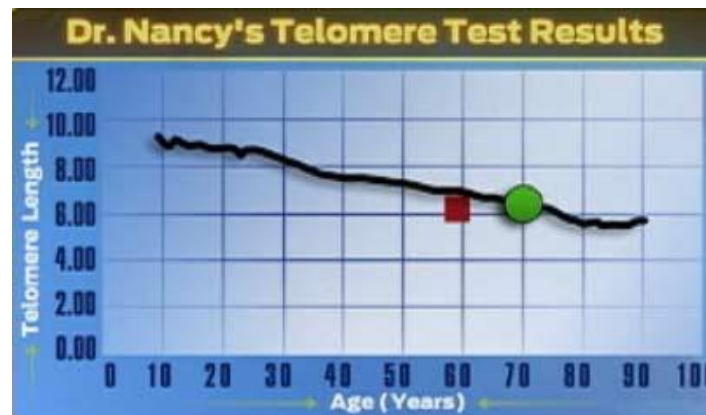
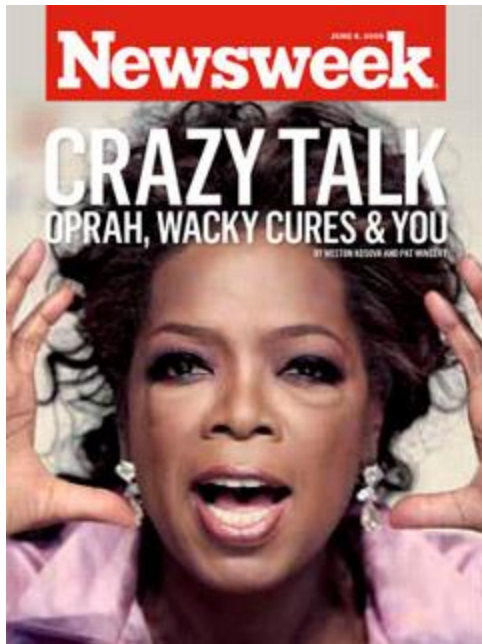


# **Telomere Talk...Pearls from 7 Years of Monitoring Patients on TA-65®<sup>®</sup>**

Joseph M. Raffaele, MD  
Co-Founder PhysioAge Medical Group  
And  
CEO PhysioAge Systems

# Telomeres and TA-65<sup>®</sup> 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



# SOCIAL SECURITY AD



Mike Keefe THE DAWG POST '98

# 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?
- How will I know that it is working?
- Will it increase my risk of cancer?
- How much should I take and for how long?

# Why Pearls? 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<sup>®</sup> .
- Co-author on first two papers describing these results.
- Monitoring hundreds of patients on TA-65<sup>®</sup> for the past 7 years
- Over 1000 telomere length measurements discussed with patients.
- Some patients with yearly follow ups up to 7 years



# 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.**



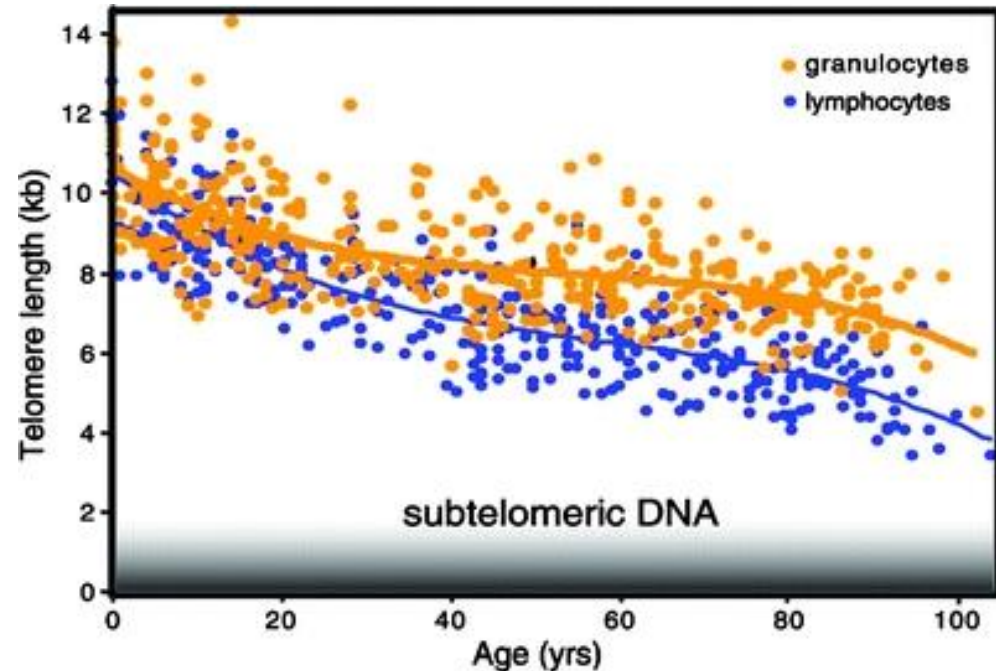
# Why Are Telomeres Important?

## 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**

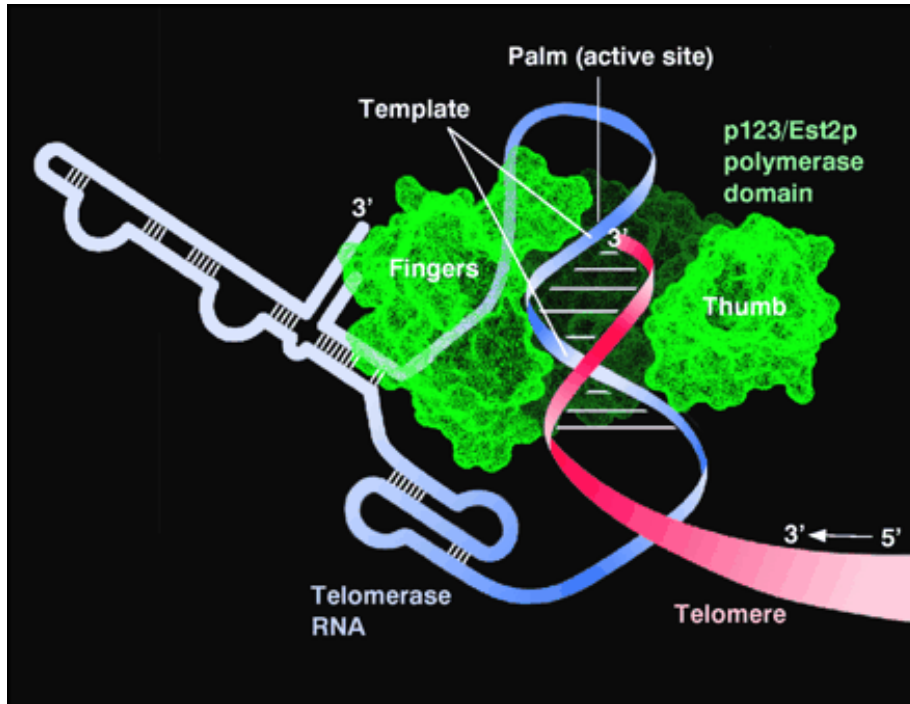
# Telomeres Basics: Age-associated Shortening

- **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*

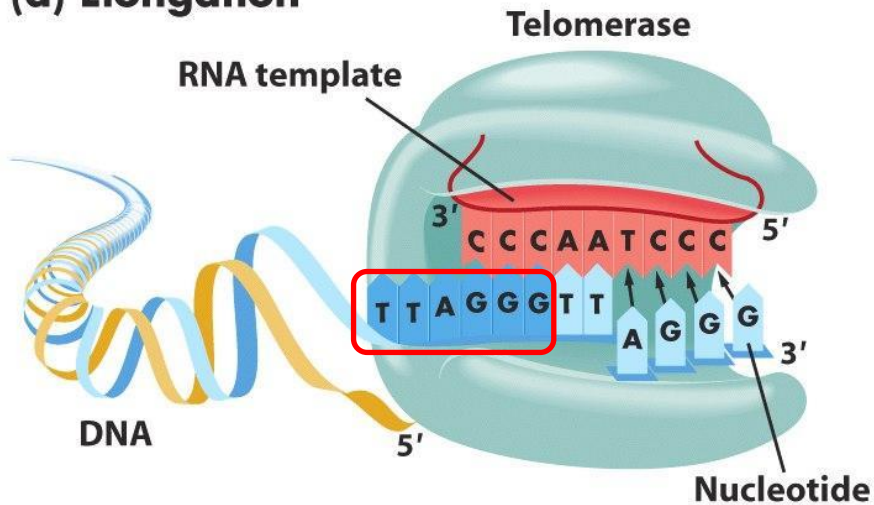
# Telomerase Basics



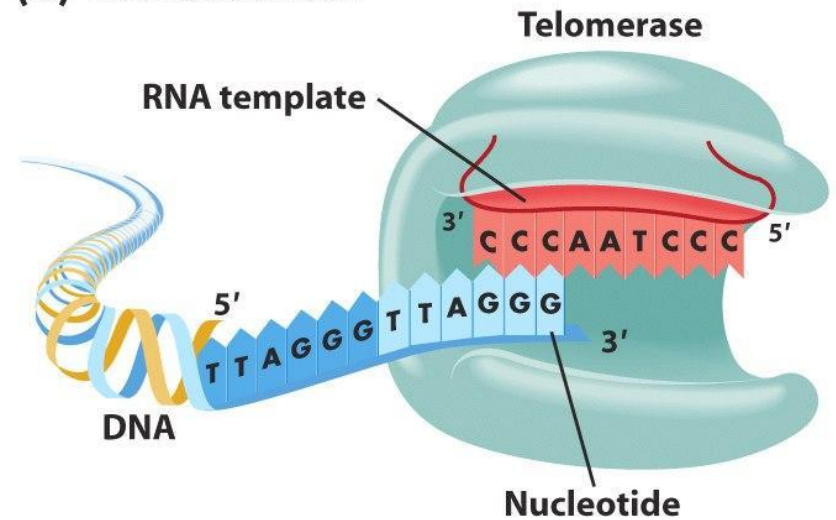
- **Discovered by Elizabeth Blackburn** in 1980—Nobel prize awarded in 2009
- **Structure:** Two components
  - hTERT: **human telomerase reverse transcriptase**, the catalytic component
  - TERC: **telomerase RNA template** component
- **Function: Lengthen telomeres**
- **Activation:**
  - Very active 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

(a) Elongation



(b) Translocation

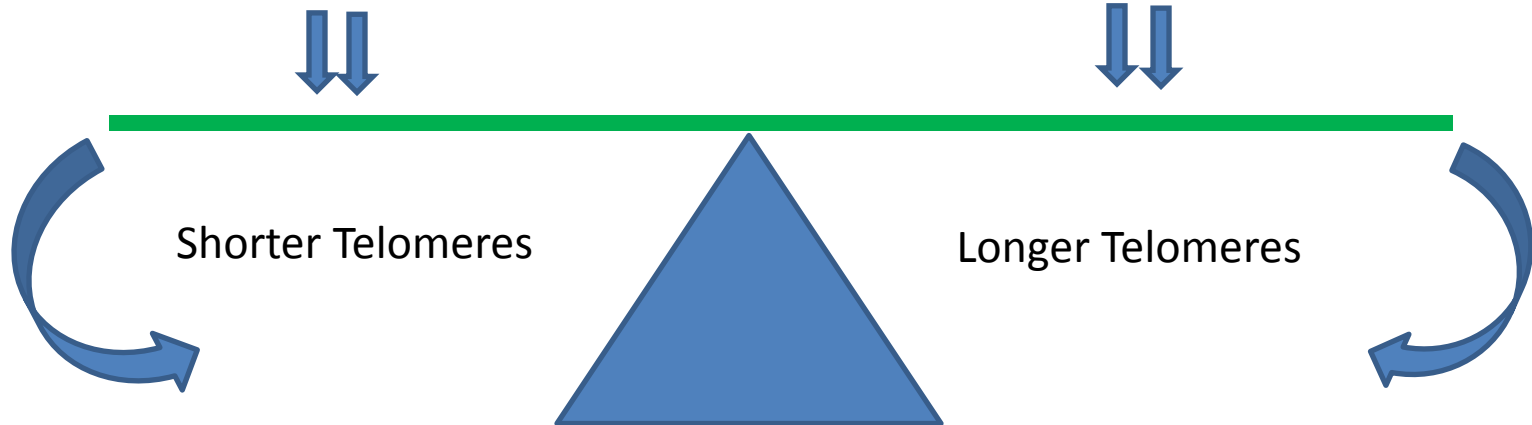




# Telomere Attrition determined by balance between loss and telomerase activity

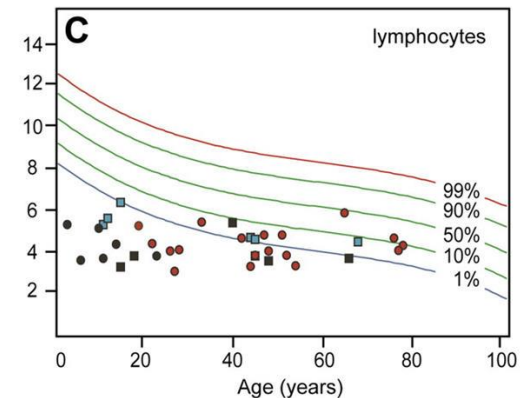
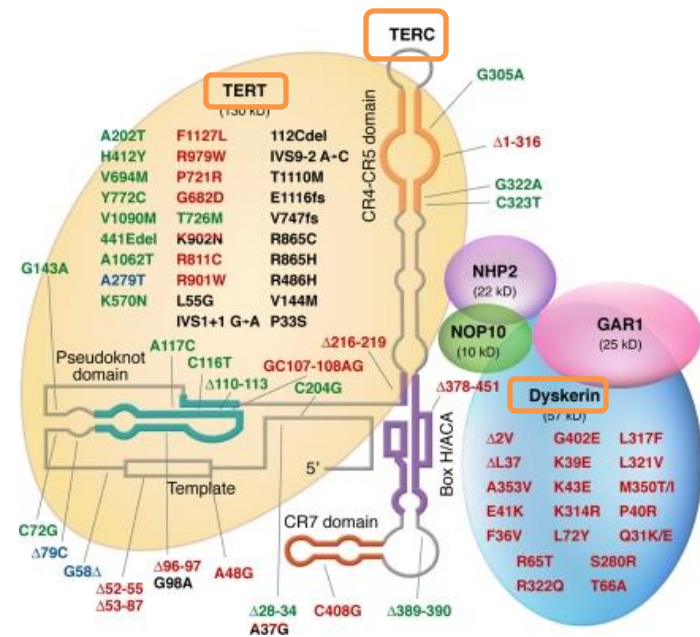
- Proliferative activity
- Oxidative stress
- Inflammation

Telomerase activity



# Genetic Telomere Diseases: Telomeropathies

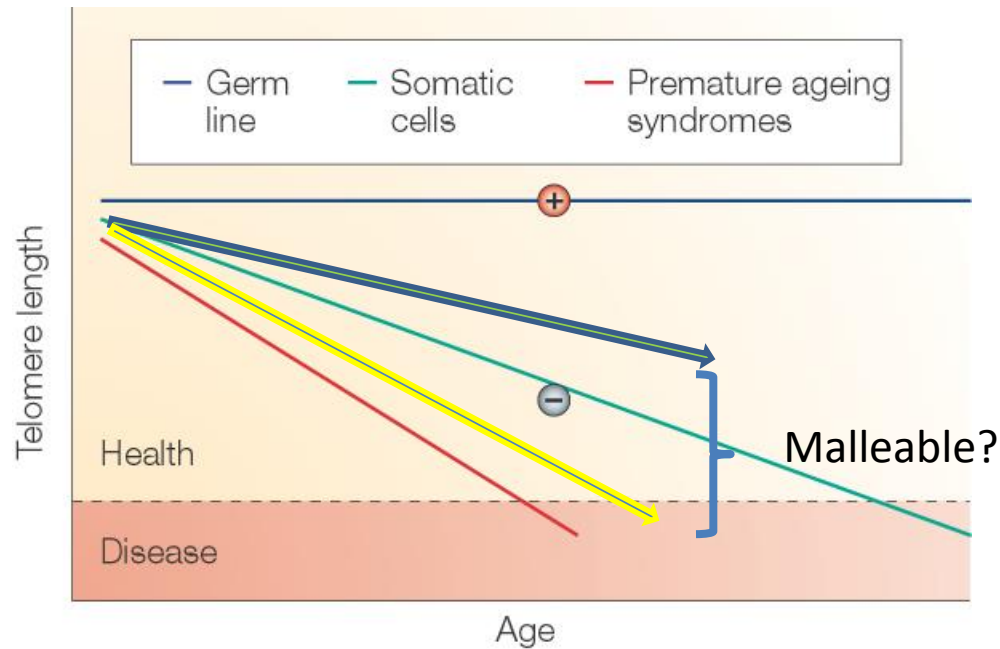
- Genetic disorders with mutations in the telomerase complex
  - Dyskeratosis congenita
    - Abnormal pigmentation, nail dystrophy, short stature, pulmonary and hepatic fibrosis, hypogonadism, bone marrow failure, increased malignancies, premature death
  - Idiopathic pulmonary fibrosis
    - Premature death from fibrosis of lungs
    - Short telomeres a risk factor (15% cases with TERT/TERC mutations)
  - Aplastic anemia
    - Shortened telomeres and premature death
    - 10% idiopathic AA pts have TERT/TERC mutations
- Extremely short telomeres



# 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
  - Progeria
  - Dyskeratosis Congenita
  - Idiopathic Pulmonary Fibrosis
  - Cri du Chat syndrome
  - Down's Syndrome
  - Fanconi's Anemia
  - Tuberous Sclerosis
  - Werner's Syndrome
  - Aplastic Anemia
  - **And, Aging Itself?**

# Differing telomere attrition rates



Adapted from Copyright © 2005 Nature Publishing Group  
Nature Reviews | Genetics




# Do You Know Your Cholesterol Level?

Are you worried about it?

## RESEARCH

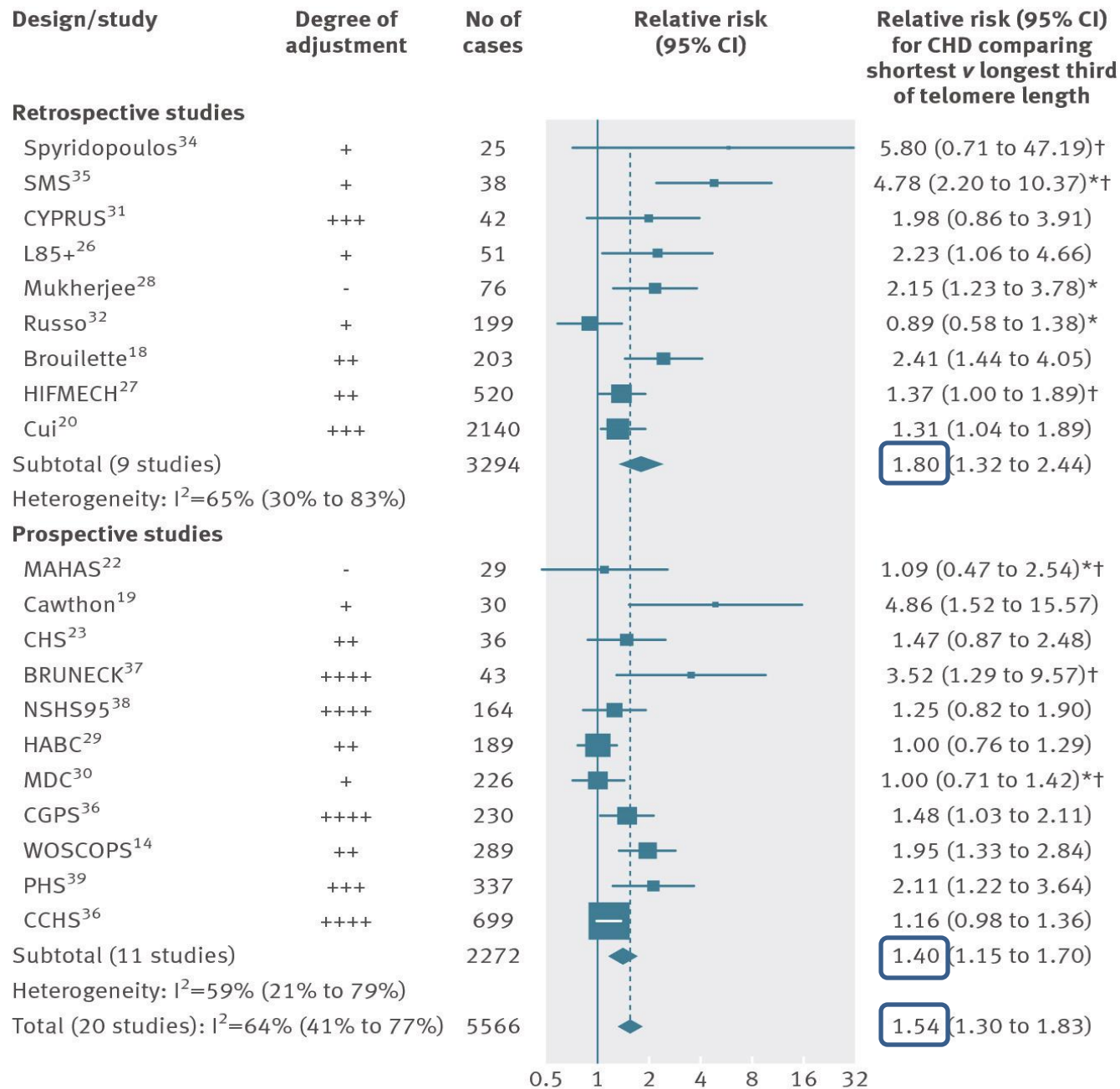
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# Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis

 OPEN ACCESS

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

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

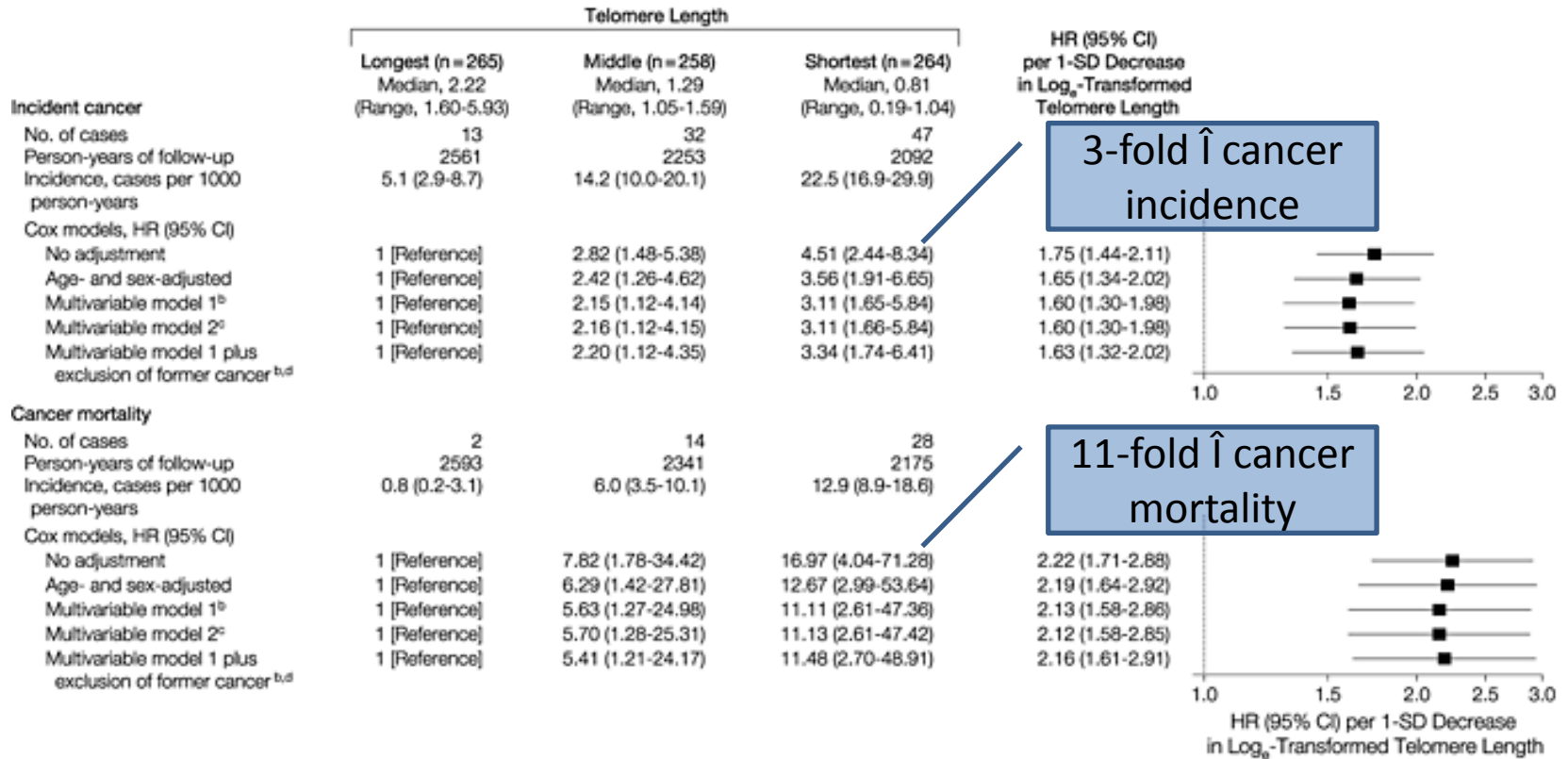


# Are You Worried About Getting Cancer?

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 Willeit, P. et al. JAMA 2010;304:69-75



# 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.



Dilbert.com DilbertCartoonist@gmail.com

THAT'S AN INDICATION  
THAT YOU VALUE WORK  
ABOVE EXERCISE.



7-29-11 ©2011 Scott Adams, Inc.

BUT YOU  
HAVE A  
COMPANY  
GYM.



THAT'S  
OUR  
SLACKER  
TRAP!



# First Age Reversal in a Mammal

## Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice

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

- 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

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

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

<sup>\*</sup>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; <sup>†</sup>Falk Cardiovascular Research Center, Stanford University School of Medicine, Stanford, California, USA; <sup>‡</sup>SpectraCell Laboratories, Inc., Houston, Texas, USA; and <sup>§</sup>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<sup>®</sup>?

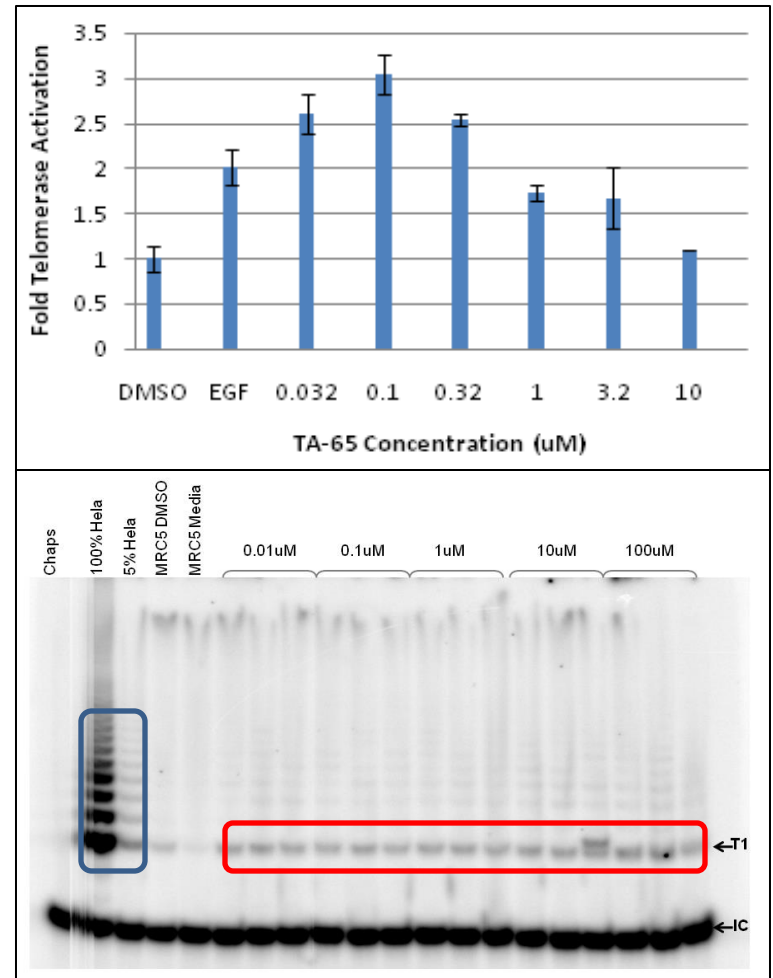
# TA-65<sup>®</sup>

## The Only Proven Commercially Available Telomerase Activation Product

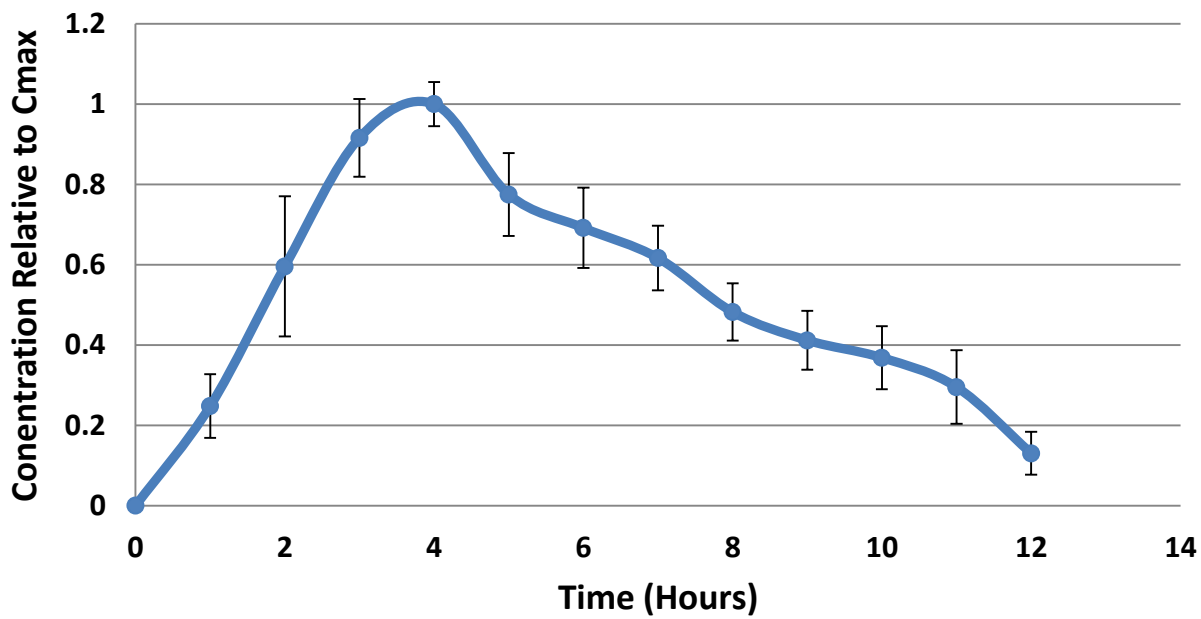
- TA-65<sup>®</sup> is a rare molecule discovered in a common medicinal plant, *astragalus membranaceus*.
- TA-65<sup>®</sup> is proven to transiently activate telomerase.
- 98% pure extract

# *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)



## Pharmacokinetic Profile of TA-65® (12 Subjects)

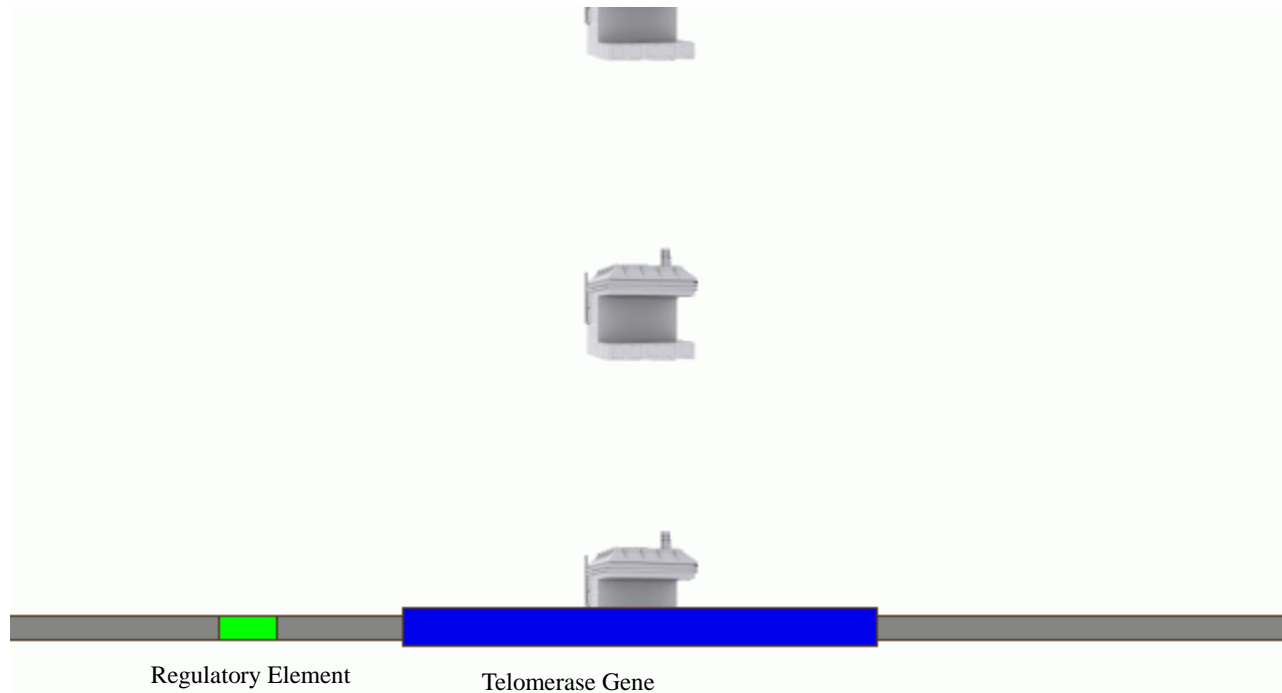


TA Sciences data

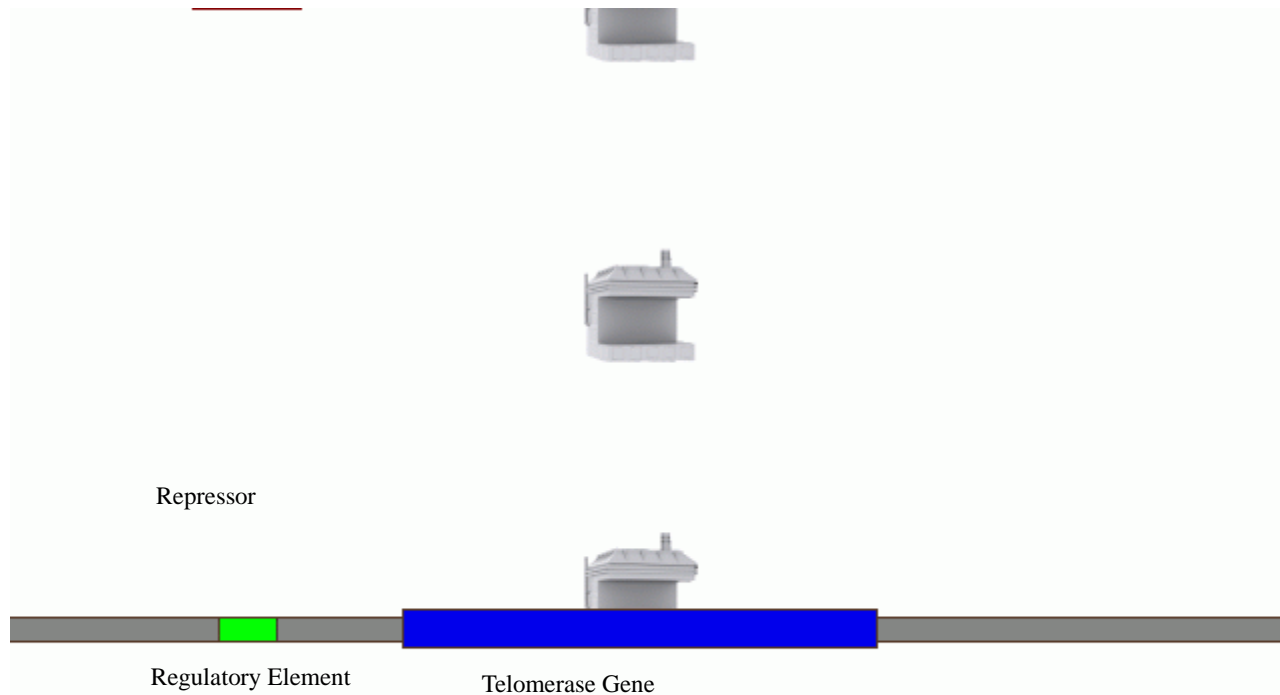


How does TA-65<sup>®</sup> Work?

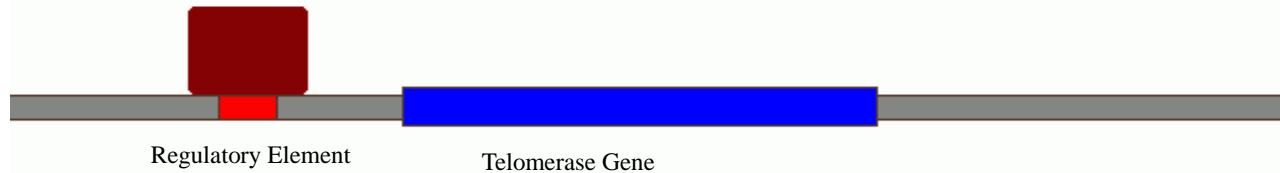
# Reproductive Cells Produce Telomerase



# The Gene is Repressed in All Other Cells



# TA-65<sup>®</sup> 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,<sup>1</sup> Kerstin Schneeberger,<sup>1</sup>  
Elsa Vera,<sup>1,2</sup> Agueda Tejera,<sup>1</sup> Calvin B. Harley<sup>3</sup> and  
Maria A. Blasco<sup>1</sup>

- 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<sup>®</sup> Clinical Research

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

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

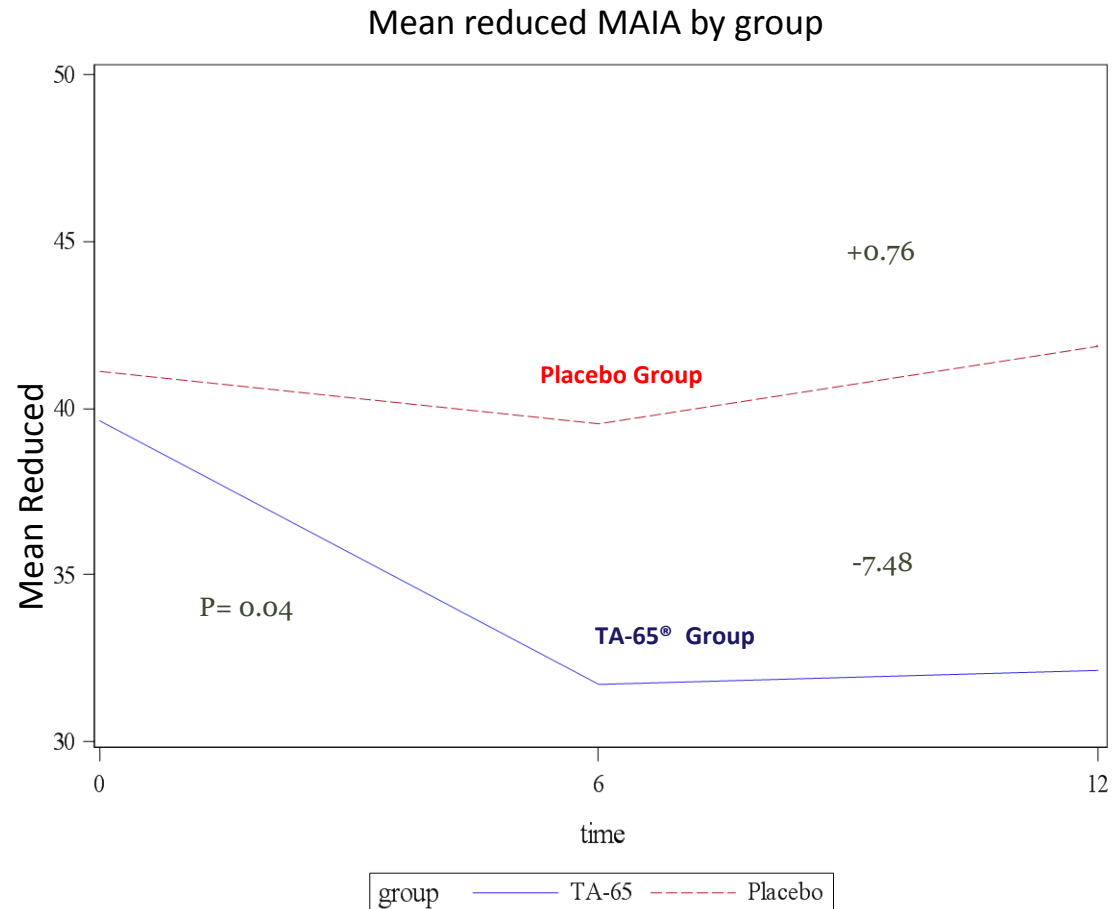
1. A Natural Product Telomerase Activator As Part of a Health Maintenance Program. Harley CB, et al. Rejuvenation Research. 2011 February;14(1):45-56.
2. A Natural Product Telomerase Activator as part of a Health Maintenance Program: Metabolic and Cardiovascular Response. Harley CB, et al. Rejuvenation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



# Evaluation of Telomerase Activator TA-65<sup>®</sup> for early ARMD ( Age Related Macular Degeneration)

Improvements in Eye Function as indicated by MAIA

- 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



# Effect of TA-65<sup>®</sup> 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<sup>®</sup> 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 *

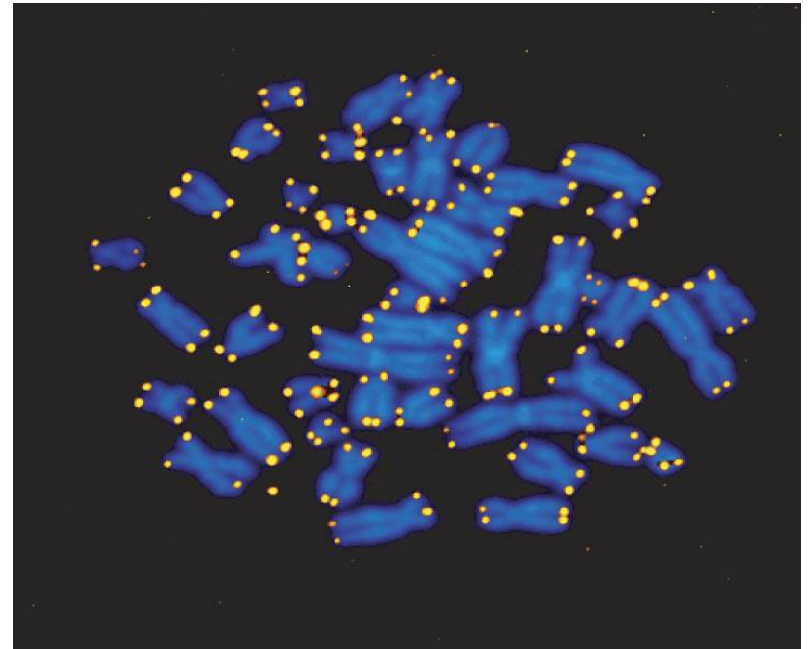
\* Statistically significant

# How Do I Know if I Need to Take TA-65<sup>®</sup> ?

- 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**

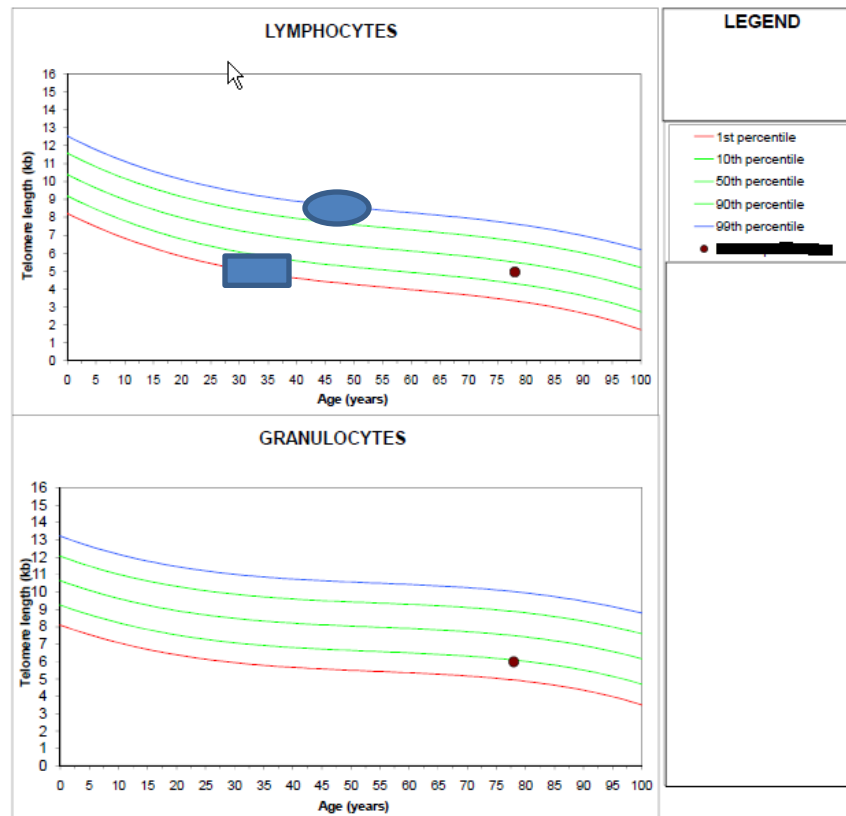


# Lymphocyte and granulocyte mean telomere length (Repeat Diagnostics)

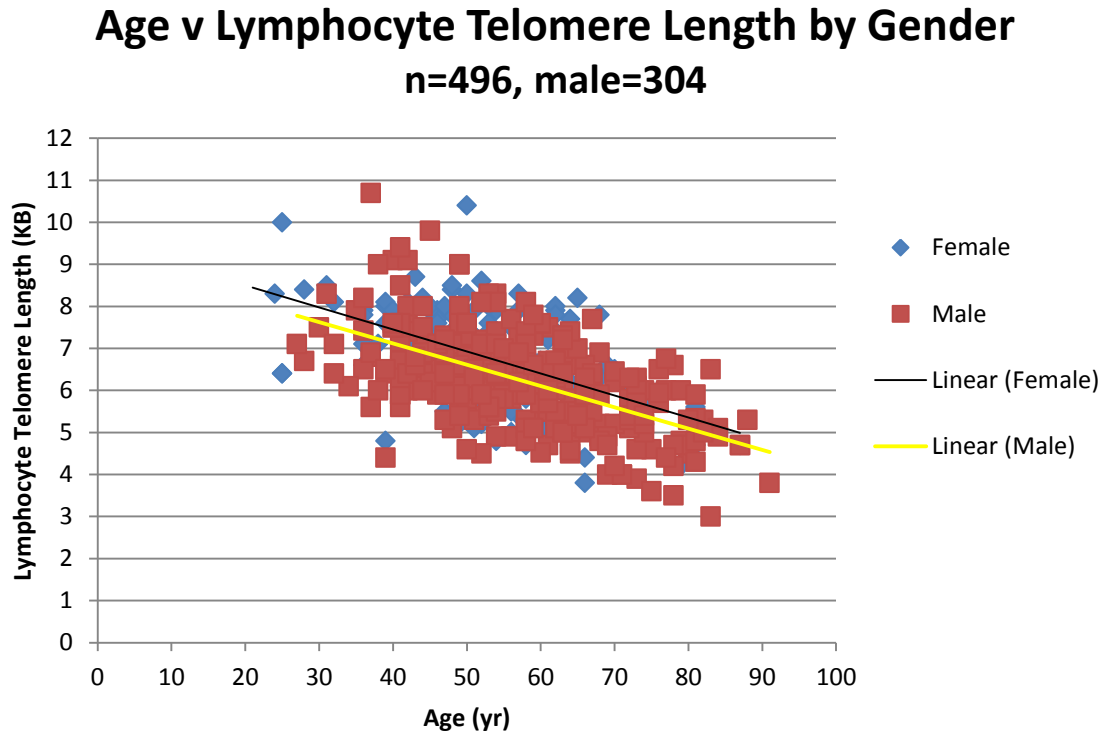
Lymphocytes			Granulocytes		
MTL (kb)	MTLN (kb)	INT	MTL (kb)	MTLN (kb)	INT
4.9	5.5	N	6.0	7.5	L

\* 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



# Cross-sectional Telomere Length Change with Age



Unpublished data PhysioAge Systems 2008-2015



# What Will I Notice if I Take TA-65<sup>®</sup> ?

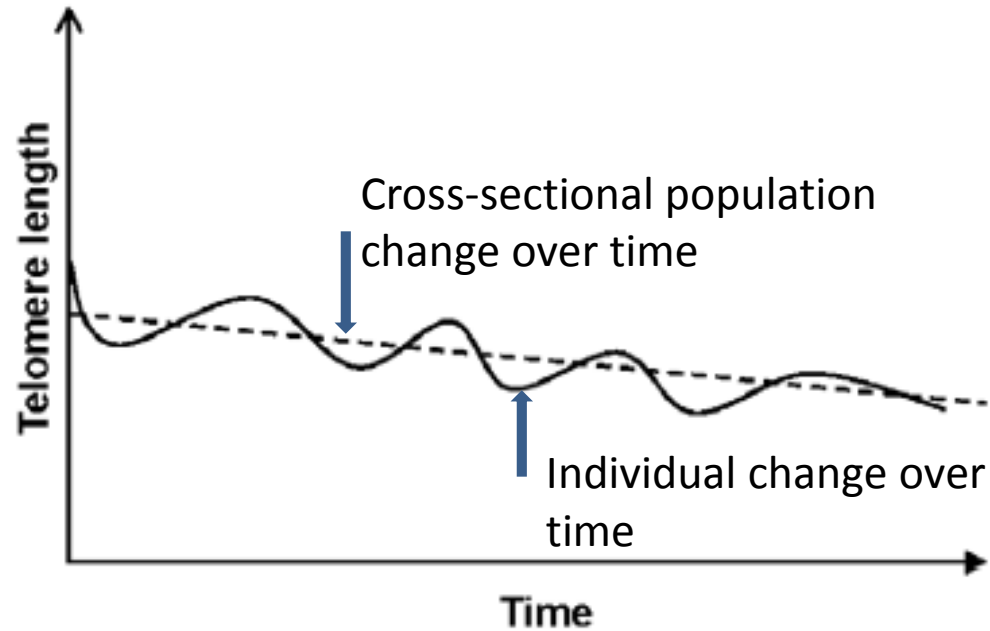
- Subjective:
  - Improved energy
  - Improved recovery from workouts in athletes
  - Decreased graying of hair
  - Better vision
  - Improved skin
- Objective:
  - Reduction in presbyopia documented for a number of people
  - Improved skin appearance and pigmentation

# 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

## Telomere Length Dynamics



**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.

## How "Reversible" Is Telomeric Aging?

Elissa Epel

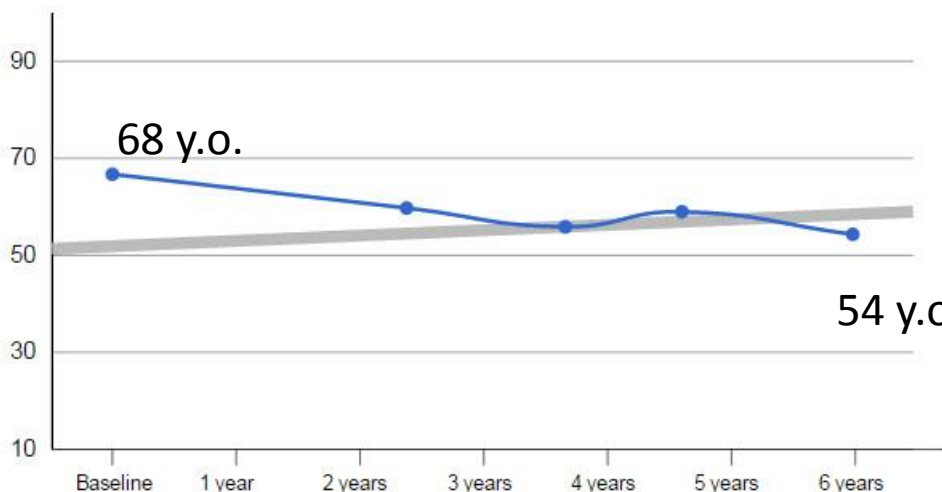
### Abstract

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”

# TelomerAge

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



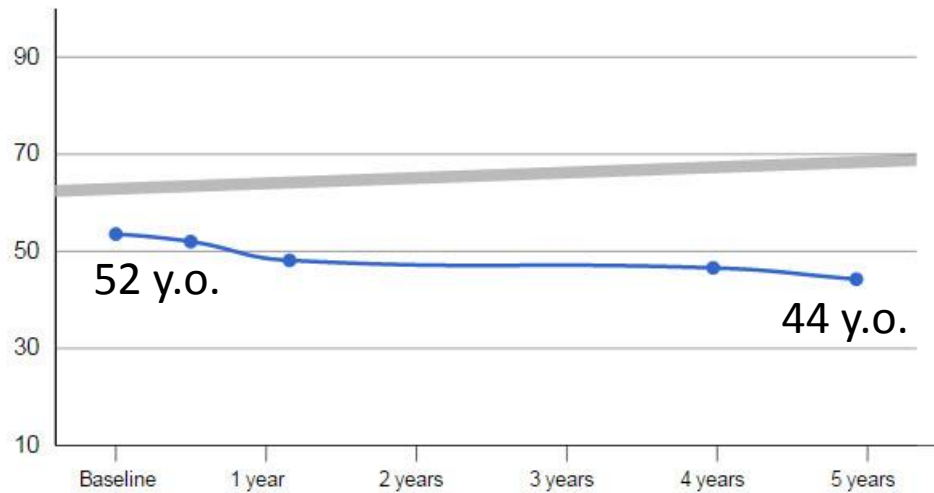
1.6 kb lymphocyte  
Telomere length  
increase over 6  
yrs

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

Name	Result	Units	Normal Range	Optimal Range	Baseline	Change	
<b>Telomere Length</b>							
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
<b>Telomere Length</b>							
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<sup>®</sup> 500 IU/D



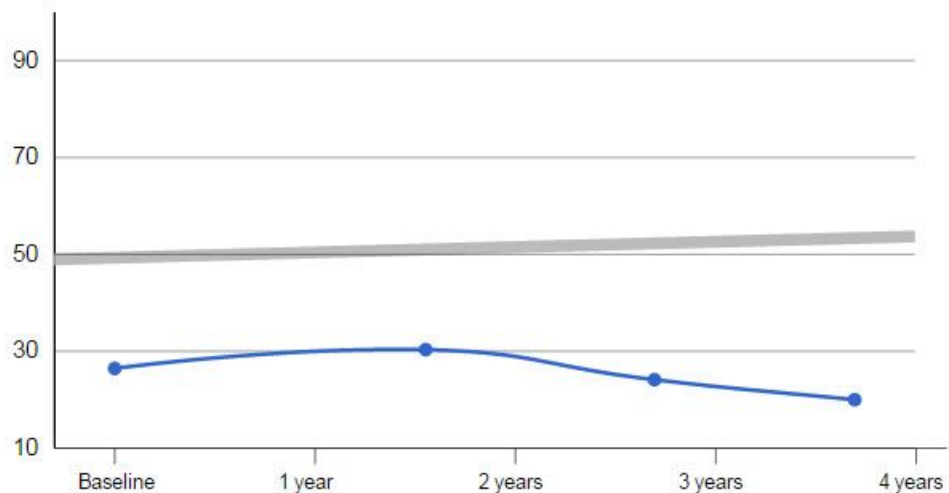
1.2 kb increase in lymphocyte telomere length over 5 years

**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
<b>Granulocyte Telomere Length</b>				8.0	7.7	8.2	8.7	8.6
<b>Lymphocyte Telomere Length</b>				6.9	7.1	7.6	7.8	8.1

# TelomerAge

53 y.o. woman on no telomerase meds



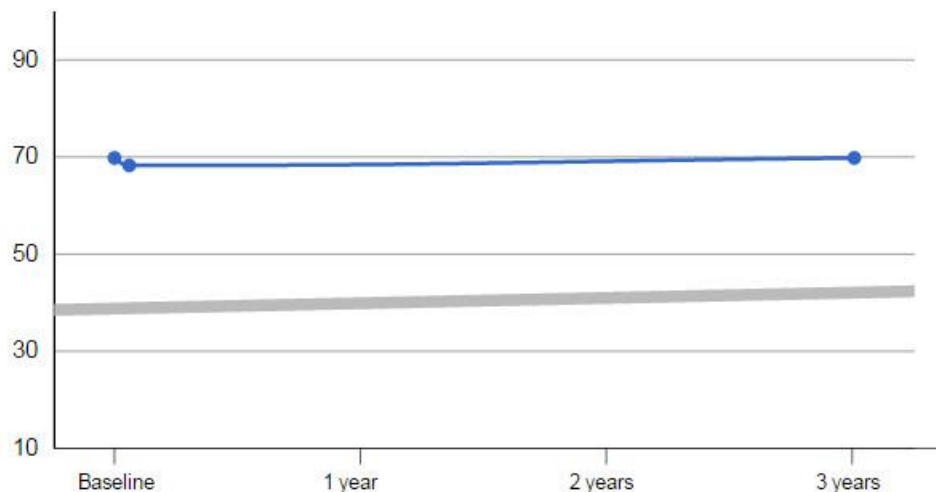
**Your TelomerAge is 20.0, 33 years younger than average for your age.**

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
<b>Granulocyte Telomere Length</b>			10.0	9.3	10.6	10.9
<b>Lymphocyte Telomere Length</b>			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
------------	---------------	---------------	---------------

<b>Granulocyte Telomere Length</b>	5.6	5.0	5.5
------------------------------------	-----	-----	-----

<b>Lymphocyte Telomere Length</b>	4.8	5.0	4.8
-----------------------------------	-----	-----	-----

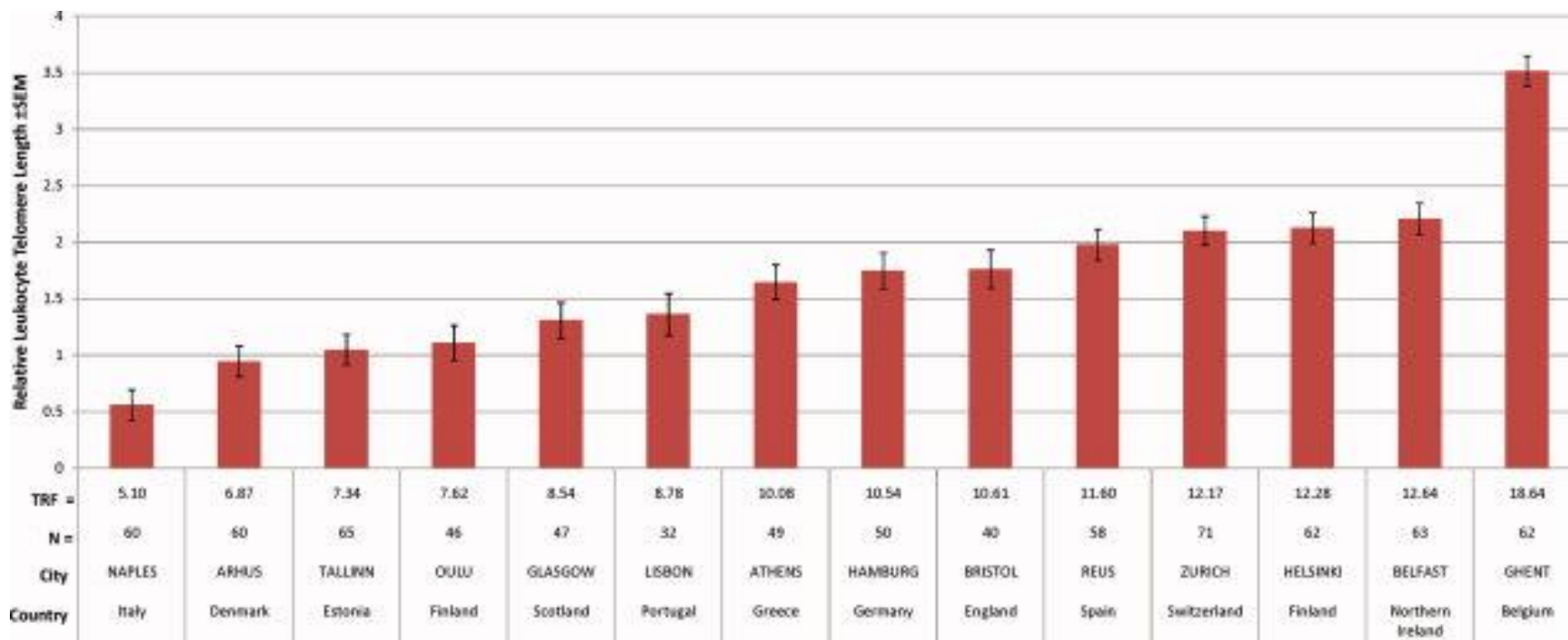
Name	Result	Units	Normal Range	Optimal Range	Baseline	Change
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## Telomere Length

Granulocyte Telomere Length	5.5	kb	5.5 - 10.0	> 9	5.6	-2 %
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Lymphocyte Telomere Length	4.8	kb	4.5 - 9.0	> 8	4.8	0 %
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## Substantial variation in qPCR measured mean blood telomere lengths in young men from eleven European countries



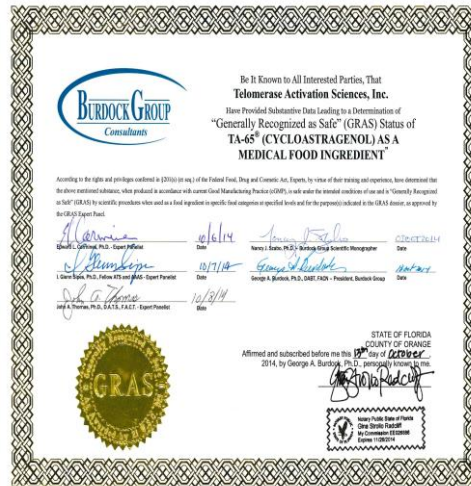
Mean telomere length can vary widely between different populations  
5.2 kb in Naples up to 18.6 kb in Ghent.

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

# 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”



- 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<sup>®</sup> 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<sup>®</sup> 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

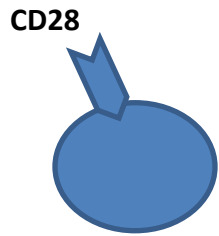
Mid-length telomeres

Very short telomeres

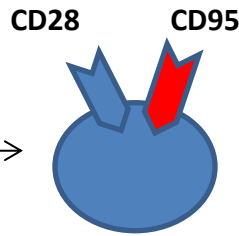
**Naïve T cell**

**Healthy T cell**

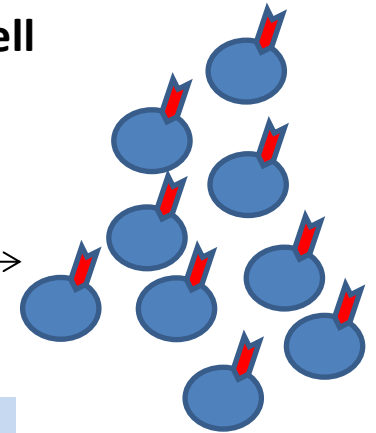
**Senescent T cell**



Antigen exposure



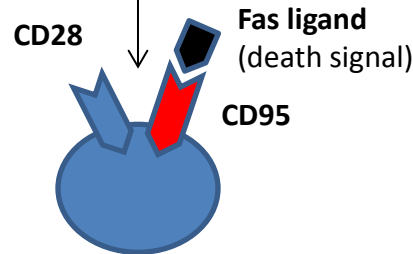
Chronic stimulation



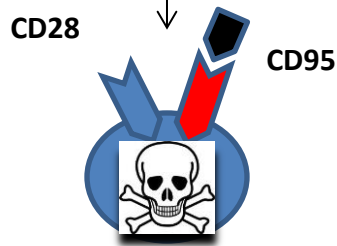
CD8+CD28+CD95-

CD8+CD28+CD95+

CD8+CD28-CD95+



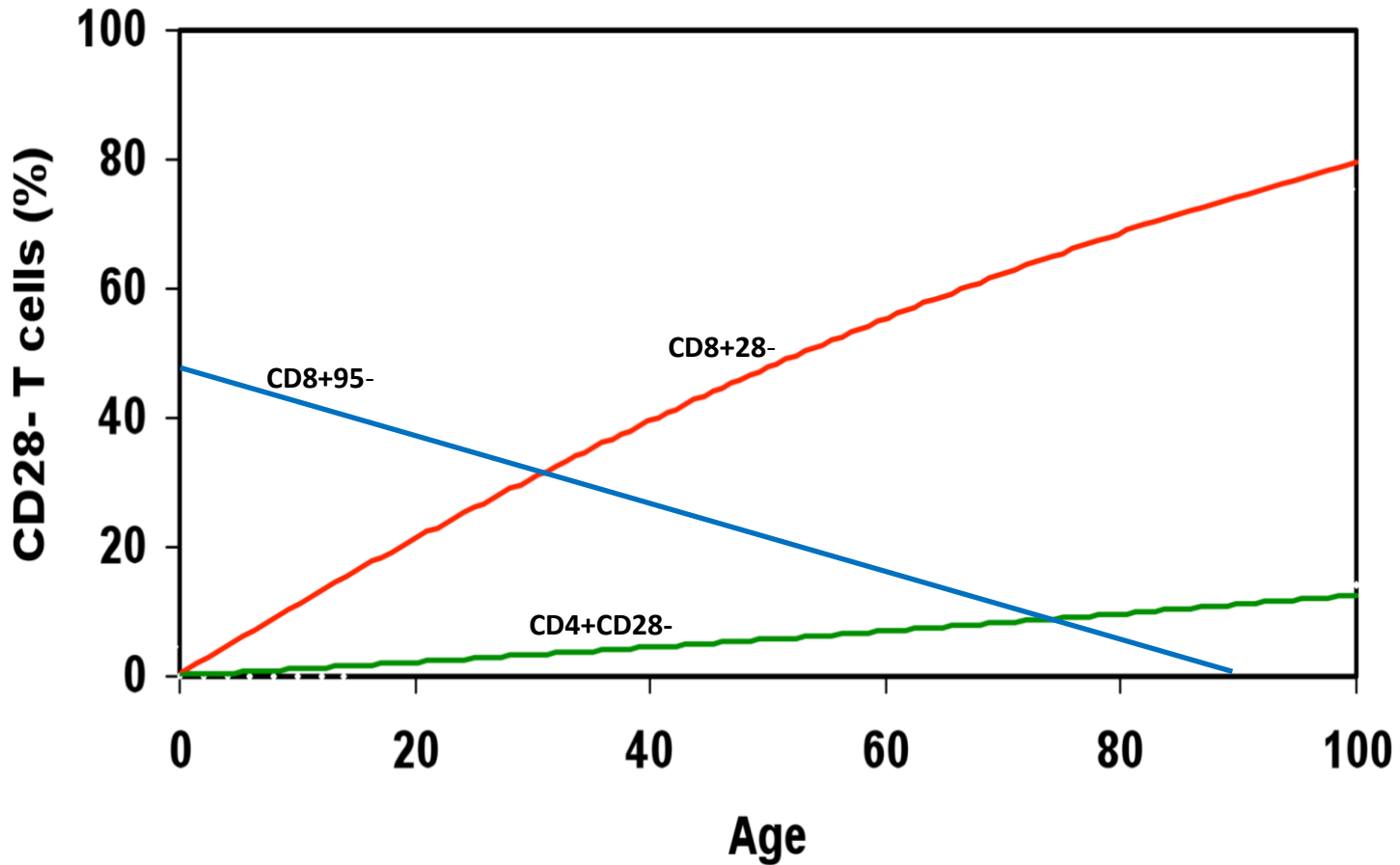
Apoptosis



Fills up “immunological space”

Clears up “immunological space”

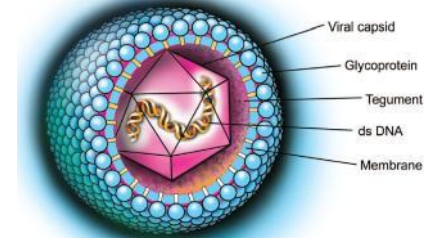
# *In vivo* change in CD28 and CD95 expression with age



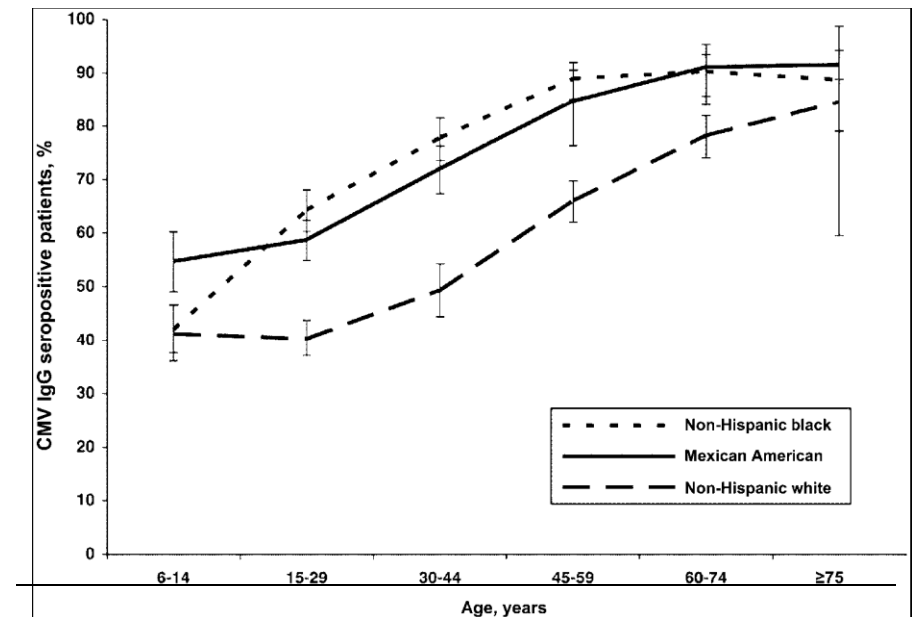
Adapted from Weng N-P 2009 *Trends Immunol*

# 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**



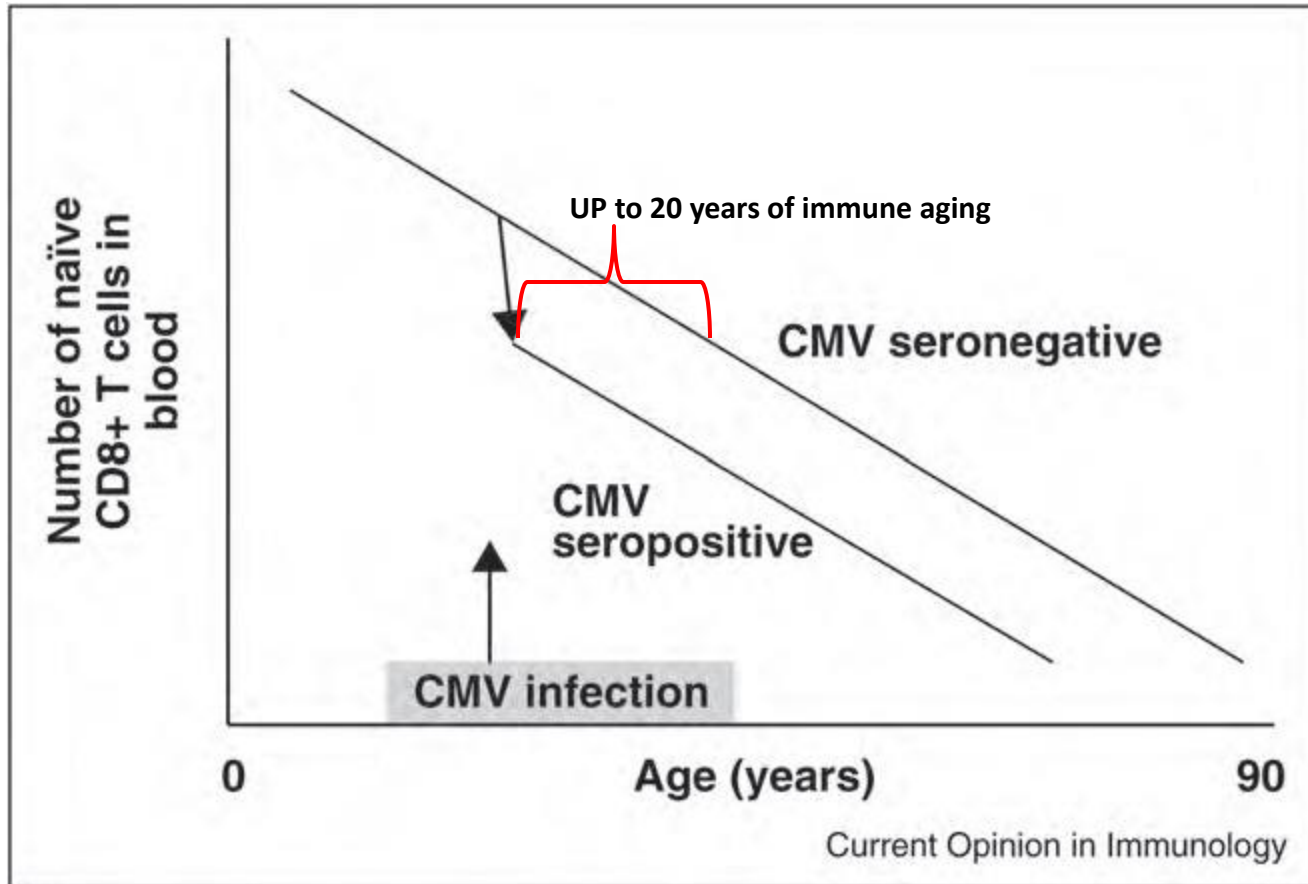
HCMV Human Cytomegalovirus



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.”

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



# Young lymphocyte subset panel

A) Hematology	Results	Reference Range
WBC (cell/ $\mu$ l)	7900	3.5 - 9.5 x 10 <sup>3</sup>
Lymphocyte (%)	23	20 - 48
Lymphocyte / $\mu$ l	1817	1078 - 2828

## B) Flow T-cell subset Analysis

Marker	%	Reference Range (%)	Abs number of (cells / $\mu$ L)	Reference Range (cell/ $\mu$ L)
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

- 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

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.



# “Youthful” lymphocyte subset panel

## A) Hematology

	<u>Results</u>	<u>Reference Range</u>
WBC (cell/ $\mu$ l)	7000	$3.5 - 9.5 \times 10^3$
Lymphocyte (%)	24	20 - 48
Lymphocyte / $\mu$ l	1680	1078 - 2828

## B) Flow T-cell subset Analysis

Marker	%	Reference Range (%)	Abs number of (cells / $\mu$ L)	Reference Range (cell/ $\mu$ L)
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

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

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.

# No accumulation of senescent T cells

## A) Hematology

	<u>Results</u>	<u>Reference Range</u>
WBC (cell/ $\mu$ l)	8900	3.5 – 9.5 x 10 <sup>3</sup>
Lymphocyte (%)	24	20 – 48
Lymphocyte / $\mu$ l	2136	1078 - 2828

## B) Flow T-cell subset Analysis

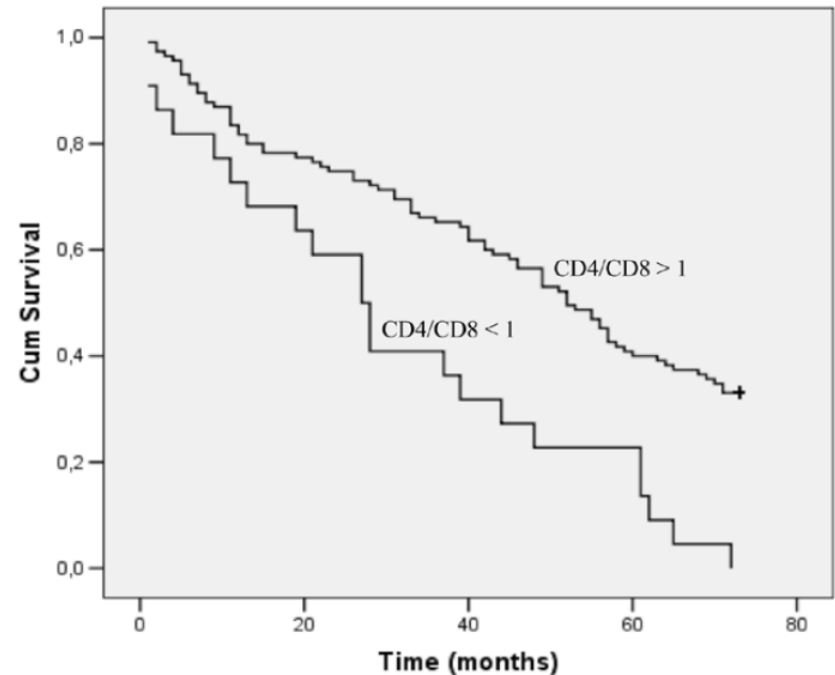
Marker	%	Reference Range (%)	Abs number of (cells / $\mu$ L)	Reference Range (cell/ $\mu$ L)
B Cell ( CD19)	10	5-22	214	74 - 447
NK Cell( CD56/16)	15	3-26	320	51 - 543
PAN T (CD3)	76	58 - 87	1623	767 - 2318
T Helper/Inducer (CD4)	63	32 – 59	1346	467 - 1350
T Suppressor/Cytotoxic (CD8)	10	13 – 38	214	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/nonagenarians followed for 6 years
- **OCTO: Immune risk profile (IRP)**
  - CD4/CD8 < 1
    - Primarily due to accumulation of CD8<sup>+</sup>CD28<sup>-</sup> 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<sup>-</sup> T cells (even if CMV<sup>+</sup>, which 83% are)
  - Have profile of a CMV<sup>-</sup> person



# Immune Risk Profile

## A) Hematology

	<u>Results</u>	<u>Reference Range</u>
WBC (cell/ $\mu$ l)	5900	3.5 – 9.5 x 10 <sup>3</sup>
Lymphocyte (%)	22	20 – 48
Lymphocyte / $\mu$ l	1298	1078 - 2828

## B) Flow T-cell subset Analysis

Marker	%	Reference Range (%)	Abs number of (cells / $\mu$ L)	Reference Range (cell/ $\mu$ L)
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

- 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

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.

# IRP Reversal after 1 year

A) Hematology	<u>Results</u>	<u>Reference Range</u>
WBC (cell/ $\mu$ l)	7600	3.5 – 9.5 x 10 <sup>3</sup>
Lymphocyte (%)	11	20 – 48
Lymphocyte / $\mu$ l	836	1078 - 2828

B) Flow T-cell subset Analysis				
Marker	%	Reference Range (%)	Abs number of (cells / $\mu$ L)	Reference Range (cell/ $\mu$ L)
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 subcomponent]) 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]

Supplemental material is available for this article.

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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<sup>®</sup> is an important, safe, and effective adjunct to a comprehensive age management program

Slides Available soon

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