Telomeres, Telomerase, and TA-65

What you need to know in 2013

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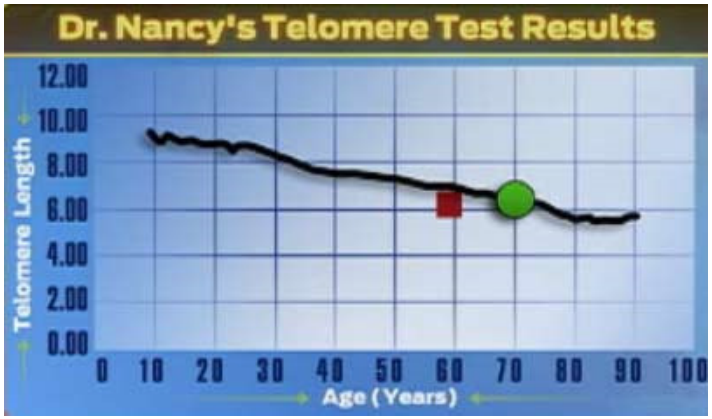
Why do I need to know about telomeres?



Exponential growth in research

Pubmed search

Search term	1990	2011	2013/total
Telomere	107	2449	1698/14,770
Telomere Aging	3	578	323/2902
Telomere CVD	2	163	88/514
Telomere cancer	19	787	491/4503

Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature. 1990;345:458–60.

Objectives

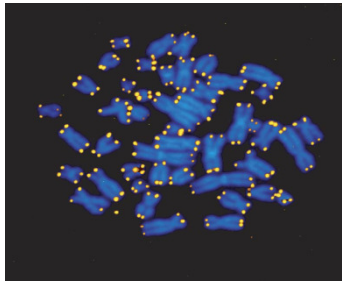
- Briefly discuss the structure and function of human telomeres and telomerase.
- Discuss replicative senescence and the telomere theory of aging
- Briefly discuss the literature demonstrating the strong association between telomere length and aging/chronic disease risk.
- Briefly review the clinically available methods for telomere length measurement (qPCR, Flow-FISH, and HT Q-FISH for short telomeres)
- Briefly discuss the currently available ways to slow telomere shortening through diet, exercise, stress reduction, antioxidants, bHRT, and small-molecule telomerase activation.
- Review the results of the first published in vivo human study of TA-65
- Discuss the practical aspects of monitoring telomerase activation therapy effectiveness and some of the pitfalls of telomere testing

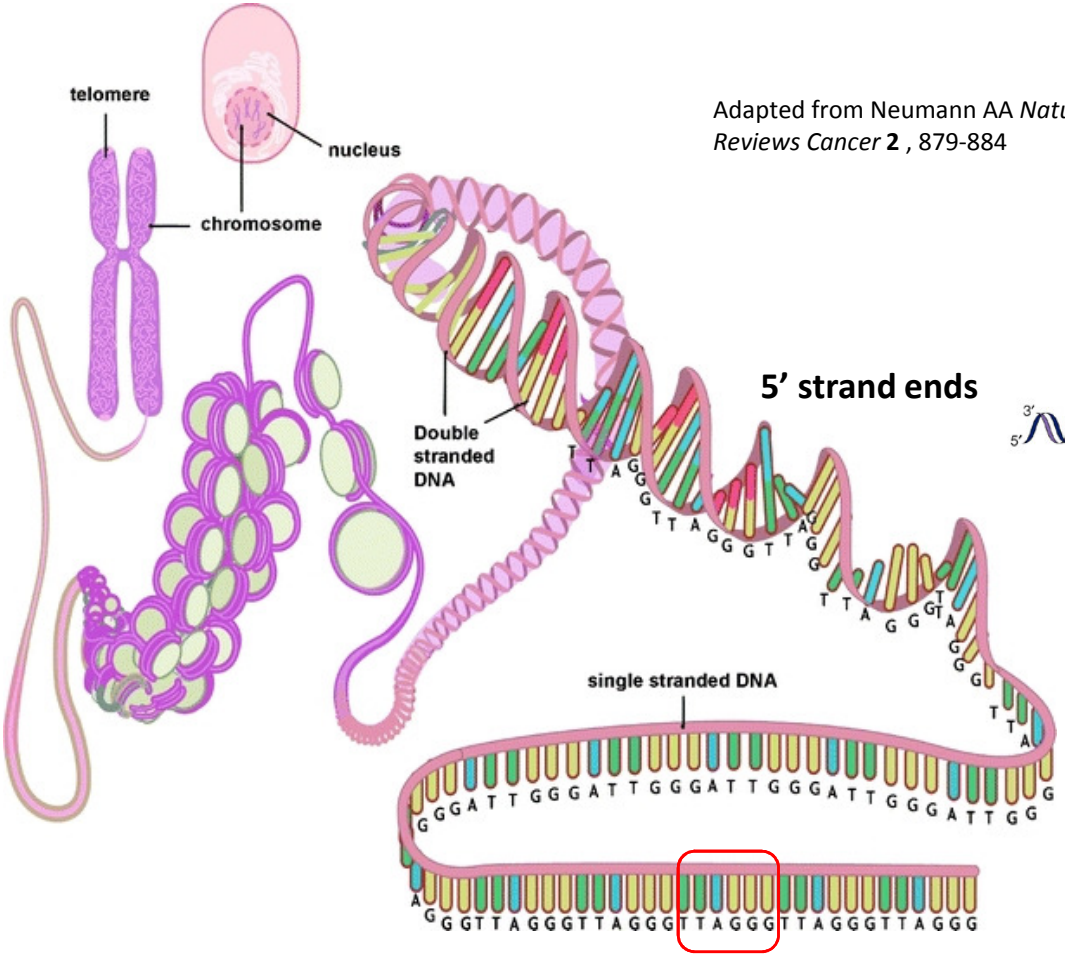
What do Telomeres do?

- 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 can not divide when telomeres get too short)

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

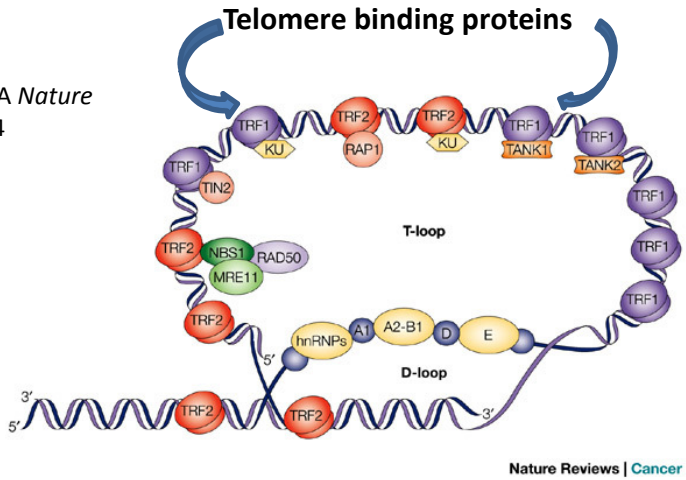
Telomere Basics: Structure

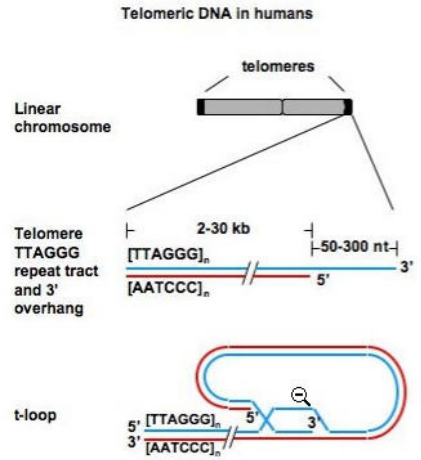




Adapted from Neumann AA *Nature Reviews Cancer* **2** , 879-884

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

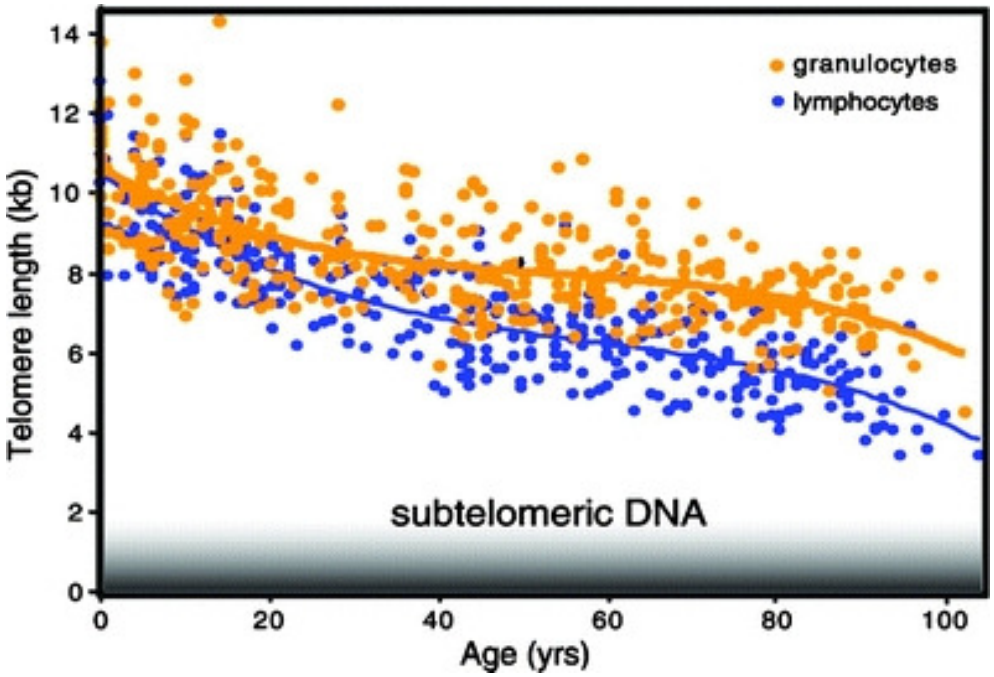




Telomere caps

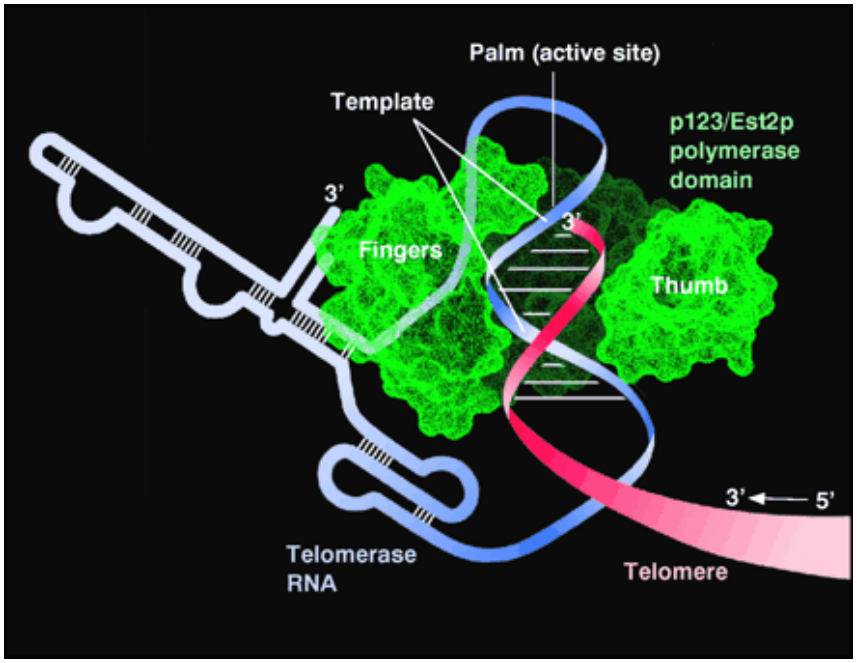
Telomeres Basics: Age-associated Shortening

- **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



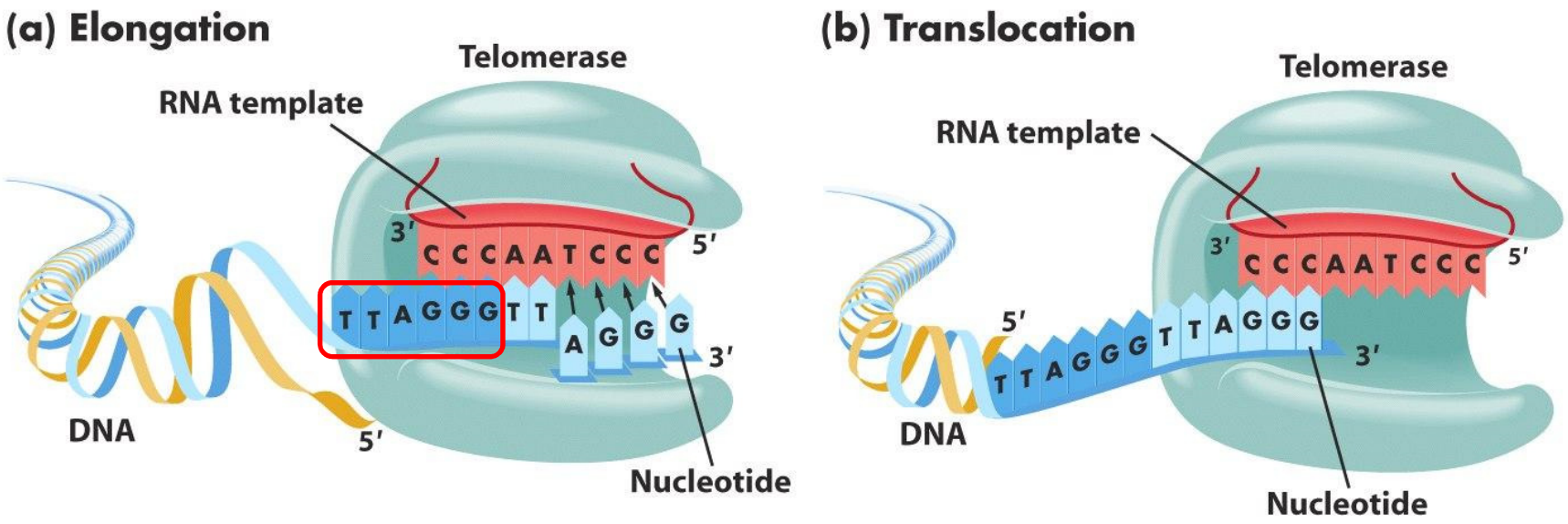
Aubert and Lansdorp 2008 *Physiol Rev*

Telomerase Basics

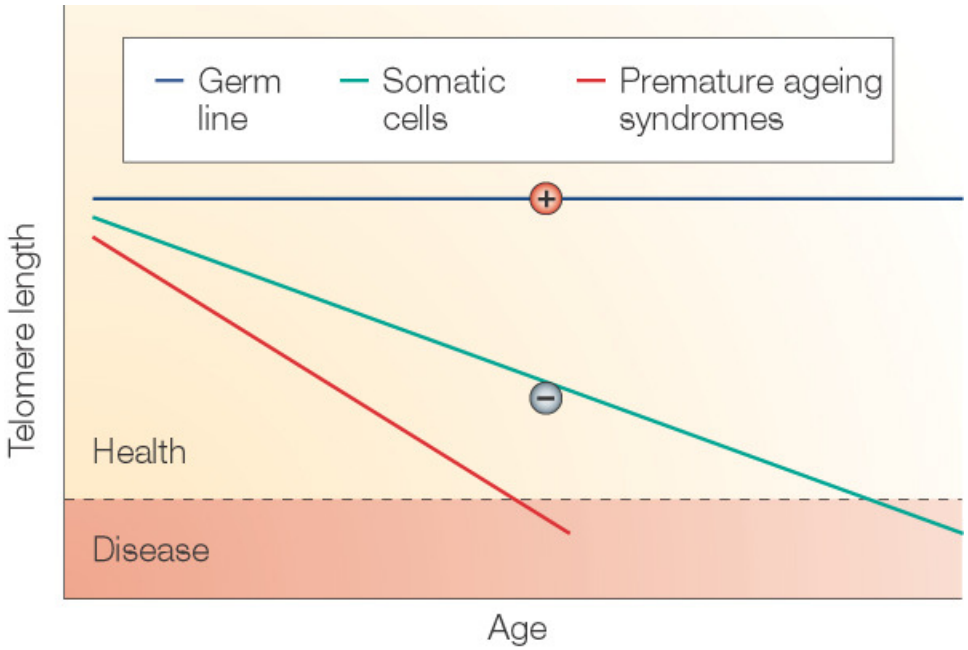


- **Discovered by Elizabeth Blackburn** in 1980—Nobel prize awarded in 2009
- **Structure**: Two components
  - hTERT: **h**uman **te**lomerase **r**everse **t**ranscriptase, the catalytic component
  - TERC: **te**lomerase **R**NA template **c**omponent
- **Function**: Lengthen telomeres
- **Activation**:
  - 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**
- **Reactivation**:
  - hTERT gene transduction
  - Small molecule hTERT transcription activator

Telomerase Basics: How it works



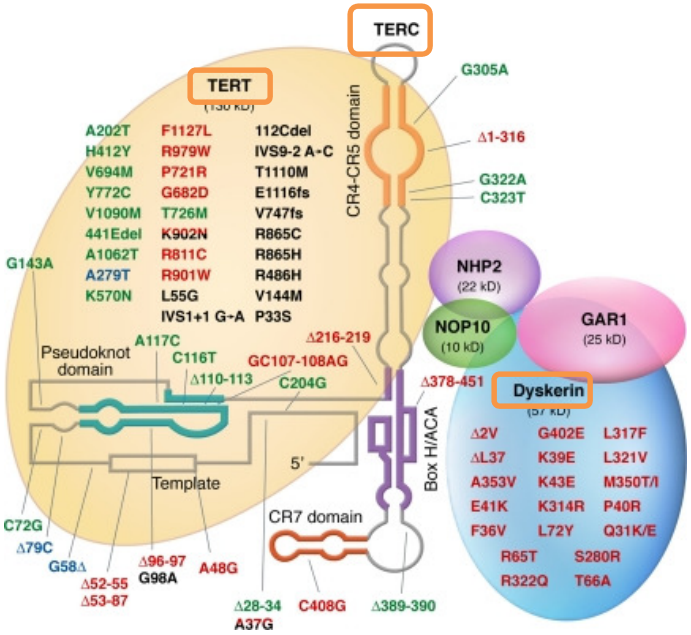
Differing telomere attrition rates

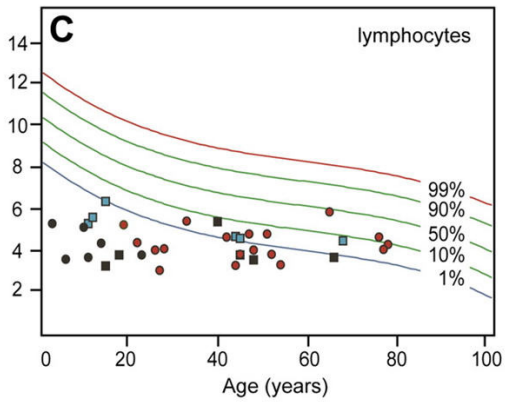


Nature Reviews | Genetics

Telomere Diseases: Telomeropathies

- Genetic disorders with mutations in 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





Senescence: Important concepts

- Senescence comes from the Latin word *senex*, meaning old man or old age
- At the level of the organism:
  - Senescence = aging
  - It is defined as the decrease over time in an organism's ability to maintain homeostasis—the condition when all its systems are in balance and the body as a whole is working as it should—in the face of stress
  - Causes an increase in morbidity and mortality
- At the level of the cell:
  - Senescence = replicative senescence
  - It is confined to mitotic cells
  - Post-mitotic cells can become damaged, but technically they don't senesce

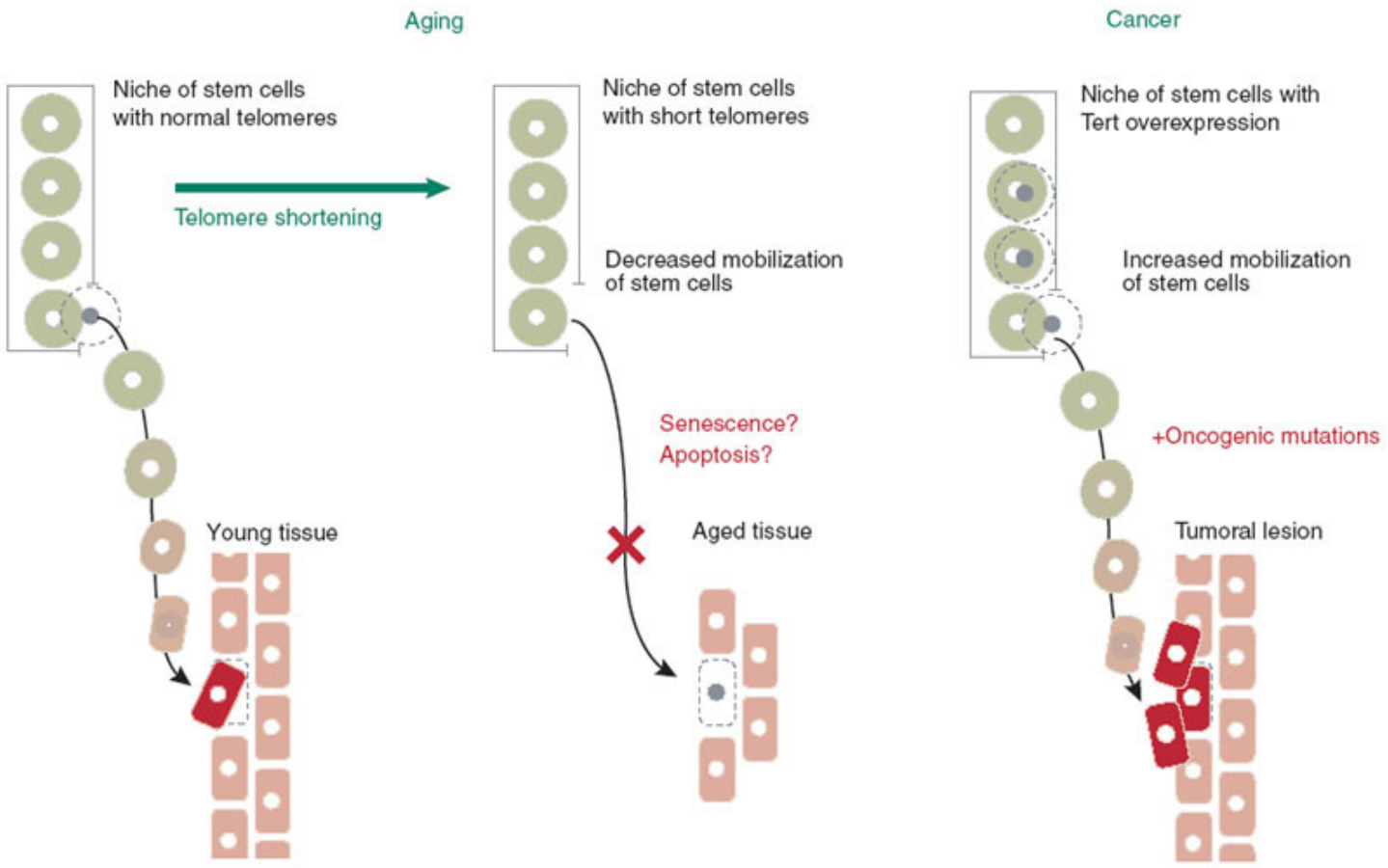
What does in vitro replicative senescence have to with human aging?

- Naïve hypothesis: cell aging = organism aging
  - The hayflick phenomenon in vitro is the same mechanism for multicellular organism aging
- Common objections:
  - Many important cells of vital tissues don't divide
    - Neurons
    - Myocytes: cardiac and skeletal
  - Tissues of old people aren't full of senescent cells (at most up to 15%)
  - Telomere lengths don't correlate very well with species lifespan: e.g., mice have very long telomeres compared to humans (>20 Kb), but they live only a few years

Tissue stem cells

- Cardiac stem cells
  - Replace damaged post-mitotic cardiac muscle
- Muscle stem cells (satellite cells)
  - Replace damage post-mitotic skeletal myocytes
- Endothelial progenitor cells
  - Replace endothelial cells
- Epidermal stem cells
  - Replace keratinocytes
- Gastrointestinal crypt cells
  - Replace gastrointestinal epithelial cells
- **Hematopoetic stem cells**
  - Replace erythrocytes, granulocytes, lymphocytes, platelets

Stem cell theory of aging



From Blasco, M 2007 Nature/Chemical Biology

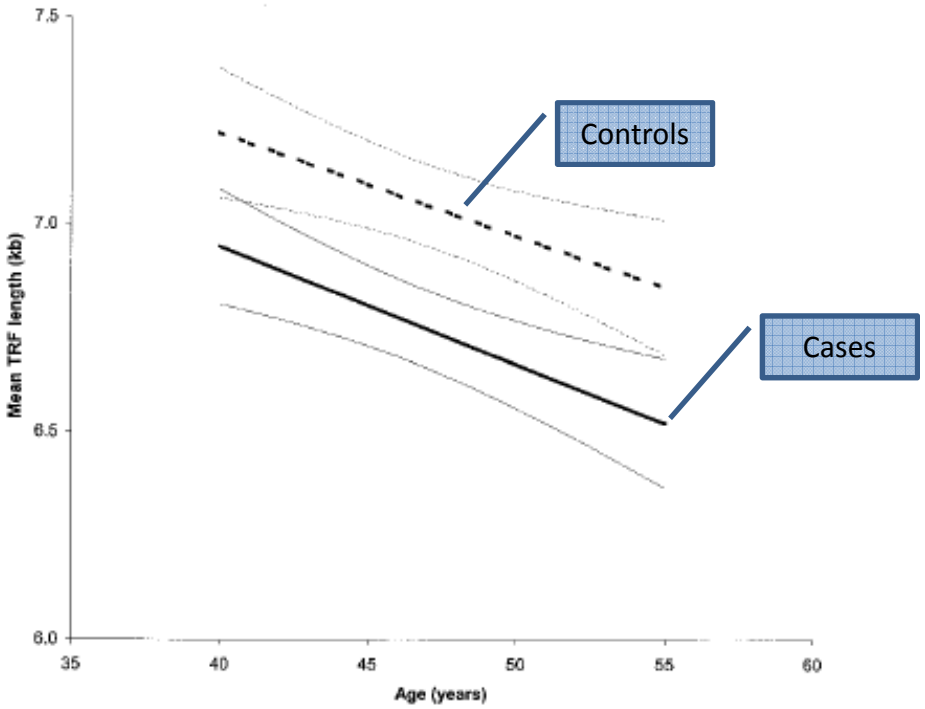
Telomerase is not an oncogene

- 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**

Telomeres and Disease Association

- **Chronic disease association**
  - Hypertension
  - Atherosclerosis
  - CVD
  - Alzheimer's dementia
  - Obesity/Diabetes
  - Metabolic syndrome
  - Cancer
  - Chronic stress
- **Mortality association**:
  - Cawthon 2003 *Lancet:* Landmark study in subjects 60 years old
    - Those with longest telomeres lived longer than shortest telomeres. Cause of death infection
  - Fitzpatrick 2011 *J Gerontol A Biol Sci Med Sci*: The Cardiovascular Health Study
    - Shortest quartile of telomere length 60% more likely to die than longest quartile. Cause again infectious

Telomere length sheds light on relationship between CVD risk factors and events



- Having shorter than average lymphocyte mean telomere length **increased the risk of premature MI roughly 3-fold**
- The difference in telomere length between cases and controls translates into **a biological age difference of 11 years**

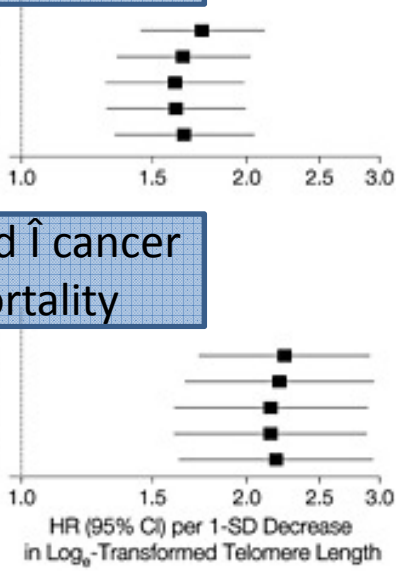
Brouilette S Arterioscler Throm Vasc Biol 2003

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

	Telomere Length			
	Longest (n = 265) Median, 2.22 (Range, 1.60-5.93)	Middle (n = 258) Median, 1.29 (Range, 1.05-1.59)	Shortest (n = 264) Median, 0.81 (Range, 0.19-1.04)	HR (95% CI) per 1-SD Decrease in Log_e-Transformed Telomere Length
Incident cancer				
No. of cases	13	32	47	
Person-years of follow-up	2561	2253	2092	
Incidence, cases per 1000 person-years	5.1 (2.9-8.7)	14.2 (10.0-20.1)	22.5 (16.9-29.9)	
Cox models, HR (95% CI)				
No adjustment	1 [Reference]	2.82 (1.48-5.38)	4.51 (2.44-8.34)	1.75 (1.44-2.11)
Age- and sex-adjusted	1 [Reference]	2.42 (1.26-4.62)	3.56 (1.91-6.65)	1.65 (1.34-2.02)
Multivariable model 1[b]	1 [Reference]	2.15 (1.12-4.14)	3.11 (1.65-5.84)	1.60 (1.30-1.98)
Multivariable model 2[c]	1 [Reference]	2.16 (1.12-4.15)	3.11 (1.66-5.84)	1.60 (1.30-1.98)
Multivariable model 1 plus exclusion of former cancer[b,d]	1 [Reference]	2.20 (1.12-4.35)	3.34 (1.74-6.41)	1.63 (1.32-2.02)
Cancer mortality				
No. of cases	2	14	28	
Person-years of follow-up	2593	2341	2175	
Incidence, cases per 1000 person-years	0.8 (0.2-3.1)	6.0 (3.5-10.1)	12.9 (8.9-18.6)	
Cox models, HR (95% CI)				
No adjustment	1 [Reference]	7.82 (1.78-34.42)	16.97 (4.04-71.28)	2.22 (1.71-2.88)
Age- and sex-adjusted	1 [Reference]	6.29 (1.42-27.81)	12.67 (2.99-53.64)	2.19 (1.64-2.92)
Multivariable model 1[b]	1 [Reference]	5.63 (1.27-24.98)	11.11 (2.61-47.36)	2.13 (1.58-2.86)
Multivariable model 2[c]	1 [Reference]	5.70 (1.28-25.31)	11.13 (2.61-47.42)	2.12 (1.58-2.85)
Multivariable model 1 plus exclusion of former cancer[b,d]	1 [Reference]	5.41 (1.21-24.17)	11.48 (2.70-48.91)	2.16 (1.61-2.91)

3-fold ↑ cancer incidence

11-fold ↑ cancer mortality

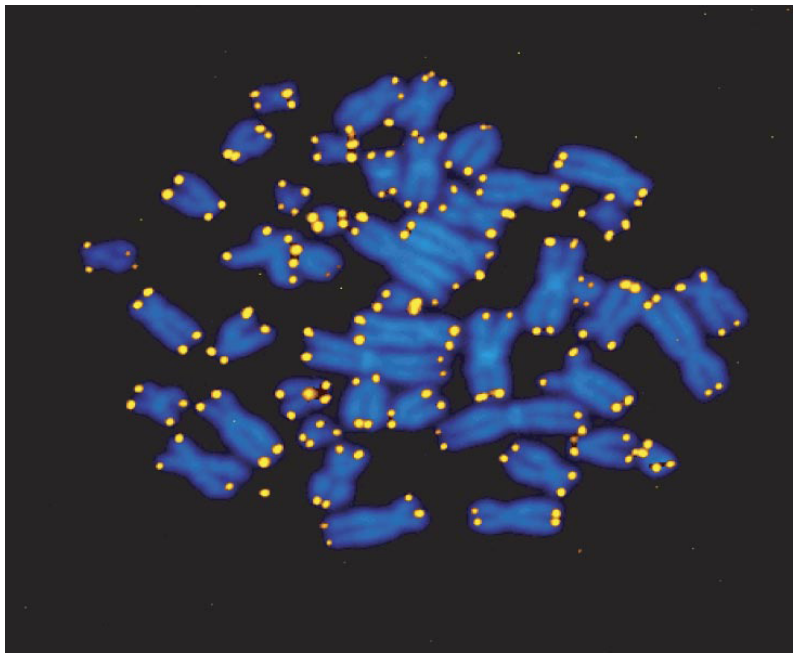


Willeit, P. et al. JAMA 2010;304:69-75

JAMA

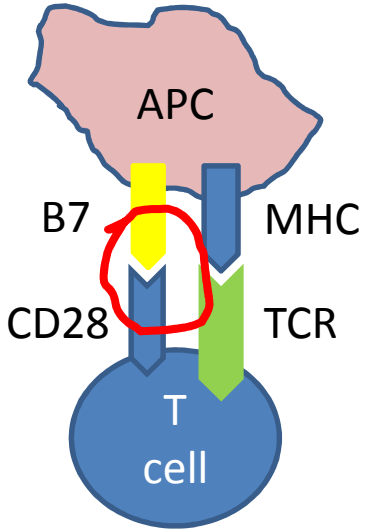
Leukocyte telomere length: Measurement techniques

- **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
    - Telome health
  - Flow-FISH: Lymphocytes and Granulocytes
    - Repeat Diagnostics
  - HT Q-FISH Percent Shortest Telomeres
    - Life Length



Replicative Senescence: Why does it happen?

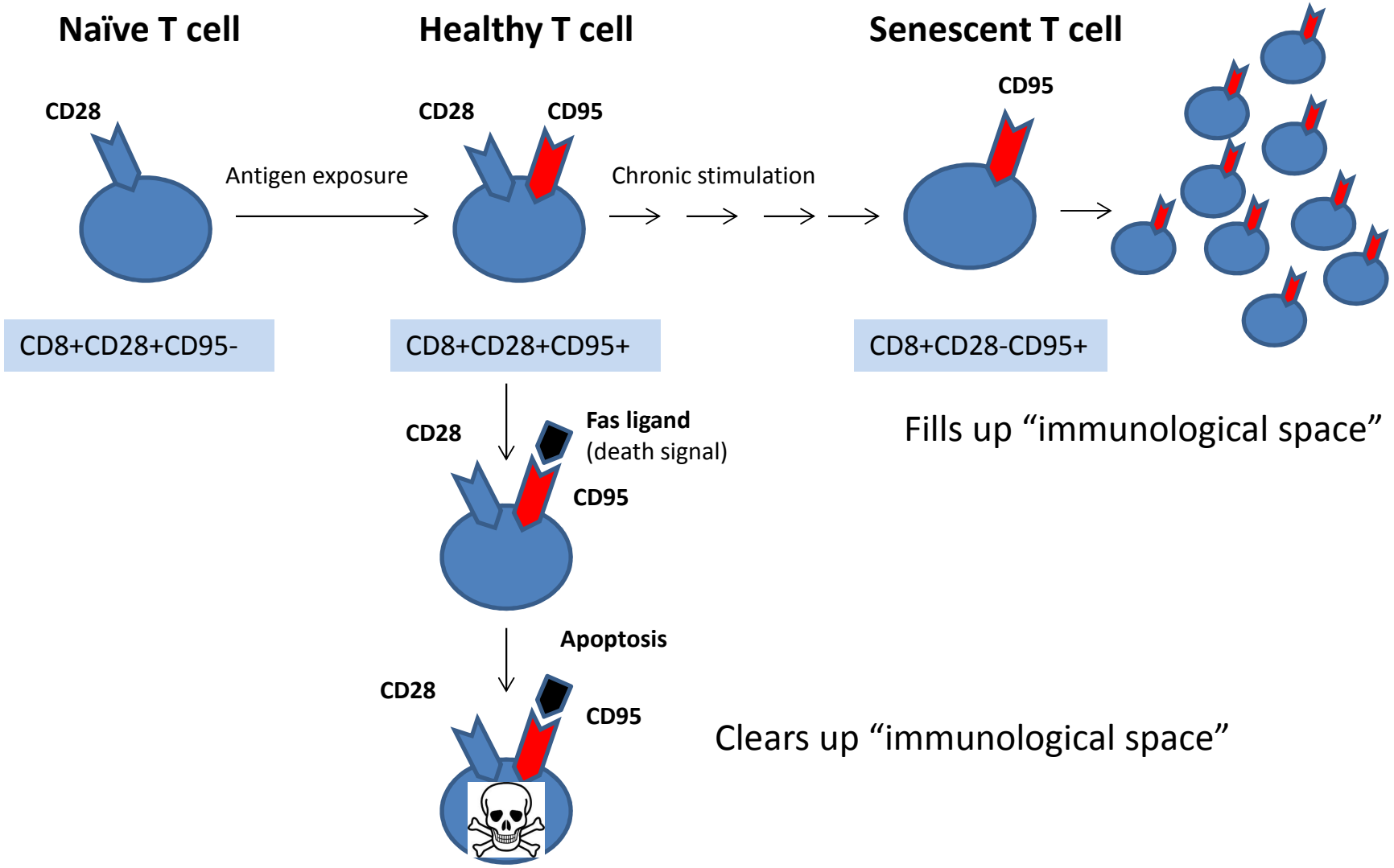
- ***In vitro* model studies**
  - Studies of cytotoxic T cells (CD8+) reveal reduced ability to proliferate after repeated stimulation
    - Stop proliferating
    - Still alive
  - **Not quiescent**
    - Resistant to apoptosis
    - Produce ↑ inflammatory cytokines (TNFα, IL-6) and ↓ INFγ
- **Major change**: Loss of CD28 expression
  - **Major co-stimulatory molecule**
    - Activation, proliferation, stabilization of mRNA, and glucose metabolism
    - All T cells express it initially



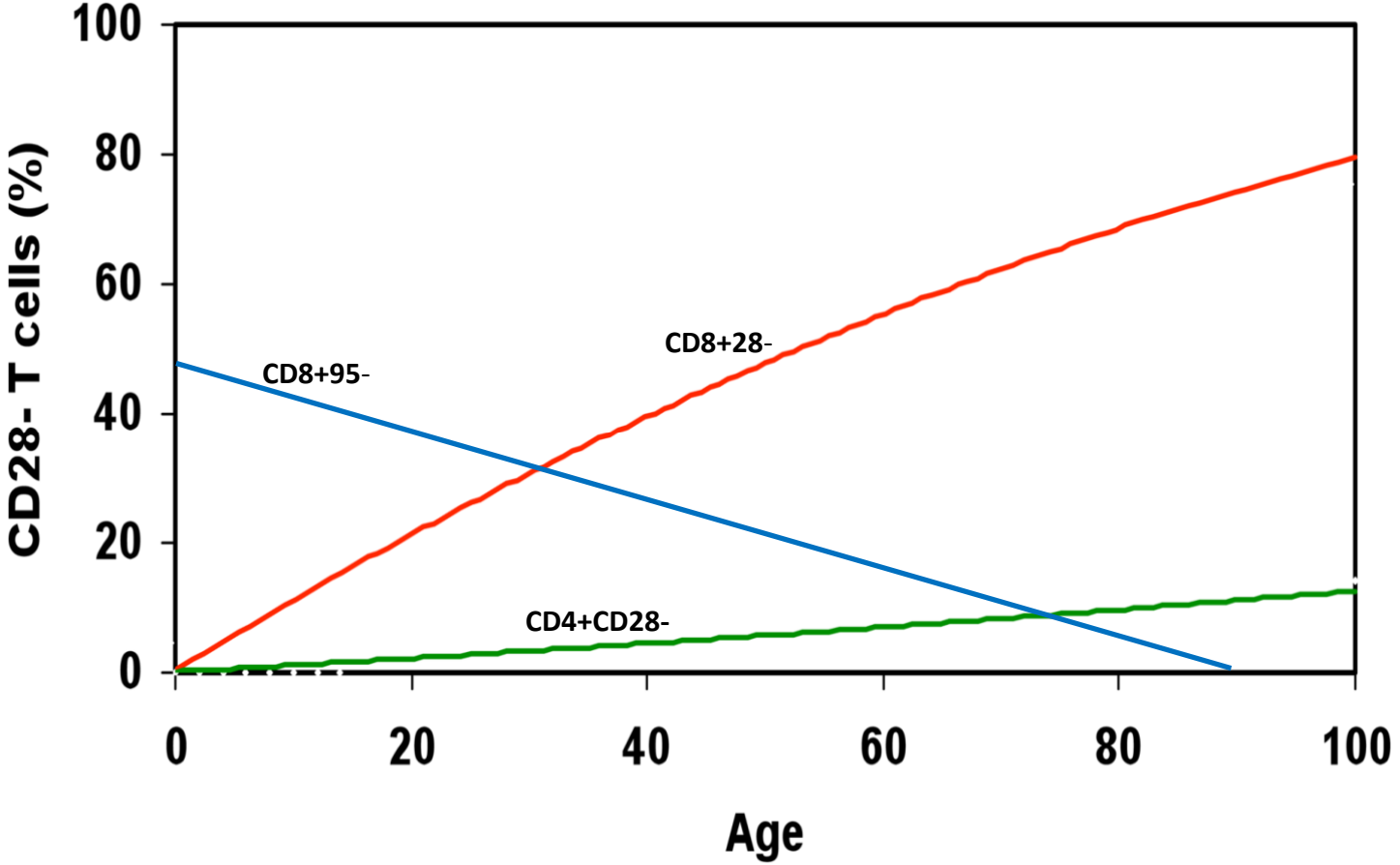
Effros RB *Immunol Rev* 2005

Loss of CD28 expression

- **Consequence**
  - **Inability to upregulate telomerase**
  - By third round of stimulation, telomerase activity gone
  - Blocking CD28 from interacting with B7 wipes out telomerase
  - Eventually whole population CD28-
  - Lead to critically short telomeres
  - **Resistance to apoptosis**
    - Accumulation of dysfunctional cells
- **Does it occur *in vivo?***
  - **Yes**, but not in mice (so mouse model not helpful)
  - **Not in everyone** (discussed later)


Naïve T cell
Healthy T cell
Senescent T cell
CD28
CD28
CD95
CD95
Antigen exposure
Chronic stimulation
CD8+CD28+CD95-
CD8+CD28+CD95+
CD8+CD28-CD95+
CD28
Fas ligand
(death signal)
CD95
Apoptosis
CD28
CD95
Fills up “immunological space”
Clears up “immunological space”

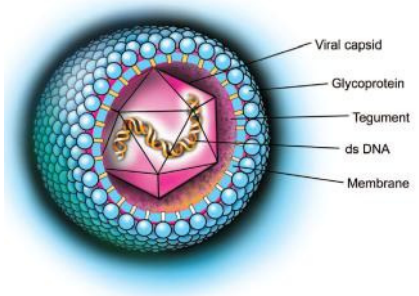
In vivo change in CD28 and CD95 expression with age



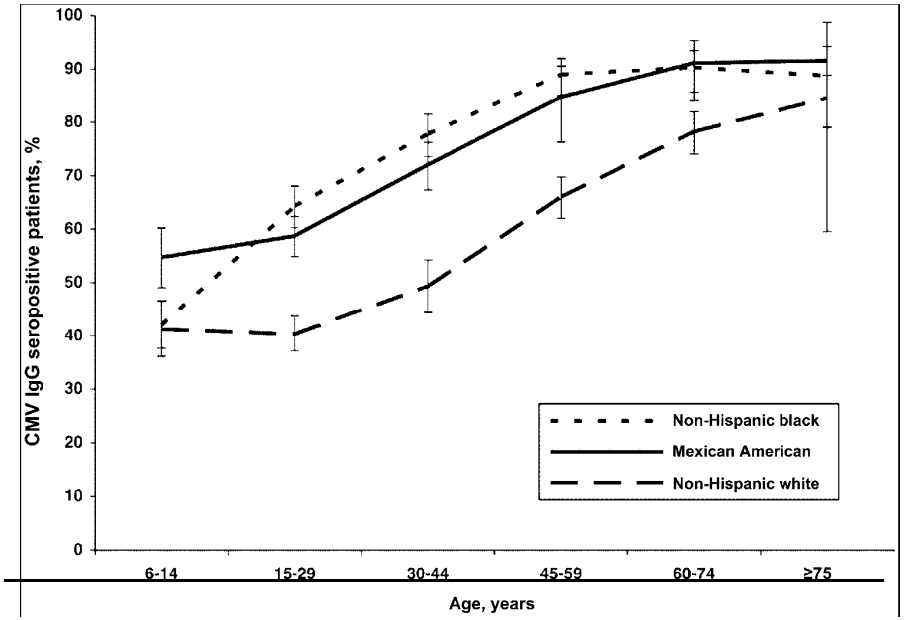
Adapted from Weng N-P 2009 *Trends Immunol*

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

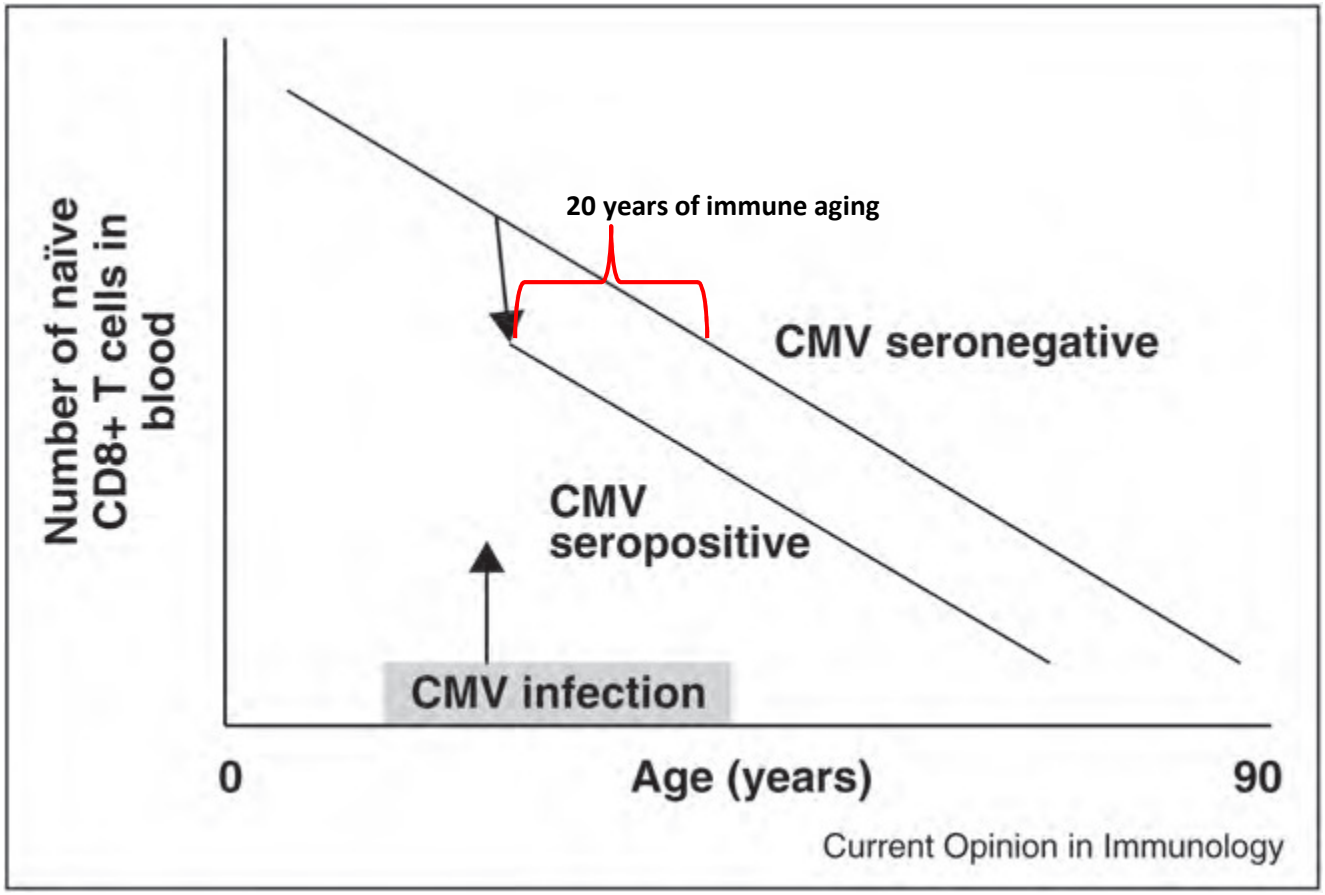


Staras, SA 2006 *CID*

“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.”

Moss P 2010 *Curr Opin Immunol*

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



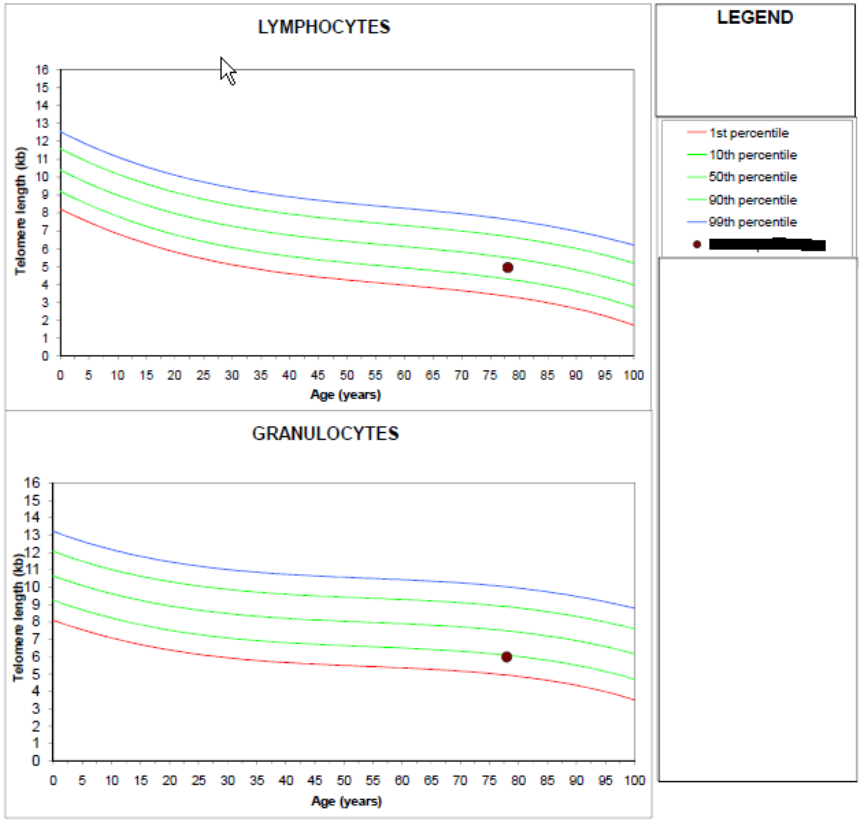
Adapted from Moss P 2010

Lymphocyte and granulocyte mean telomere length gap

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



Strategies for telomere maintenance

- 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*
- 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*

First published study of the effect of telomerase activation in humans

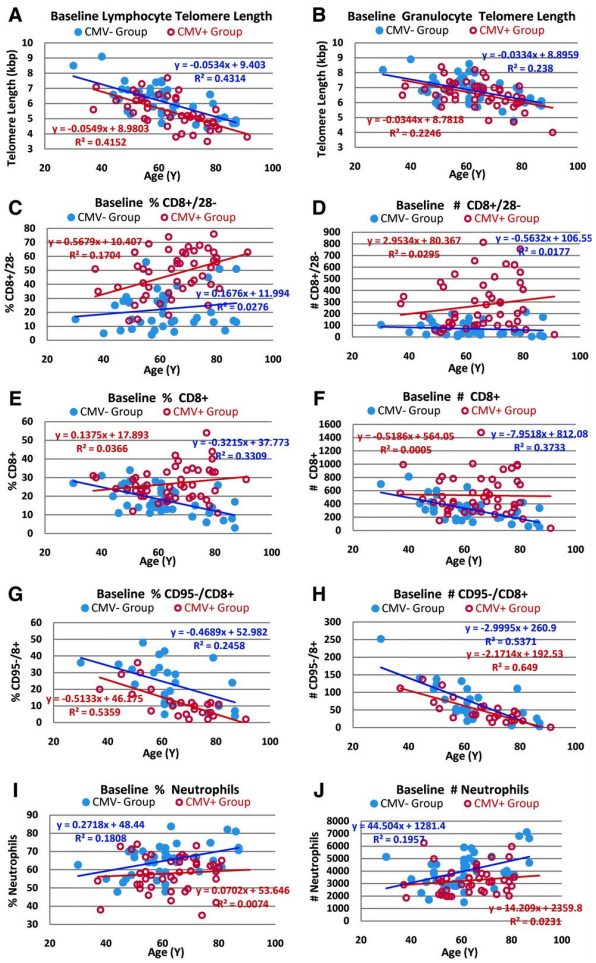
A Natural Product Telomerase Activator As Part of a Health Maintenance Program

Calvin B. Harley,[1,6] Weimin Liu,[2] Maria Blasco,[3] Elsa Vera,[3] William H. Andrews,[4] Laura A. Briggs,[4] and Joseph M. Raffaele[5]

- **Subjects**:
  - In 2007, a commercial age-management program was launched
  - Voluntarily participated and signed Customer Acknowledgement Form
  - Baseline n=114; 63 ± 12 years, range 30-87; 72% male
  - High socioeconomic status, 54% CMV seropositive
  - Evaluable # at 3, 6, 9, 12 months; 43, 59, 27, 37 subjects
- **Intervention**:
  - **TA-65:** >95% pure single chemical entity isolated from a proprietary extract of the dried root of *Astragalus membranaceous* and formulated into 5-10 mg capsules. Some subjects increased their dose to 25-50 mg after several months on the protocol.
  - **Comprehensive dietary supplement pack**, 2 a day

Rejuvenation Research 2010

Cross-sectional Change with Age by CMV



- Lymphocyte telomere length
  - Declines at similar rate (0.05 kb/yr)
  - CMV pos ≈ 10 years older
    - 5.43 vs 6.11 kb
- Granulocyte telomere length
  - Decline at similar rates
  - Not sig different by CMV status
    - 6.56 vs 6.83
- $CD28^-$: increased only in CMV^+
- Total CD8+:
  - No increase in CMV^+
  - Decrease in CMV^- (↓ CD28+)
- Naïve T cells ($CD95^-$)
  - Decrease in CMV^- and CMV^+
  - Difference in % at all ages due to increased senescent T cells in CMV^+ subjects
- Neutrophils
  - Only increase in CMV^-

CMV Causes an Unhealthy Remodeling of Immune System

CMV Positive

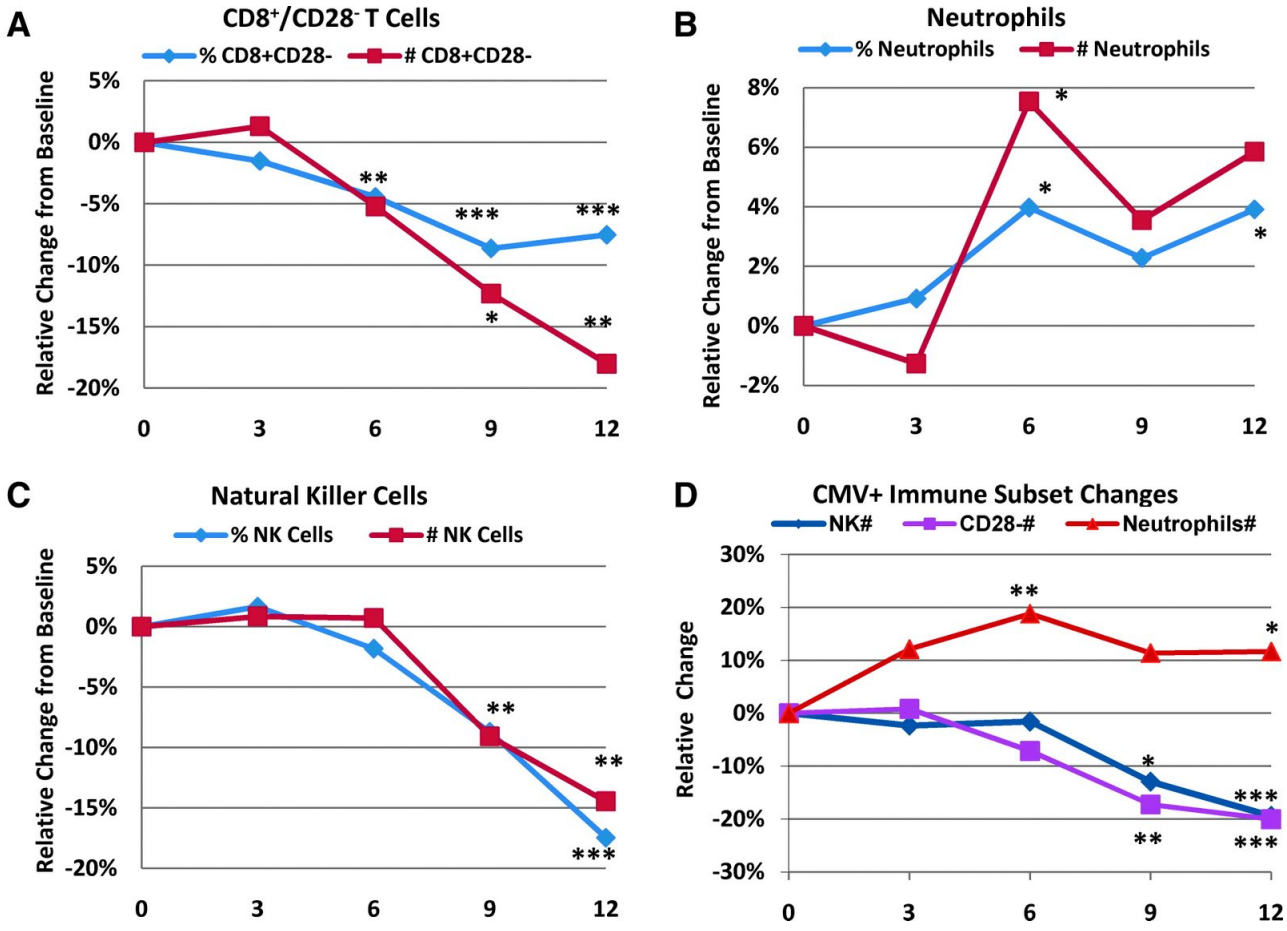
- **Different**
  - Increased $CD28^-$ cytotoxic T cells upon initial infection and then gradual increase
  - No increase in neutrophils with age—contrary to literature
  - CD4/CD8 decreases
- **Similar**
  - Decline in $CD28^+$
  - $CD95^-$ (naïve) T cells decrease with age
  - $CD19^+$ (B cells) decrease with age

CMV Negative

- **Different**
  - No change or decrease $CD28^-$ T cells
  - Novel finding: Increase in neutrophils only in negative
  - CD4/CD8 increases
- **Similar**
  - Decline in $CD28^+$ cells
  - $CD95^-$ T cells decrease with age
  - $CD19^+$ (B cells) decrease with age

Effect of 12 months of the Protocol: Healthy

Remodeling of the Immune System



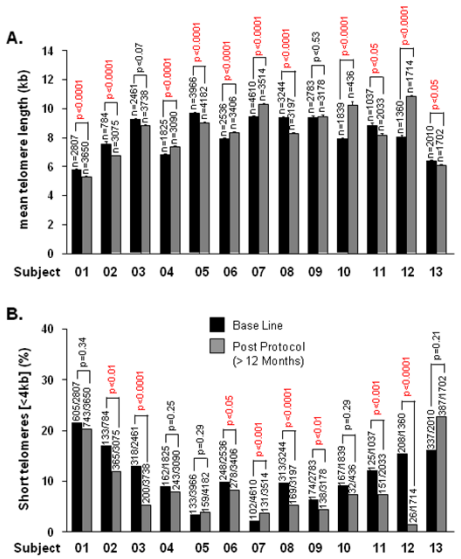
*p<0.1, ** p<0.05, ***p<0.01

Study Summary

- **The 1st year of program**
  - Comprehensive dietary supplement
  - A small molecule telomerase activator
- **Effected a remodeling of the immune system towards a more youthful profile**
  - Significantly reduced the number of senescent T cells
    - 5-20 year “reversal of immune aging” in CMV^+ subjects
  - Increased neutrophils
    - Toward CMV^- level
  - Decreased NK cells
    - Reversal of age-associated increase expected for both CMV^+ and CMV^-
- **No significant adverse effects**

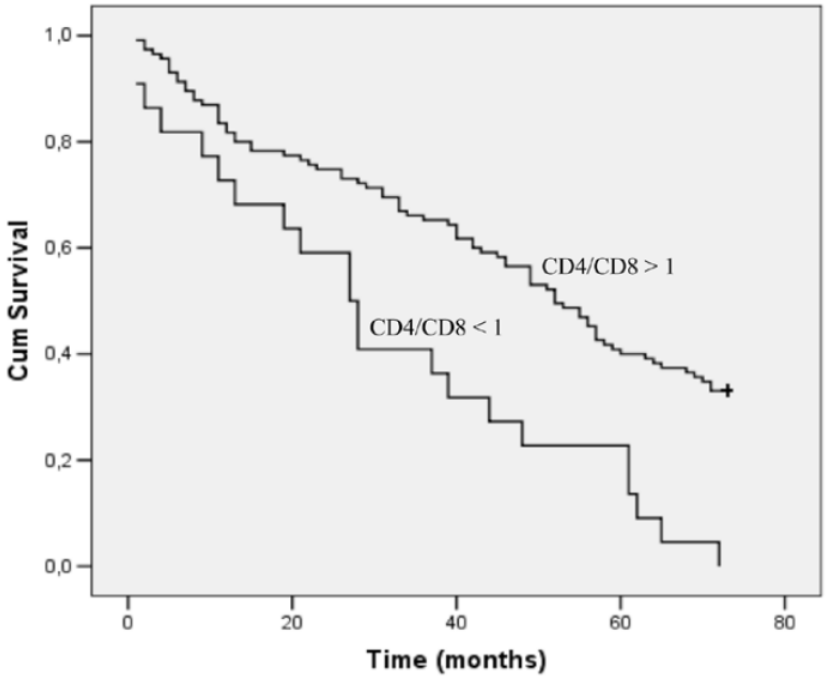
Exact Mechanism?

- **Unclear:**
  - Observational study
  - Two interventions/no control
    - Telomerase activation
    - Oxidative stress reduction
      - Antioxidants, vitamin D, B-complex, etc.
- **Mean telomere length**: No change
  - Non-significant decrease even though 40% increased
- **Shortest telomere**
  - Subset of 13 subjects: decrease in percent nuclei with shortest telomeres (<4 kb)
  - Shortest telomere in a cell
    - Triggers senescent phenotype



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

Further evidence

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 activity in haploinsufficient (Terc-/+) mouse fibroblasts and lengthens short telomeres, but not in Terc-/- fibroblasts

- 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

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

Proof of principle

“Accumulating evidence implicating telomere damage as a driver of age-associated organ decline and disease risk and the marked reversal of systemic degenerative phenotypes in adult mice observed here support the development of regenerative strategies designed to
restore telomere integrity.”

Telomeres and Aging: Conclusions

- Telomere shortening is a fundamental aspect of the aging process and all diseases of aging *in humans.*
- Many interventions to slow attrition: lifestyle, diet, supplement, and hormone strategies.
- TA-65 can improve certain aspects of immune aging and possibly the percent of critically short telomeres, particularly in CMV positive individuals.
- Significant adverse effects have not been detected

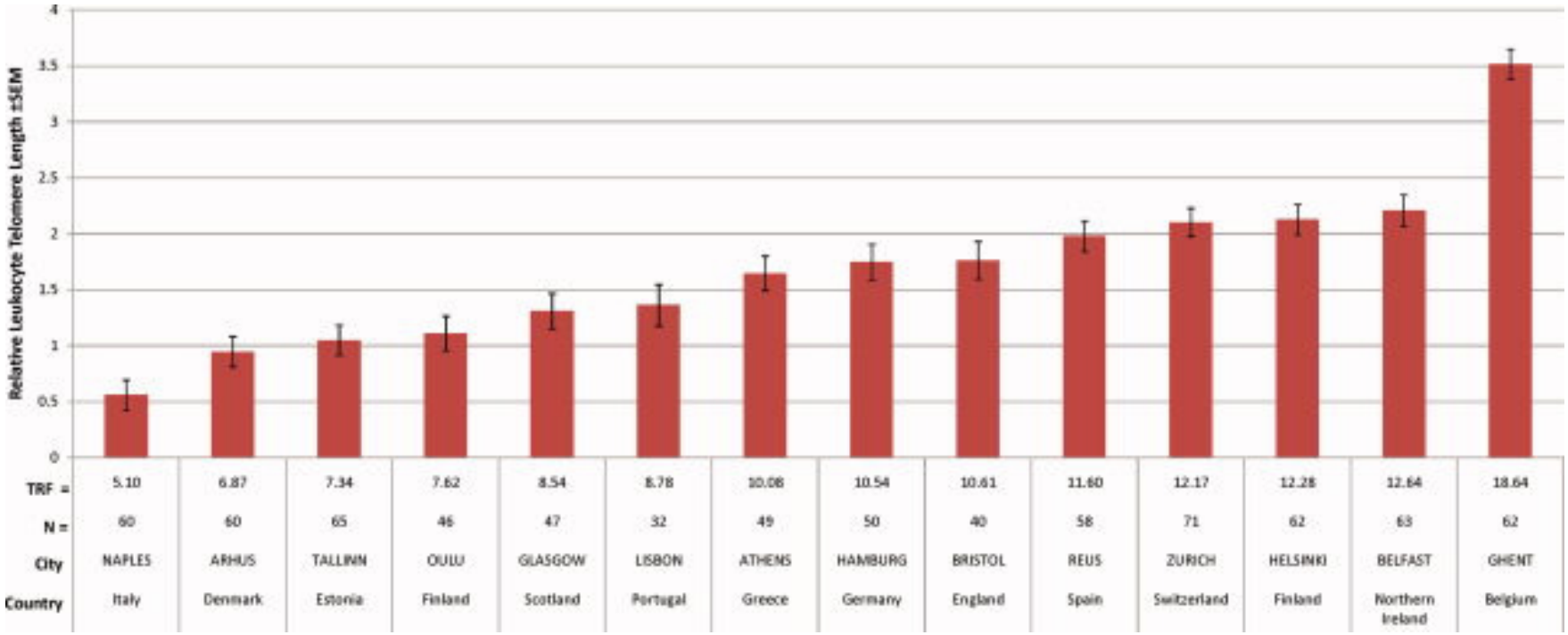
Active Studies with TA-65

- **Healthy CMV+**
  - RCT DB n=120 men and women 55-75 y.o.
  - 1 year of TA-65, high dose/lower dose, placebo
  - Fully enrolled Feb 2013 with 6 month analysis expected Sept 2013
  - Telomere length: median and percent short
  - Lymphocyte subsets: senescent T-cells primary endpoint
- **Metabolic Syndrome**
  - DB PCT crossover, n=45, results expected Fall 2013
- **Age-related Macular Degeneration**
  - Enrollment starts this Summer

Who to start? How to monitor?

- Measure telomere length
- Monitor telomere length annually
- Test for CMV seropositivity
- Measure lymphocyte subsets
  - Senescent suppressor cells (CD28-)
  - Naïve suppressor cells
  - CD4/CD8 (Immune risk profile)
- Start with 250 IU

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

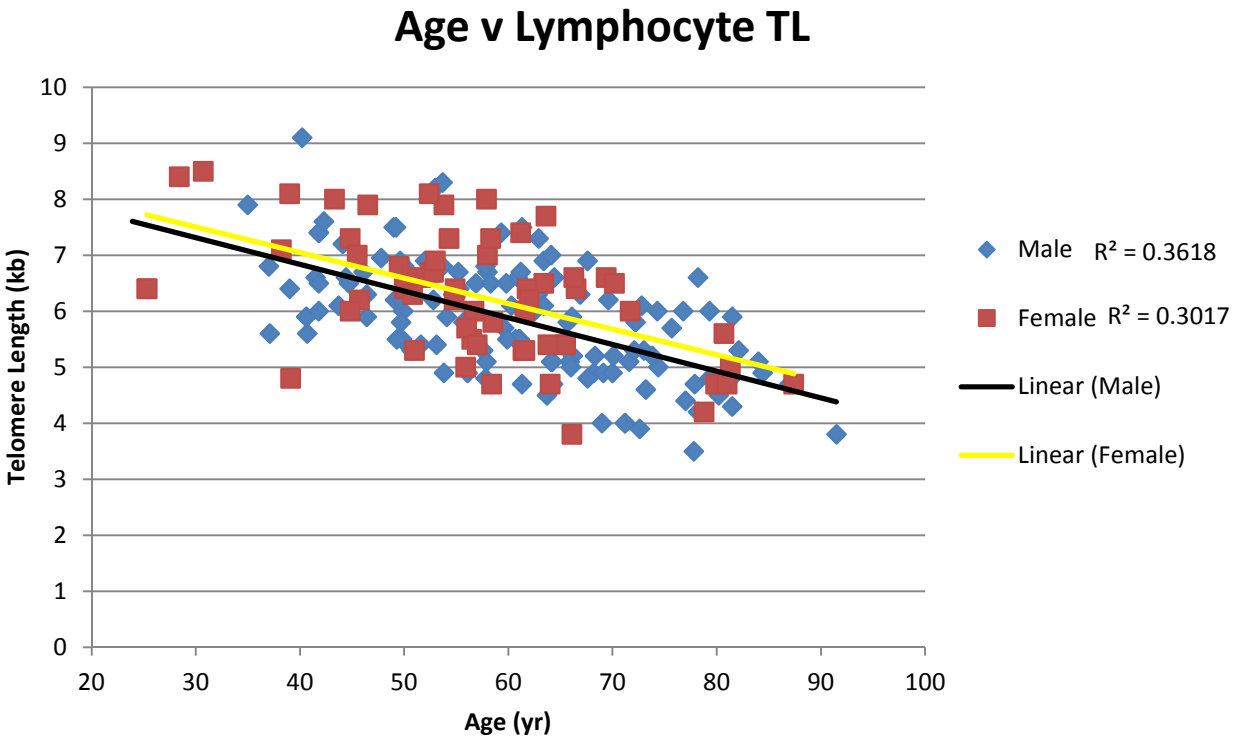


Mean telomere length can vary widely between different populations
5.2 kb in Naples up to 18.6 kb in Ghent.

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

Lymphocyte Telomere Length



PhysioAge Medical Group
unpublished data 2007-2013

Telomere Length Dynamics

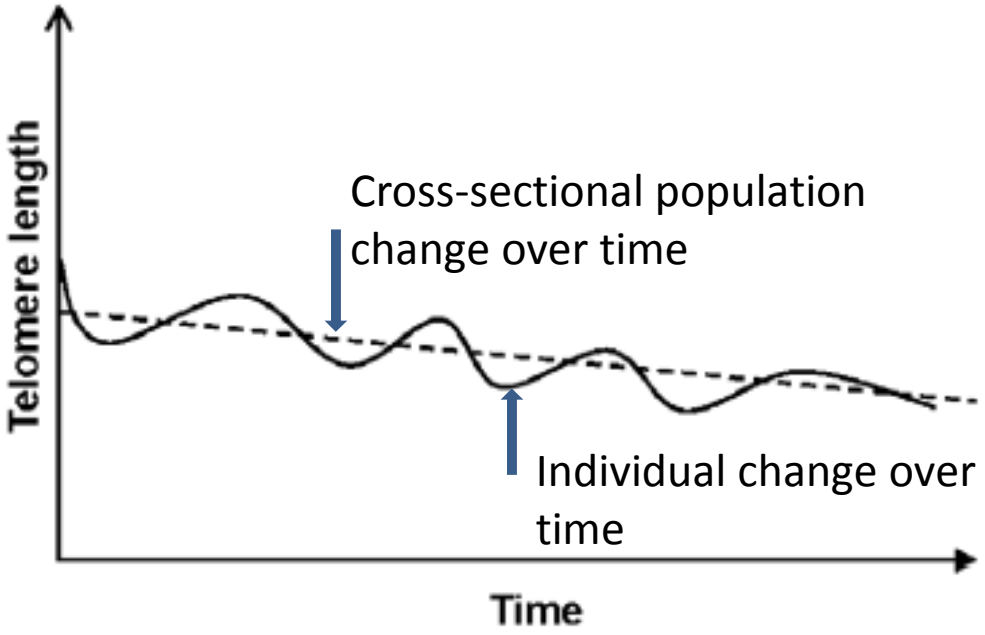


Figure 6. The oscillation hypothesis. Hypothetical illustration of RTL changes over time at the individual (solid line) and population (dotted line) level, based on the collected data from the present study and the literature.

Svenson U PloS ONE 2011

MiniReview

Cancer Prevention Research

How "Reversible" Is Telomeric Aging?

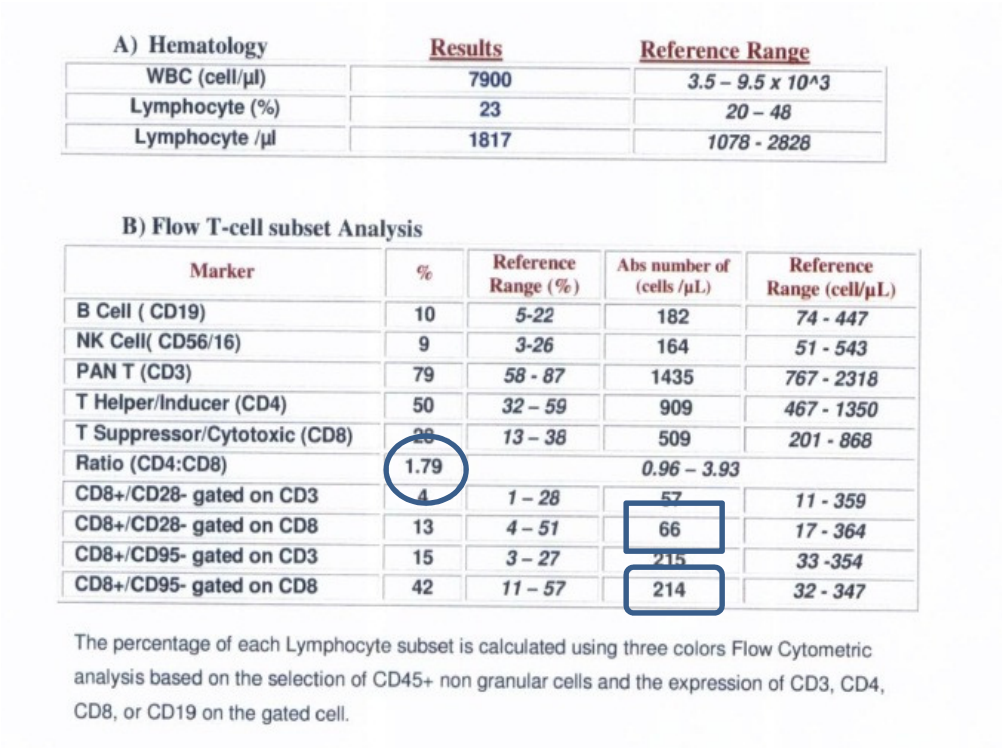
Elissa Epel

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"

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

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

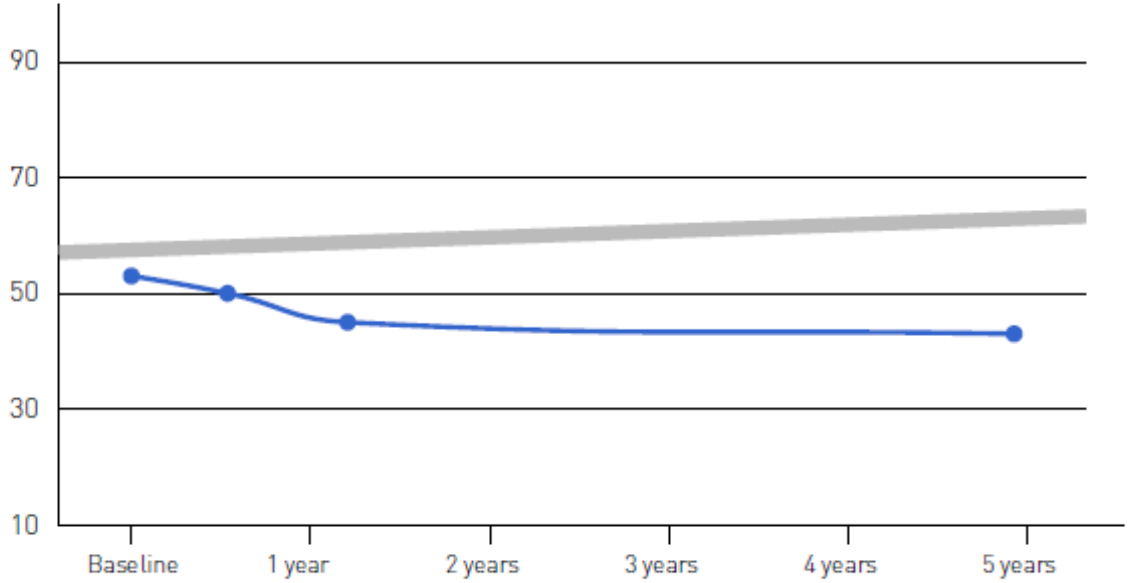
Marker	%	Reference Range (%)	Abs number of (cells /µL)	Reference Range (cell/µL)
B Cell (CD19)	17	5-22	142	74 - 447
NK Cell(CD56/16)	15	3-26	125	51 - 543
PAN T (CD3)	65	58 - 87	543	767 - 2318
T Helper/Inducer (CD4)	35	32 – 59	293	467 - 1350
T Suppressor/Cytotoxic (CD8)	28	13 – 38	234	201 - 868
Ratio (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

TelomerAge

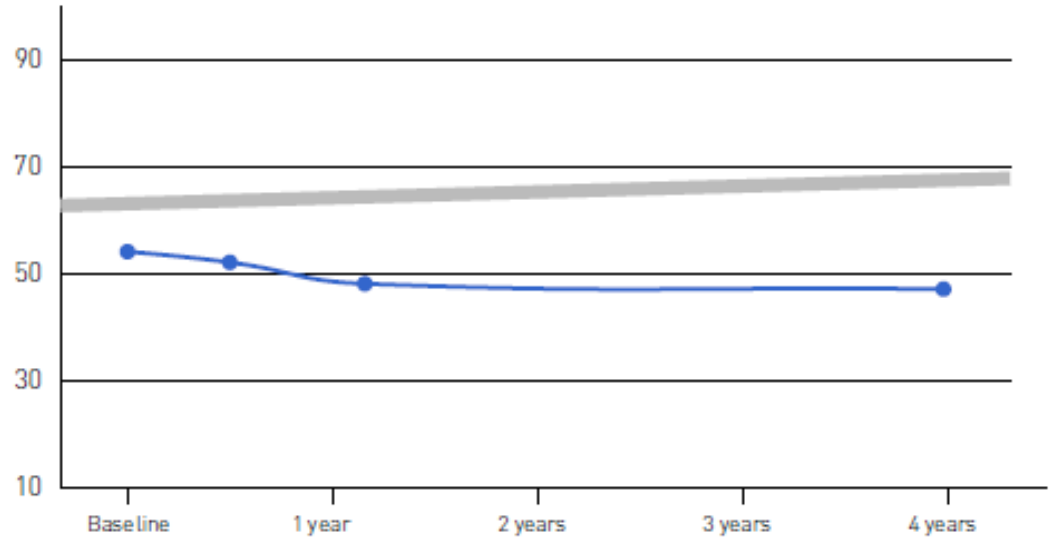
62 y.o. CMV- female on TA-65 500 IU/D.



Telomere Length				
Granulocyte Length	8.10	8.30	8.50	9.20
Lymphocyte Length	7.00	7.40	8.00	8.30

TelomerAge

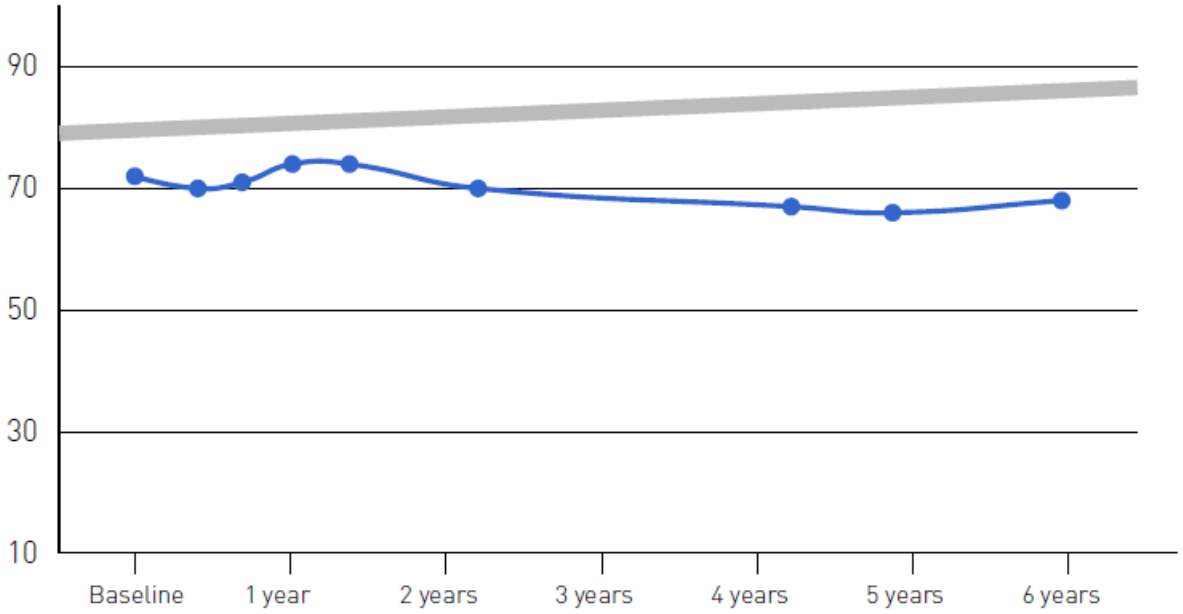
63 y.o. CMV- male on TA-65 500 IU/D



Visit Date	10/20 2008	04/20 2009	12/16 2009	10/10 2012
Telomere Length				
Granulocyte Length	8.00	7.70	8.20	8.70
Lymphocyte Length	6.90	7.10	7.60	7.80

TelomerAge

79 y.o. CMV+ male on TA-65 1000 IU/D



Visit Date	03/20 2007	08/15 2007	11/27 2007	03/24 2008	08/05 2008	06/03 2009	03/21 2011	06/08 2011	02/01 2012	03/04 2013
Granulocyte Length	6.30	6.70	6.60	6.10	6.60	6.70		6.80	7.20	6.30
Lymphocyte Length	4.50	4.80	4.70	4.30	4.30	4.80		5.20	5.30	5.00

Thank You
Questions?