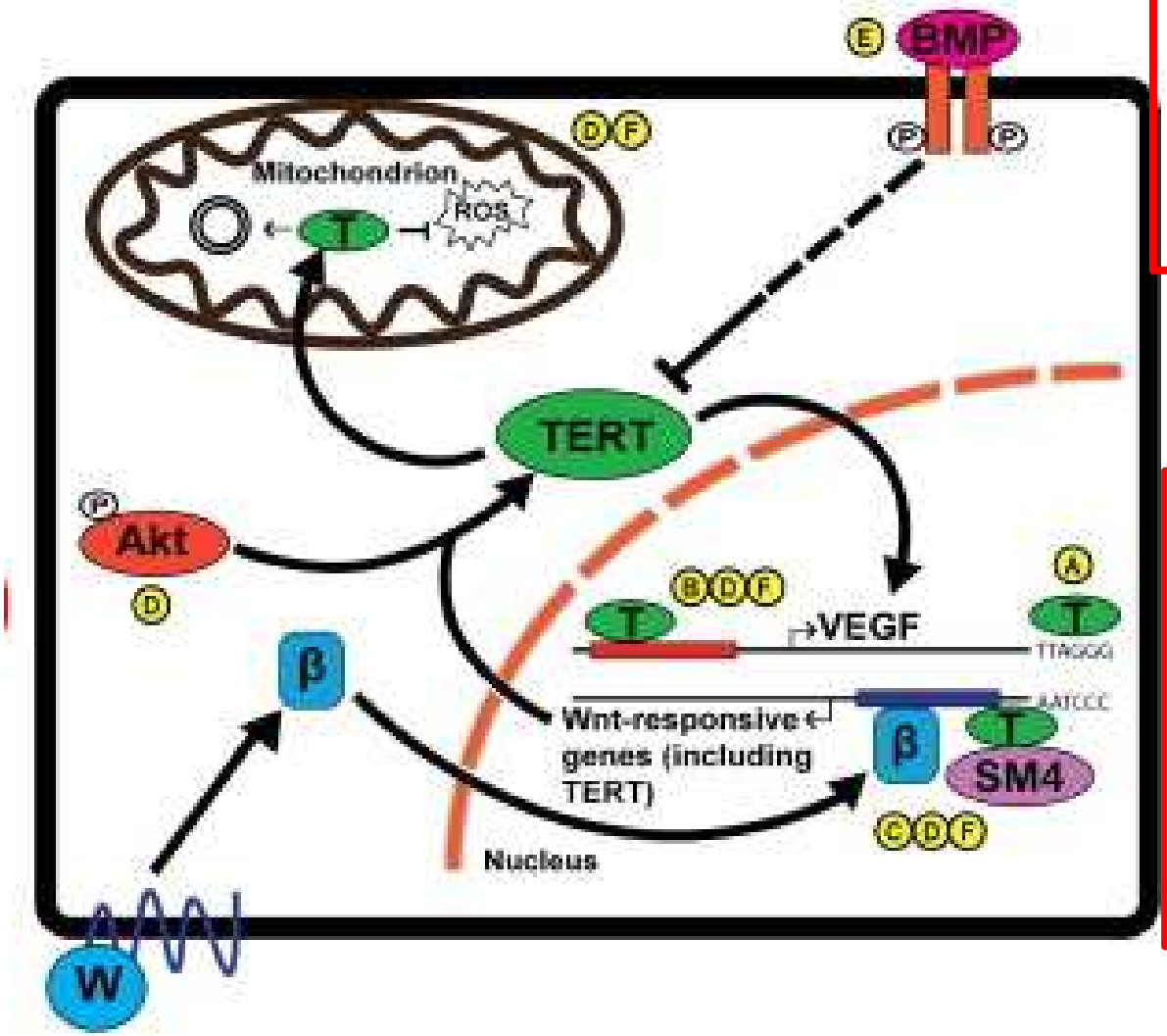
These features of ALT telomeres may lead to displacement of TRF2

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).



Under conditions of oxidative stress, TERT may translocate to mitochondria and regulate the expression of mitochondrial genes

TERT targets the expression of vascular endothelial growth factor (VEGF),

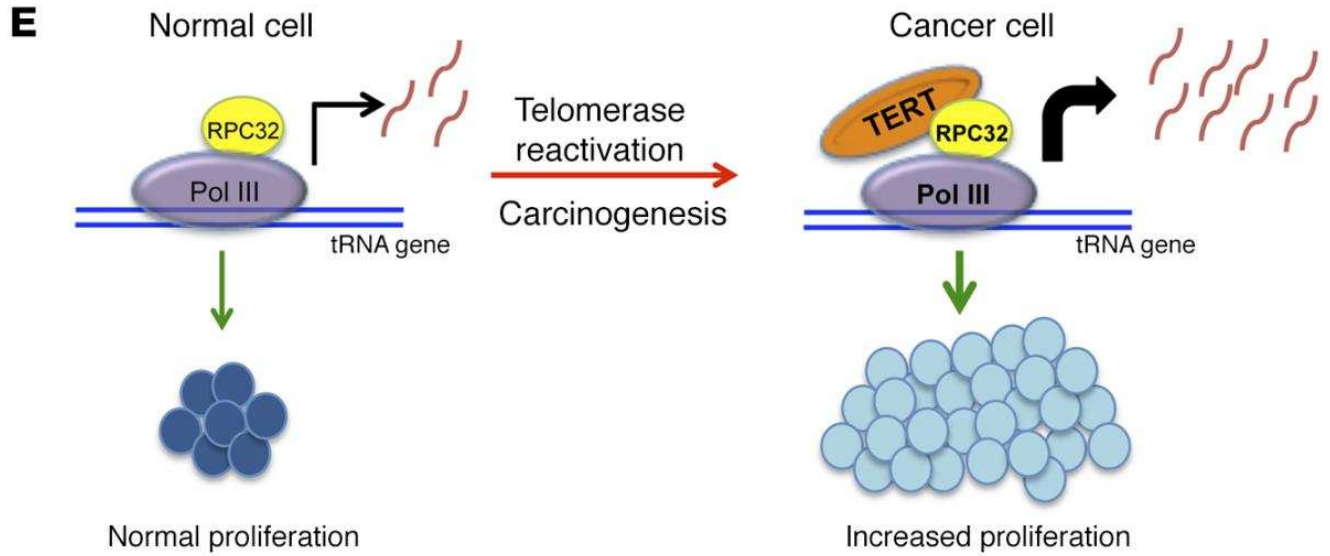
as well as Wnt:β-catenin axis genes (in association with chromatin remodeler SMARCA4 (SM4).

Extratelomeric functions of TERT

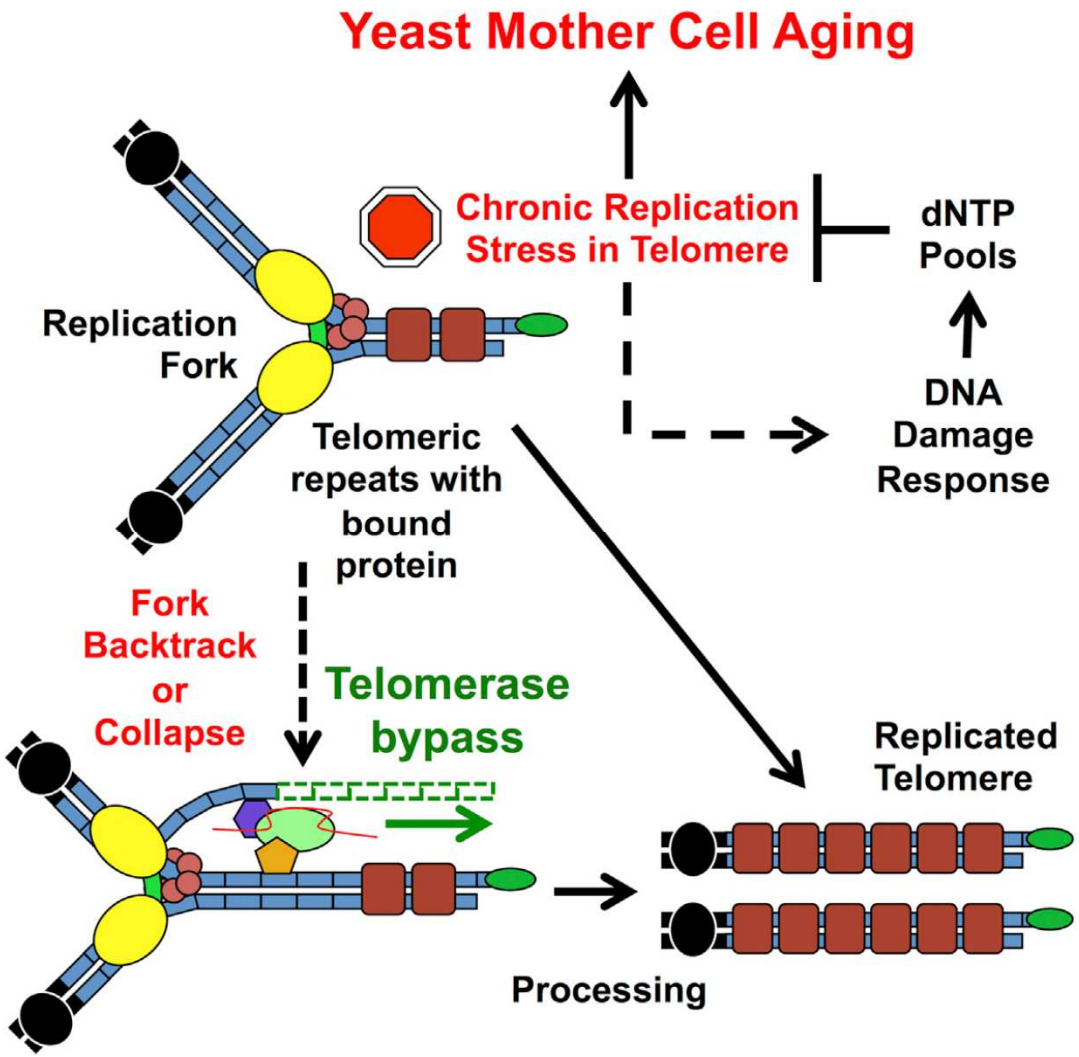
coupled to cancer cell dissemination and tumor formation.

Means to the ends: The role of telomeres and telomere processing machinery in metastasis
Biochimica et Biophysica Acta (BBA) - Reviews on Cancer, 2016

TERT associates with the RPC32 subunit of RNA pol III and augments tRNA expression in breast cancer

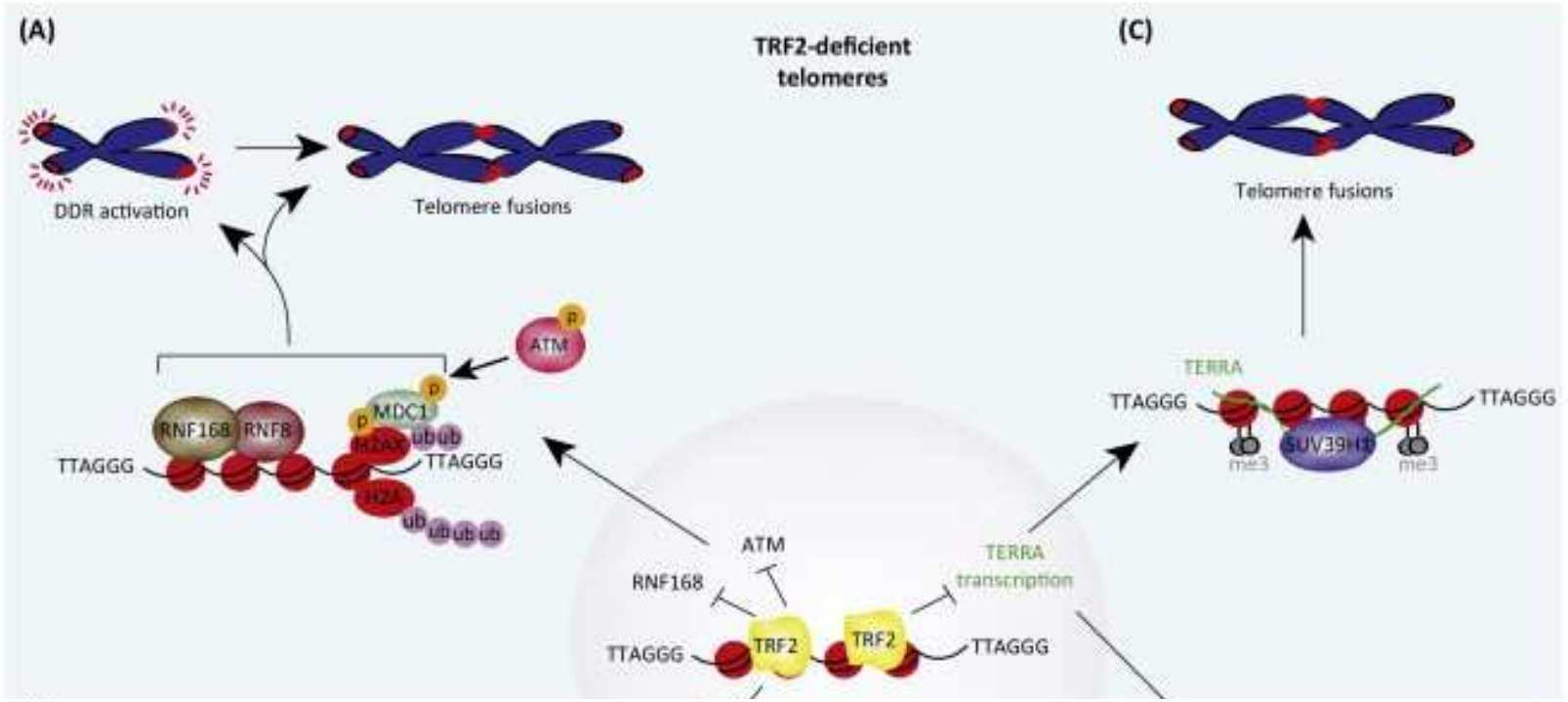


J Clin Invest. 2016;126(10):4045-4060



even early after early telomerase inactivation yeast mother cells show transient DNA damage response (DDR)

before critical telomere shortening, telomerase is continuously required to respond to transient DNA replication stress in mother cells and that a lack of telomerase accelerates otherwisenormal aging.



TERRA-mediated recruitment of SUV39H1 to TRF2-devoid telomeres leads to telomere fusions

TRF2 inhibits the recruitment to or activation at telomeres of ATM, RNF168 (E3 ubiquitin protein ligase) as well as the transcription of telomeric transcripts, TERRA (telomeric repeat-containing RNA).

Figure 4. TRF2 (telomere repeat-binding factor 2) inhibits end-processing pathways by regulating the chromatin environment at telomeres...

ARTICLE

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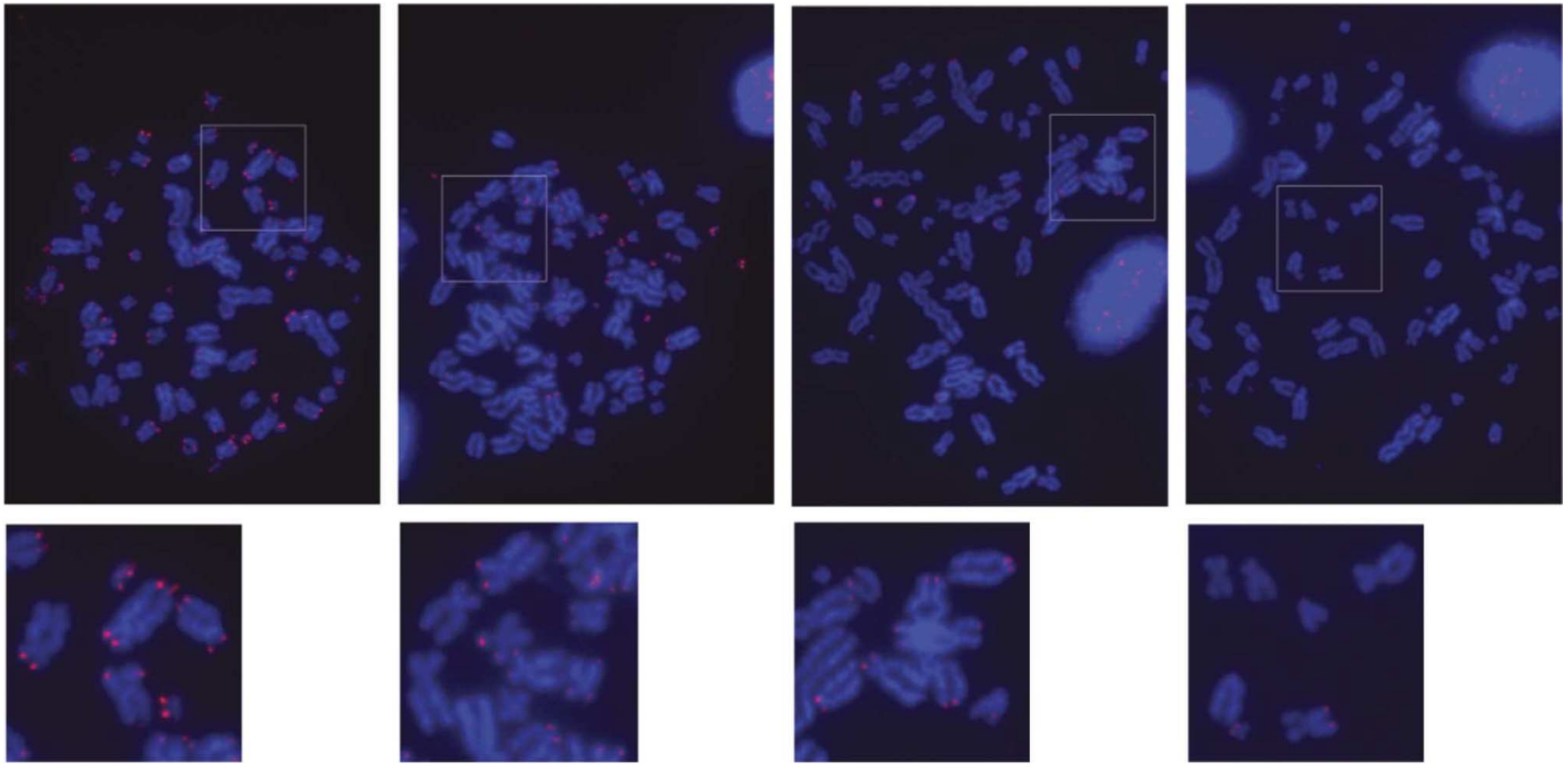
DOI: 10.1038/ncomms12534

Telomeric RNAs are essential to maintain telomeres

Juan José Montero[1,*], Isabel López de Silanes[1,*], Osvaldo Graña[2] & Maria A. Blasco[1]

Telomeres are transcribed generating long non-coding RNAs known as TERRA. Deciphering the role of TERRA has been one of the unsolved issues of telomere biology in the past decade. This has been, in part, due to lack of knowledge on the TERRA loci, thus preventing functional genetic studies. Here, we describe that long non-coding RNAs with TERRA features are transcribed from the human 20q and Xp subtelomeres. Deletion of the 20q locus by using the CRISPR-Cas9 technology causes a dramatic decrease in TERRA levels, while deletion of the Xp locus does not result in decreased TERRA levels. Strikingly, 20q-TERRA ablation leads to dramatic loss of telomere sequences and the induction of a massive DNA damage response. These findings identify chromosome 20q as a main TERRA locus in human cells and represent the first demonstration in any organism of the essential role of TERRA in the maintenance of telomeres.

WT KO for the Chr20q-TERRA locus



Q-FISH images

Deletion of the 20q-TERRA locus decreases telomere length