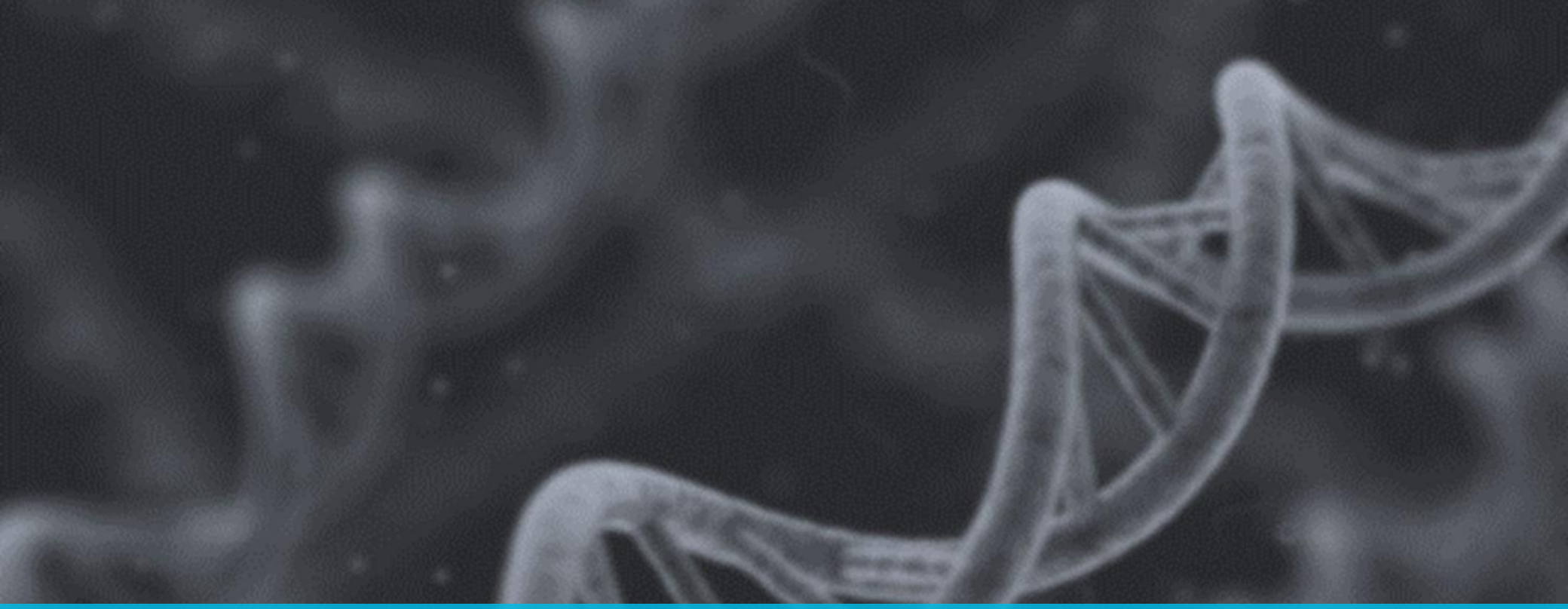


2016

Module II: Clinical Presentation to Telomere Testing

LIFE LENGTH

Index

Why should you care about telomeres?

Adverse consequences of short telomeres

- ✓ Loss of tissue renewal capacity
- ✓ Failure of stem cells to divide in sufficient numbers
- ✓ Poor immune response

Short telomeres accelerate the onset of age-related diseases

- ✓ Cardiovascular Disease
- ✓ Metabolic Syndromes incl. Diabetes Type 2
- ✓ Certain Cancers
- ✓ Central Nervous System Diseases
- ✓ Arthritis & Osteoporosis

Cancer: Prognosis and risk stratification

TAT as the reference test for use by physicians in personalized and functional medicine

CANCER

- Short telomeres have been shown to be a risk factor for certain types of cancer:

  - ✓ Breast
  - ✓ Ovarian
  - ✓ Uterine
  - ✓ Prostate
  - ✓ Skin cancers

This is especially relevant in individuals with family history

Recent study by Harvard / Northwestern University suggest that abnormal telomere attrition rates changes may predict cancer

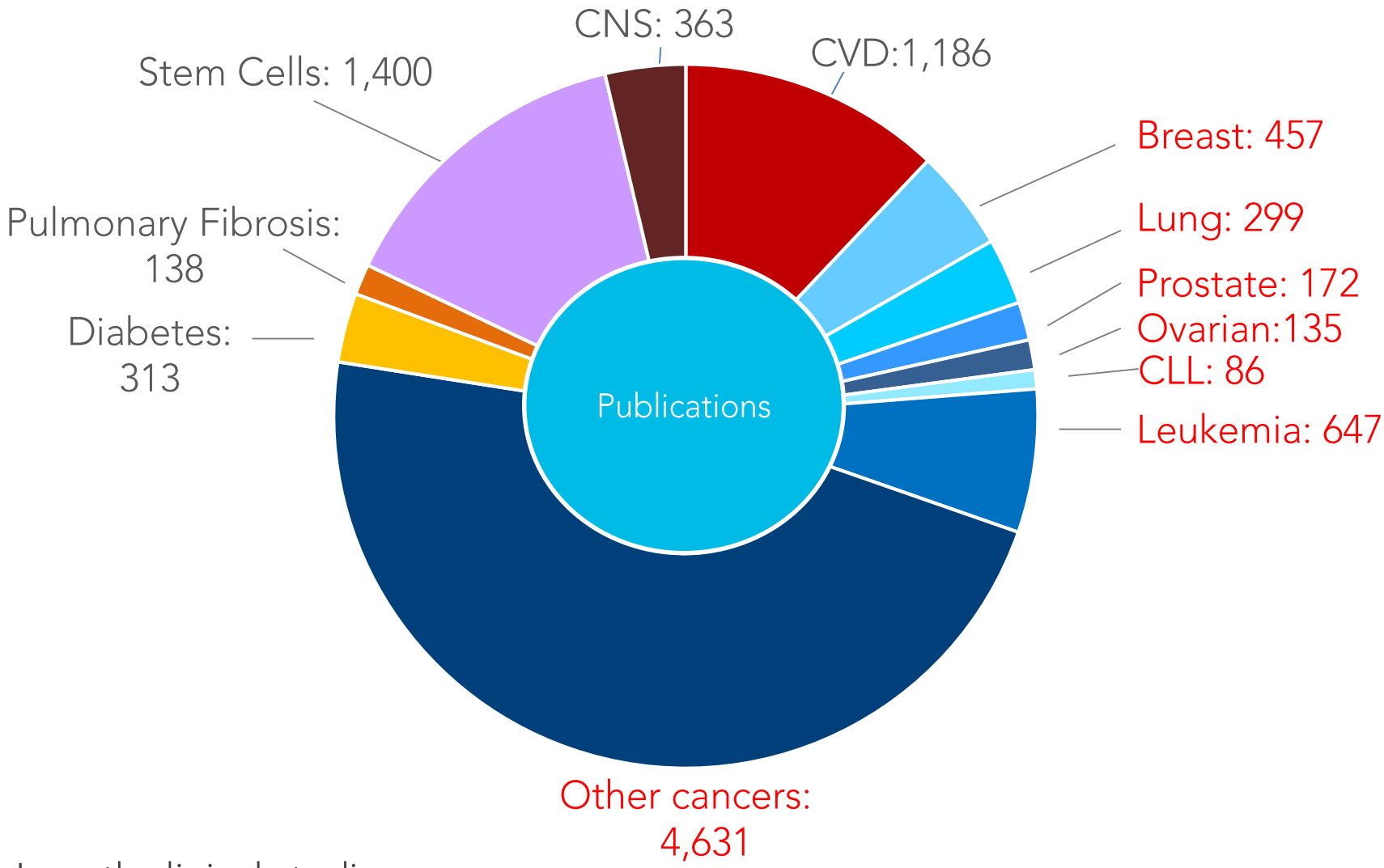
TAT as the reference test for use by physicians in personalized and functional medicine

CANCER

- Telomere analysis will help detect high risk individuals with short telomeres - more frequent check-ups at an earlier age
- Patients with BRCA1/2 and short telomeres develop breast cancer at a younger age than those with longer telomeres
- Individuals with short telomeres have been demonstrated to have higher probability of developing secondary cancers

Several studies are being conducted to evaluate the potential clinical use of telomeres in non-small cell lung cancer

~6,500 Telomere - Cancer Articles in Pub Med



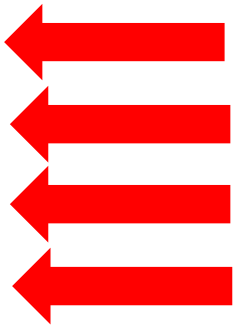
— Life Length clinical studies

TAT as an important new tool for pharma and biotech R&D and clinical trials

Selected Client Examples	Study Purpose	
MD Anderson Cancer Centre	Evaluate TL distribution in mice with telomere dysfunction	
MAJOR PHARMA	Phase II Clinical trial for a cancer drug	
Universidad Complutense de Madrid - Hospital Clinico San Carlos	Prognosis in Lung cancer patients	
The Jackson Laboratory	Confidential	
The Welcome Trust Sanger Institute	Murine embryonic fibroblast - telomere attrition during culture	
Clínica CEMTRO	Autologous Transplantation of chondrocytes	
University of Turku	Mouse sperm quality	
MAJOR PHARMA	Phase II Clinical trial for a cancer drug (different indication)	
Celltex	Personal medicine based on stem cell therapy	
Clasado	Nutraceutical efficacy testing	

TAT as an important new tool for pharma and biotech R&D and clinical trials

Client	Collaboration Purpose
Instituto José Carreras	Reprograming of iPSC from leukemia cells
Hospital Universitario Niño Jesús	Clinical validation of TAT in solid tumors prognosis in children
Hospital Universitario Niño Jesús	Clinical validation of TAT in hematopoietic transplant in children
SENS Research Foundation	Cancer drug screening



Selected References

- Telomere length and risk of incident cancer and cancer mortality.
- Fifteen-year follow-up of association between telomere length and incident cancer and cancer mortality.
- Short telomeres are frequent in hereditary breast tumors and are associated with high tumor grade.
- Change in peripheral blood leukocyte telomere length and mortality in breast cancer survivors.
- Telomere shortening is an early somatic DNA alteration in human prostate tumorigenesis.
- Shorter telomere length is associated with increased ovarian cancer risk in both familial and sporadic cases.
- Extensive telomere erosion in the initiation of colorectal adenomas and its association with chromosomal instability.
- Telomere length and risk of melanoma, squamous cell carcinoma, and basal cell carcinoma.
- Genetic Anticipation Is Associated with Telomere Shortening in Hereditary Breast Cancer
- Shorter telomeres associate with a reduced risk of melanoma development


Cardiovascular Disease

TAT as the reference test for use by physicians in personalized and functional medicine

CARDIOVASCULAR DISEASE

- Reduced telomere lengths are found in patients with cardiovascular risk factors such as:
  - ✓ Atherosclerosis
  - ✓ Hypertension
  - ✓ Obesity
  - ✓ Diabetes (especially Type 2)
  - ✓ Smoking
  - ✓ Physical inactivity
  - ✓ Stress
  - ✓ Chronic infections

Shorter telomeres have been associated with poor survival

A positive effect on telomere length is found with increased physical activity, statin treatment, and higher blood levels of omega-3 fatty acids

TAT as the reference test for use by physicians in personalized and functional medicine

CARDIOVASCULAR DISEASE

- Telomere length has now been demonstrated to be an independent risk factor for CVD
- Telomere testing is being used to complement traditional cardiovascular tests in order to provide a more comprehensive analysis and even identify the risks of developing cardiovascular problems at an earlier stage
- Telomere measurement can identify high risk individuals with short telomeres

The **BMJ** has published a study of more than 43,000 participants which concluded that telomere length is an independent risk factor for cardiovascular disease

Selected References

- Telomere shortening over 6 years is associated with increased subclinical carotid vascular damage and worse cardiovascular prognosis in the general population.
- The roles of senescence and telomere shortening in cardiovascular disease.
- Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis.
- Decreased leukocyte telomere length (LTL) is associated with stroke but unlikely to be causative.
- Short leukocyte telomere length predicts incidence and progression of carotid atherosclerosis in American Indians: the Strong Heart Family Study.
- Short telomeres are associated with increased carotid atherosclerosis in hypertensive subjects.
- Telomeres, atherosclerosis, and the hemothelium: the longer view.
- Association of leukocyte telomere length with circulating biomarkers of the renin-angiotensin-aldosterone system: the Framingham Heart Study.
- Association of shorter telomere length with essential hypertension in Indian population.


Metabolic diseases

TAT as the reference test for use by physicians in personalized and functional medicine

METABOLIC DISEASES

- Telomere analysis can be used to detect individuals at risk of developing metabolic diseases, particularly at a younger age, such as Type 2 Diabetes
- Recent studies demonstrate that short telomere length is associated with future development of Type 2 Diabetes independently of the known risk factors
- These studies show that individuals in the lowest quartile of leucocyte telomere length had twice the risk of developing diabetes than those with longer telomeres

Selected References

- Association between telomere length and type 2 diabetes mellitus: a meta-analysis.
- Leucocyte telomere shortening in relation to newly diagnosed type 2 diabetic patients with depression.
- White blood cells telomere length is shorter in males with type 2 diabetes and microalbuminuria.
- Shortened telomere length in white blood cells of patients with IDDM.
- Leucocyte telomere length and risk of type 2 diabetes mellitus: new prospective cohort study and literature-based meta-analysis


Infertility

Infertility

- It has been shown that telomere homeostasis is compromised in spermatocytes from patients with idiopathic infertility.
- Telomeres are relevant to meiosis as they promote pairing and synapsis between homologous chromosomes by the formation of the bouquet. Short and/or damaged telomeres have been often related to many processes related to infertility

- Gametogenesis disruption
- Impairment of nuclear organization in spermatozoa
- Incidence of aneuploidy gametes
- Reproductive aging

Infertility

- Studies in the reproductive field have increasingly focused on telomere length, especially those studies focused on finding an association between the telomere lengths in embryos or cumulus cells and IVF treatment outcomes.

- One study showed that fertile women have oocytes with longer telomere lengths than those of infertile women. This suggests that the telomere length in human cumulus cells is important for oocyte maturation and embryo development.

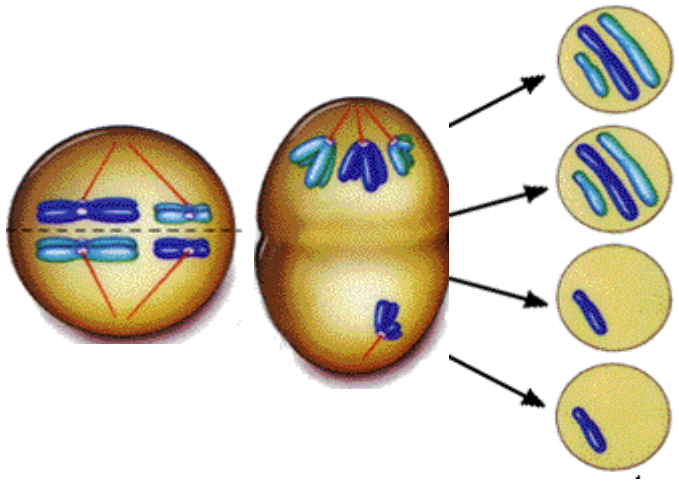
- On the other hand the sperm telomere length (STL) can also affect the IVF treatment. This is the first study to examine the possible role of STL in the development of embryos for IVF humans.

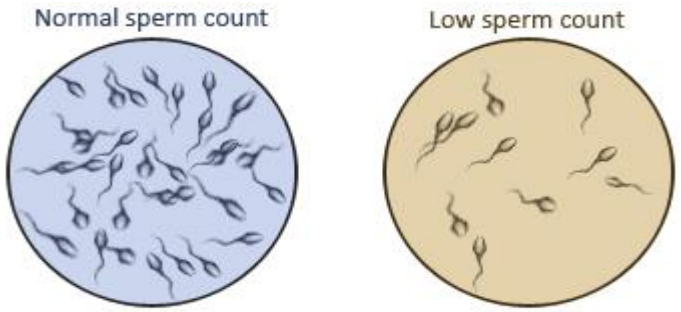
Female Reproductive Aging

- Researchers have demonstrated shorter telomeres in oocytes from women who did not conceive after in vitro fertilization (IVF) compared with those who did and in oocytes from cycles that produced fragmented embryos.
- Another study demonstrated a direct relationship between short telomere length in polar bodies and aneuploidy embryos from patients undergoing IVF.
- A more recent study found that women with diminished ovarian reserve had shorter telomeres and lower telomerase activity in their granulosa cells.

Male Reproductive Aging

- The adult human ovary does not contain oogonia, so females are born with a fixed cohort of oocytes, whereas testes in the adult male maintain spermatogonia capable of replicating germ cells throughout adult life.
- Unlike telomere length in oocytes, which decreases with the age of the female, telomere length in sperm increases with age. Telomere lengthening in human sperm presumably arises from the continuous action of telomerase, which is expressed at high levels in spermatogonia.
- Compromised telomere length in sperm may contribute to segregation error, apoptosis with reduced sperm count, and reduced fertility.





Potential uses for TAT® in infertility

TELOMERE ANALYSIS TECHNOLOGY

1. To identify current and potential future infertility problems in men and women who are trying to have children (and where short telomeres might be a co-biomarker of why they are having difficulty) and for those who are postponing having children and who might benefit by freezing eggs or sperm so that in 5 or 10 years when they want to have children, they have this "insurance" in place.

2. To select the best donors.

3. To check that oocyte is suitable for IVF: Measurement of telomere length in oocytes and polar bodies from women could be used to predict risk of reproductive failure and aneuploidy in embryos and identify fertilized oocytes with relatively low risk of reproductive failure for in vitro fertilization.

Infertility

- All this evidence suggests that telomere testing has the potential to be a fundamental biomarker for infertility.

- We envision our TAT as a companion biomarker for all couples undergoing IVF treatments.

- We look forward to contributing to carrying out the kind of clinical studies that will demonstrate that telomere length measurement can be valuable in contributing to diagnose and predict infertility problems.

Selected References

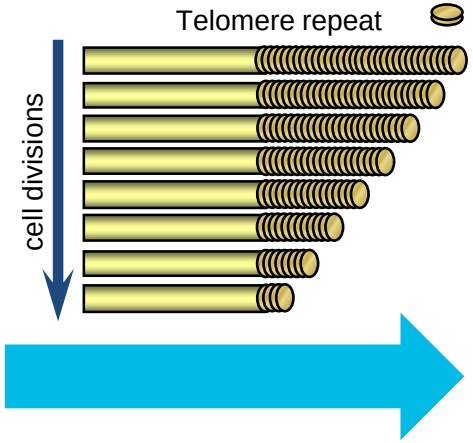
- Telomere homeostasis is compromised in spermatocytes from patients with idiopathic infertility.
- Sperm telomere length is positively associated with the quality of early embryonic development.
- Telomeres and human reproduction.
- In young men sperm telomere length is related to sperm number and parental age.
- Oocyte competency is the key to embryo potential.
- Extended fertility and longevity: the genetic and epigenetic link.

Stem Cells: Quality Control and Characterization

TAT for Quality Control and Stem Cell Characterization

- Mesenchymal stem cells (MSCs) occur at low frequency in tissues and must be expanded in vitro to obtain sufficient numbers for research and therapeutic applications.
- Long-term culture of mesenchymal stem cells (MSC) has major impact on cellular characteristics and differentiation potential.

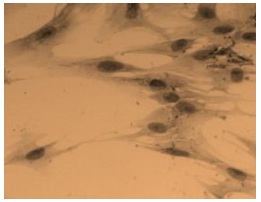
Stem cells culture: determine number of cell replication (doublings) at different conditions; media, hypoxia, seeding densities, donor ages, drug treatment



Standardize telomere attrition rate (TAR), optimize ex vivo conditions, enhance quality control for proliferation capacity, tumorigenesis

Quantify telomere length variation by Telomere Analysis Technology® TAT and telomerase activity by Q-TRAP

Baseline data: cell proliferation



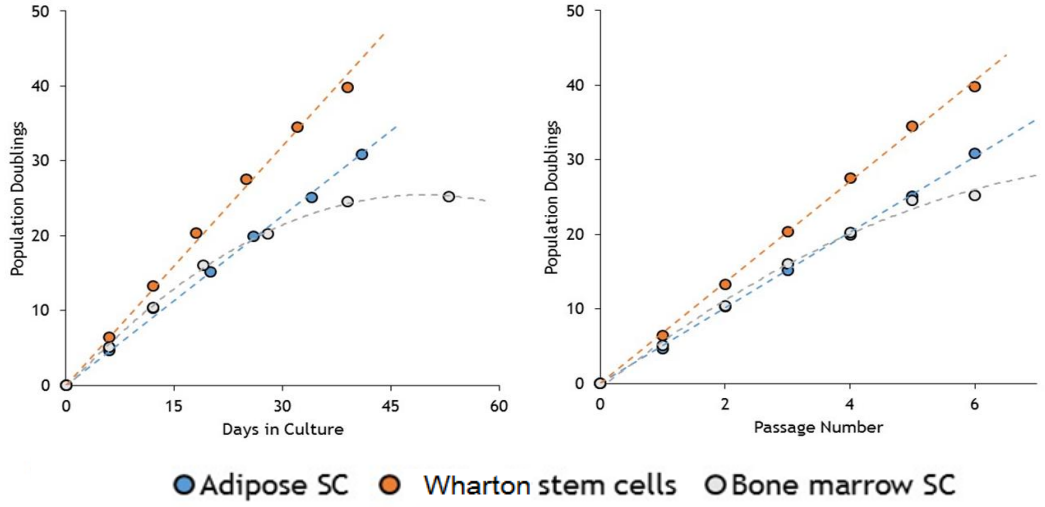
Stem cell *ex vivo*

- Ad-MSCs
- BM-MSCs
- Umbilical-SCs

Proliferative capacity *in vitro* (non immortalized)

Variables to monitor

- Serum free
- % O2
- Donor age
- Growth and differentiation factors



- Different kinetics depending on cell source.
- In this assay BM-MSC reached senescence earlier than adipose or Wharton stem cells.

Selected References

- Enhanced telomere rejuvenation in pluripotent cells reprogrammed via nuclear transfer relative to induced pluripotent cells.
- Telomere stability and telomerase in mesenchymal stem cells
- Telomere regulation in pluripotent stem cells
- Genomic instability in human stem cells
- Inducible immortality in hTERT-human mesenchymal stem cells
- An *in vitro* expansion score for tissue engineering applications with human bone marrow-derived mesenchymal stem cells
- The aging signature: a hallmark of induced pluripotent stem cells?
- Biosafety evidence for human dedifferentiated adipocytes

Diet, Nutrition & Obesity

TAT as the reference test for use by physicians in personalized and functional medicine

DIET, NUTRITION & OBESITY

- Increasing abdominal adiposity is accompanied by accelerated telomere attrition.
- A healthy diet positive contributes to telomere length, including:
  - ✓ Vegetables
  - ✓ Legumes
  - ✓ Fruits
  - ✓ High ratio of monounsaturated fat/saturated fat
- A healthy diet improves healthspan
- Telomere measurement can be used to monitor the benefits of a healthy lifestyle

Longer telomeres were associated with greater adherence to the Mediterranean diet

Selected References

- Body mass index and leukocyte telomere length dynamics among older adults
- Mediterranean diet and telomere length in Nurse's Health Study: population based cohort study
- Effect of vegetable consumption on the association between peripheral leucocyte telomere length and hypertension
- Nutrition and lifestyle in healthy aging: the telomere challenge

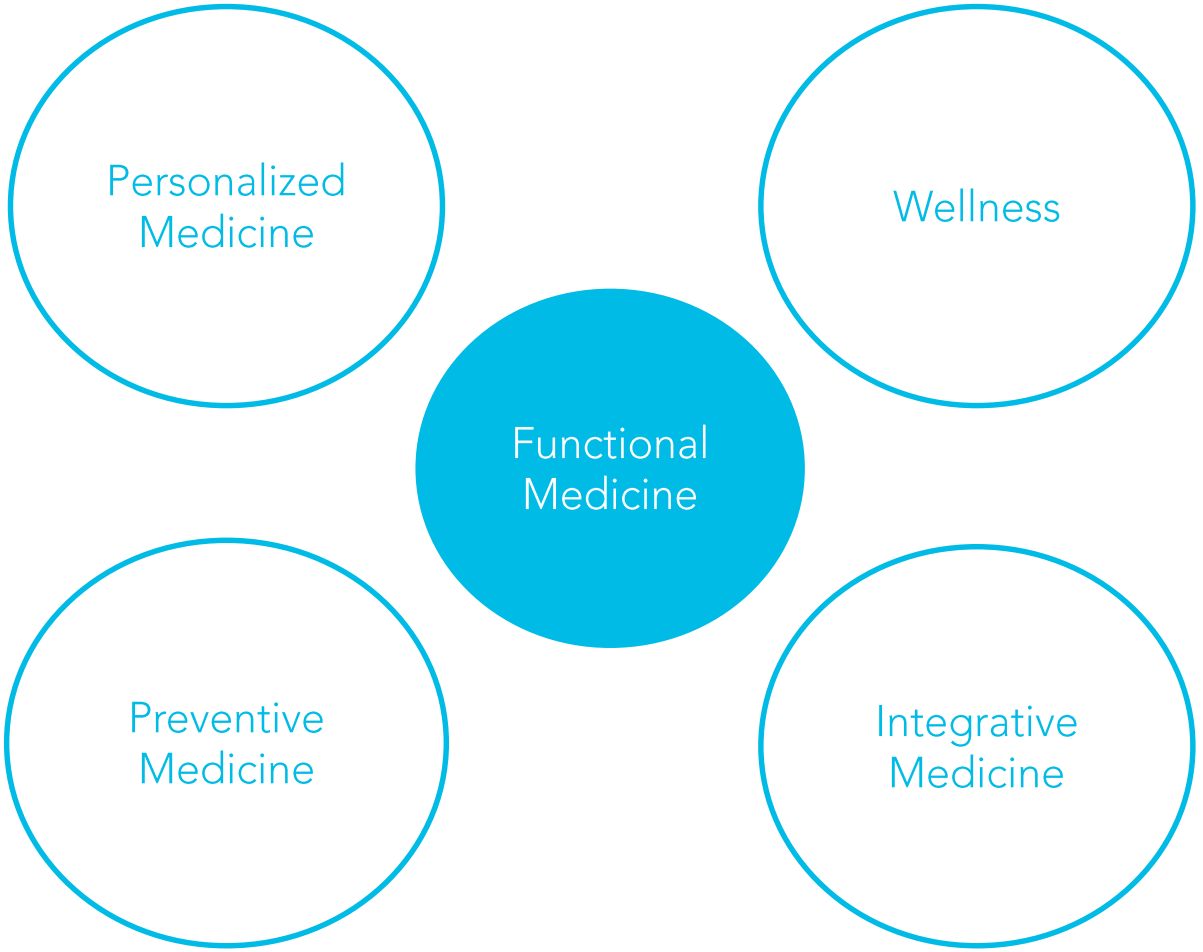

Age Management Medicine

TAT as the reference test for use by physicians in personalized and functional medicine

An invaluable tool in age management medicine

- To risk stratify patients and identify individuals with accelerated biological aging
- To enable early intervention-lifestyle modifications to reduce stress, inflammation, oxidative damage and other inducers of accelerated telomere loss
- To provide therapeutic interventions to slow down or reverse telomere loss (supplements, stem cells, optimal donor selection for transplants)
- To complement other testing (traditional and genetic) for the predictive diagnosis of disease and to take action accordingly
- To measure longitudinally patient's cellular/biological aging over time to monitor the effectiveness of wellness/anti-aging programs

TAT as the reference test for use by physicians in personalized and functional medicine



Selected References

- Association between alcohol consumption in healthy midlife and telomere length in older men. The Helsinki Businessmen Study.
- Shortened telomeres in individuals with abuse in alcohol consumption.
- Longer leukocyte telomeres are associated with ultra-endurance exercise independent of cardiovascular risk factors.
- The power of exercise: buffering the effect of chronic stress on telomere length.
- Associations between rotating night shifts, sleep duration, and telomere length in women.
- Short sleep duration is associated with shorter telomere length in healthy men: findings from the Whitehall II cohort study.
- Cellular aging and restorative processes: subjective sleep quality and duration moderate the association between age and telomere length in a sample of middle-aged and older adults.
- Obesity, cigarette smoking, and telomere length in women.

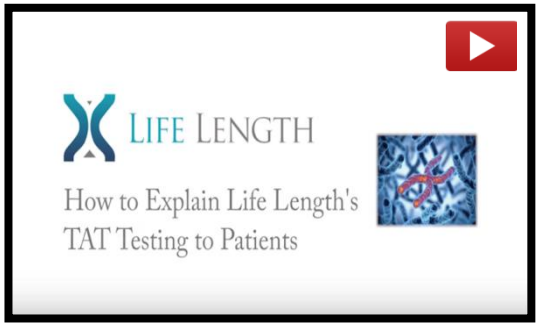
Selected References

- Inverse association between adiposity and telomere length: The Fels Longitudinal Study.
- Short leukocyte telomere length is associated with obesity in American Indians: the Strong Heart Family study.
- Body mass index and leukocyte telomere length in adults: a systematic review and meta-analysis.
- Telomere shortening in smokers with and without COPD.
- Telomere length loss due to smoking and metabolic traits.
- Impact of Intrauterine Tobacco Exposure on Fetal Telomere Length.
- Mediterranean diet, telomere maintenance and health status among elderly.
- Higher serum vitamin D concentrations are associated with longer leukocyte telomere length in women.
- Association of marine omega-3 fatty acid levels with telomeric aging in patients with coronary heart disease.

Continue your education online

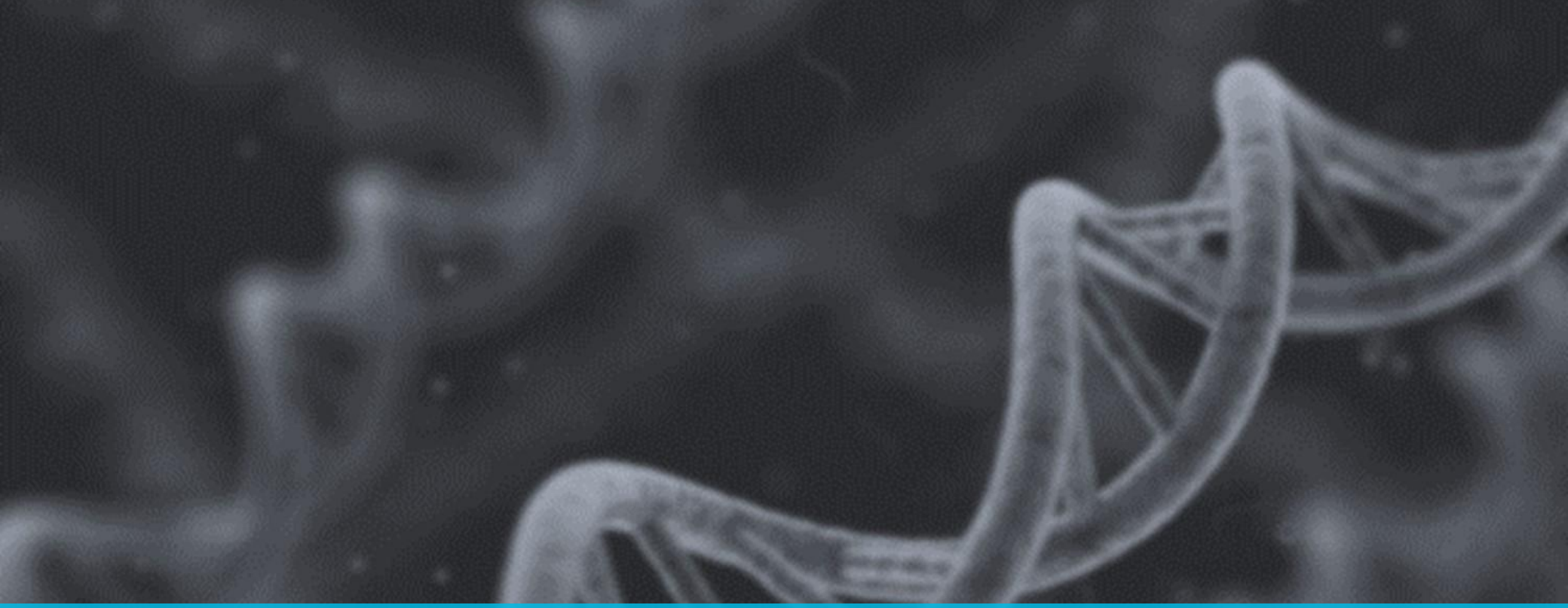
- Visit our website: www.lifelength.com
  - In our website, you will find in our "classroom" Telomeres 101 extensive educational materials, presentations and references to clinical articles by pathology.

- The three links below are videos that further explain how to use TAT test and its clinical value and applications:



(Note: these videos are in the Life Length channel of YouTube)

- For more information : Life Length Corporate Dropbox - Telomere Studies



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www.lifelength.com
info@lifelength.com