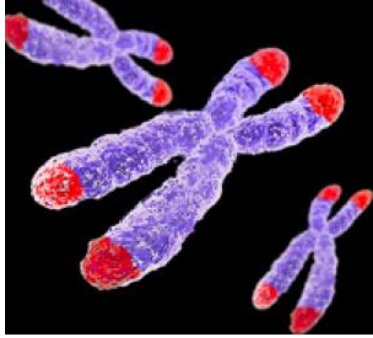


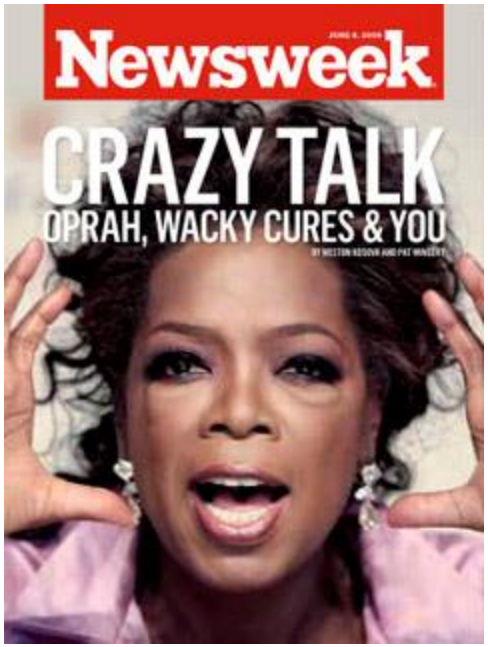
Telomere Talk: 9 Years of Researching and Monitoring Patients on TA-65® ®

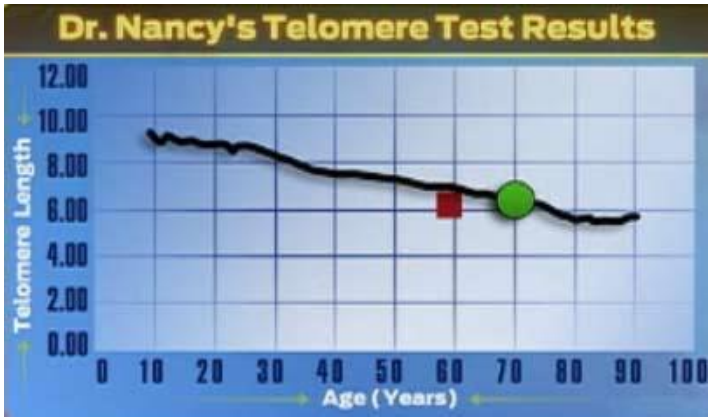
Joseph M. Raffaele, MD

Raffaele Medical

CEO PhysioAge Systems

Telomeres and TA-65® are in the News!





HOW TO AGE BACKWARD

The results are in: Take these steps to start turning back the clock now.

A PILL WITH PROMISE Of course, anytime there's an effective diet, there's sure to be a pill promising the same—or better—results. Enter **TA-65**, a nutritional supplement that proponents claim can not only slow telomere shortening but actually lengthen existing ones. Available without a prescription, TA-65 is derived from astragalus root extract, a powerful antioxidant, and is said to work by activating the enzyme telomerase, which counteracts telomere shortening.

Joseph Raffaele, an internist and cofounder of PhysioAge Medical Group in New York, has studied TA-65 since 2007 and says his research shows that people who take TA-65 have "a decrease in blood pressure, cholesterol, and fasting glucose as well as an increase in bone density." Ron Rothenberg, founder and medical director of the California HealthSpan Institute in

Lifespan ≠ Healthspan

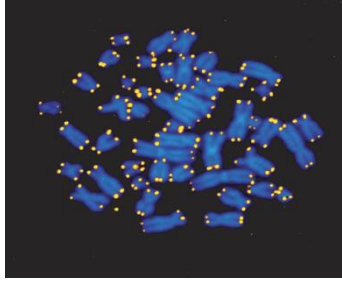
Common Questions Asked about TA-65®

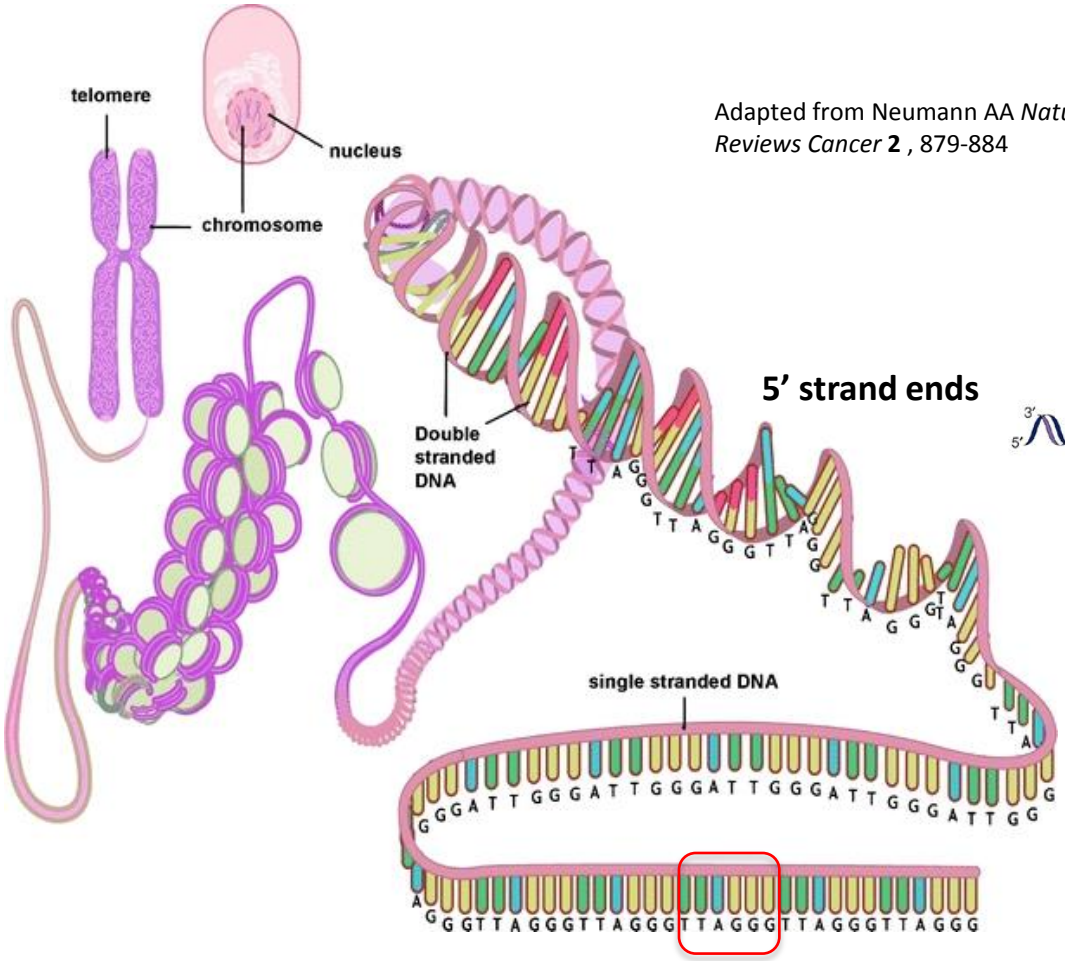
- What are Telomeres?
- What do Telomeres do?
- Why are they important?
- What can be done to keep them healthy?
- What is TA-65® ?
- How do I know if I need to take it?
- What will I feel when I take it?
- What research supports its effectiveness?
- How will I know that it is working?
- Will it increase my risk of cancer?
- How much should I take and for how long?

Bona Fides

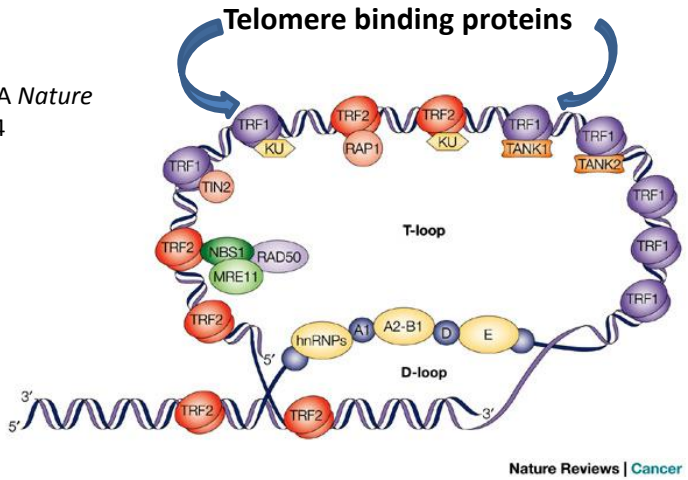
- Practicing age management medicine for 20 years.
- Original MD to work with TA Sciences on first two observational studies on initial cohort of humans on TA-65® .
- Co-author on first two papers describing these results.
- Monitoring hundreds of patients on TA-65® for the past 7 years
- Over 1500 telomere length measurements discussed with patients.
- Recently co-authored 1 year RCT with TA-65

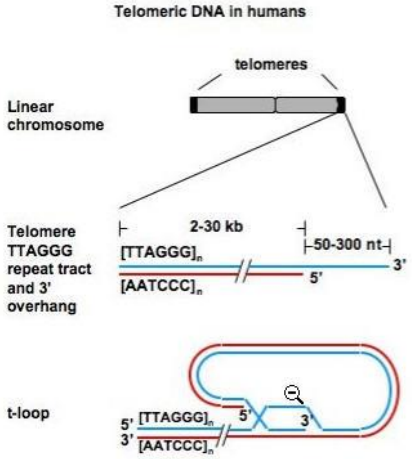
What Are Telomeres?





Adapted from Neumann AA *Nature Reviews Cancer* **2** , 879-884





Telomere caps

Adapted from Oeseburg Eur J Physiol (2010) 459:259–268

What do Telomeres do?

- Serve as chromosome end-caps to protect the integrity of our genes.
- Keep chromosomes from degrading to prevent fusion and massive genomic instability.
- Allow cells to replicate (cells cannot divide when telomeres get too short)

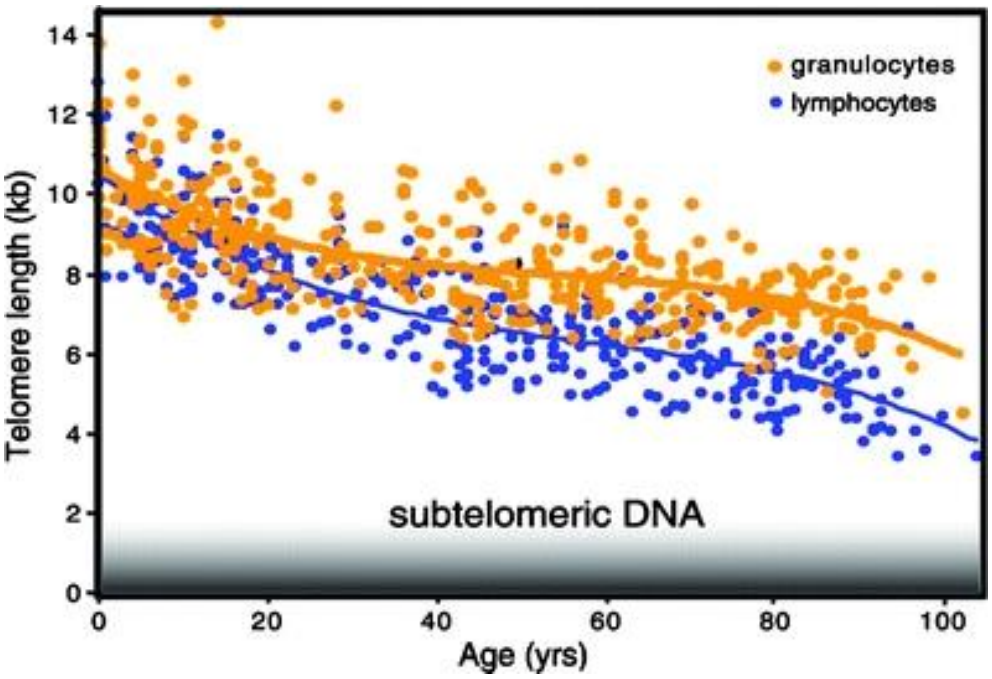
Bottom Line: Telomeres protect cells from DNA mutations, senescence and death.

Why Are Telomeres Important?
Short Answer for Some Patients

- Because they get shorter with age, diseases, and unhealthy activities/lifestyle/diet
- This causes trouble!
- You get old faster and die younger!
- They are a molecular clock of your rate of aging
- Indicator of your longevity potential
  - **A Biological 401K**

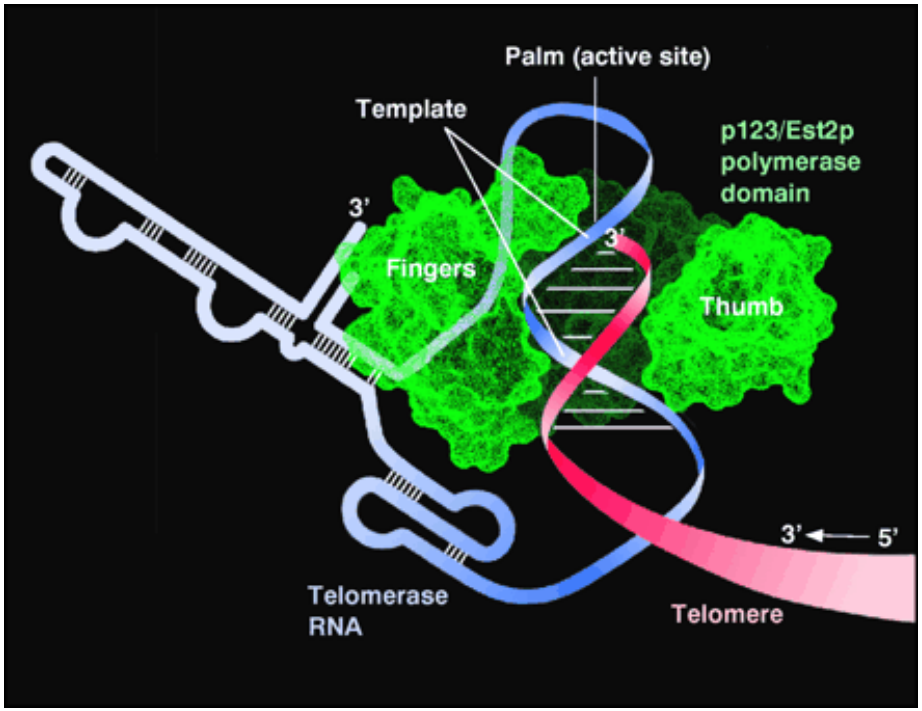
Telomeres Basics: Age-associated Shortening

- **Aging: lose 30-60 base-pairs per year**
  - **Cell division**:
    - **Lose 100 base-pairs per division**
    - Mostly in stem cells and highly proliferative tissues (BM, WBC, gut, skin, etc.)
  - **Oxidative stress**:
    - Increases loss with each division
    - GGG portion of TTAGGG repeat very susceptible to free radicals
  - **End-replication problem**:
    - Cannot fully replicate lagging (3') strand
    - Need Telomerase



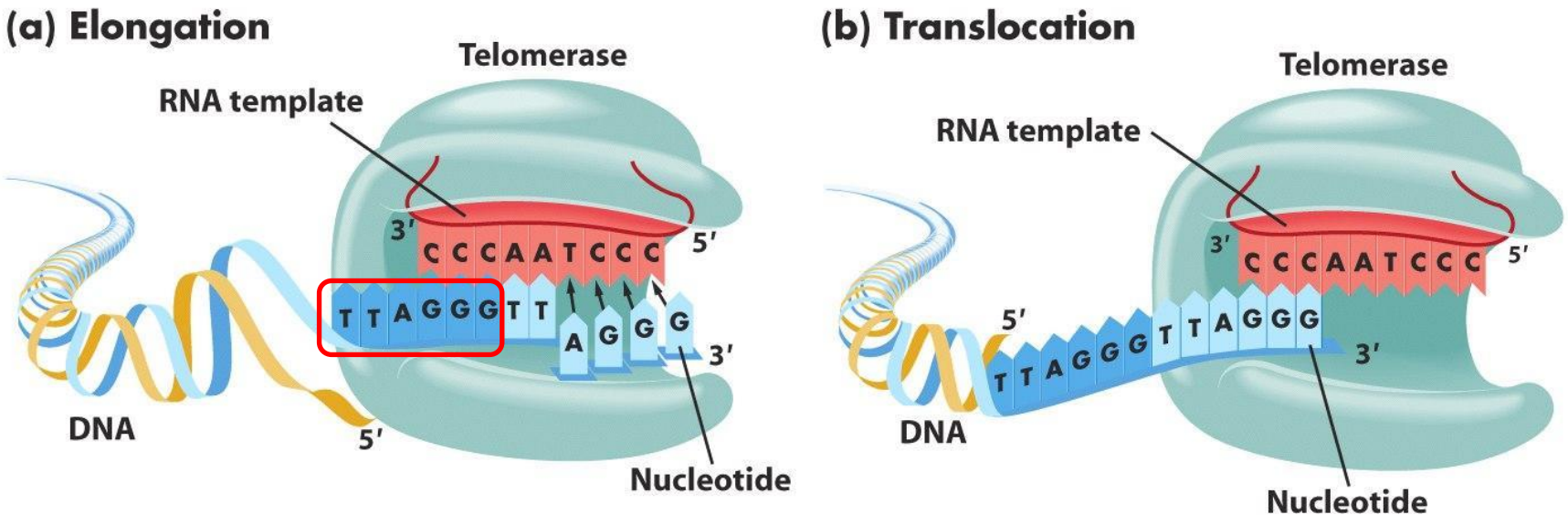
Aubert and Lansdorp 2008 *Physiol Rev*

Telomerase Basics



- **Discovered by Elizabeth Blackburn** in 1980—Nobel prize awarded in 2009
- **Structure**: Two components
  - hTERT: **h**uman **te**lomerase **r**everse **t**ranscriptase, the catalytic component
  - TERC: **te**lomerase **R**NA template **c**omponent
- **Function**: **Lengthen telomeres**
- **Activation**:
  - Very active during embryogenesis
  - Repressed before birth
  - Repressed during adult life in most tissues except those with rapid turnover—immune, gut, skin.
  - **Adult activity insufficient to maintain telomere length**
  - **Birth marks beginning of slow telomere erosion**

Telomerase Basics: How it works



Telomere Length Determinants

Inherited Length

- “Telotype” inherited trait
- Heritability rate 0.36-0.84
- Paternal age is factor: older men pass on longer telomeres

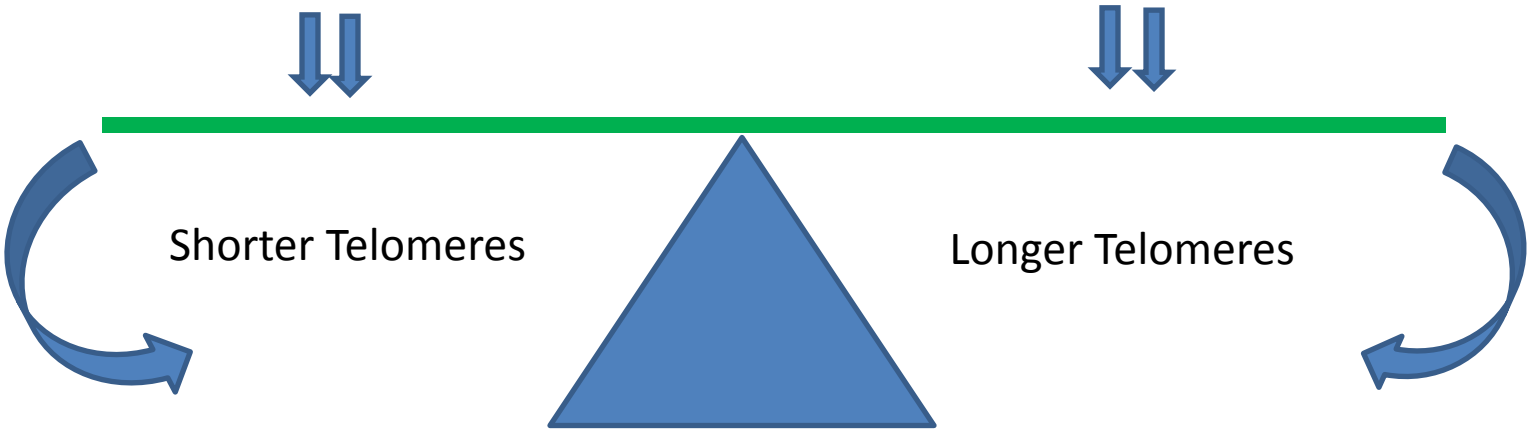
Attrition Rate

- Telomerase activity
- Proliferative activity
- Oxidative stress

Telomere Attrition determined by balance between loss and telomerase activity

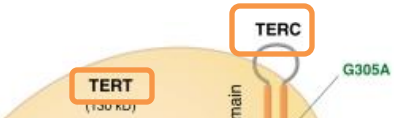
- Proliferative activity
- Oxidative stress
- Inflammation

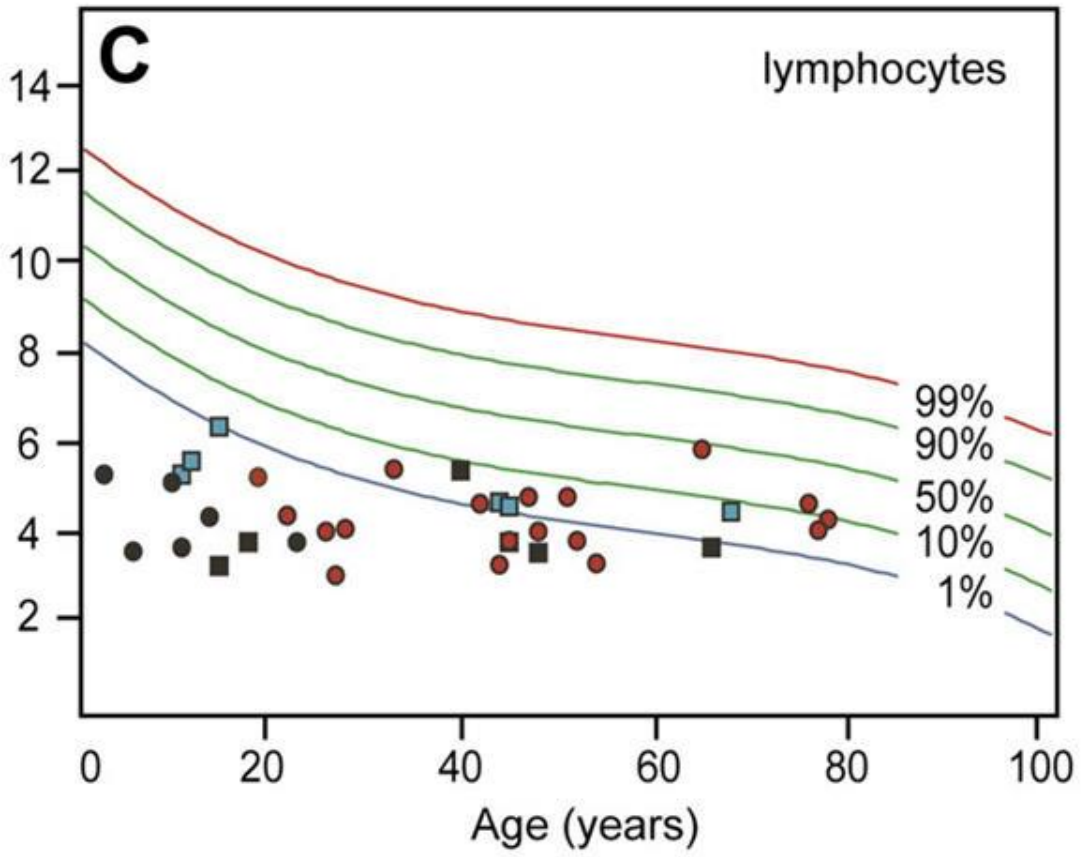
Telomerase activity



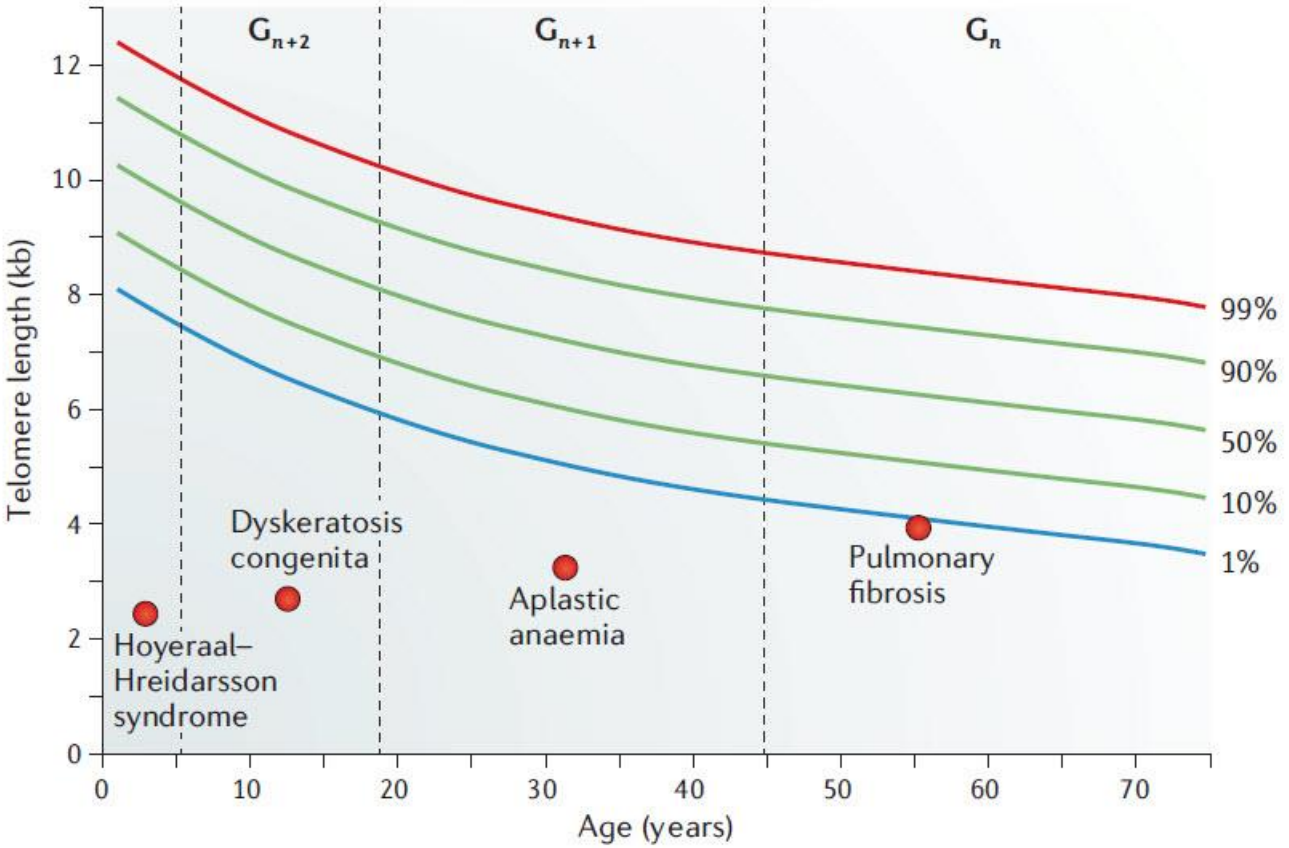
Genetic Telomere Diseases: Telomeropathies

- Genetic disorders with mutations in the telomerase complex: **Dyskerin, TERT, TE**
  - Dyskeratosis congenita
    - Abnormal pigmentation, nail dystro stature, pulmonary and hepatic fibr hypogonadism, bone marrow failur malignancies, premature death
  - Aplastic anemia
    - Shortened telomeres and prer death
    - 10% idiopathic AA pts have TE mutations
  - Idiopathic pulmonary fibros
    - Premature death from fibrosis
    - Short telomeres a risk factor (with TERT/TERC mutations)
- Extremely short telomere



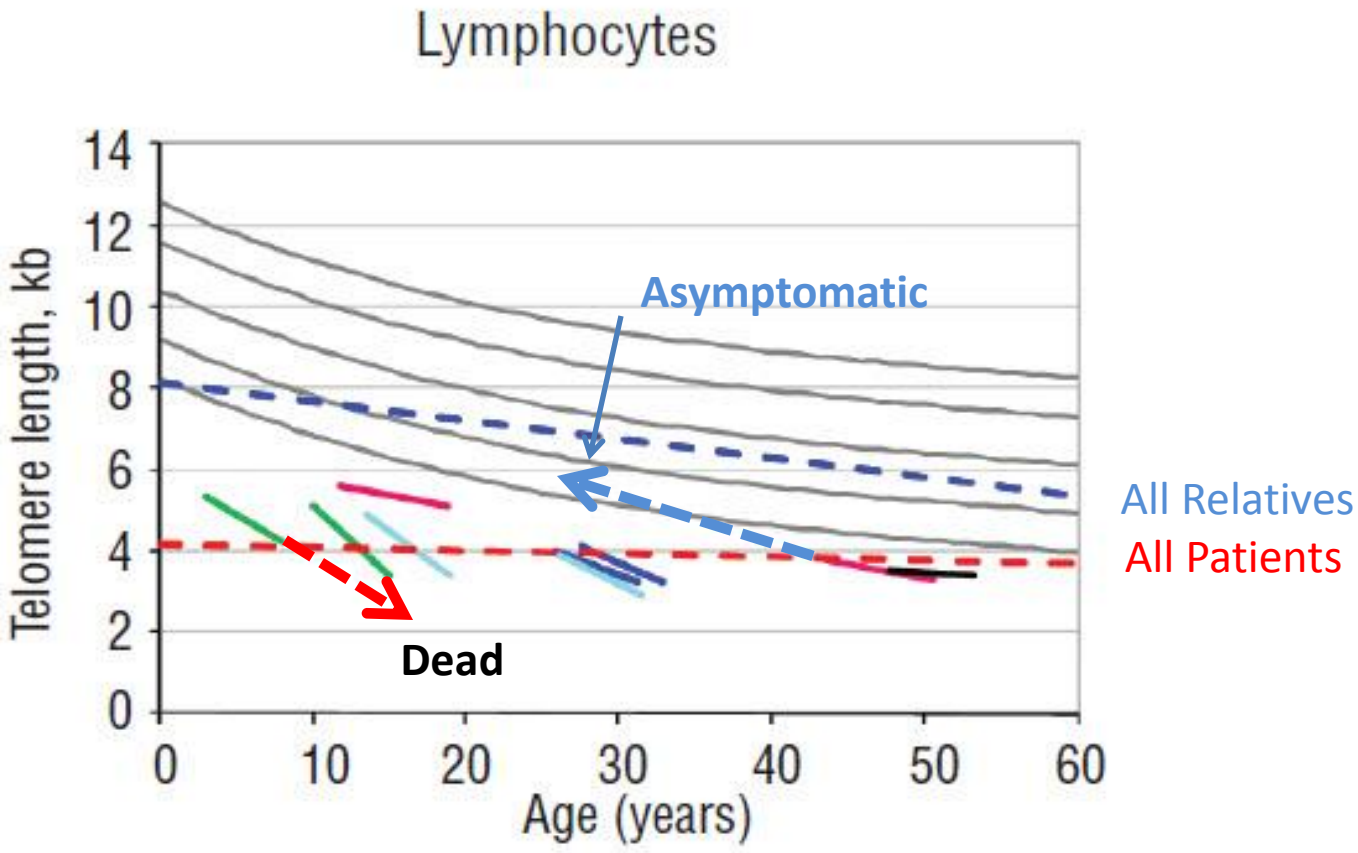


Genetic Anticipation of Age of Onset and Clinical Manifestations



Adapted from Armanios, M, Blackburn EH. The Telomere Syndromes, Nature Reviews Genetics, 2012:13:693-704

Telomere Attrition Rate in Telomeropathies



Adapted from Alter BP 2012 *Haematologica; 97(3)*

Telomere Syndrome Diseases

- Cardiovascular
- Cancer
- COPD
- Alzheimer's
- Degenerative Disc Disease
- Osteoarthritis
- Rheumatoid Arthritis
- Osteoporosis
- General Immunity
- Skin Aging
- Macular Degeneration
- Liver Cirrhosis

- Muscular Dystrophy
- Cell & Tissue Transplants
- AIDS
- Dyskeratosis Congenita
- Aplastic Anemia
- Idiopathic Pulmonary Fibrosis
- Cri du Chat syndrome
- Down's Syndrome
- Fanconi's Anemia
- Tuberous Sclerosis
- Progeria
- Werner's Syndrome
- **And, Aging Itself?**

Armanios, M, Blackburn EH. The Telomere Syndromes, Nature Reviews Genetics, 2012:13:693-704

Do You Know Your Cholesterol Level?

Are you worried about it?

thebmj

CrossMark

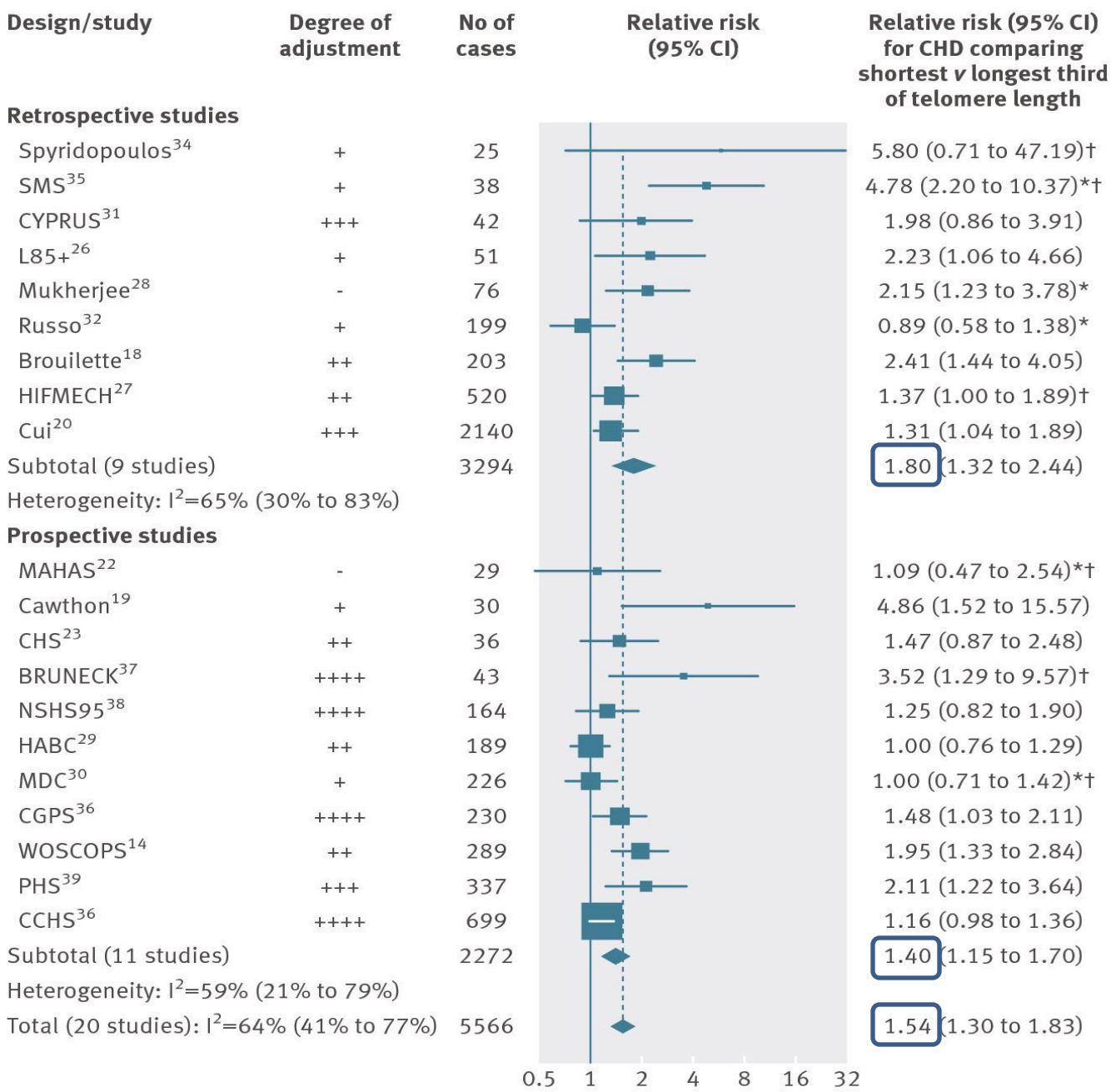
BMJ 2014;349:g4227 doi: 10.1136/bmj.g4227 (Published 8 July 2014)

RESEARCH

Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis

Philip C Haycock *postdoctoral research assistant*[1 2], Emma E Heydon *doctoral candidate*[1], Stephen Kaptoge *senior research associate*[1], Adam S Butterworth *university lecturer*[1], Alex Thompson *senior epidemiologist*[1 3], Peter Willeit *research associate*[1 4]

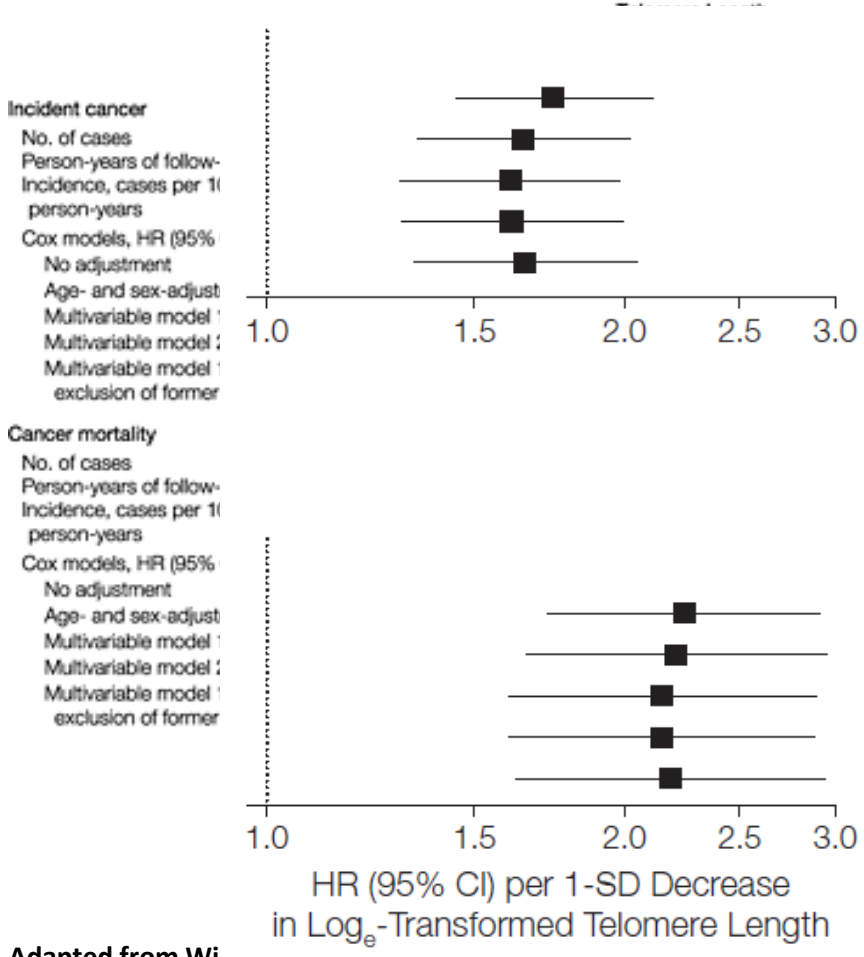
[1]Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, Institute of Public Health, University of Cambridge, Strangeways Research Laboratory, Cambridge, UK; [2]Medical Research Council Integrative Epidemiology Unit, School of Social and Community Medicine, University of Bristol, Bristol, UK; [3]Roche, Welwyn Garden City, UK; [4]Department of Neurology, Innsbruck Medical University, Austria

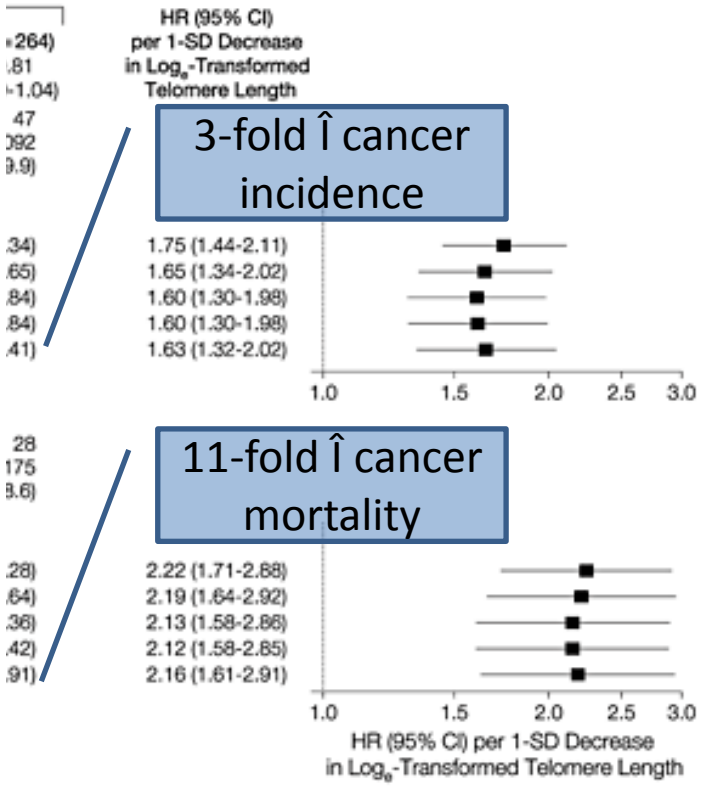

Design/study
Degree of adjustment
No of cases
Relative risk (95% CI)
Relative risk (95% CI) for CHD comparing shortest v longest third of telomere length
Retrospective studies
Spyridopoulos[34] + 25 5.80 (0.71 to 47.19)†
SMS[35] + 38 4.78 (2.20 to 10.37)*†
CYPRUS[31] +++ 42 1.98 (0.86 to 3.91)
L85+[26] + 51 2.23 (1.06 to 4.66)
Mukherjee[28] - 76 2.15 (1.23 to 3.78)*
Russo[32] + 199 0.89 (0.58 to 1.38)*
Brouilette[18] ++ 203 2.41 (1.44 to 4.05)
HIFMECH[27] ++ 520 1.37 (1.00 to 1.89)†
Cui[20] +++ 2140 1.31 (1.04 to 1.89)
Subtotal (9 studies) 3294 1.80 (1.32 to 2.44)
Heterogeneity: I²=65% (30% to 83%)
Prospective studies
MAHAS[22] - 29 1.09 (0.47 to 2.54)*†
Cawthon[19] + 30 4.86 (1.52 to 15.57)
CHS[23] ++ 36 1.47 (0.87 to 2.48)
BRUNECK[37] ++++ 43 3.52 (1.29 to 9.57)†
NSHS95[38] ++++ 164 1.25 (0.82 to 1.90)
HABC[29] ++ 189 1.00 (0.76 to 1.29)
MDC[30] + 226 1.00 (0.71 to 1.42)*†
CGPS[36] ++++ 230 1.48 (1.03 to 2.11)
WOSCOPS[14] ++ 289 1.95 (1.33 to 2.84)
PHS[39] +++ 337 2.11 (1.22 to 3.64)
CCHS[36] ++++ 699 1.16 (0.98 to 1.36)
Subtotal (11 studies) 2272 1.40 (1.15 to 1.70)
Heterogeneity: I²=59% (21% to 79%)
Total (20 studies): I²=64% (41% to 77%) 5566 1.54 (1.30 to 1.83)
0.5
1
2
4
8
16
32

Are Your Worried About Getting Cancer?

Have you had all your routine cancer screening tests?

Association of Telomere Length With Cancer Incidence and Mortality Between 1995 and 2005 in the Bruneck Study (N = 787)

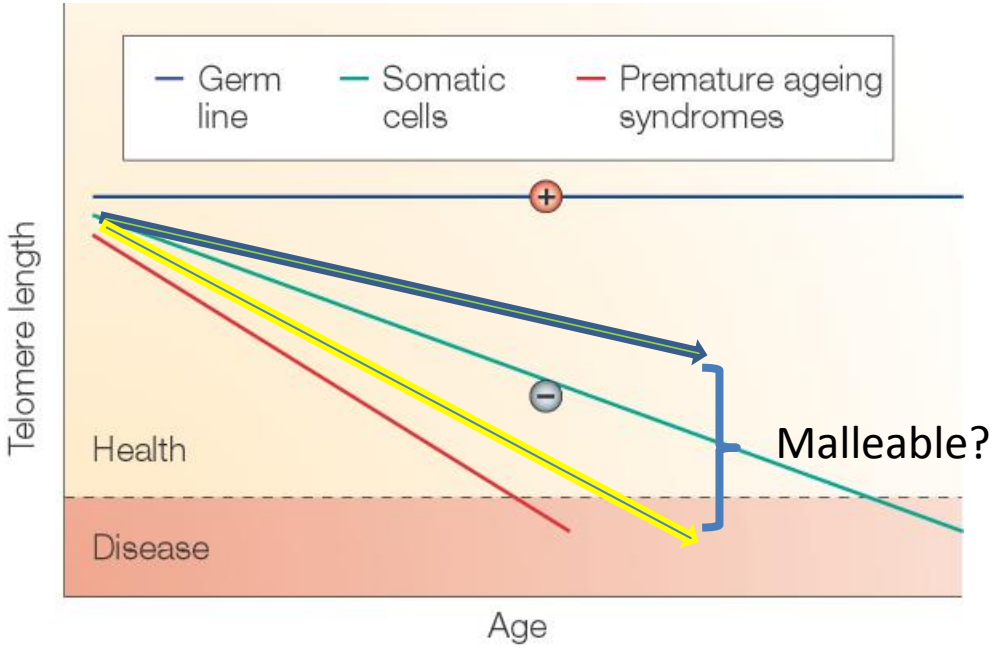




Adapted from Wi

JAMA

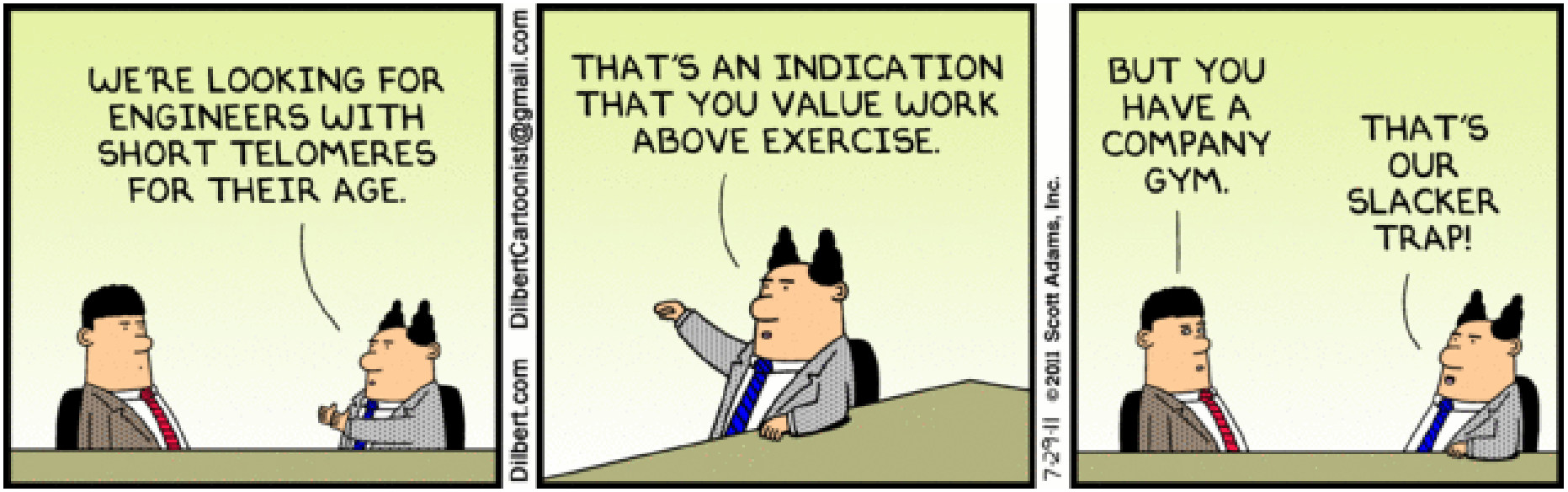
Differing telomere attrition rates



Adapted from
Nature Reviews | Genetics

How Do I Keep My Telomeres Healthy?

- **Lifestyle**
  - Stress reduction Epel ES 2004 *PNAS*
  - Exercise
    - Mitigates effect of perceived stress Puterman E 2010 *PloS One*
  - Weight loss Valdez AM 2005 *Lancet*
  - Smoking cessation
  - Avoidance of CMV
- **Diet**
  - Omega-3 FA intake Farzaneh-Far R 2010 *JAMA*
  - Low fat intake
- **Supplements**
  - Vitamin D Richards BJ 2007 *Am J Clin Nutr*
  - Antioxidants
- **Hormones**
  - Estradiol increases telomerase activation (TA) Calado RT 2009 *Blood*
  - Cortisol decreases TA Choi J 2008 *Brain Behav Immun*
  - IGF-1 increase TA Moverare-Skrtic S 2009 *JCEM*


WE'RE LOOKING FOR ENGINEERS WITH SHORT TELOMERES FOR THEIR AGE.
THAT'S AN INDICATION THAT YOU VALUE WORK ABOVE EXERCISE.
BUT YOU HAVE A COMPANY GYM.
THAT'S OUR SLACKER TRAP!
Dilbert.com DilbertCartoonist@gmail.com
7-29-11 ©2011 Scott Adams, Inc.

First Age Reversal in a Mammal

Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice

Mariela Jaskelioff[1], Florian L. Muller[1], Ji-Hye Paik[1], Emily Thomas[1], Shan Jiang[1], Andrew C. Adams[2], Ergun Sahin[1], Maria Kost-Alimova[1], Alexei Protopopov[1], Juan Cadiñanos[1], James W. Horner[1], Eleftheria Maratos-Flier[2] & Ronald A. DePinho[1]

- Telomerase Activation was used to change old mice back to young adults.
- Brain, spleen and reproductive organs were all rejuvenated;
- Resulting in increased neurons and new viable sperm cells.
- Sense of smell returned.
- None of the mice developed cancer.

2011 DePinho et al

The FASEB Journal article fj.14-259531. Published online January 22, 2015.

The FASEB Journal • Research Communication

Transient delivery of modified mRNA encoding TERT rapidly extends telomeres in human cells

John Ramunas,[*,1] Eduard Yakubov,[†,1,2] Jennifer J. Brady,[*] Stéphane Y. Corbel,[*] Colin Holbrook,[*] Moritz Brandt,[*] Jonathan Stein,[‡] Juan G. Santiago,[§] John P. Cooke,[†,2] and Helen M. Blau[*,3]

[*]Baxter Laboratory for Stem Cell Biology, Department of Microbiology and Immunology, Institute for Stem Cell Biology and Regenerative Medicine, Clinical Sciences Research Center, Stanford University School of Medicine, Stanford, California, USA; [†]Falk Cardiovascular Research Center, Stanford University School of Medicine, Stanford, California, USA; [‡]SpectraCell Laboratories, Inc., Houston, Texas, USA; and [§]Department of Mechanical Engineering, Stanford University, Stanford, California, USA

Gene Therapy, Knockouts, and RNA therapies: Not Ready for Prime Time

An orally absorbable molecule that can transiently activate telomerase

What is TA-65®?

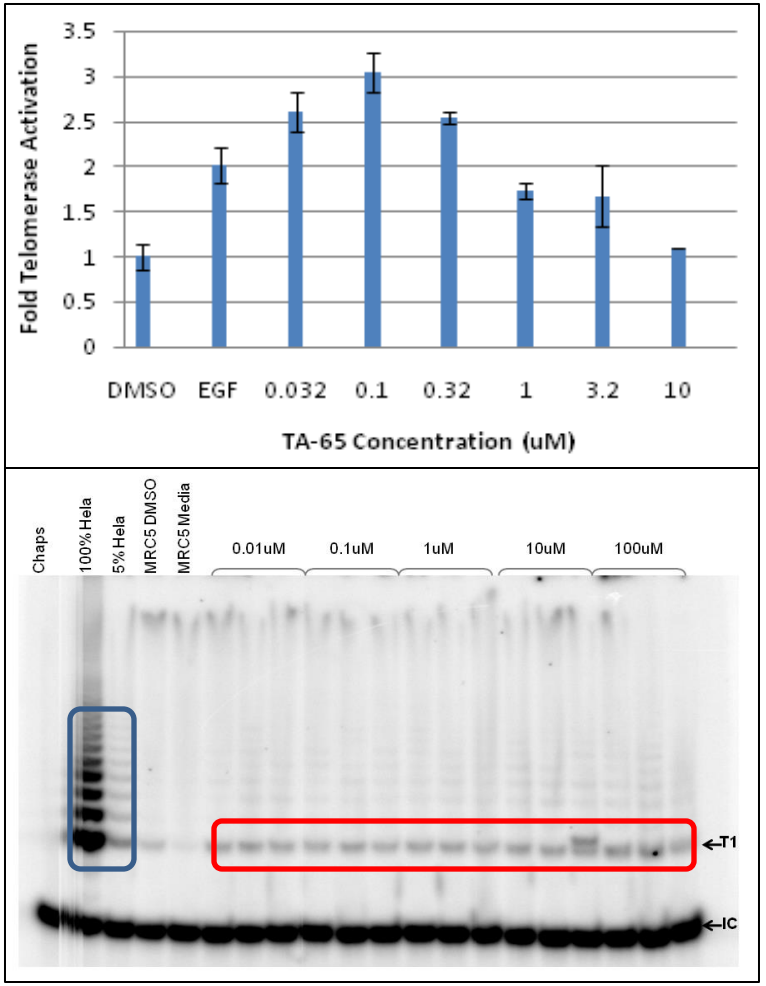
TA-65®

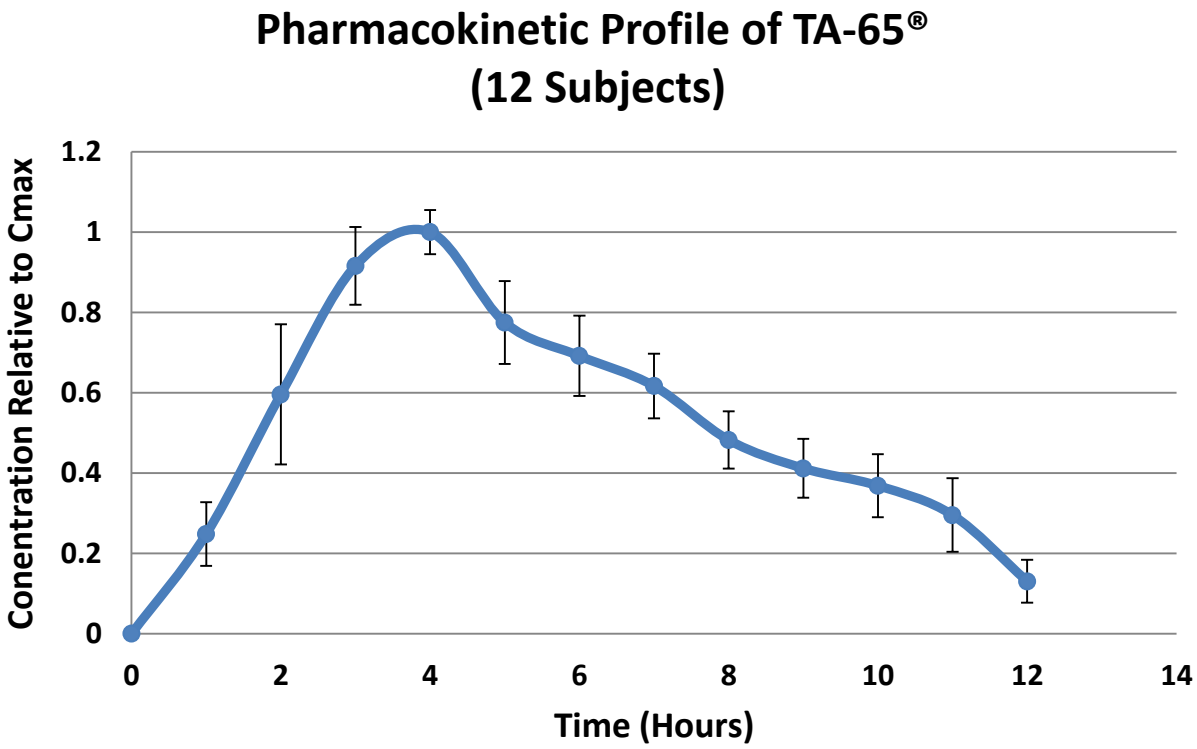
The Only Proven Commercially Available Telomerase Activation Product

- TA-65® is a rare molecule discovered in a common medicinal plant, *astragalus membranaceus*.
- TA-65® is proven to transiently activate telomerase.
- 98% pure extract

In Vitro proof: telomerase activator

- **Activates telomerase**
  - **TRAP Assay**
    - 3-fold increase at 0.1 mcMol
    - Moderate telomerase activator
  - Neonatal foreskin keratinocytes (top)
  - Fetal lung fibroblasts (bottom)
- **Serum levels**
  - Pharmacokinetic studies in humans in range equivalent to middle concentration after single dose (unpublished data)

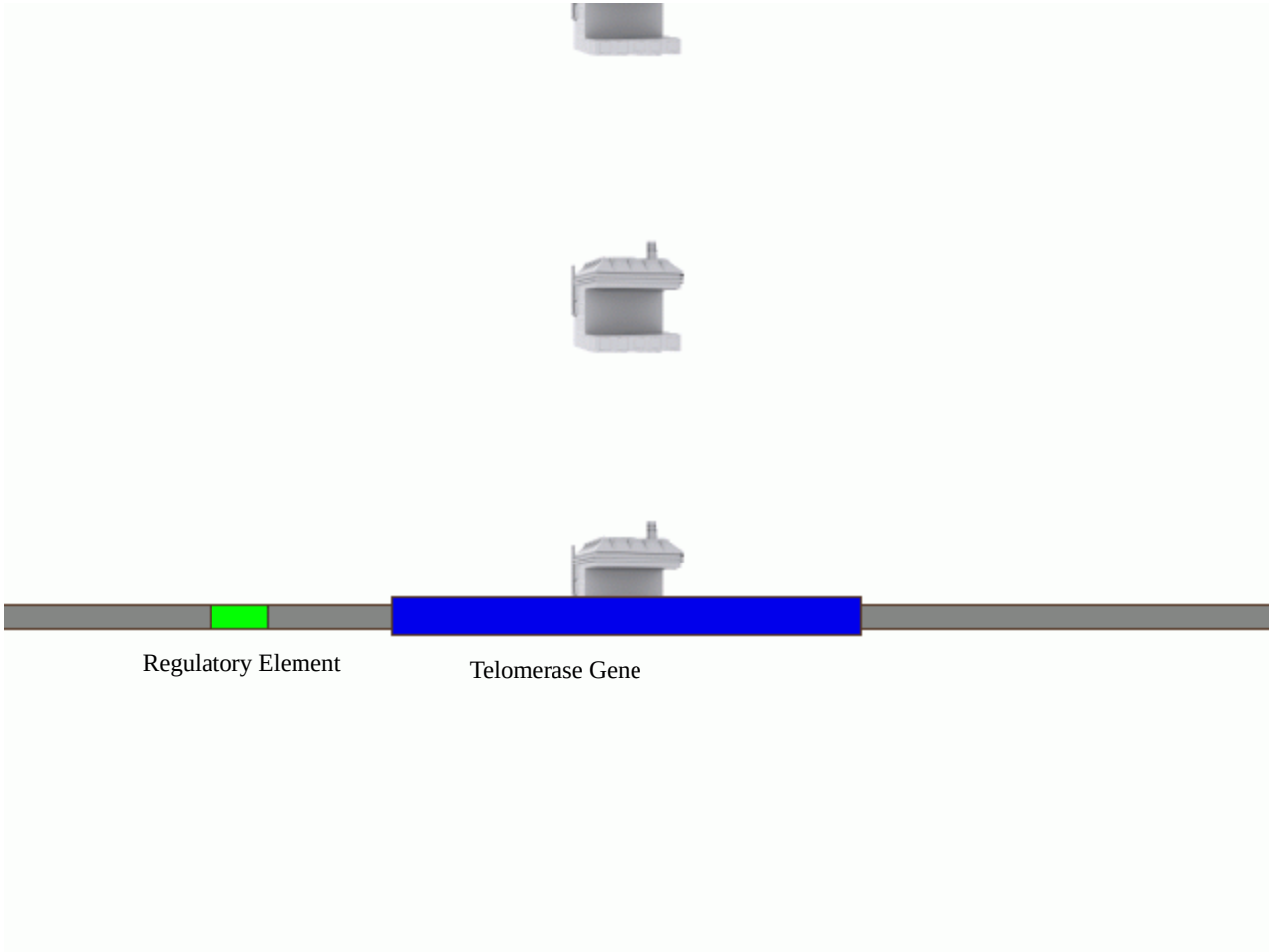




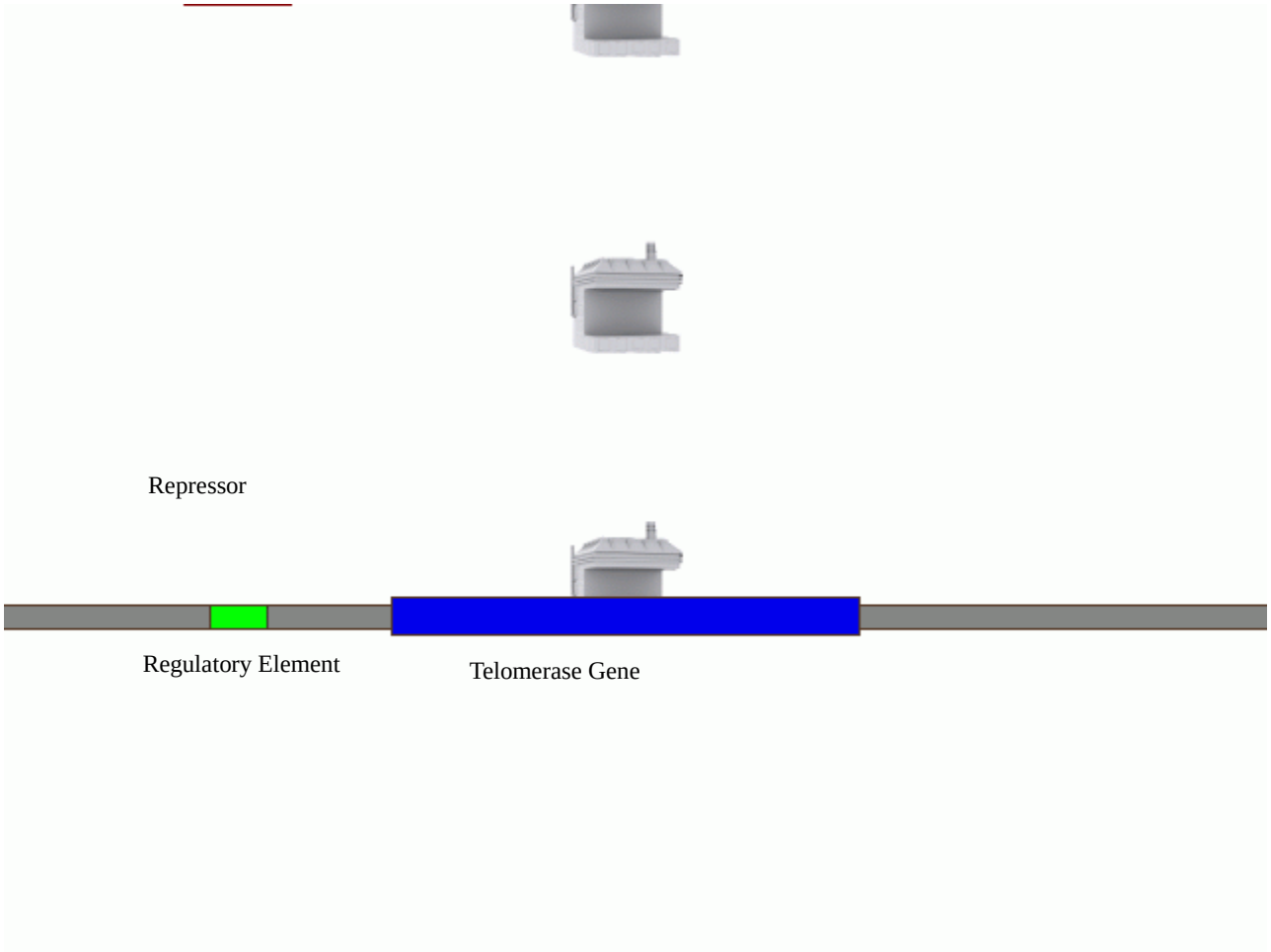
TA Sciences data

How does TA-65® Work?

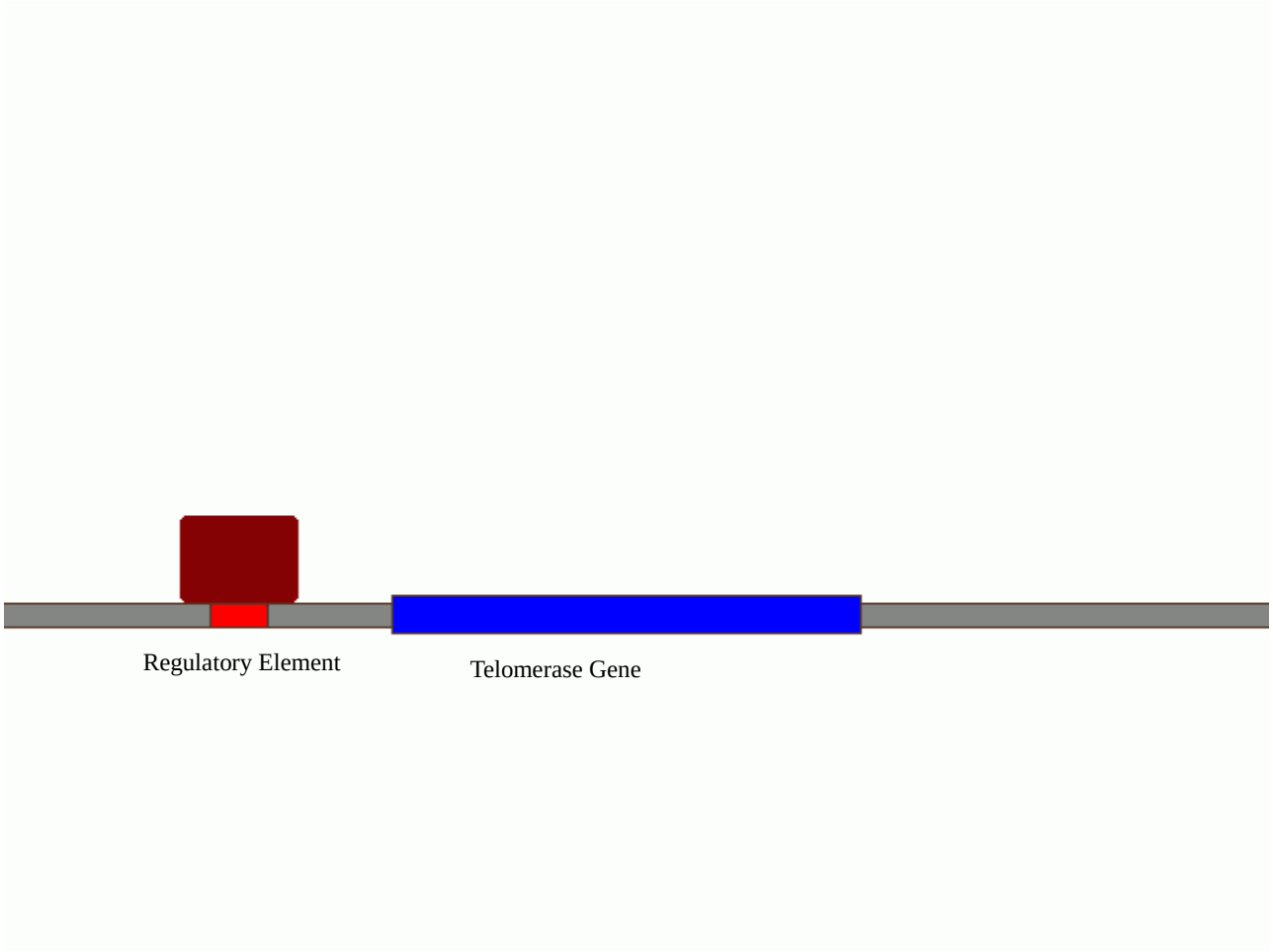
Reproductive Cells Produce Telomerase



The Gene is Repressed in All Other Cells



TA-65® Removes the Repressor



Further evidence: *In Vivo*

The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence

Aging Cell 2011

Bruno Bernardes de Jesus,[1] Kerstin Schneeberger,[1] Elsa Vera,[1,2] Agueda Tejera,[1] Calvin B. Harley[3] and Maria A. Blasco[1]

- TA-65® activates telomerase in certain tissues when added to mouse diet and rescues short telomeres. Preferentially activates telomerase in cells with shortest telomeres.

- TA-65® improves the healthspan in female mice without affecting longevity or increasing cancer incidence
  - Improved glucose metabolism, hair regrowth, liver health, bone density

"In short, this study provides proof-of-principle that health improvements are possible through treatment with a small molecule telomerase activator without any detectable deleterious effects"

TA-65® Clinical Research

Published clinical research in humans showed that people taking TA-65® ® experienced improvement in certain biomarkers of aging, including:

- **Decreased percentage of short telomeres[1]**
- **Healthy number of neutrophils among CMV+ subjects[1]**
- **Reduced percentage of non-functioning senescent cytotoxic T cells[1]**
- **Overall "more youthful" immune cell profile[1]**
- **Reduction in fasting blood glucose and improvement in insulin sensitivity[2]**
- **Reduction in total and LDL cholesterol[2]**
- **Reduction in systolic and diastolic blood pressure[2]**
- **Reduction in homocysteine, a key marker of inflammation[2]**
- **Increase in bone mineral density[2]**

1. A Natural Product Telomerase Activator As Part of a Health Maintenance Program. Harley CB, et al. Rejuvenation Research. 2011 February;14(1):45-56.
2. A Natural Product Telomerase Activator as part of a Health Maintenance Program: Metabolic and Cardiovascular Response. Harley CB, et al. Rejuvenation Research. 2013 October;16(5):386-395.

Accepted for publication in *Clinical ophthalmology*

Evaluation of an Oral Telomerase Activator for Early Age-Related Macular Degeneration - A Pilot Study

Coad Thomas Dow, M.D.[1, 3] and Calvin B. Harley[2]

[1]McPherson Eye Research Institute, University of Wisconsin, Madison
And Chippewa Valley Eye Clinic, Eau Claire, Wisconsin
[2]Independent telomere biology consultant, Murphys, CA, 95247
[3]Corresponding Author: 2715 Damon Street, Eau Claire, Wisconsin 54701
Phone: 715-834-8471; Email: ctdow@me.com

Abstract

Purpose: Telomere attrition and corresponding cellular senescence of the retinal pigment epithelium (RPE) contribute to the changes of age-related macular degeneration (AMD). Activation of the enzyme telomerase can add telomeric DNA to RPE chromosome ends and has been proposed as a treatment for AMD. We report for the first time the use of a small molecule, oral telomerase activator (TA-65) in a randomized controlled study of subjects with a serious medical condition. This study, focusing on early macular degeneration, provides a model for the use of telomerase activator in age-related disease.

Methods: Thirty-eight patients were randomly assigned to a one-year, double-blinded, placebo-controlled interventional study with arms for oral TA-65 or placebo. Macular functions via micro-perimetry were the primary measured outcomes.

Results: The macular function in the arm receiving TA-65 showed significant improvement relative to the placebo control. The improvement was manifest at six months and was maintained at one year: macular threshold sensitivity, measured as average dB (logarithmic decibel scale of light attenuation) improved 0.97 dB compared to placebo (p=0.02) and percent reduced thresholds lessened 8.2% compared to the placebo arm (p=0.04).

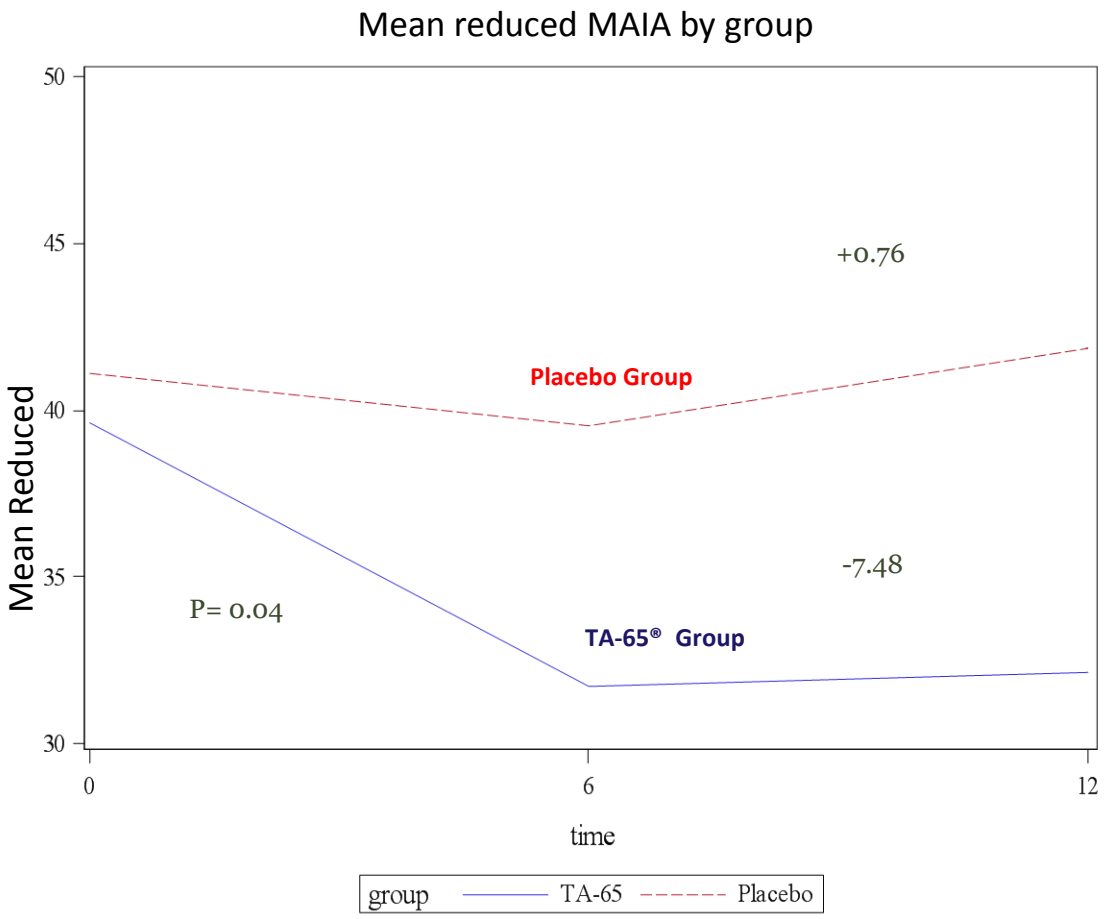
Conclusion: The oral telomerase activator significantly improved the macular function of treatment subjects compared to controls. Although this study was a pilot and a larger study is being planned, it is noteworthy in that it is, to our knowledge, the first randomized placebo controlled study of a safe telomerase activator supplement.

Key words: drusen, macular degeneration, micro-perimetry, senescence, telomerase activation, telomere

Evaluation of Telomerase Activator TA-65® for early ARMD
(Age Related Macular Degeneration)

- Randomized, double-blind, placebo controlled study; Men and Women (52-83 years old); N=38
- All patients diagnosed with early age related macular degeneration
- 12 Months Study

Improvements in Eye Function as indicated by MAIA



2016: First Human RCT with TA-65

REJUVENATION RESEARCH
Volume XX, Number XX, 2016
Mary Ann Liebert, Inc.
DOI: 10.1089/rej.2015.1793

Original Article

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

Laura Salvador,[1] Gunasekaran Singaravelu,[2] Calvin B. Harley,[3] Peter Flom,[4] Anitha Suram,[2] and Joseph M. Raffaele[5]

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 high dose of TA-65 (1000 U) showed a trend of improvements in TL compared with that of the placebo group; however, the improvements did not reach statistical significance. TL changes in the low-dose group were similar for both median and 20th percentile TLs. The findings suggest that TA-65 can lengthen telomeres in a statistically and possibly clinically significant manner.

Effect of TA-65® on Telomere Length in Humans

- Study was conducted in Barcelona, Spain.
- Randomized, double-blind, placebo controlled study; Men and Women (50-84 years old); N=97
- Clinic visit at every 3 months with telomere length testing and routine blood tests

TA-65® Group
Increase in median telomere length

Time (months)	Increase in length (base pairs)
3 months	+384 (± 195) bp *
6 months	+158 (± 164) bp
9 months	+526 (± 167) bp *
12 months	+533 (± 183) bp *

Placebo Group
Decrease in median telomere length

Time (months)	Decrease in length (base pairs)
3 months	-24 (± 106) bp
6 months	none
9 months	-170 (± 106) bp *
12 months	-288 (± 101) bp *

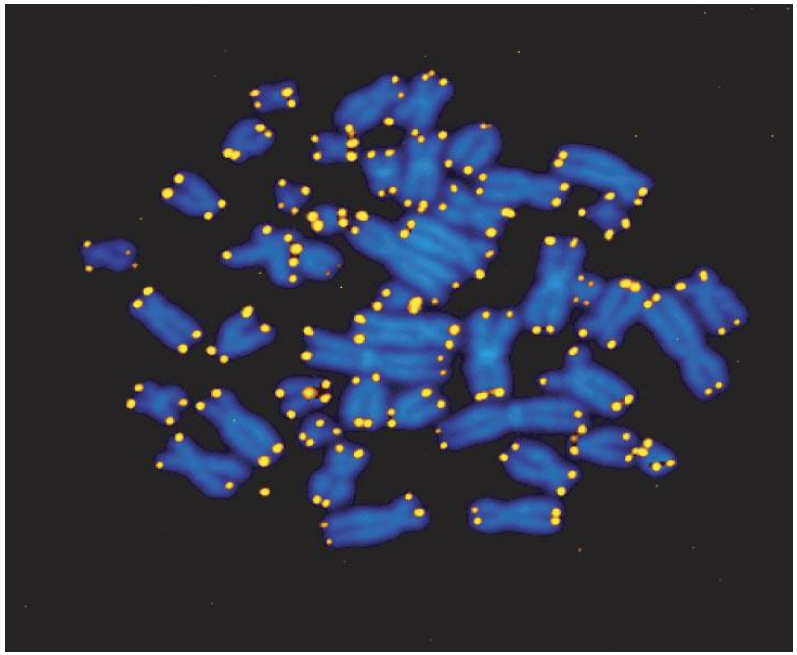
* Statistically significant

How Do I Know if I Should Take TA-65® ?

- Theoretically, everyone can benefit from telomerase activation after birth because it is suppressed
- By 40 years old, most have had significant telomere shortening
- BUT, there is great variability in telomere length!!
- My Answer: Measure telomere length

Leukocyte telomere length: Measurement techniques

- **How to measure**
  - TRF: Terminal restriction fragment
  - Q-PCR: Quantitative polymerase chain reaction
  - Q-FISH: Quantitative-florescence in situ hybridization
  - Flow-FISH: Florescent in situ hybridization and flow cytometry
    - Multiple Cell Types
- **Available commercially**
  - Q-PCR: Leukocytes
    - Spectracell
    - Telomere Diagnostics
  - HT Q-FISH Percent Shortest Telomeres
    - Life Length
  - **Flow-FISH: Lymphocytes and Granulocytes**
    - **Repeat Diagnostics**



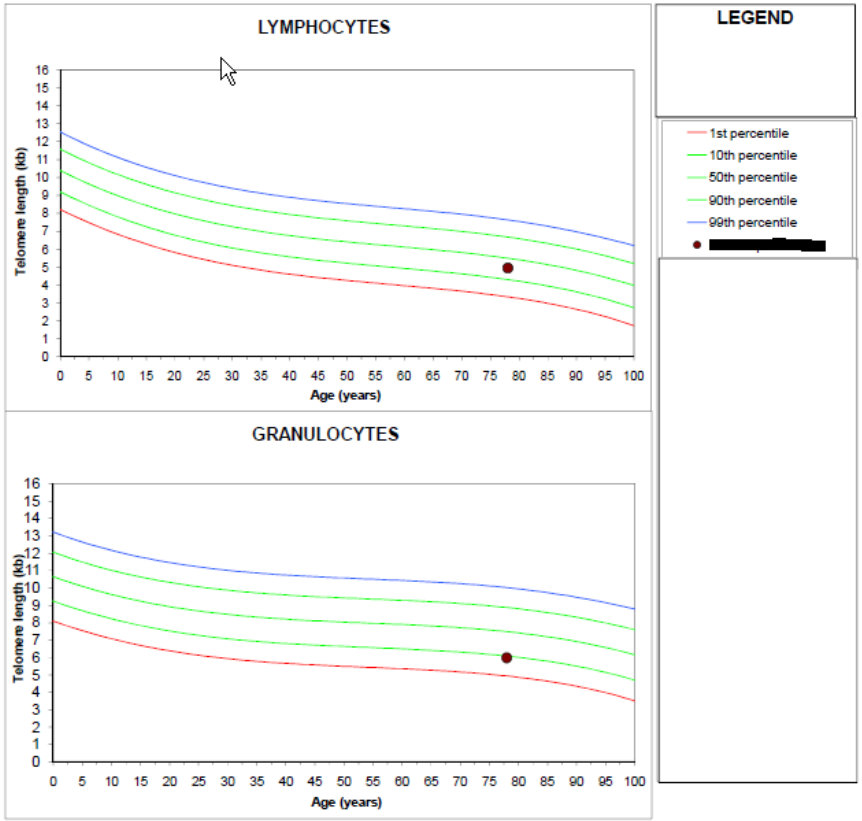
Lymphocyte and granulocyte median telomere length (Repeat Diagnostics)

- 0.2-3 kb resolution
- 3.3% inter-assay CV
- 1.6% intra-assay CV

Lymphocytes			Granulocytes		
MTL (kb)	MTLN (kb)	INT	MTL (kb)	MTLN (kb)	INT
4.9	5.5	N	6.0	7.5	L

* MTL = Client Median Telomere Length
*** MTLN = Normal MTL at age (50th percentile)
**** INT = Telomere length interpretation

VH = Very High
H = High
N = Normal
L = Low
VL = Very Low



Practical Clinical Telomerase Therapy

Pearls and Pitfalls of Treating Patients with TA-65

What Will I Notice if I Take TA-65® ?

- Subjective:
  - Improved energy
  - Improved recovery from workouts in athletes
  - Decreased graying of hair
  - Better vision
  - Improved skin
- Objective:
  - Reduction in presbyopia documented for a number of people
  - Improved skin appearance and pigmentation

How Do I Know If it is Working? (*In Me)*

- Monitor your telomere length annually or semi-annually
- There is fluctuation, but over time you will see a trend
- More like 401K (or moving average) than day trading
- Like looking for long-term effect of a blood pressure or cholesterol lowering medication
- Except: No immediate marker of effect

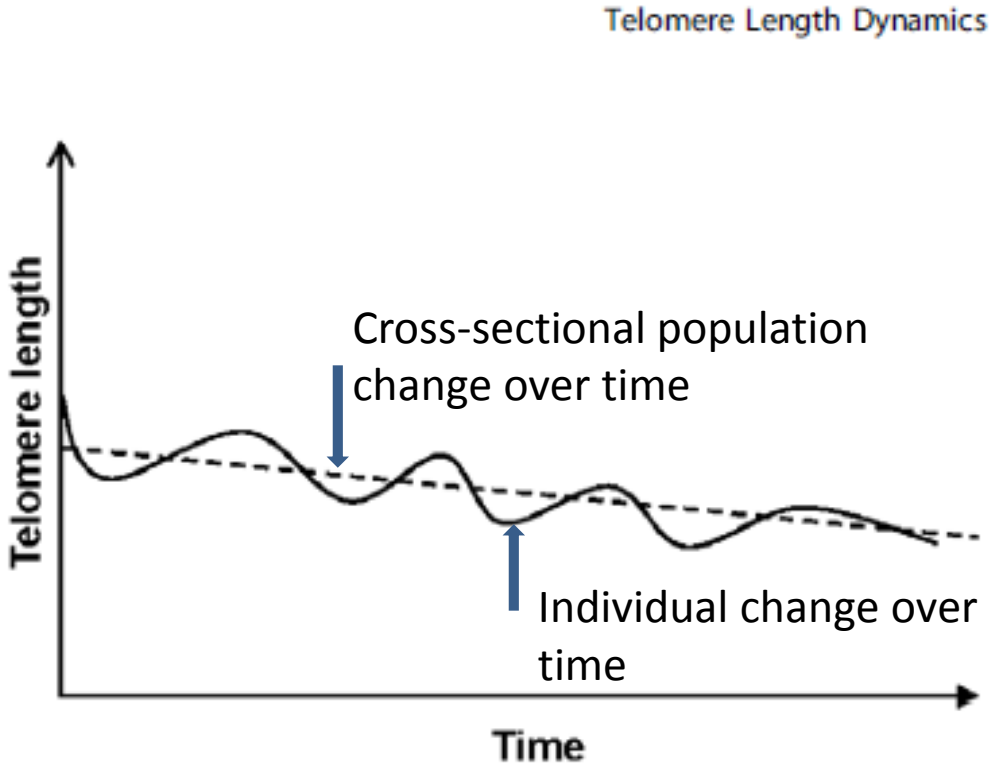


Figure 6. The oscillation hypothesis. Hypothetical illustration of RTL changes over time at the individual (solid line) and population (dotted line) level, based on the collected data from the present study and the literature.

Svenson U PloS ONE 2011

MiniReview

Cancer Prevention Research

How "Reversible" Is Telomeric Aging?

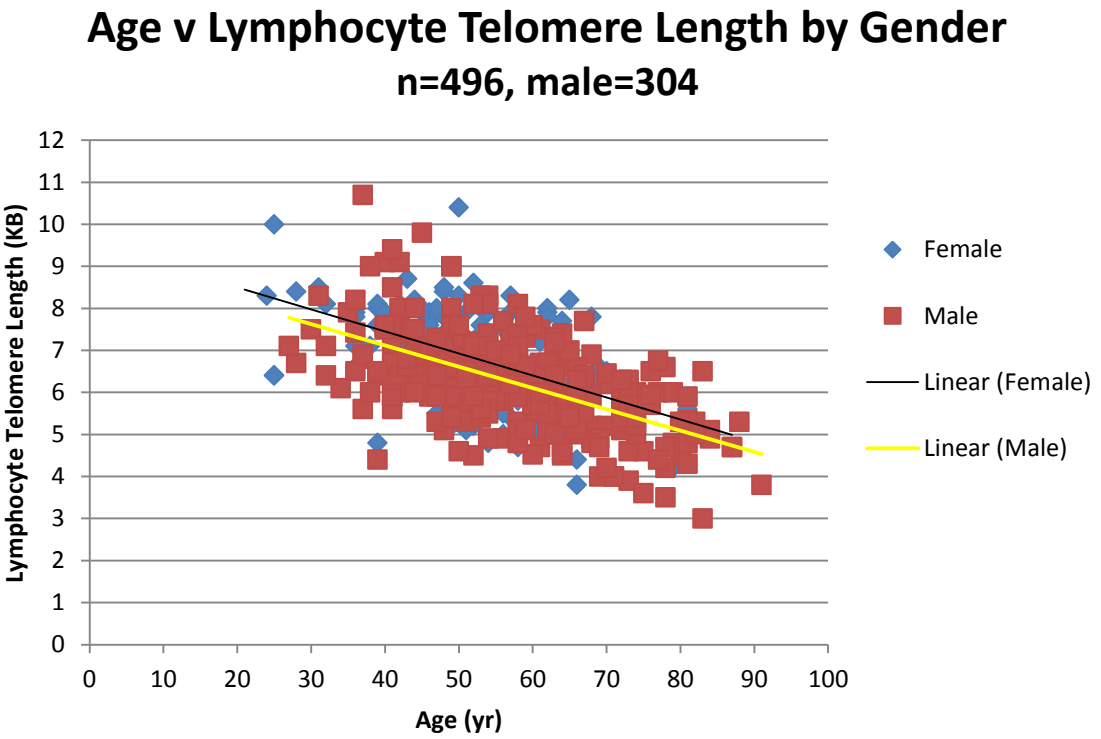
Elissa Epel

Abstract

A critical question in human health is the malleability of telomere length. Telomere length, sampled at one point during adult life, is predictive of certain types of cancer and other immune and metabolic-related diseases. We now know from basic studies that the telomere/telomerase maintenance system plays a causal role in accelerating biologic aging and promoting disease processes. One can develop short telomeres for a multitude of reasons. Historical factors such as genetics, prenatal conditions, and early adversity, contribute to adult telomere length; however, current stress and lifestyle are also associated. If these modifiable predictors are causal factors in telomere shortening, there is a tremendous opportunity to improve maintenance and possibly even lengthen telomeres with behavioral interventions. This minireview discusses our current understanding of telomere lengthening and questions facing the field. Several small-scale stress reduction/wellness studies show promising findings, suggesting that cell aging can be slowed or reversed *in vivo* over short periods. Moreover, possible mechanisms are discussed, that take into account actual telomeric lengthening, such as that which occurs through telomerase-mediated elongation, or mechanisms resulting in "pseudo-telomeric lengthening" as might occur from changes in cell type distribution. There is a strong need for more translational clinical to bench research to address mechanistic questions in experimental models. In addition, well-designed intervention research that examines both telomeres and potential mediators of change can further enhance our understanding of malleability, mechanism, and clinical implications of telomere lengthening. *Cancer Prev Res; 5(10); 1163–8.* ©2012 AACR.

"Pseudo-telomeric lengthening and shortening"

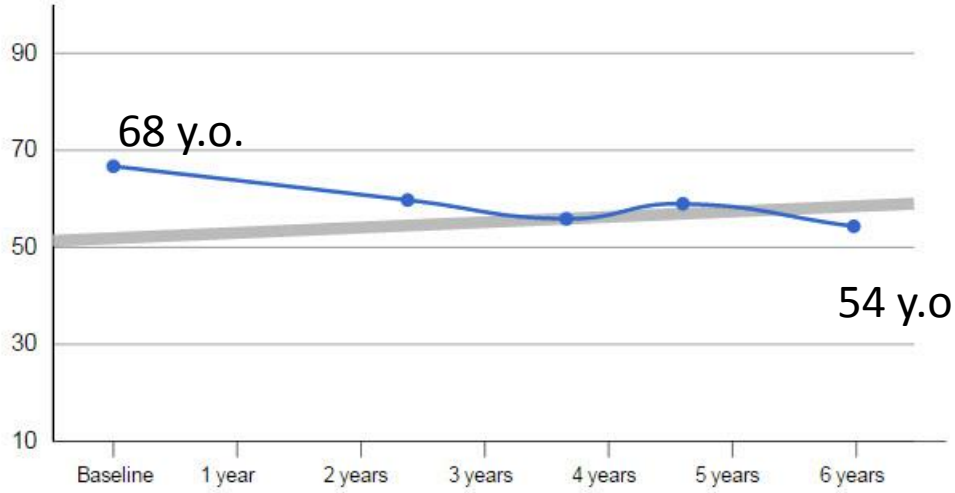
Cross-sectional Telomere Length Change with Age



Unpublished data PhysioAge Systems 2008-2015

TelomerAge

58 y.o. woman on TA-65® 500 IU/D for 6 years



1.6 kb lymphocyte Telomere length increase over 6 yrs

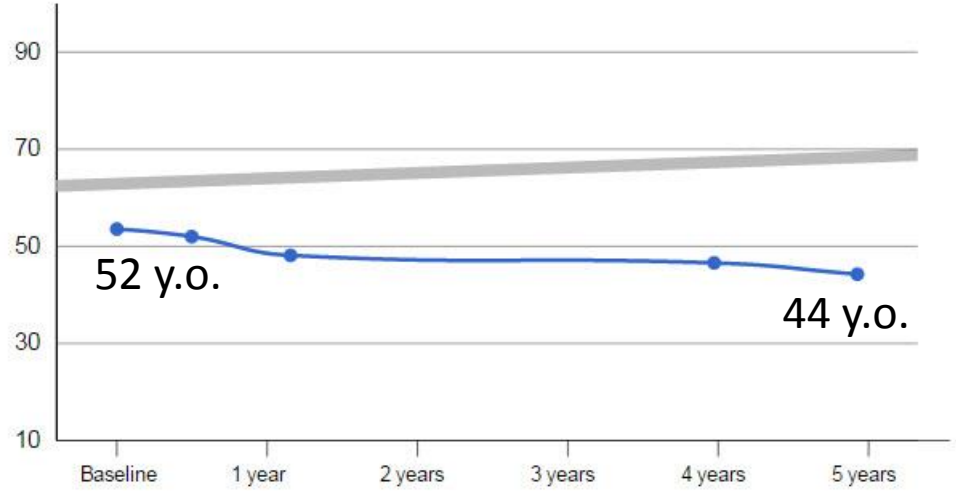
Your TelomerAge is 54.3, 4 years younger than average for your age.

Name	Result	Units	Normal Range	Optimal Range	Baseline	Change
Telomere Length						
Granulocyte Telomere Length	7.3	kb	5.5 - 10.0	> 9	7.0	4 %
Lymphocyte Telomere Length	6.8	kb	4.5 - 9.0	> 8	5.2	31 %

Visit Date	04-23 2008	09-08 2010	12-20 2011	11-28 2012	04-16 2014
Telomere Length					
Granulocyte Telomere Length	7.0	6.8	7.6	6.9	7.3
Lymphocyte Telomere Length	5.2	6.1	6.6	6.2	6.8

TelomerAge

68 y.o. male on TA-65® 500 IU/D



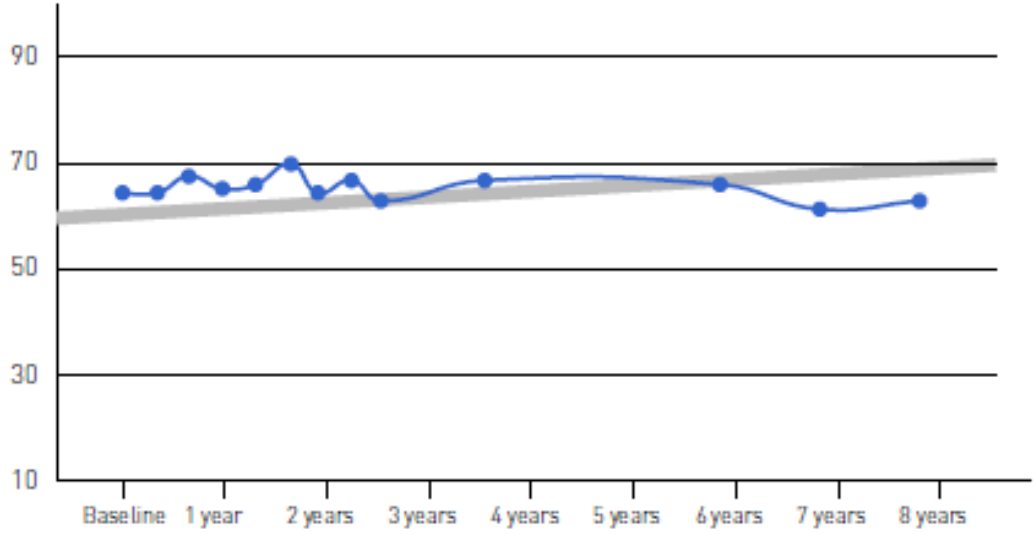
1.2 kb increase in lymphocyte telomere length over 5 years

Your TelomerAge is 44.3, 24 years younger than average for your age.

Name	Result	Units	Normal Range	Optimal Range	Spectrum	Baseline	Change
Granulocyte Telomere Length	8.6	kb	5.5 - 10.0	> 9		8.0	8 %
Lymphocyte Telomere Length	8.1	kb	4.5 - 9.0	> 8		6.9	17 %

Visit Date	10-20 2008	04-20 2009	12-16 2009	10-10 2012	09-23 2013
Granulocyte Telomere Length	8.0	7.7	8.2	8.7	8.6
Lymphocyte Telomere Length	6.9	7.1	7.6	7.8	8.1

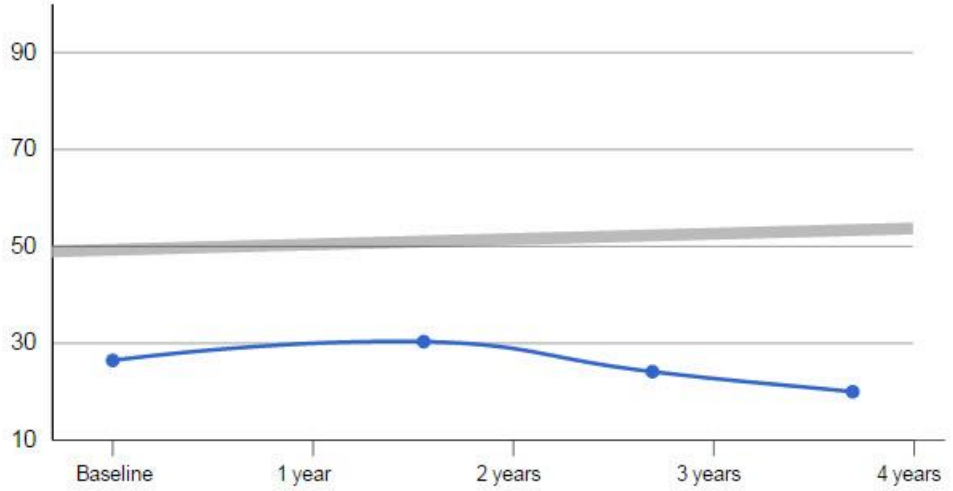
TelomerAge



Your TelomerAge is 62.8, 6 years younger than average for your age.

TelomerAge

53 y.o. woman on no telomerase meds



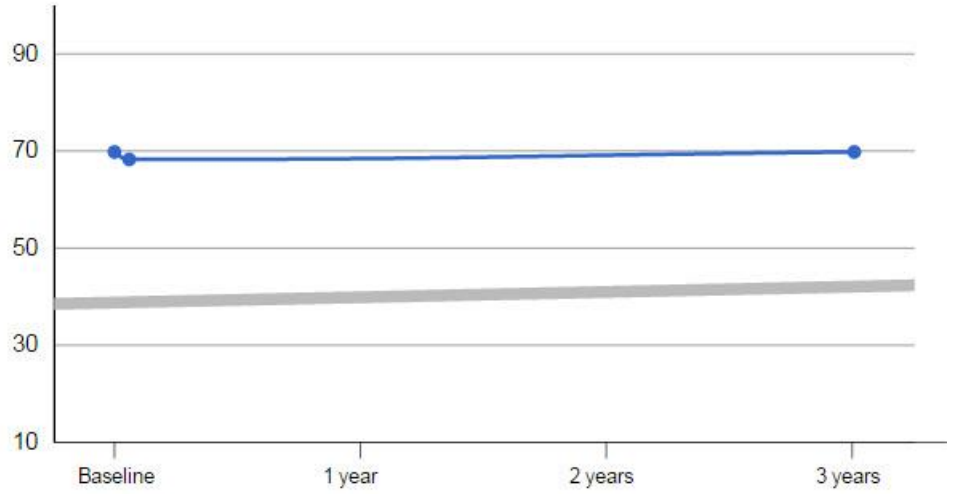
Your TelomerAge is 20.0, 33 years younger than average for your age.

Granulocyte Telomere Length	10.9	kb	5.5 - 10.0	> 9	10.0	9 %
Lymphocyte Telomere Length	11.3	kb	4.5 - 9.0	> 8	10.4	9 %

Visit Date	02-04 2010	08-25 2011	10-15 2012	10-15 2013
Granulocyte Telomere Length	10.0	9.3	10.6	10.9
Lymphocyte Telomere Length	10.4	9.9	10.7	11.3

TelomerAge

42 y.o. woman on 500 IU/D TA-65®

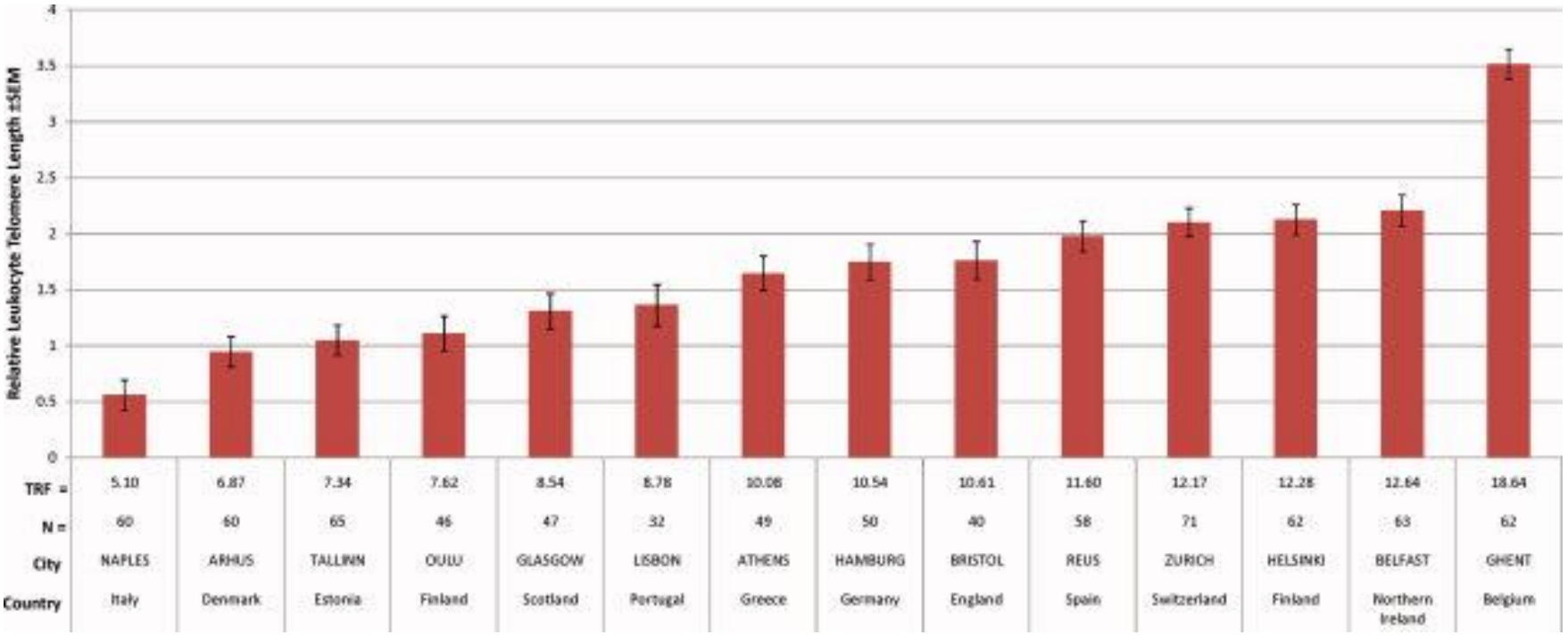


Your TelomerAge is 69.8, 28 years older than average for your age.

Visit Date	02-07 2012	02-29 2012	02-10 2015
Granulocyte Telomere Length	5.6	5.0	5.5
Lymphocyte Telomere Length	4.8	5.0	4.8

Name	Result	Units	Normal Range	Optimal Range	Baseline	Change
Telomere Length						
Granulocyte Telomere Length	5.5	kb	5.5 - 10.0	> 9	5.6	-2 %
Lymphocyte Telomere Length	4.8	kb	4.5 - 9.0	> 8	4.8	0 %

Substantial variation in qPCR measured mean blood telomere lengths in young men from eleven European countries



Mean telomere length can vary widely between different populations
5.2 kb in Naples up to 18.6 kb in Ghent.

Rate of change is more important than a single TL determination!

American Journal of Human Biology
Volume 23, Issue 2, pages 228-231, 10 JAN 2011 DOI: 10.1002/ajhb.21126
http://onlinelibrary.wiley.com/doi/10.1002/ajhb.21126/full#fig1

Safety Questions

- What are the risks?
- How much testing has been done?
- Can it increase my risk of cancer?

TA-65® "Generally Recognized As Safe"

Be It Known to All Interested Parties, That

Telomerase Activation Sciences, Inc.

Have Provided Substantive Data Leading to a Determination of

"Generally Recognized as Safe" (GRAS) Status of

TA-65® (CYCLOASTRAGENOL) AS A MEDICAL FOOD INGREDIENT*

According to the rights and privileges confirmed in (201(s) et seq.) of the Federal Food, Drug and Cosmetic Act, Experts, by virtue of their training and experience, have determined that the above mentioned substance, when produced in accordance with current Good Manufacturing Practice (cGMP), is safe under the intended conditions of use and is "Generally Recognized as Safe" (GRAS) by scientific procedures when used as a food ingredient in specific final categories at specified levels and for the purpose(s) indicated in the GRAS dossier, as approved by the GRAS Expert Panel.

Edward L. Carlson, Ph.D. - Expert Panelist — Date 10/6/14

Nancy J. Szabo, Ph.D. - Burdock Group Scientific Monographer — Date

I. Glenn Sipes, Ph.D., Fellow ATS and IAAS - Expert Panelist — Date 10/7/14

George A. Burdock, Ph.D., DABT, FACN - President, Burdock Group — Date

John A. Thomas, Ph.D., DATS, F.A.C.T. - Expert Panelist — Date 10/8/14

GRAS

STATE OF FLORIDA
COUNTY OF ORANGE

Affirmed and subscribed before me this 17th day of October, 2014, by George A. Burdock, Ph.D., personally known to me.

Notary Public State of Florida
Gina Strollo Radcliff
My Commission EE108598
Expires 11/26/2014

- An independent expert panel has determined TA-65® to be Generally Recognized as Safe (GRAS)
- T.A. Sciences® provided extensive animal & human clinical data to support the status

Transient vs. Permanent Telomerase Activation

- Permanent Telomerase Activation in somatic cells is associated with unhealthy cell growth.
- Transiently activating telomerase is the key to enhancing telomere length safely.
  - TA-65® utilizes a safe pathway for transient Telomerase Activation that ceases approximately 12 hours after taking a capsule.

Telomerase is not an oncogene

- Cancer cell ≠ and immortalized cell
- Both have unlimited proliferation because of telomerase activation
- Cancer cells: oncogenic mutation
  - Lose function and control of cell cycle
  - Have altered morphology/nuclear changes
- Normal cells: without oncogenic mutations
  - Normal function and morphology
- Gene transduction with the catalytic component of hTERT on fibroblasts, epithelial cells, and keratinocytes
  - Unlimited proliferation and normal function
  - **When transplanted into immunodeficient mice: NO altered growth and NO tumorigenesis**

TA-65®
Published Safety Conclusions

- *Rejuvenation Research Journal*: author, Cal Harley, Sept. 2010

  Safety findings: No adverse events occurred among the subjects taking the telomerase activator

- "The Telomerase Activator elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence"…..author, Maria Blasco, "Aging Cell" April 2011

- Over 10,000 people using TA-65® , some for over 8 years, with no significant adverse effects.

How Much and for How Long?

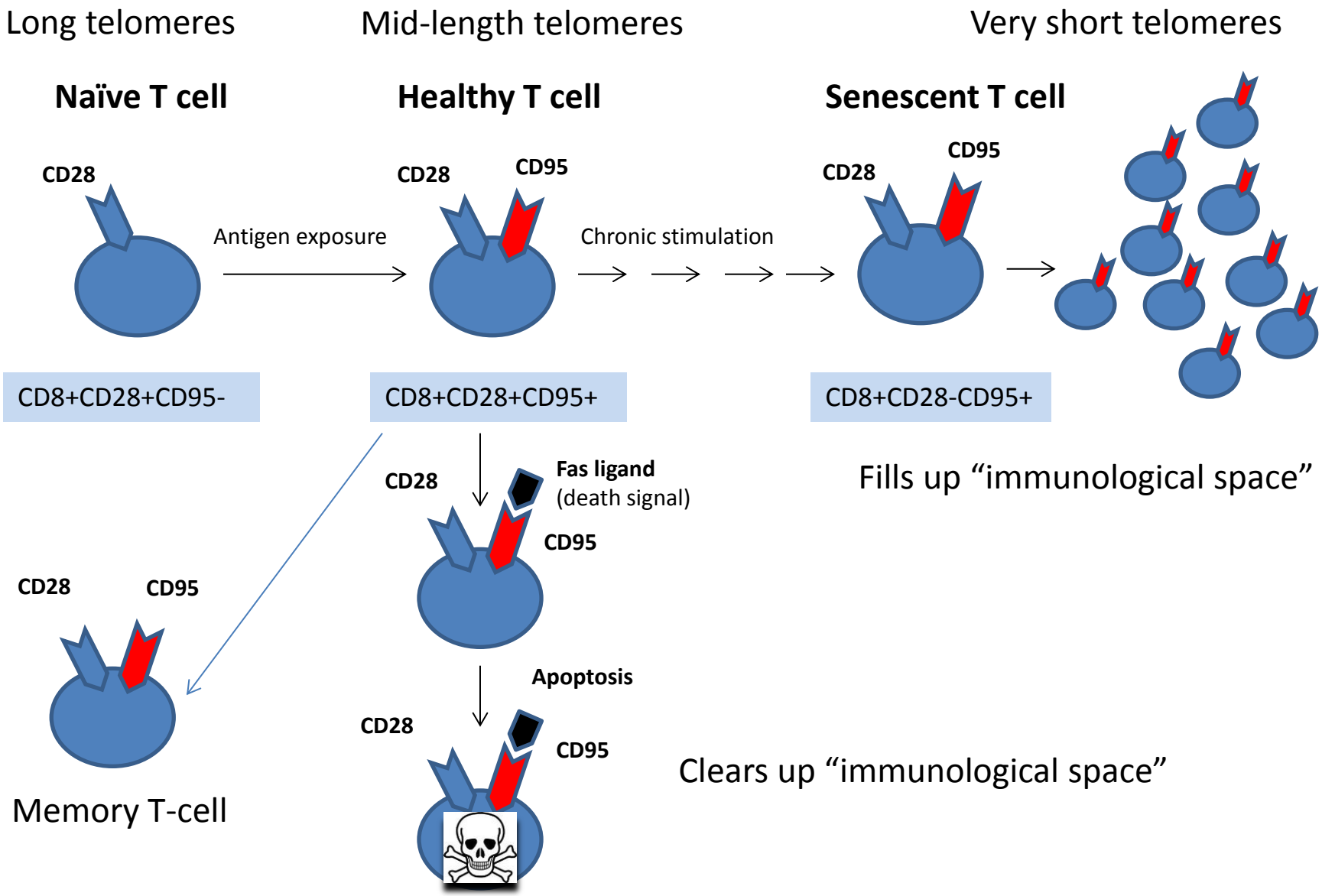
- Get baseline telomere length measurements
- Start with 250 IU a day—1 capsule
- Look for subjective effects
- Recheck telomeres annually or semi-annually
- Can increase to 2 capsules a day or 1 twice a day
- May take it for many years or take breaks

Conclusions

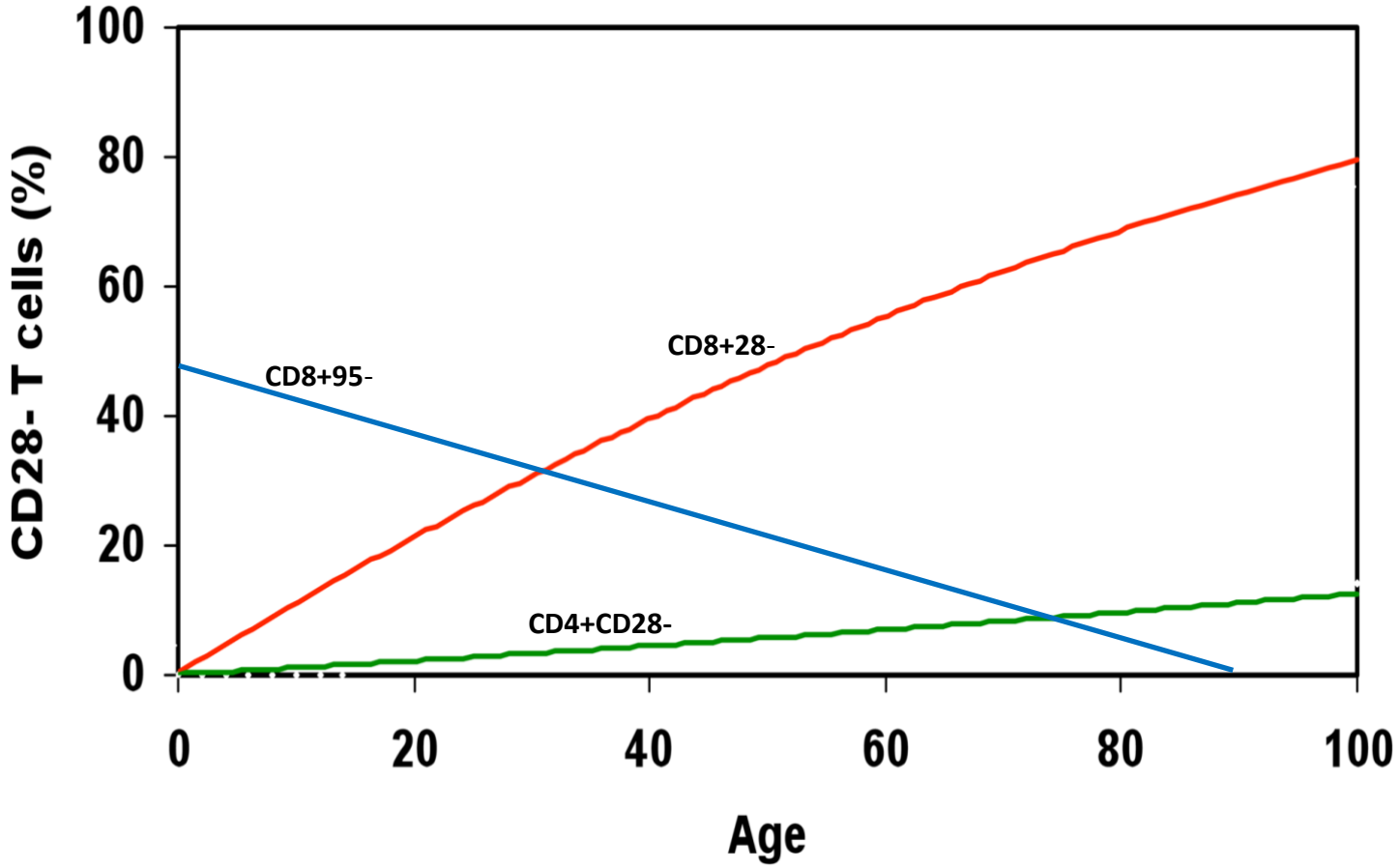
- Mind your telomeres
- Their health is essential for yours!
- Healthy diet, exercise, supplements, and hormone optimization are essential
- TA-65® is an important, safe, and effective adjunct to a comprehensive age management program

Dose Adjustments: Beyond Telomeres

- Lymphocyte subset panel
- UCLA Clinical Immunology Laboratory
- CD28- and CD95- Suppressor T-cell Counts
- OR: CD4/8, Helper-to-Suppressor Ratio
- Responds sooner to therapeutic dose


Long telomeres
Mid-length telomeres
Very short telomeres
Naïve T cell
Healthy T cell
Senescent T cell
CD28
CD28
CD95
CD28
CD95
Antigen exposure
Chronic stimulation
CD8+CD28+CD95-
CD8+CD28+CD95+
CD8+CD28-CD95+
Fills up "immunological space"
CD28
Fas ligand
(death signal)
CD95
CD28
CD95
Apoptosis
CD28
CD95
Clears up "immunological space"
Memory T-cell

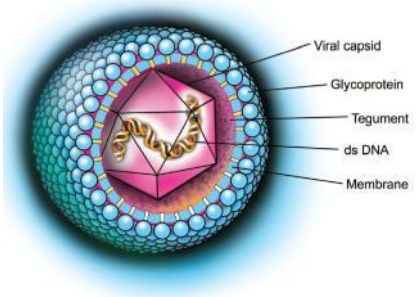
In vivo change in CD28 and CD95 expression with age



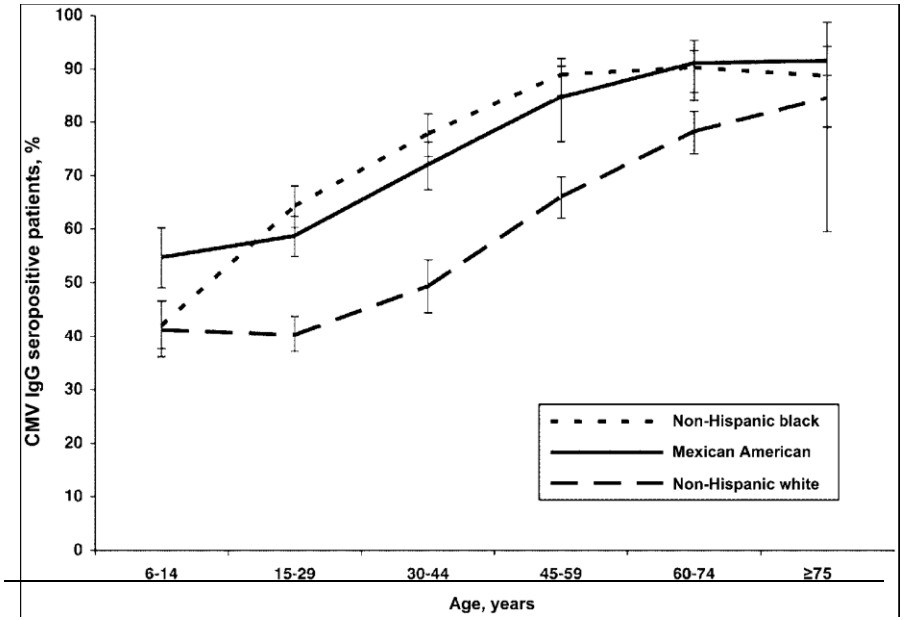
Adapted from Weng N-P 2009 *Trends Immunol*

Cytomegalovirus: Chronic Immune stressor

- **Ubiquitous herpesvirus**
  - In same family with EBV and VZV
  - Seroprevalence 30-90% in industrialized countries
  - 55% seroprevalence in the US
  - 30% by age 10, then about 1% seroconversion/yr
  - By 80 years, 90% are CMV+
- **Primary infection:**
  - Usually asymptomatic but can cause mononucleosis
- **Remains latent in monocytes and endothelial cells lifelong**
  - Requires continual surveillance by cytotoxic T cells
- **Makes it difficult to differentiate effects of CMV from aging on immune system**



HCMV Human Cytomegalovirus

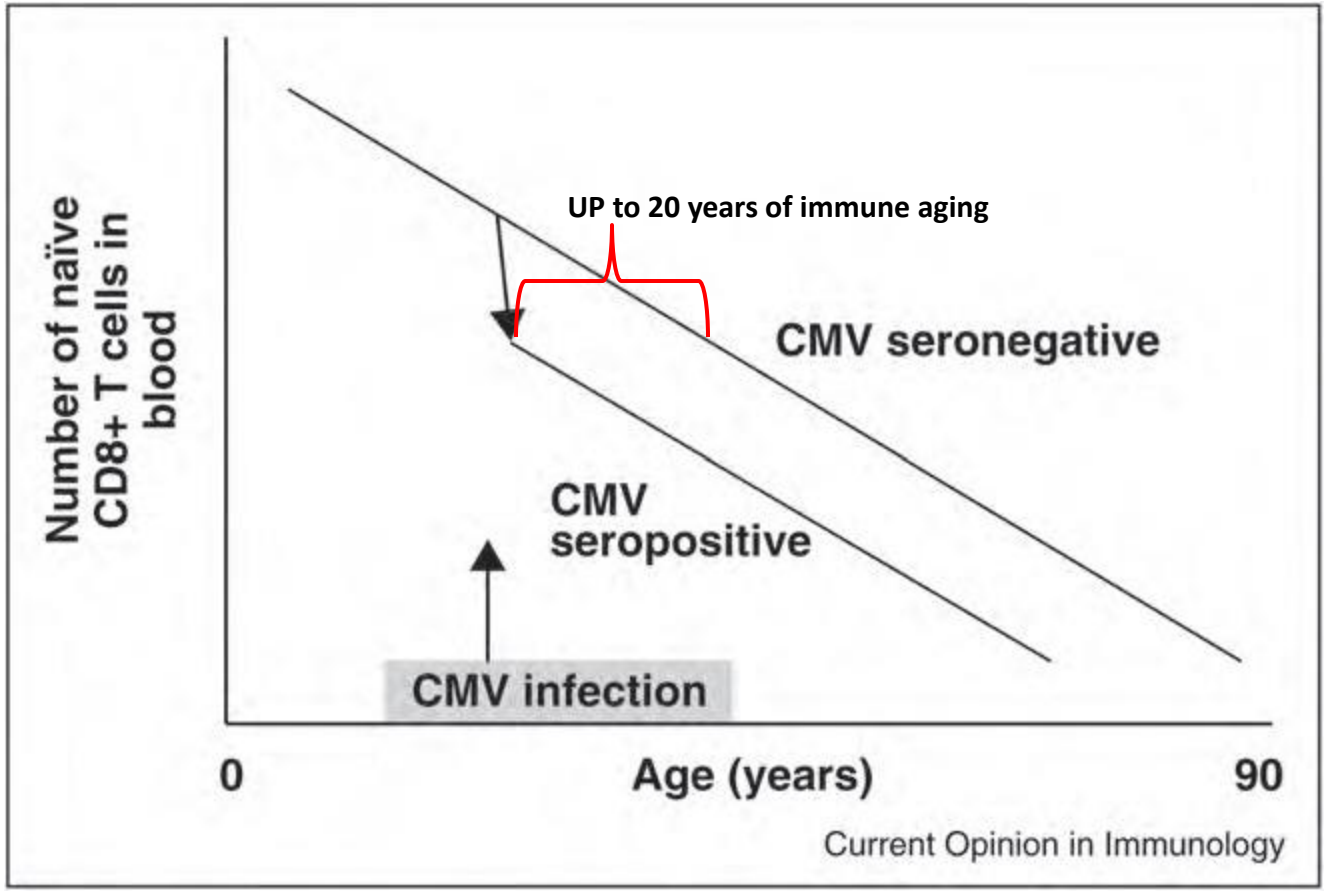


Staras, SA 2006 *CID*

"CMV is arguably the most immunodominant antigen to which the human immune system will be exposed and after infection the host must maintain a very large memory T cell compartment to suppress viral replication."

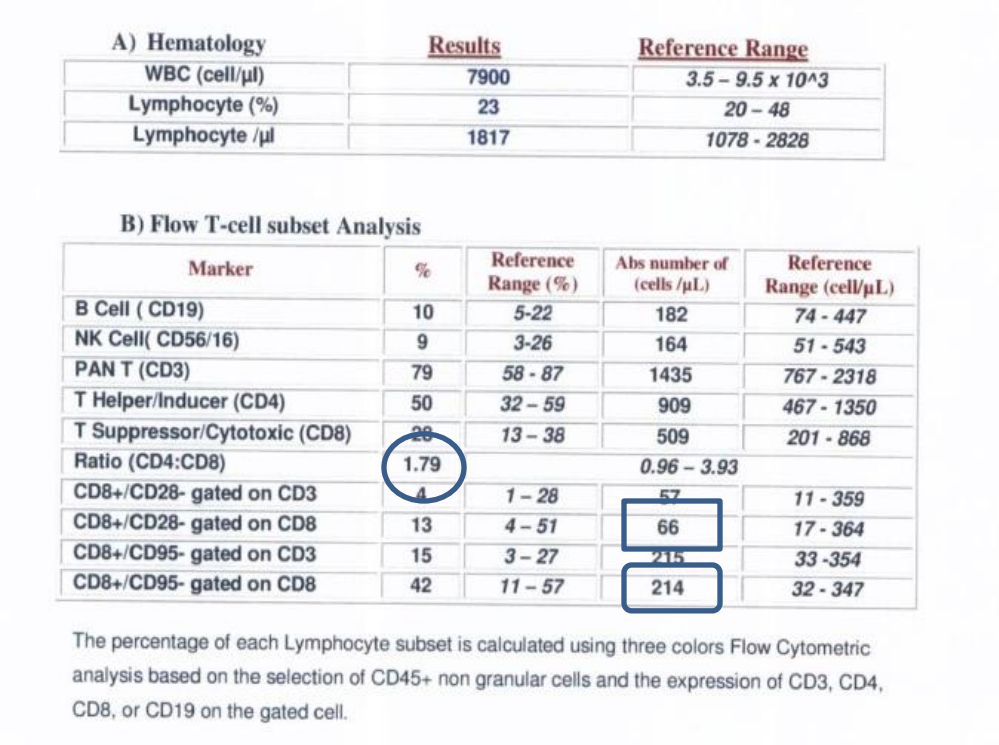
Moss P 2010 *Curr Opin Immunol*

Effect of CMV on number of naïve CD8+ T cells with age



Adapted from Moss P 2010

Young lymphocyte subset panel



A) Hematology

	Results	Reference Range
WBC (cell/µl)	7900	3.5 – 9.5 x 10^3
Lymphocyte (%)	23	20 – 48
Lymphocyte /µl	1817	1078 - 2828

B) Flow T-cell subset Analysis

Marker	%	Reference Range (%)	Abs number of (cells /µL)	Reference Range (cell/µL)
B Cell (CD19)	10	5-22	182	74 - 447
NK Cell(CD56/16)	9	3-26	164	51 - 543
PAN T (CD3)	79	58 - 87	1435	767 - 2318
T Helper/Inducer (CD4)	50	32 – 59	909	467 - 1350
T Suppressor/Cytotoxic (CD8)	28	13 – 38	509	201 - 868
Ratio (CD4:CD8)	1.79		0.96 – 3.93	
CD8+/CD28- gated on CD3	4	1 – 28	57	11 - 359
CD8+/CD28- gated on CD8	13	4 – 51	66	17 - 364
CD8+/CD95- gated on CD3	15	3 – 27	215	33 -354
CD8+/CD95- gated on CD8	42	11 – 57	214	32 - 347

The percentage of each Lymphocyte subset is calculated using three colors Flow Cytometric analysis based on the selection of CD45+ non granular cells and the expression of CD3, CD4, CD8, or CD19 on the gated cell.

- **28 y.o. female**, CMV-
- Relatively low senescent T cell count (66 cells)
- Higher naïve T cell count (214)
- CD4:CD8 around 2
- B cells normal
- NK cells low normal

“Youthful” lymphocyte subset panel

A) Hematology	Results	Reference Range
WBC (cell/µl)	7000	3.5 – 9.5 x 10^3
Lymphocyte (%)	24	20 – 48
Lymphocyte /µl	1680	1078 - 2828

B) Flow T-cell subset Analysis

Marker	%	Reference Range (%)	Abs number of (cells /µL)	Reference Range (cell/µL)
B Cell (CD19)	12	5-22	202	74 - 447
NK Cell(CD56/16)	5	3-26	84	51 - 543
PAN T (CD3)	82	58 - 87	1378	767 - 2318
T Helper/Inducer (CD4)	55	32 – 59	924	467 - 1350
T Suppressor/Cytotoxic (CD8)	21	13 – 38	353	201 - 868
Ratio (CD4:CD8)	2.62		0.96 – 3.93	
CD8+/CD28- gated on CD3	3	1 – 28	41	11 - 359
CD8+/CD28- gated on CD8	12	4 – 51	42	17 - 364
CD8+/CD95- gated on CD3	9	3 – 27	124	33 -354
CD8+/CD95- gated on CD8	34	11 – 57	120	32 - 347

The percentage of each Lymphocyte subset is calculated using three colors Flow Cytometric analysis based on the selection of CD45+ non granular cells and the expression of CD3, CD4, CD8, or CD19 on the gated cell.

- 50 y.o. very healthy woman, CMV-
- Similar profile as 28 y.o., except slightly lower naïve T cells

No accumulation of senescent T cells

A) Hematology	Results	Reference Range
WBC (cell/µl)	8900	3.5 – 9.5 x 10^3
Lymphocyte (%)	24	20 – 48
Lymphocyte /µl	2136	1078 - 2828

B) Flow T-cell subset Analysis

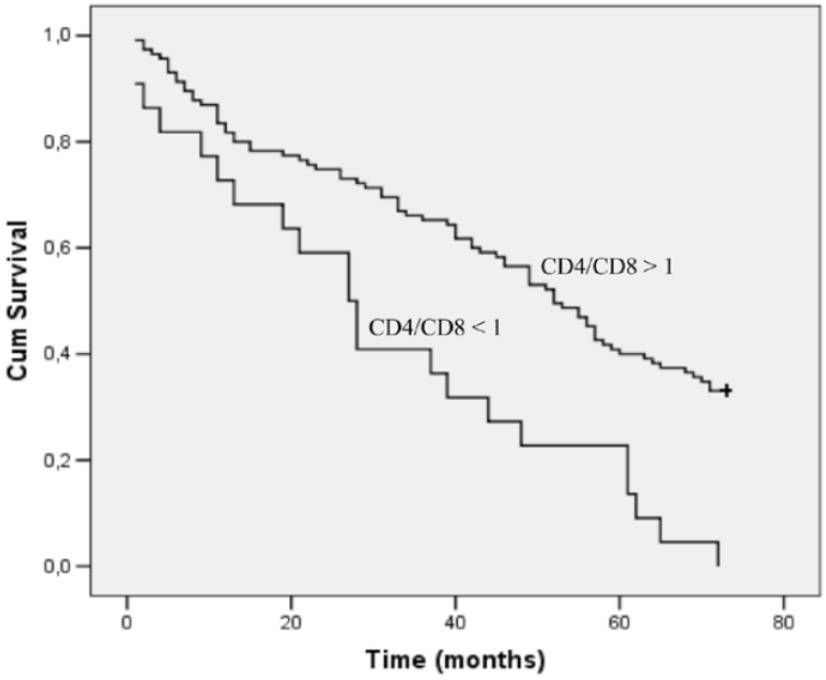
Marker	%	Reference Range (%)	Abs number of (cells /µL)	Reference Range (cell/µL)
B Cell (CD19)	10	5-22	214	74 - 447
NK Cell(CD56/16)	15	3-26	320	51 - 543
PAN T (CD3)	76	58 - 87	1623	767 - 2318
T Helper/Inducer (CD4)	63	32 – 59	1346	467 - 1350
T Suppressor/Cytotoxic (CD8)	10	13 – 38	214	201 - 868
Ratio (CD4:CD8)	6.30		0.96 – 3.93	
CD8+/CD28- gated on CD3	1	1 – 28	16	11 - 359
CD8+/CD28- gated on CD8	10	4 – 51	21	17 - 364
CD8+/CD95- gated on CD3	4	3 – 27	65	33 -354
CD8+/CD95- gated on CD8	33	11 – 57	71	32 - 347

The percentage of each Lymphocyte subset is calculated using three colors Flow Cytometric analysis based on the selection of CD45+ non granular cells and the expression of CD3, CD4, CD8, or CD19 on the gated cell.

- 50 y.o. healthy male, CMV-
- Low CD28-
- Preserved CD4+
- Normal aging of naïve T cell count
- High CD4:CD8

Effect of Senescent T cells on Mortality in the Very Old

- **Longitudinal Swedish OCTO/NONA studies**
  - Started in 1998
  - Cohort of octo/nonagenerians followed for 6 years
- **OCTO: Immune risk profile (IRP)**
  - CD4/CD8 < 1
    - Primarily due to accumulation of CD8+CD28- senescent T cells
  - Low B cells
  - CMV positive
- **NONA: 16% of cohort in IRP**
  - **100% IRP vs 67% non-IRP individuals deceased after 6 years**
- **Now 95-100 y.o.**
  - No centenarians ever in IRP
  - Don't accumulate $CD28^-$ T cells (even if CMV^+, which 83% are)
  - Have profile of a CMV^- person



Wikby *Immunosenescence* 2007

Immune Risk Profile

A) Hematology	Results	Reference Range
WBC (cell/µl)	5900	3.5 – 9.5 x 10^3
Lymphocyte (%)	22	20 – 48
Lymphocyte /µl	1298	1078 - 2828

B) Flow T-cell subset Analysis

Marker	%	Reference Range (%)	Abs number of (cells /µL)	Reference Range (cell/µL)
B Cell (CD19)	18	5-22	234	74 - 447
PAN T (CD3)	58	58 - 87	753	767 - 2318
T Helper/Inducer (CD4)	27	32 – 59	350	467 - 1350
T Suppressor/Cytotoxic (CD8)	29	13 – 38	376	201 - 868
Ratio (CD4:CD8)	0.93		0.96 – 3.93	
CD8+/CD28- gated on CD3	34	1 – 28	256	11 - 359
CD8+/CD28- gated on CD8	69	4 – 51	259	17 - 364
CD8+/CD95- gated on CD3	4	3 – 27	30	33 -354
CD8+/CD95- gated on CD8	7	11 – 57	26	32 - 347

The percentage of each Lymphocyte subset is calculated using three colors Flow Cytometric analysis based on the selection of CD45+ non granular cells and the expression of CD3, CD4, CD8, or CD19 on the gated cell.

- 84 y.o. male, very healthy, active with h/o early stage PCA rx'd xrt/seeds, CMV+.
- CD4:CD8 = 0.93, inverted
- Low naïve T cell
- Senescent cytotoxic T cells 69% and 259 count

IRP Reversal after 1 year

A) Hematology	Results	Reference Range
WBC (cell/µl)	7600	3.5 – 9.5 x 10^3
Lymphocyte (%)	11	20 – 48
Lymphocyte /µl	836	1078 - 2828

B) Flow T-cell subset Analysis

Marker	%	Reference Range (%)	Abs number of (cells /µL)	Reference Range (cell/µL)
B Cell (CD19)	17	5-22	142	74 - 447
NK Cell(CD56/16)	15	3-26	125	51 - 543
PAN T (CD3)	65	58 - 87	543	767 - 2318
T Helper/Inducer (CD4)	35	32 – 59	293	467 - 1350
T Suppressor/Cytotoxic (CD8)	28	13 – 38	234	201 - 868
Ratio (CD4:CD8)	1.25		0.96 – 3.93	
CD8+/CD28- gated on CD3	26	1 – 28	141	11 - 359
CD8+/CD28- gated on CD8	62	4 – 51	145	17 - 364
CD8+/CD95- gated on CD3	5	3 – 27	27	33 -354
CD8+/CD95- gated on CD8	11	11 – 57	26	32 - 347

The percentage of each Lymphocyte subset is calculated using three colors Flow Cytometric analysis based on the selection of CD45+ non granular cells and the expression of CD3, CD4, CD8, or CD19 on the gated cell.

- Treatment:
  - Comprehensive supplement pack
  - oral telomerase activator derived from astragalus root
- CD4:CD8 went from 0.93 to 1.25 and CD28- count from 259 to 145 (~ 40% reduction)
- Theoretically a significant reduction in 6 yr mortality

New Function of Telomeres

Telomere position effect: regulation of gene expression with progressive telomere shortening over long distances

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While global chromatin conformation studies are emerging, very little is known about the chromatin conformation of human telomeres. Most studies have focused on the role of telomeres as a tumor suppressor mechanism. Here we describe how telomere length regulates gene expression long before telomeres become short enough to produce a DNA damage response (senescence). We directly mapped the interactions adjacent to specific telomere ends using a Hi-C (chromosome capture followed by high-throughput sequencing) technique modified to enrich for specific genomic regions. We demonstrate that chromosome looping brings the telomere close to genes up to 10 Mb away from the telomere when telomeres are long and that the same loci become separated when telomeres are short. Furthermore, expression array analysis reveals that many loci, including noncoding RNAs, may be regulated by telomere length. We report three genes (ISG15 [interferon-stimulated gene 15 kd], DSP [Desmoplakin], and C1S [complement component 1s subcomplement]) located at three different subtelomeric ends (1p, 6p, and 12p) whose expressions are altered with telomere length. Additionally, we confirmed by in situ analysis (3D-FISH [three-dimensional fluorescence in situ hybridization]) that chromosomal looping occurs between the loci of those genes and their respective telomere ends. We term this process TPE-OLD for "telomere position effect over long distances." Our results suggest a potential novel mechanism for how telomere shortening could contribute to aging and disease initiation/progression in human cells long before the induction of a critical DNA damage response.

[*Keywords*: chromatin; replicative aging; senescence; cancer; age-dependent gene expression; telomerase; chromosome looping]

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Conclusions

- Mind your telomeres
- Their health is essential for yours!
- Healthy diet, exercise, supplements, and hormone optimization are essential
- TA-65® is an important, safe, and effective adjunct to a comprehensive age management program

Slides Available

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