A4M | MEDICINE REDEFINED

MODULE II

PEPTIDE THERAPY CERTIFICATION

UNDERSTANDING CELLULAR SENESCENCE AND PEPTIDE THERAPY WITH CASES

JOSEPH CLEAVER MD

A4M – LONGEVITY CONFERENCE
LAS VEGAS
DECEMBER 2025

JOSEPH CLEAVER MD
BIO AND DISCLOSURES

- CANCER CHECK LABS – CHAIRMAN LONGEVITY COMMITEE
- Cell Surgical Network – Speaker
- Stem Cell Conference - Speaker
- International Peptide Society A4M – Teaching staff
- Paradigm Wellness Medical Group LLC - Founder
- TRT DALLAS LLC – MEN'S HEALTH
- Boutique Wellness PLLC – MED SPA - Medical Director
- Locanda Renaissance LLC – Medical Education – Cofounder
- George Washington School of Medicine – Adjunct Clinical Professor
- Trentech BIO – Scientific Advisory Board (DISCLOSURE)
- Luminary LLC – Medical Advisory Board (DISCLOSURE)
- ONE HEALTH BIOSCIENCES LLC – MEDICAL/SCIENTIFIC ADVISOR
- TA Sciences – Speaker (DISCLOSURE)
- Triple Helix labs – Director of Klotho Therapies

JOSEPH CLEAVER MD
PROFESSIONAL CAREER TIMELINE

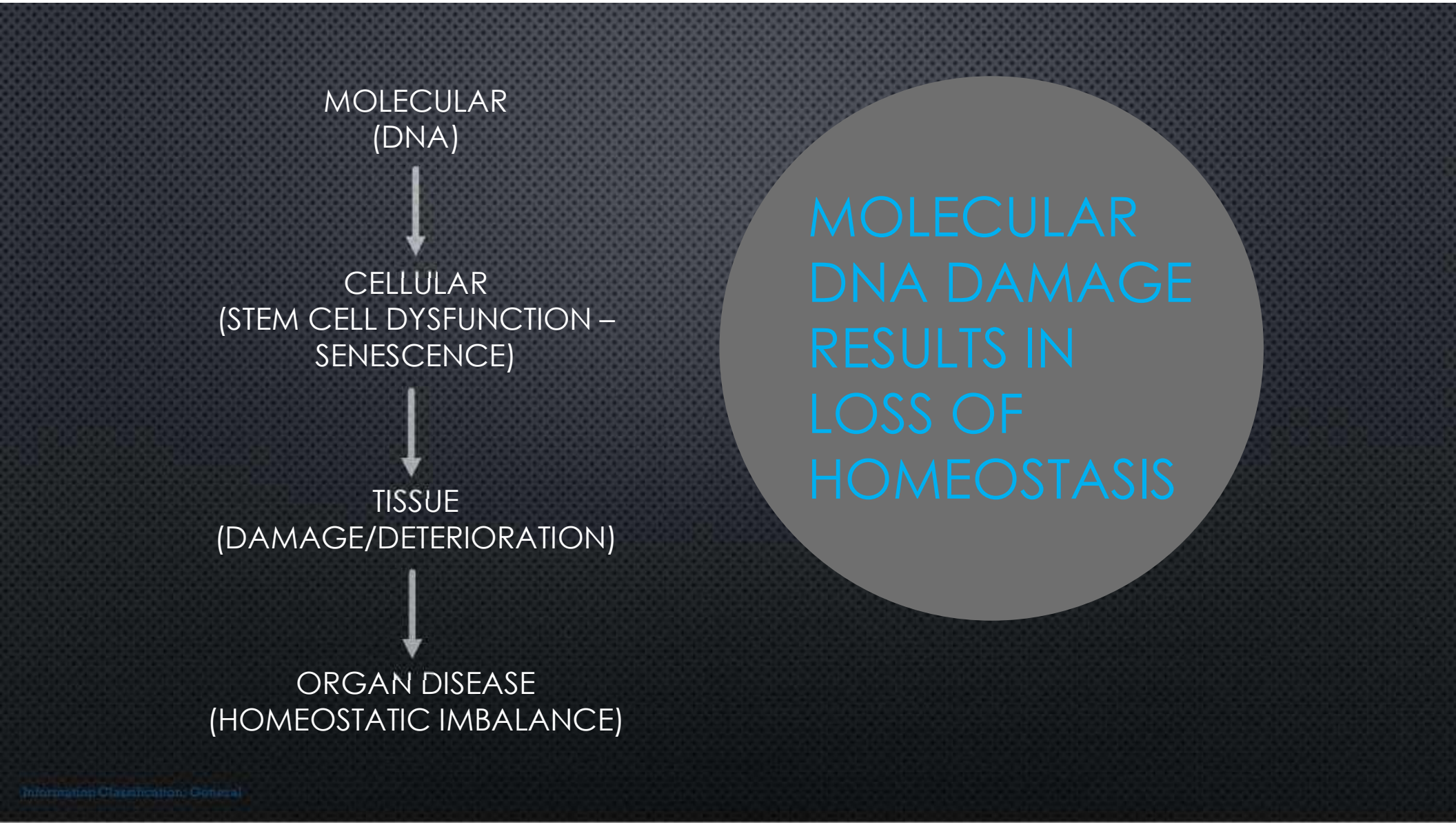
- BOARD CERTIFIED INTERNAL MEDICINE/ANTIAGING LONGEVITY MEDICINE RHEUMATOLOGY/IMMUNOLOGY
- 30 YEARS FUNCTIONAL REGENERATIVE MEDICINE
- 25 YEARS – ANTIAGING LONGEVITY REGEN AESTHETICS CLINICAL PRACTICE FOCUS ON TREATMENT PROTOCOL DEVELOPMENT
- 18 YEARS PEPTIDE EDUCATION AND THERAPIES
- 15 YEARS - STEM CELL/EXOSOME LONGEVITY PROTOCOLS
- 10 YEARS PURSUIT – KLOTHO THERAPIES

DISCLAIMER

- Statements made are for educational purposes and have not been evaluated by the US Food and Drug Administration (FDA). Information provided and contained herein is not intended to diagnose, treat, cure, or prevent any disease.

WHAT IS AGING?

THE MOST INSIDIOUS AND RELENTLESS DEGENERATIVE DISEASE KNOWN TO MAN CHARACTERIZED BY MOLECULAR DNA DAMAGE, INFLAMMAGING, MITOCHONDRIAL DYSFUNCTION, TELOMERE SHORTENING, LOSS OF KLOTHO PROTEIN, LEADING TO CELL SENESCENCE AND STEM CELL FAILURE, RESULTING IN TISSUE DAMAGE, PHYSIOLOGIC DETERIORATION WITH LOSS OF HOMEOSTASIS ALL LED BY AGING CLOCKS/IMMUNE SENESCENCE



DDR (DNA DAMAGE RESPONSE) AGING AT THE MOLECULAR LEVEL

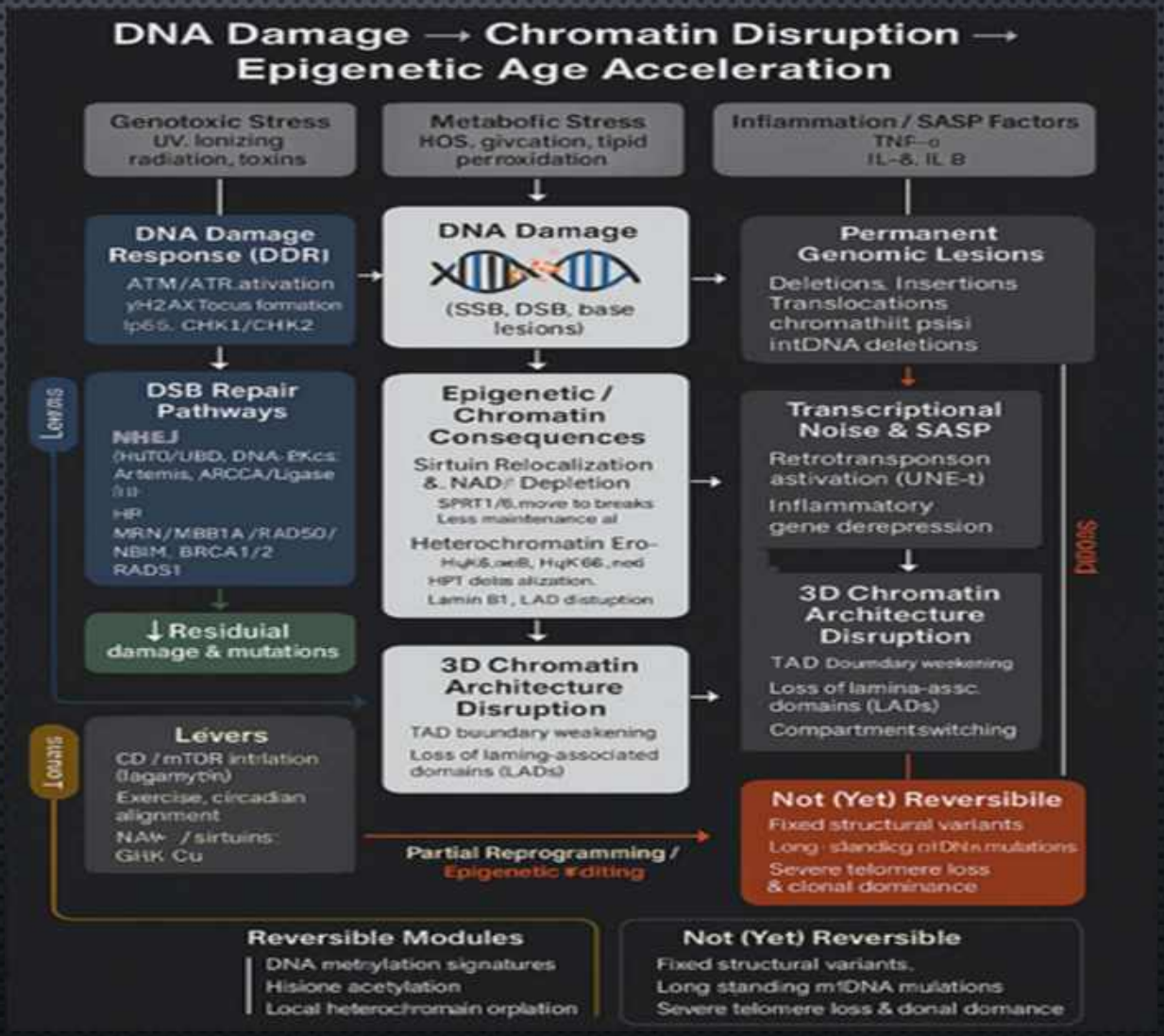
- LEADS TO CELL SENESCENCE AND SASP CELLS (ZOMBIE CELLS)
- STEM CELL FAILURE
- ACCELERATED INFLAMMAGING
- TELOMERE SHORTENEING
- DETERIORATING HEALTH
- SHORTENED LIFESPAN

Molecular Aspects of Senescence and Organismal Ageing—DNA Damage Response, Telomeres, Inflammation and Chromatin Natalia Sławi ´ nska and Renata Krupa *Int. J. Mol. Sci. 2021, Published: 8 January 2021

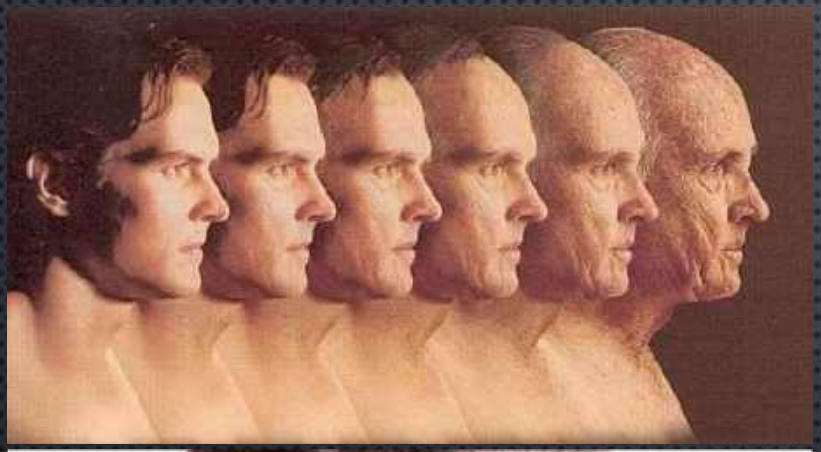
Laboratory of Medical Genetics, Faculty of Biology and Environmental Protection, University of Lodz, 90-236 Lodz, Poland; natalia.slawinska@edu.uni.lodz.pl Correspondence: renata.krupa@biol.uni.lodz.pl

MOLECULAR DNA DAMAGE DDR

- IRREPAIRABLE DOUBLE STRANDED BREAKS?



THE EFFECTS OF UNTREATED AGING

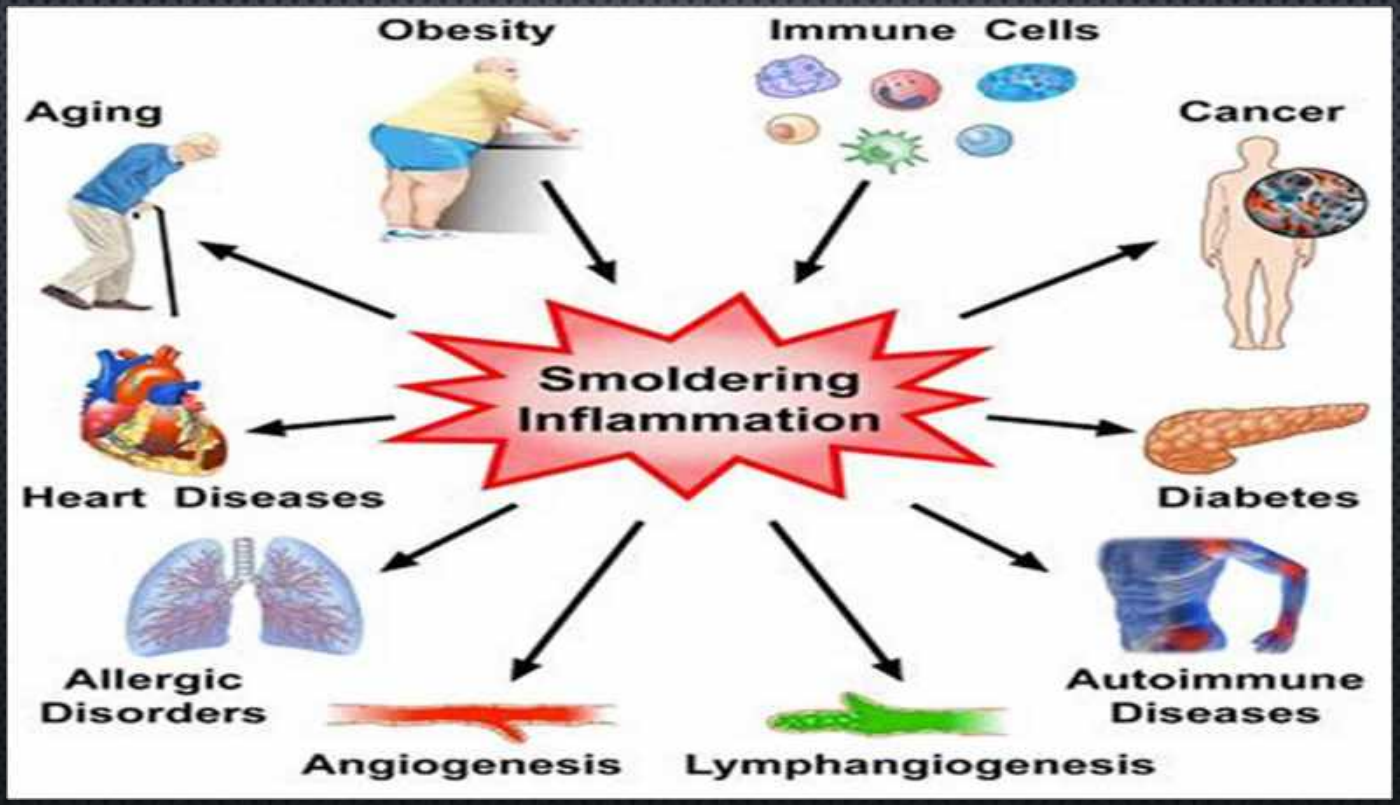


78 years old

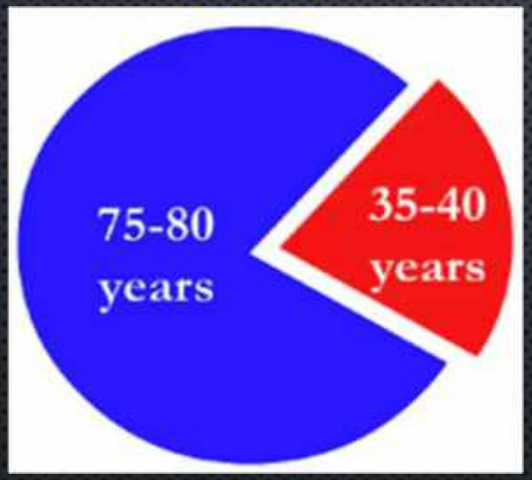
POOR LIFESTYLE CHOICES
HORMONE DEFICIENCY
MITOCHONDRIAL HEALTH
DNA DAMAGE MANAGEMENT
LONGEVITY PROTEINS - KLOTHO
TELOMERE MANAGEMENT
CELL SENESCENCE MANAGEMENT

LEADS TO INFLAMMAGING
CELL DYSFUNCTION
CELL SENESCENCE,
STEM CELL FAILURE AND
ACCELERATED AGING

INFLAMMAGING



REASONABLE LONGEVITY GOAL – 120 YEARS
POTENTIAL INCREASE IN HUMAN LIFESPAN



CANCER
HEART DISEASE
DIABETES
ALZHEIMERS
NEUROLOGIC
KIDNEY FAILURE
LUNG COPD
SKIN AGING

INFLAMMAGING
DNA DAMAGE
EPIGENETICS
MITOCHONDRIA LOSS
TELOMERE LOSS
KLOTHO LOSS
SENESCENCE
STEM CELL LOSS

ENVIRONMENT
TOXINS
RADIATION
PESTICIDES
HEAVY METALS
LATENT INFECTION
MICROPLASTICS
EMFs

LIFESTYLE
STRESS
DIET
SEDENTARY
SLEEP

AVERAGE LIFESPAN
PREMATURE AGING

BIOLOGICAL RESERVE

Kavinson etal

KEY COMPONENTS TO ANTIAGING THERAPY IS 120 IN REACH NOW?

PEPTIDES

TELOMERES

STEM CELLS
EXOSOMES
NK

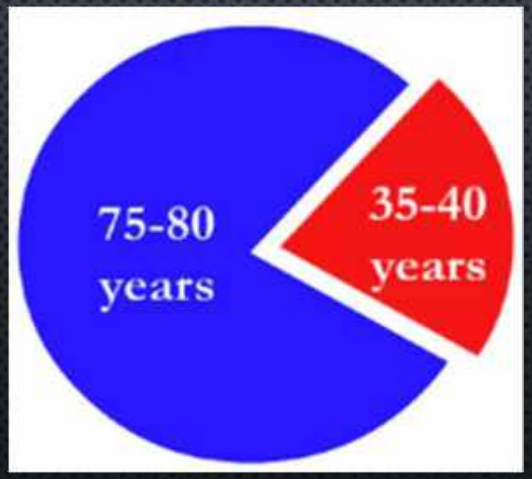
DNA DAMAGE

SENESCENCE MANAGEMENT

MITOCHONDRIA

RESTORE KLOTHO

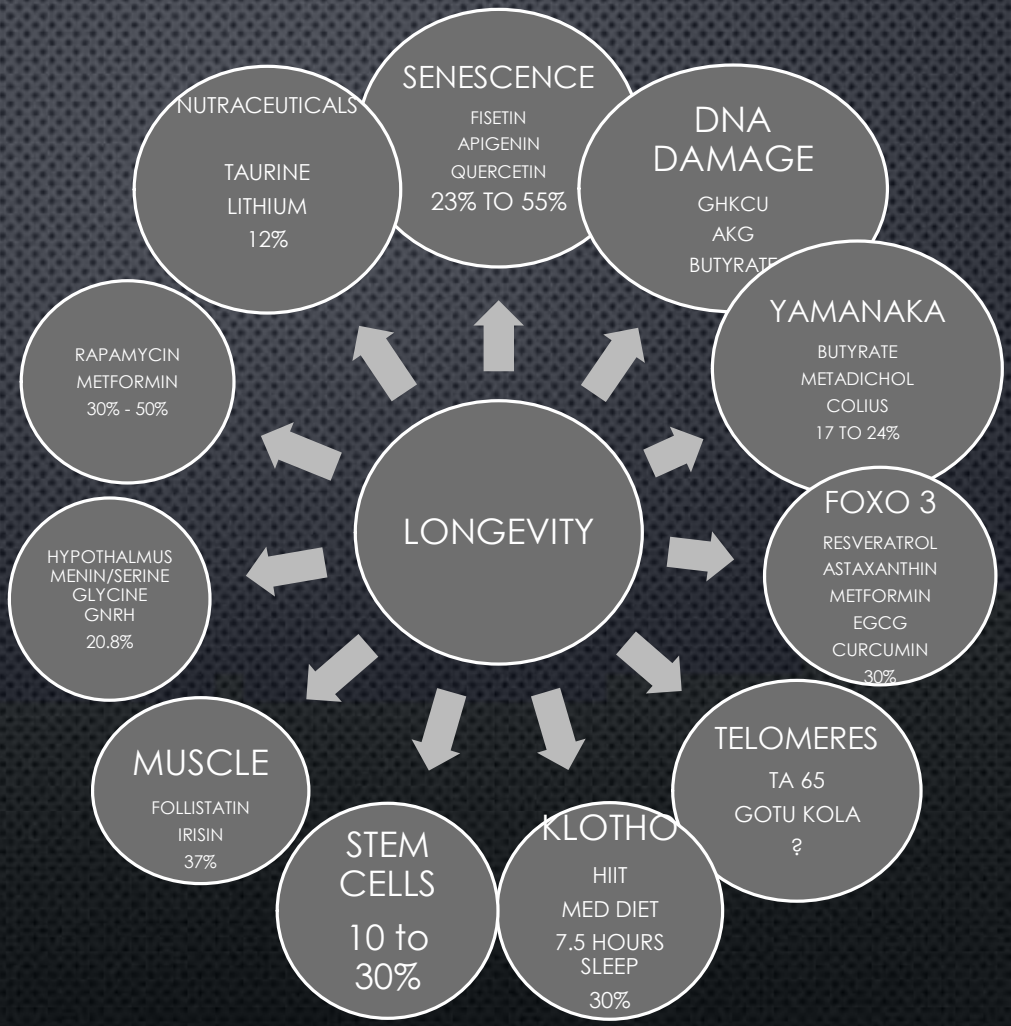
LIFESTYLE HORMONES

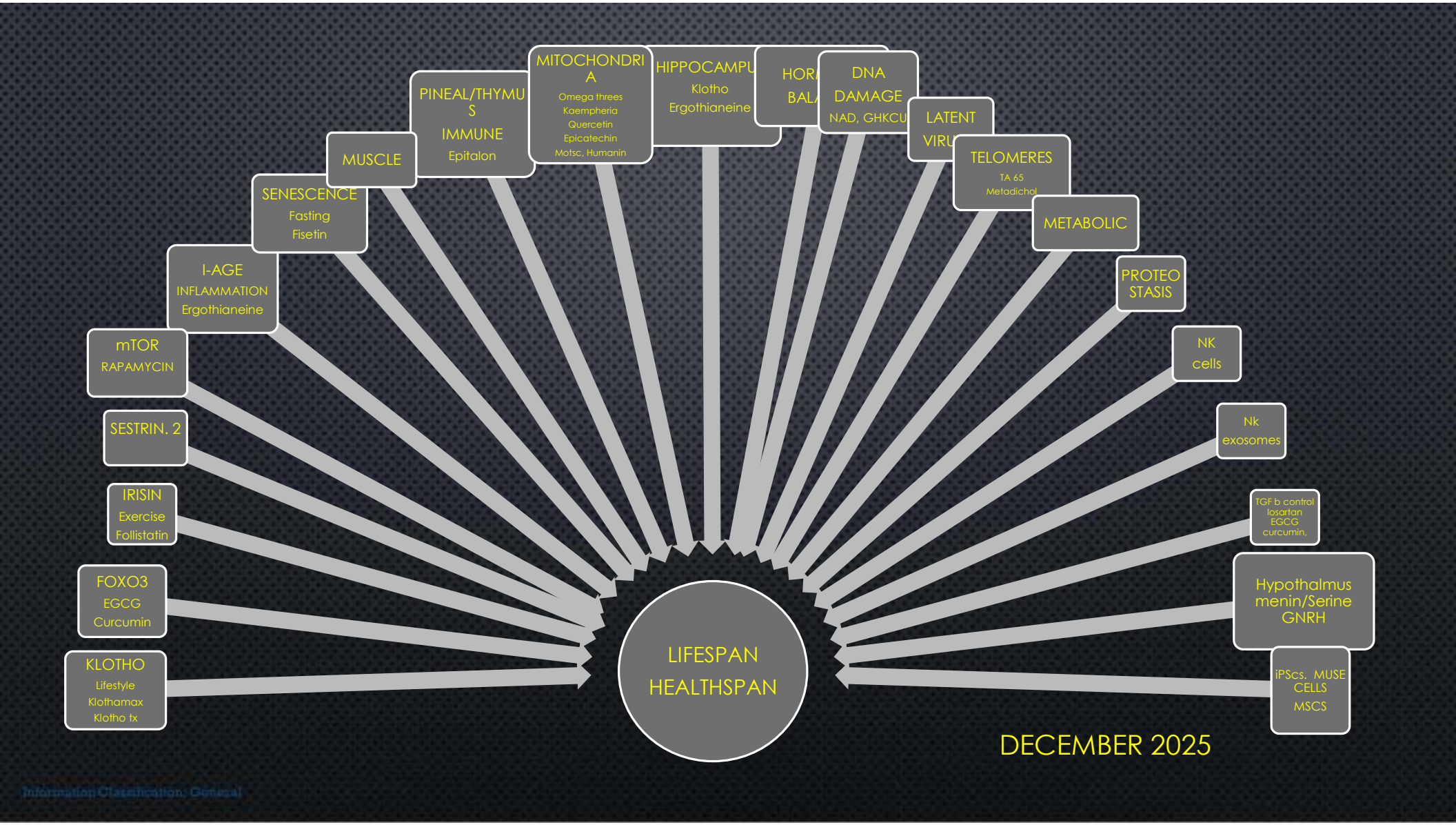


BIOLOGICAL RESERVE

PARADIGM

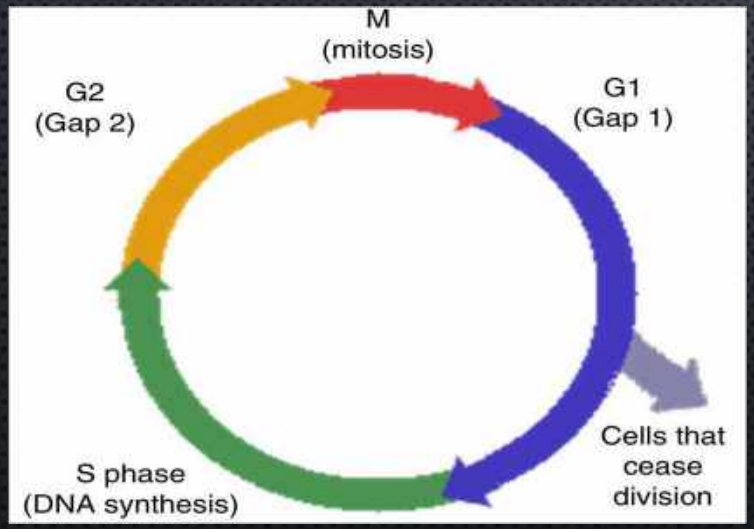
LIFE EXTENSION PERCENTAGE WORMS, FLIES, MAMMALS

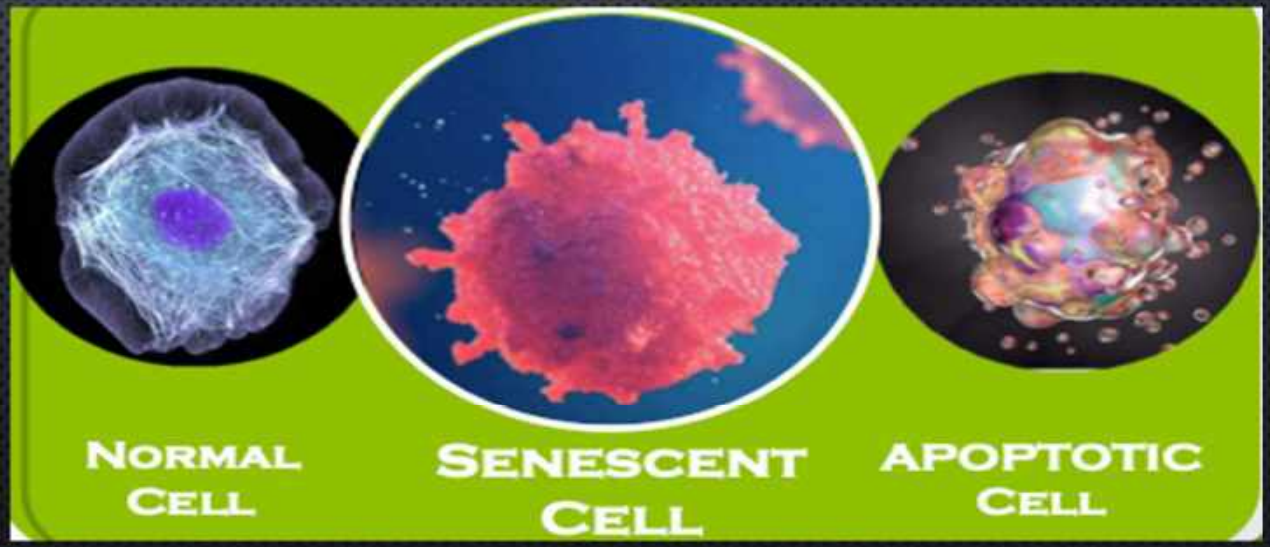




What is a senescent cell?

- Cell cycle arrest phase G1
- Can't divide but still viable
- Apoptosis does not occur





Cellular Senescence as a Therapeutic Target for Age-Related Diseases: A Review

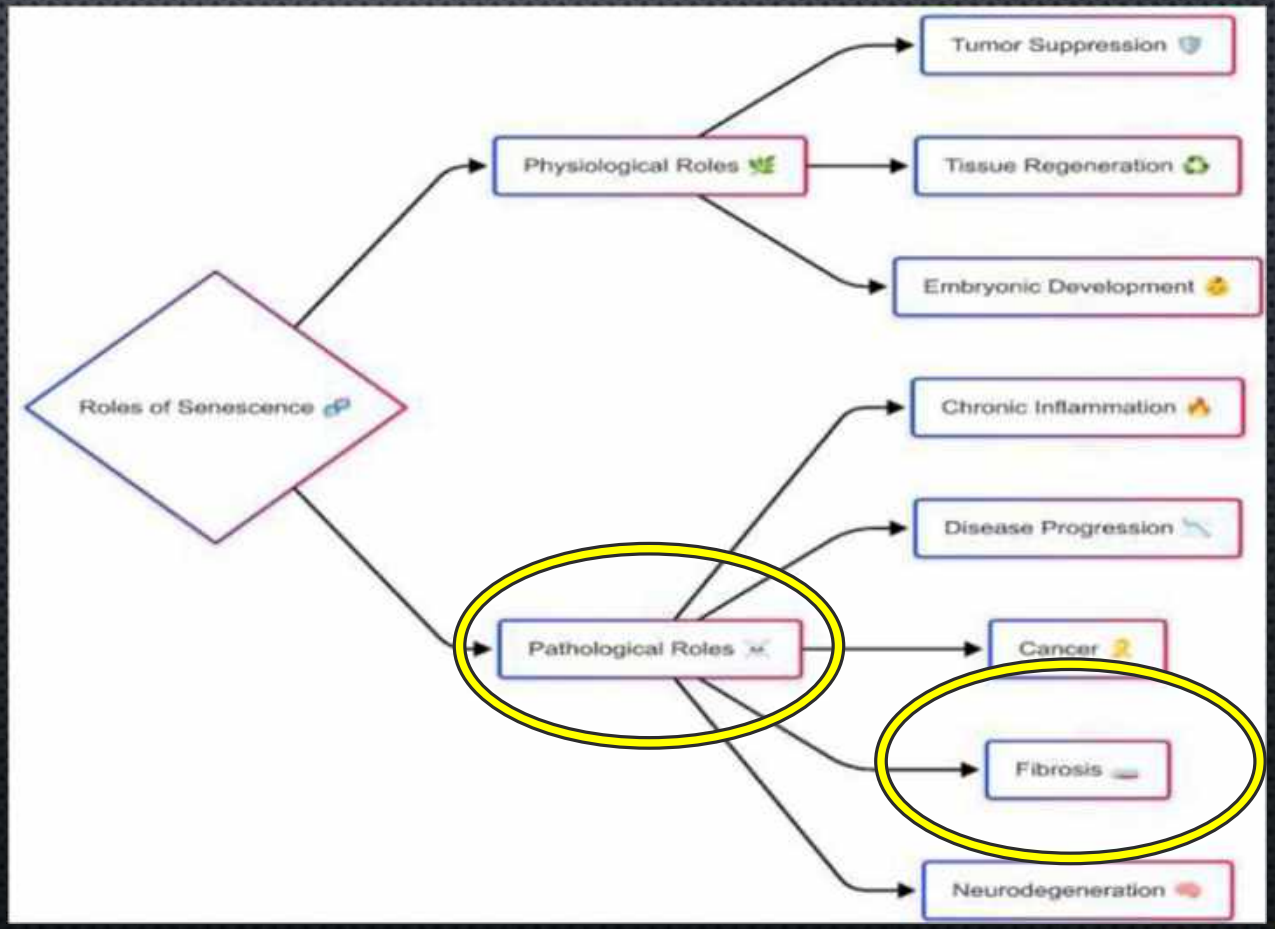
Mateo Amaya-Montoya,[1] Agustín Pérez-Londoño,[1] Valentina Guatibonza-Garcia,[1] Andrea Vargas-Villanueva,[1] and Carlos O. Mendivil[1,2]

ROLES OF SENESCENCE

Programmed

Stress induced

Replicative (Age)



REPLICATIVE SENESCENCE

Programmed

Stress induced

Replicative (Age)

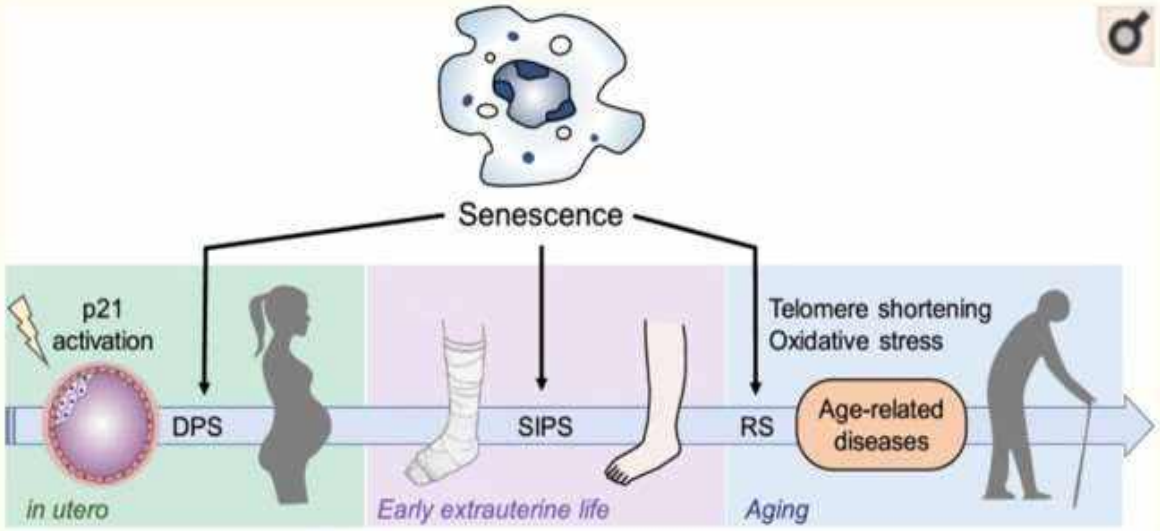


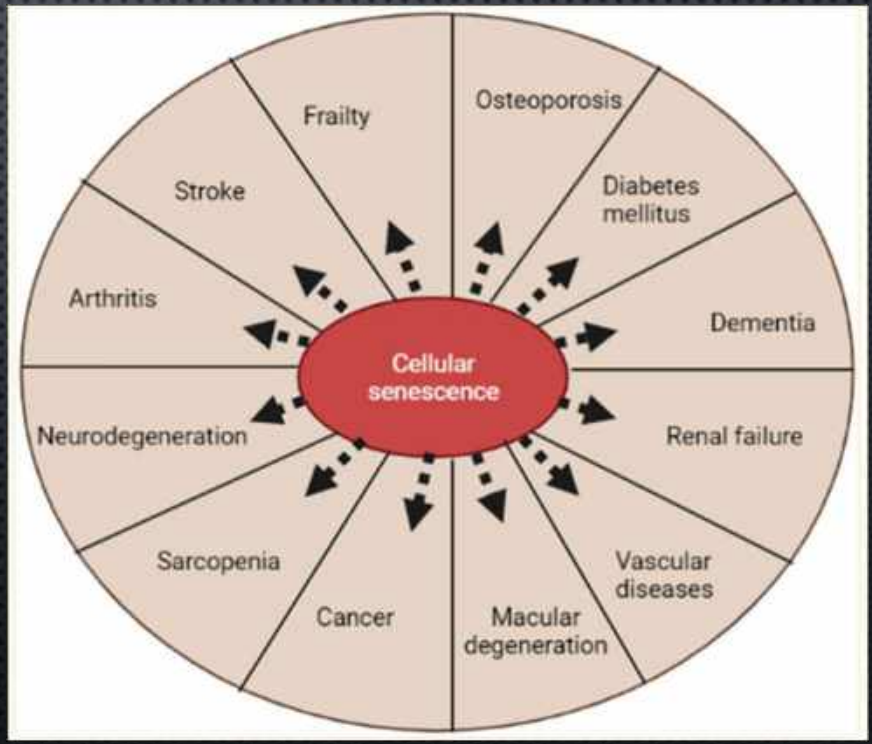
Fig. 4 Programmed Stress Replicative

Functions of cellular senescence throughout the life span. DPS developmentally programmed senescence, SIPS stress-induced premature senescence, RS replicative senescence

REPLICATIVE SENESCENCE

AGING CAUSES CELL DYSFUNCTION IN VARIOUS SYSTEMS IN THE BODY AT VARIOUS ONSETS AND RATES

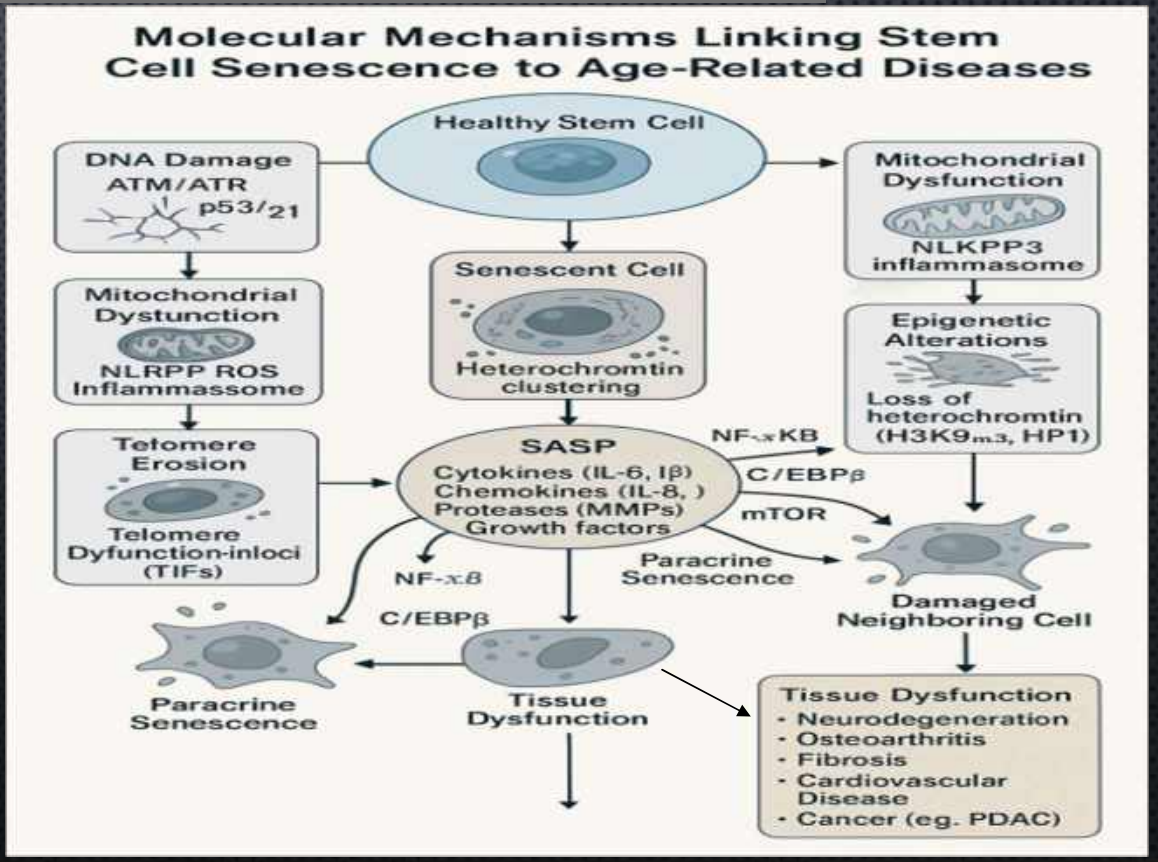
- SKIN AGING – WRINKLES/SAGGING
- HAIR LOSS
- PHYSICAL/SEXUAL HEALTH
- AUTO IMMUNE
- IMMUNE SENESCENCE



SENESCENT CELLS - SASP

SENESCENT ASSOCIATED SECRETORY PHENOTYPE

- Phenotypic and Genotypic changes – morphology, chromatin remodeling
- Metabolic changes and paracrine signaling
- Immunosenescence- immune system no longer efficient in clearing senescent cells



Cellular Senescence in Ageing, Age–Related Disease and Longevity

Article in Current Vascular Pharmacology · December 2013

2018 Jan 2; 217(1): 65–77.
doi: 10.1083/jcb.20170809 PMCID: PMC5748990 PMID: 29114066
Review
Senescence and aging: Causes, consequences, and therapeutic avenues
Domhnall McHugh[1,2] and Jesús Gil[1,2]

TIME CLOCKS OF AGING

- IMMUNE SYSTEM/PINEAL/THYMUS (TRIAD)
- HIPPOCAMPUS
- HYPOTHALMUS

THE IMMUNE SYSTEM

IMMUNOSENESCENCE

Published: 12 May 2021
An aged immune system drives senescence and ageing of solid organs
Matthew J. Yousefzadeh, Rafael R. Flores, […]Laura J. Niedernhofer

IMMUNE SYSTEM AGING IMMUNOSENESCENCE

- IMMUNE STRESSORS - VIRAL STRESS, OXIDATIVE STRESS
- DNA DAMAGE TO IMMUNE CELLS
- Elevation of circulating amounts of pro-inflammatory cytokines
- TNF-a, IL-1β IL,-6 MMPs released by resting leukocytes
- REACTIVATED T CELLS – CAUSE INFLAMMAGING

CAUSES OF IMMUNE SYSTEM AGING IMMUNOSENESCENCE

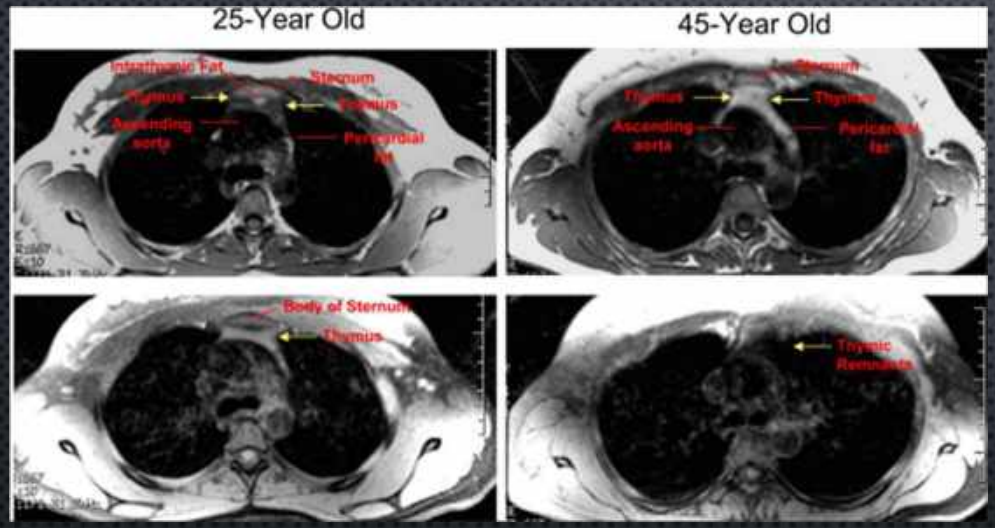
- Ultraviolet radiation
- Alcohol
- Smoking
- Pollution
- Microplastics – 40 POUNDS IN A LIFETIME
- Heavy metals
- Lack of exercise
- Stress/cortisol
- Latent viruses
- Covid 19 and long covid

IMMUNOSENESCENCE DRIVES AGEING OF SOLID ORGANS

- INDUCED IMMUNE SENESCENCE STUDY - Mouse study
- DELETED Ercc1 - encodes a crucial DNA repair protein –
  - induce immune senescence only
- Resulted in non-lymphoid organs showing increased senescence and damage
- **IMMUNE SYSTEM SECONDARY INDUCER OF SENESCENCE within other tissues**.
- **IMMUNE SYSTEM FUNCTION LOST**
  - Decreased ability to fight infection AND cancers
  - Susceptibility to chronic diseases…heart , lung, kidney, brain, etc

Published: 12 May 2021An aged immune system drives senescence and ageing of solid organsMatthew J. Yousefzadeh, Rafael R. Flores, […]Laura J. Niedernhofer

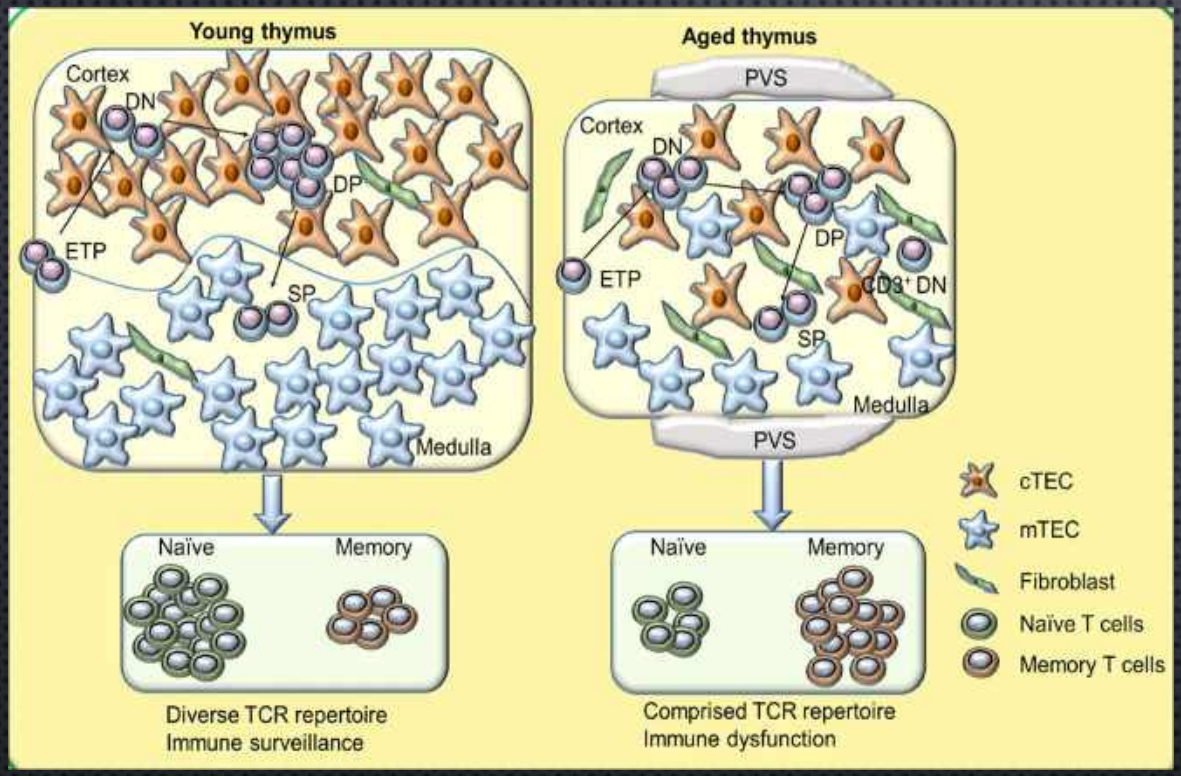
LATENT VIRUSES AND REACTIVATION OF VIRUSES ACCELERATE TELOMERE ATTRITION, THYMIC DYSFUNCTION AND EXTRINSIC AGING LEADS TO IMMUNOSENESCENCE



THYMUS AND IMMUNE SENESCENCE

- LOSE 3% OF THYMUS PER YEAR TO AGE 45
- THEN SLOWS TO 1% LOSS PER YEAR

Curr Opin Immunol. 2010 Aug; 22(4): 521–528 Thymic Fatness and Approaches to Enhance Thymopoietic Fitness in Aging Vishwa Deep Dixit1



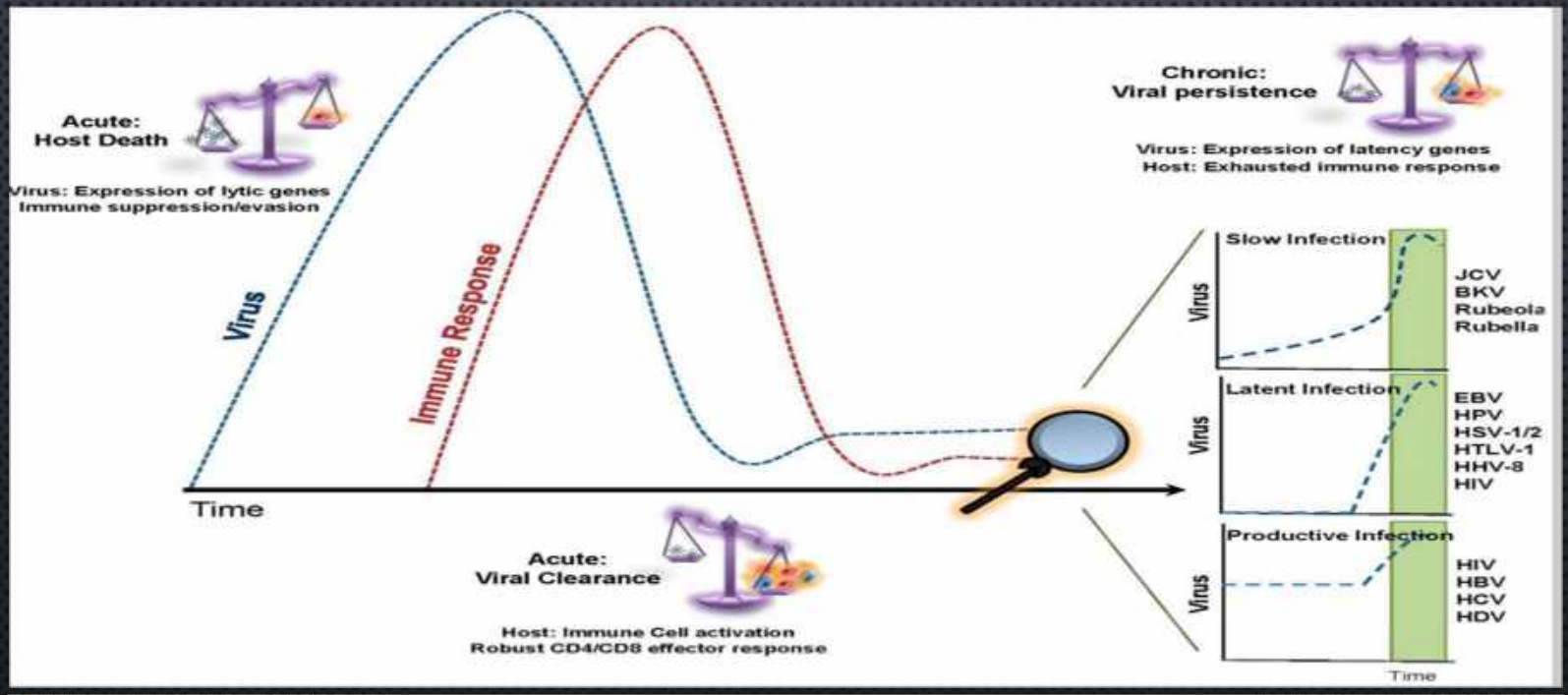
- REDUCTION IN THYMIC CELLULARITY AND THYMIC STROMAL MICROENVIRONMENT DISRUPTION, INCLUDING
- LOSS OF DEFINITE CORTICAL-MEDULLARY JUNCTIONS,
- REDUCTION OF CORTICAL THYMIC EPITHELIAL CELLS
- LOSS MEDULLARY THYMIC EPITHELIAL CELLS, FIBROBLAST
- EXPANSION AND INCREASE IN PERIVASCULAR SPACE

THYMUS AND IMMUNE SENESCENCE

1 July 2022 Age-related thymic involution: Mechanisms and functional impact Zhanfeng Liang1,2,

LATENT VIRUSES AND REACTIVATION OF VIRUSES ACCELERATE EXTRINSIC AGING AND IMMUNOSENESENCE ?

CHRONIC VIRAL INFECTIONS REACTIVATION OF LATENT VIRUS

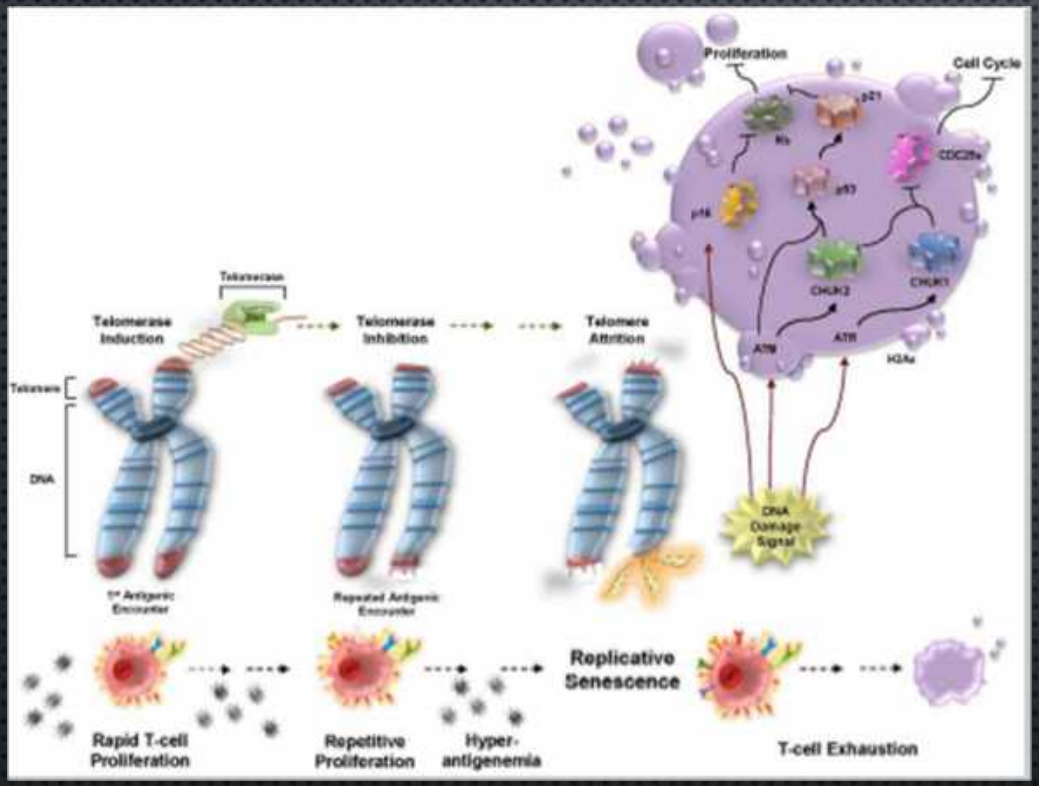


Viruses. 2017 Oct; 9(10): 289.
Published online 2017 Oct 5. doi: 10.3390/v9100289
PMCID: PMC5691640
PMID: 28981470

Telomere Dynamics in Immune Senescence and Exhaustion Triggered by Chronic Viral Infection

Marcia Bellon and Christophe Nicot*

TELOMERES, SENESCENCE, AND CHRONIC VIRAL INFECTIONS



Viruses. 2017 Oct; 9(10): 289.
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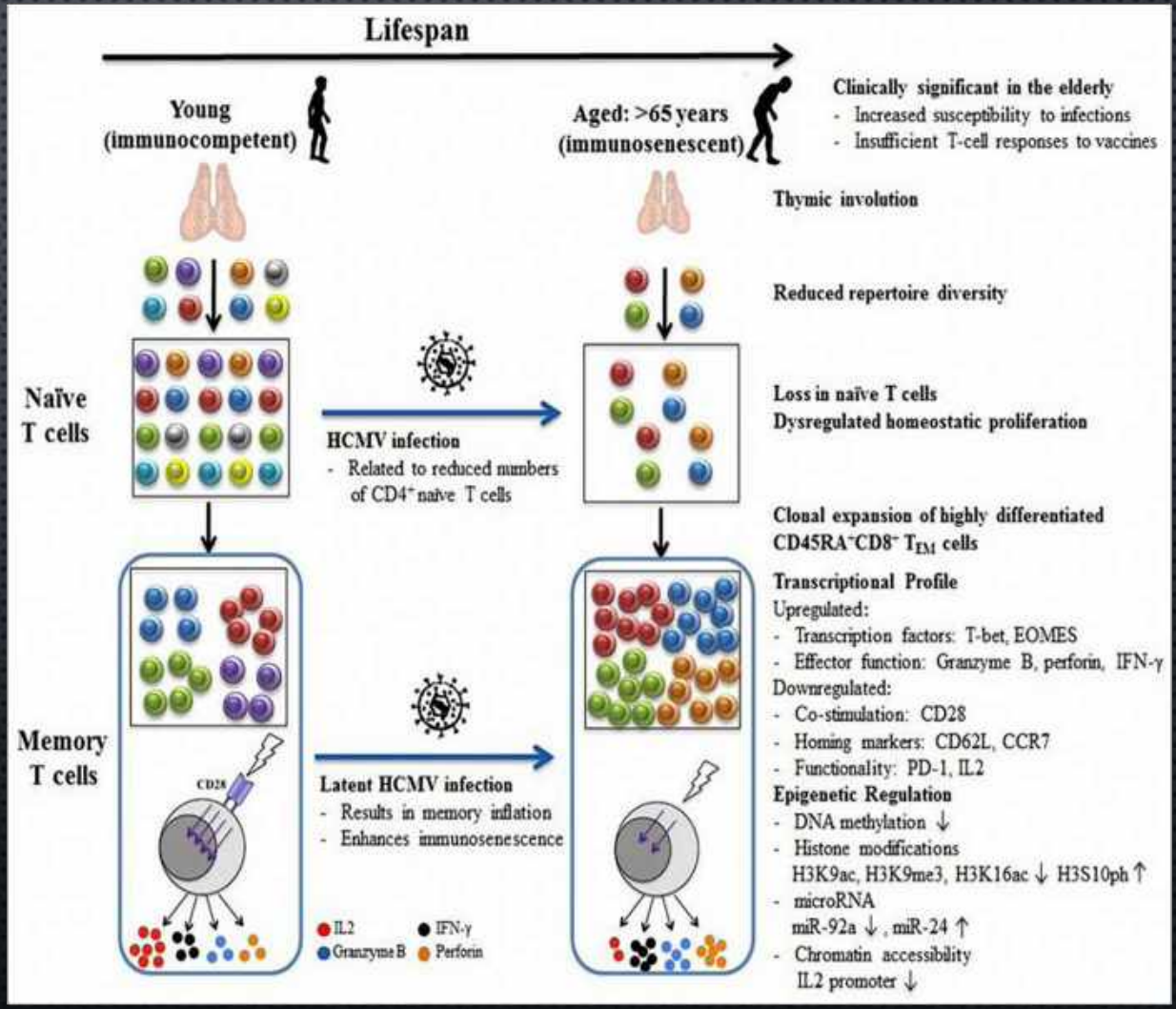
Telomere Dynamics in Immune Senescence and Exhaustion Triggered by Chronic Viral Infection

Marcia Beilon and Christophe Nicot*
Author information ▸ Article notes ▸ Copyright and License information Disclaimer

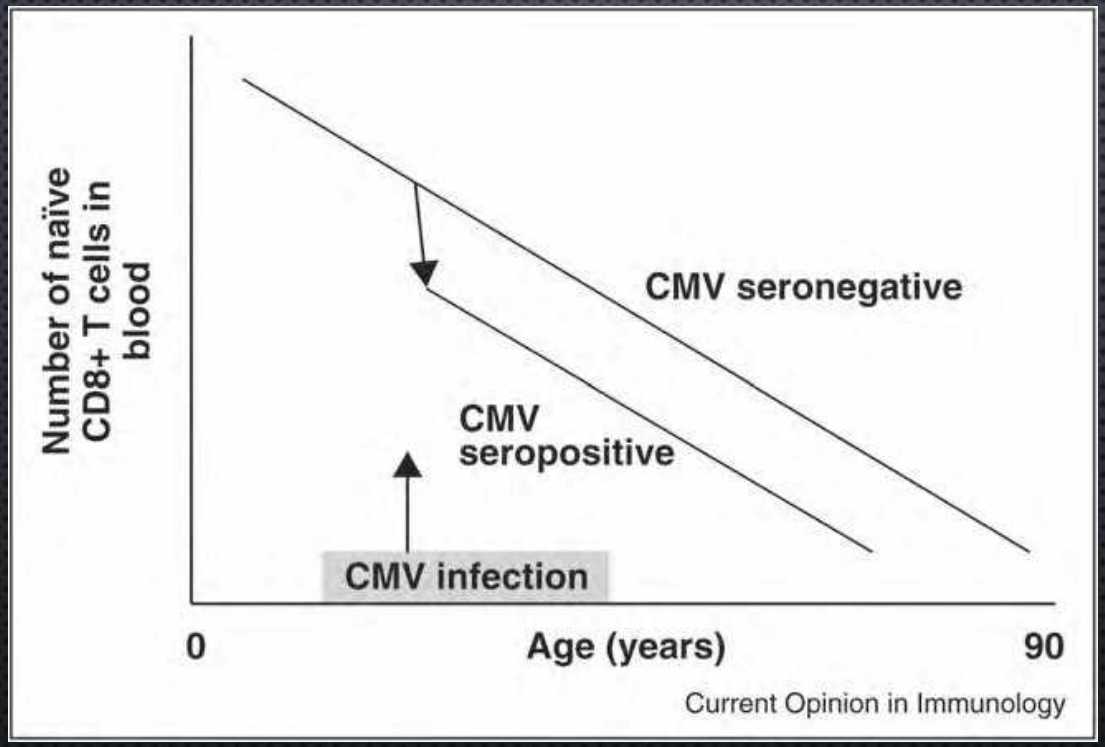
- TELOMERE ATTRITION AND DNA DAMAGE RESPONSE (DDR) DURING CHRONIC VIRAL INFECTION.
- TELOMERE ATTRITION, CAUSED BY CHRONIC EXPOSURE TO VIRAL ANTIGEN, IS EXACERBATED OVER TIME (DOTTED ARROWS).
- ONGOING HYPER-ANTIGENEMIA RESULTS IN TELOMERE CRISIS
- RESULTS IN T-CELL EXHAUSTION, INHIBITION OF T-CELL PROLIFERATION, AND CELL CYCLE ARREST.
- EVENTUAL OUTCOME IS PROGRAMMED CELL DEATH (APOPTOSIS) OR REPLICATIVE SENESCENCE

Cytomegalovirus:

- CMV ACCELERATES THYMIC INVOLUTION AND IMMUNOSENESCENCE



CMV Effect On Number Of Naïve CD8+ T Cells With Age



Up to 20 years of immune aging

Adapted from Moss P. The emerging role of cytomegalovirus in driving immune senescence: a novel therapeutic opportunity for improving health in the elderly. *Curr. Opin. Immunol.* **22**, 529–534 (2010).

TELOMERE ATTRITION INCREASES WITH NUMBER OF HERPES VIRUSES

- 400 SUBJECTS, AGES 53-76, FOLLOWED FOR 3 YEARS
- CMV, HSV-1, HSV-6 ALL CONTRIBUTE TO TELOMERE ATTRITION
- NO ASSOCIATION FOR EBV ALONE
- THE MORE CONCOMITANT INFECTIONS, THE STEEPER THE LOSS

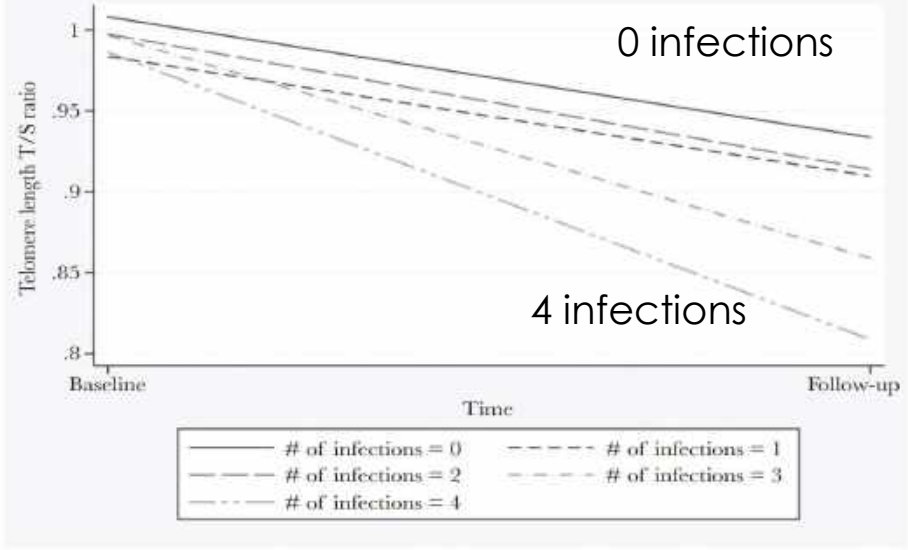
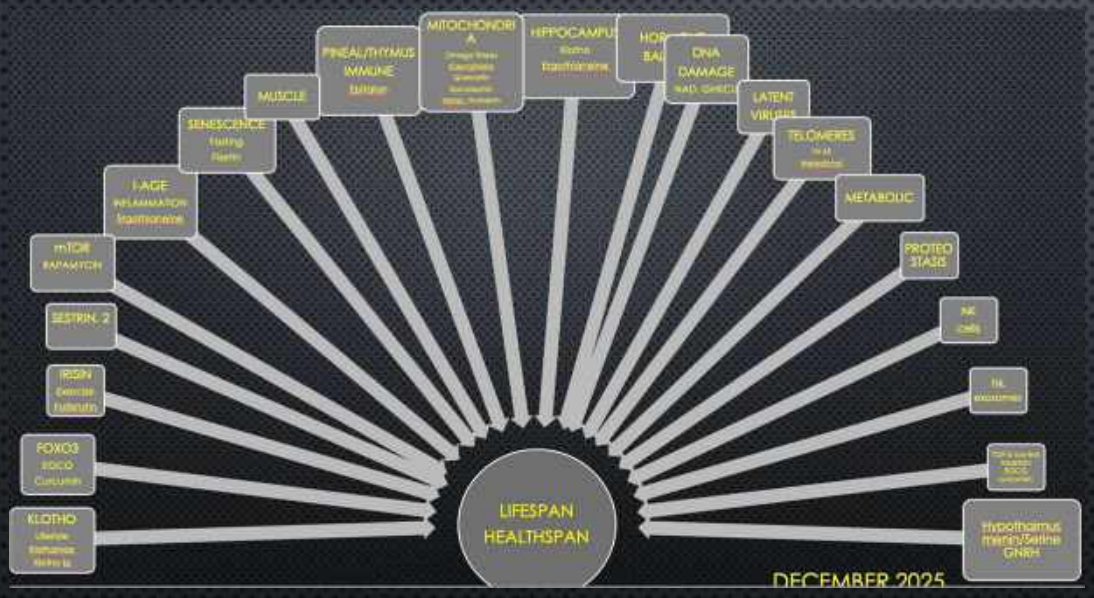


Figure 1. Number of herpesvirus infections at baseline and mean leukocyte telomere length (LTL) change (telomere [T]/single copy gene [S] ratio) over 3 years.

Dowd, J. B. *et al.* Persistent Herpesvirus Infections and Telomere Attrition Over 3 Years in the Whitehall II Cohort. *J. Infect. Dis.* **216**, 565–572 (2017).

WHERE DO WE START?
WHAT THERAPIES ARE CURRENTLY AVAILABLE?



- PEPTIDES
- BIOREGULATORS
- DNA DAMAGE – THERAPIES
- SENOPREVENTIVES
- SENOLYTICS
- MITOCHONDRIAL
- SIRTUIN THERAPIES
- KLOTHO THERAPIES
- STEM CELL THERAPY
- TELOMERE/ TELOMERASE MANAGEMENT
- STEM CELLS
- EXOSOMES
- PHOTOBIOMODULATION

KEY COMPONENTS TO ANTIAGING THERAPY IS 120 IN REACH NOW?

PEPTIDES

TELOMERES

ERADICATE LATENT VIRUS

SENESCENCE MANAGEMENT

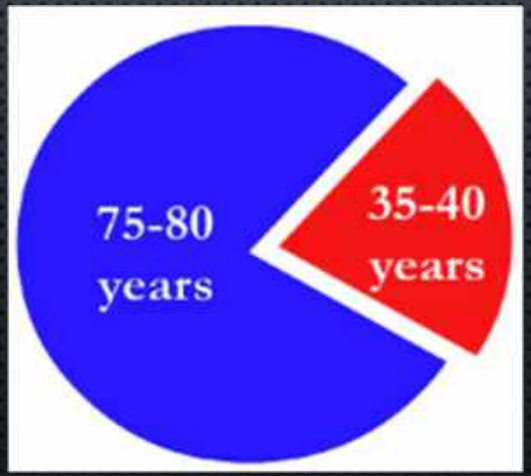
MITOCHONDRIA

STEM CELL

RESTORE KLOTHO

DNA DAMAGE

HORMONES LIFESTYLE

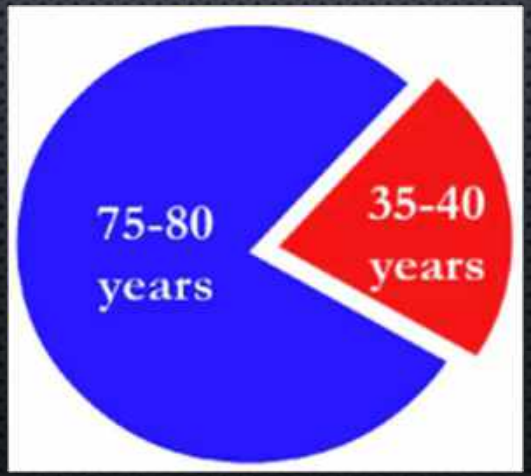


BIOLOGICAL RESERVE

MAXIMIZE STEM CELLS
MINIMIZE SENESCENT CELLS

MAXIMIZE STEM CELLS

MINIMIZE SENESCENT CELLS



BIOLOGICAL RESERVE

PEPTIDES
GOALS

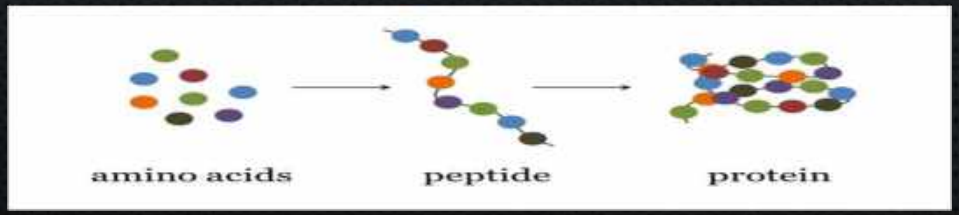
- Define peptides
- How they work
- Classes of peptides
- Side effects
- Uses in LONGEVITY/Functional/Regenerative medicine – PROTOCOL INTEGRATION

SOURCING PEPTIDES

- 503A/503B COMPOUNDING PHARMACY
- ONLINE B TO B – B TO C - LYOPHILIZED
- COMPASSIONATE USE
- OFFICE COMPOUNDING

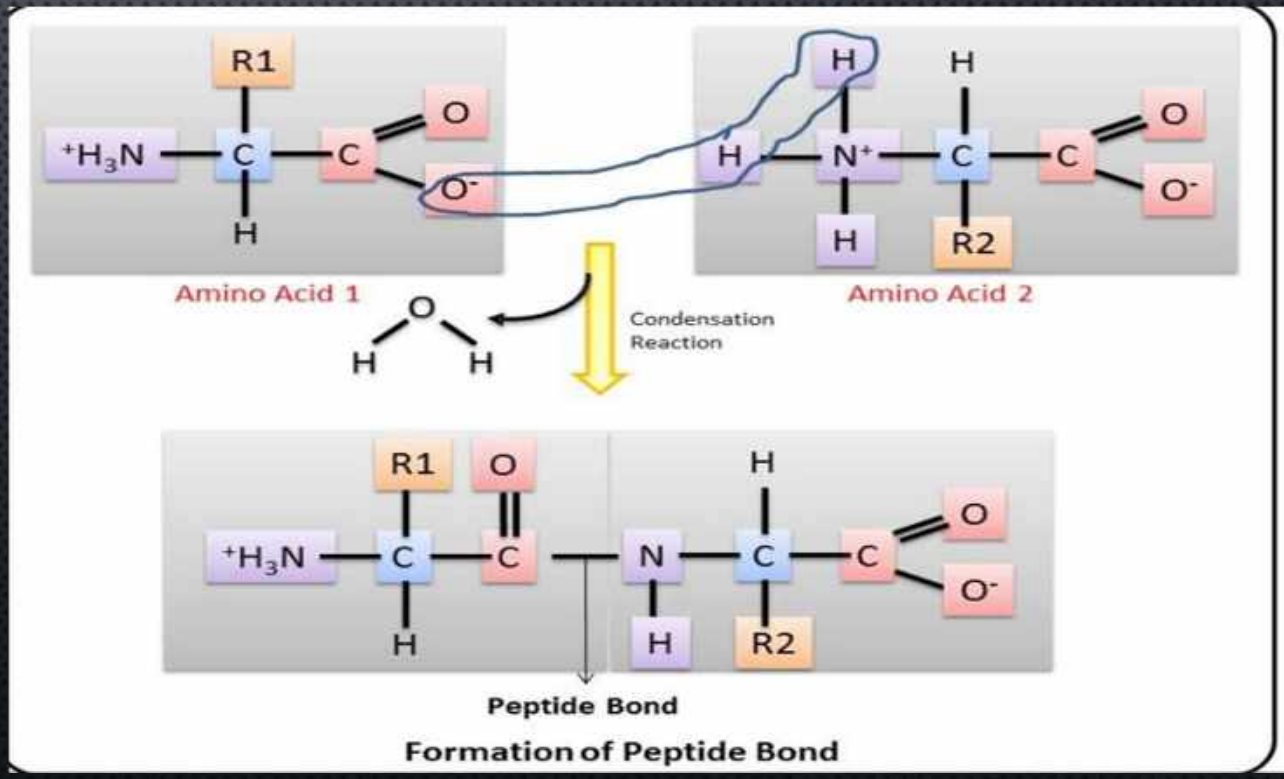
PEPTIDES 101
DEFINE

- **SHORT AMINO ACID SEQUENCES**
  - TYPICALLY LESS THAN 50 AMINO ACIDS
  - 50 TO 100 AA IS POLYPEPTIDE
  - ACT AS NATURAL BIO-REGULATORS
- **PEPTIDE (PEPTIDE BOND)** IS AMIDE LINKAGE FORMED BY THE REACTION BETWEEN A-CARBOXYL GROUP OF ONE AMINO ACID AND A-AMINO GROUP OF ANOTHER AMINO ACID WITH THE ELIMINATION OF WATER MOLECULE.



PEPTIDES 101

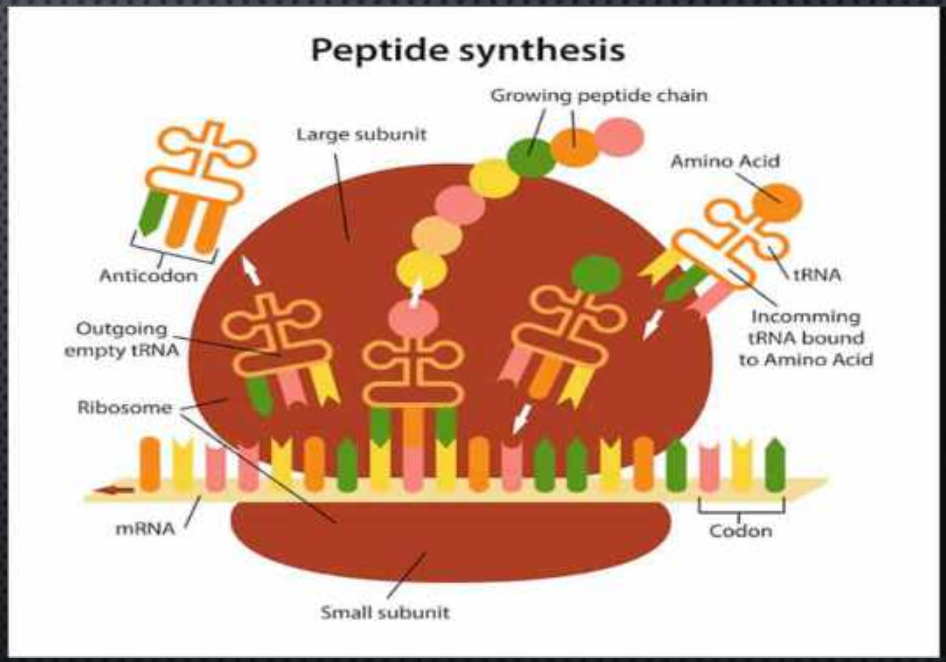
- **PEPTIDE (PEPTIDE BOND)** IS AMIDE LINKAGE FORMED BY THE REACTION BETWEEN
- **A-CARBOXYL GROUP** OF ONE AMINO ACID AND **A-AMINO GROUP** OF ANOTHER AMINO ACID WITH THE ELIMINATION OF WATER MOLECULE.



Formation of Peptide Bond

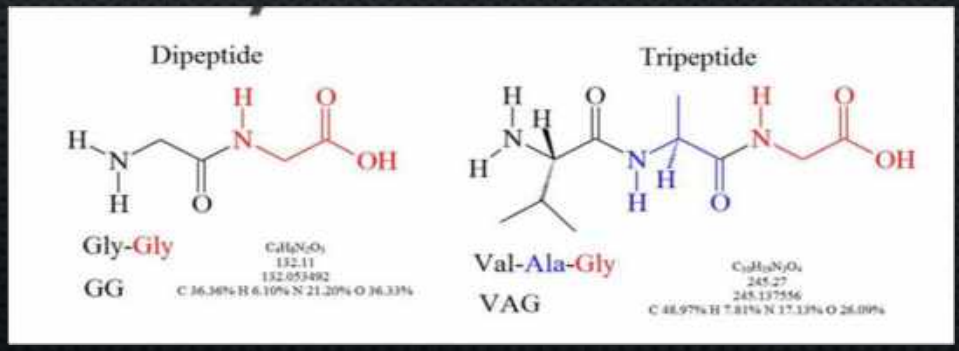
PEPTIDES 101

- PRESENT IN ALL CELLS
- NATURALLY OCCURRING PEPTIDES ARE SYNTHESIZED THROUGH TRANSLATION OF MRNA.



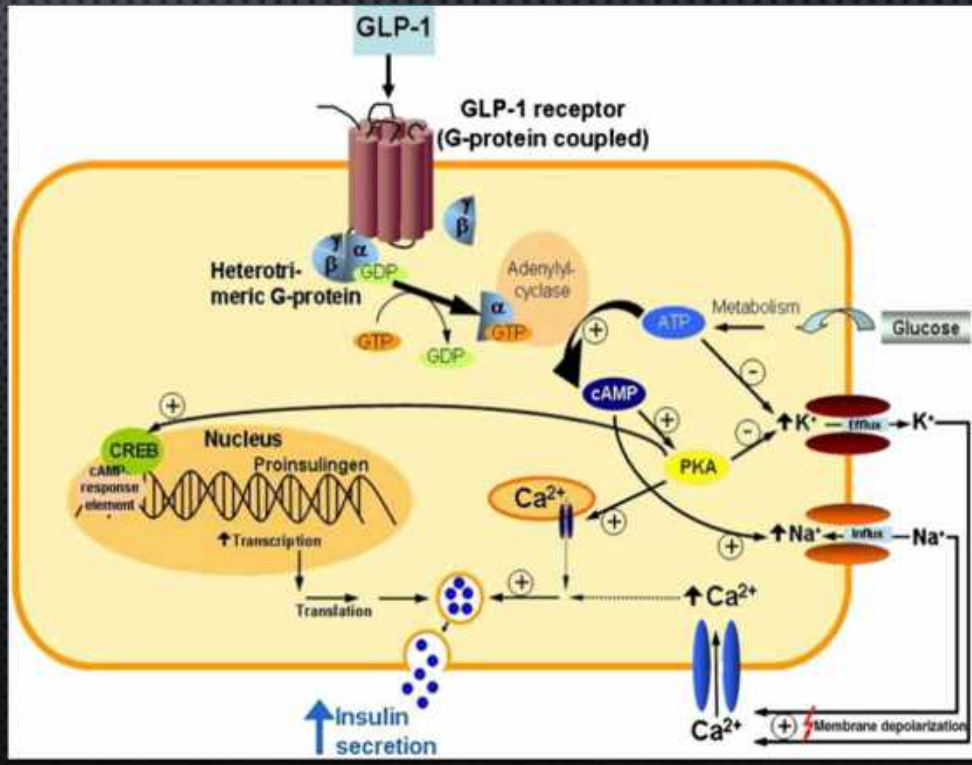
PEPTIDES 101

- MOST PEPTIDES ARE CURRENTLY SYNTHESIZED
- COMPUTATIONAL MODELING – CREATION AND SYNTHESIS
- BIOMIMETIC - COMBINATION IS UNLIMITED
- THOUSANDS IDENTIFIED



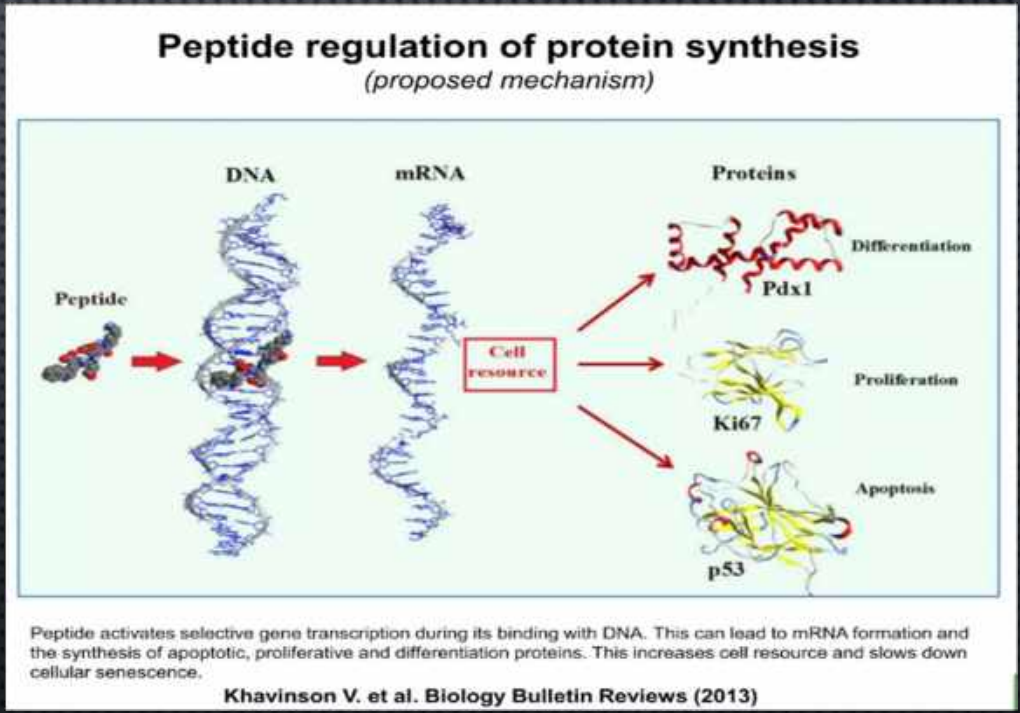
PEPTIDES 101 – HOW THEY WORK

- PEPTIDES ARE **SIGNALING PROTEINS**
- BIND TO **SPECIFIC CELL SURFACE RECEPTORS – INTRACELLULAR**
- **CPP – CELL PENETTRATING PEPTIDES**
- **WIDE RANGE OF BIOLOGICAL ACTIVITY REGULATE FUNCTIONS OF THE ENDOCRINE, NERVOUS, AND IMMUNE SYSTEMS. THE MECHANISM ACTION -- REGULATE GENE EXPRESSION AND PROTEIN SYNTHESIS**
- CONTROL A VAST NUMBER OF CELLULAR PATHWAYS AND FUNCTIONS INCLUDING ACTIONS ON:
  - **GROWTH FACTORS**
  - **STEM CELLS**
  - **HORMONES**
  - **MITOCHONDRIA, METABOLISM**
  - **BRAIN NEUROTRANSMITTERS**
  - **ANTIMICROBIAL ACTION**
  - **CANCER THERAPY**
  - **IMMUNE FUNCTION**
  - **CELL SENESCENCE**



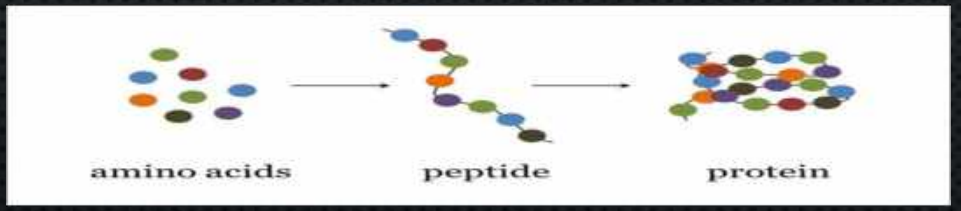
PEPTIDES 101 – MECHANISM OF ACTION

- INTRACELLULAR PATHWAYS (CELL SURFACE)
- INTERCELLULAR – BIOREGULATORS
- CELL PENETRATING PEPTIDES ESP CANCER
- RAPID CELLULAR AND PHYSIOLOGIC RESPONSE
- TARGETED EFFECTS – LOWER SIDE EFFECTS
- MANY HAVE PLEOTROPHIC MECHANISMS OF ACTION



PEPTIDES 101
EMERGING FRONTIERS

- LONGEVITY AND SENOLYTICS
- CROSS CELL MEMBRANES – BYPASSING CELL SURFACE RECEPTORS
- CONJUGATION – INCREASED HUMORAL IMMUNE RESPONSE
- (MAPS) MULTI ANTIGENIC PEPTIDES – INC IMMUNE RESPONSE
- VACCINE OPTIMIZATION
- CANCER THERAPIES
- **NEW FORMULATIONS**
  - TRANSDERMAL
  - INTRANASAL
  - PELLETS
  - ORAL (SNAC) (LIPOSOMAL, NANNO)
  - SUBLINGUAL



Protein protocols handbook p 679; Jan 1996

MOST POPULAR USES FOR PEPTIDES

- Anti-aging treatments – mitochondria, immune, DNA damage, telomeres, stem cell activation
- Immune modulation – longevity, autoimmune
- Skincare products
- Sports performance, MSK injury
- Body building – muscle health
- Hair loss therapy
- Treatment of sexual dysfunction
- Weight loss and Diabetes blood sugar control
- GUT/BRAIN support
- Biohacking

TARGET SPECIFIC PEPTIDES LONGEVITY AND CELL SENESCENCE

- **DDR - DNA DAMAGE/TELOMERES/STEM CELL HEALTH** – GHK, GHKCu
- **IMMUNE SYSTEM HEALTH, PINEAL, THYMUS –** SECRETOGOGUES, THYMULIN, TA1,BPC 157
- **LATENT VIRAL THERAPY** – BPC 157, TA1,LL 37
- **MITOCHONDRIA** – SS 31, MOTSc HUMANIN
- GROWTH HORMONE SECRETOGOGUES – SEMORELIN, IPAMORELIN
- **TELOMERE LENGTH/PINEAL** – EPITALON- ORAL, SQ
- **SENOLYTIC** – FOXO 4 DRI

TARGET SPECIFIC PEPTIDES MAXIMIZE STEM CELL HEALTH

- MOTSc - SS 31 - HUMANIN

- BPC 157
- TA1 THYMOSIN ALPHA ONE
- GHK, GHKCu
- EPITALON

TARGET SPECIFIC PEPTIDES MINIMIZE SENESCENT CELLS – SENOLYTICS/SENOMORPHICS

- **FOXO 4 – DRI**
- **SS 31**

Mitochondrial Peptides → Mitochondria → Stem Cell Fate

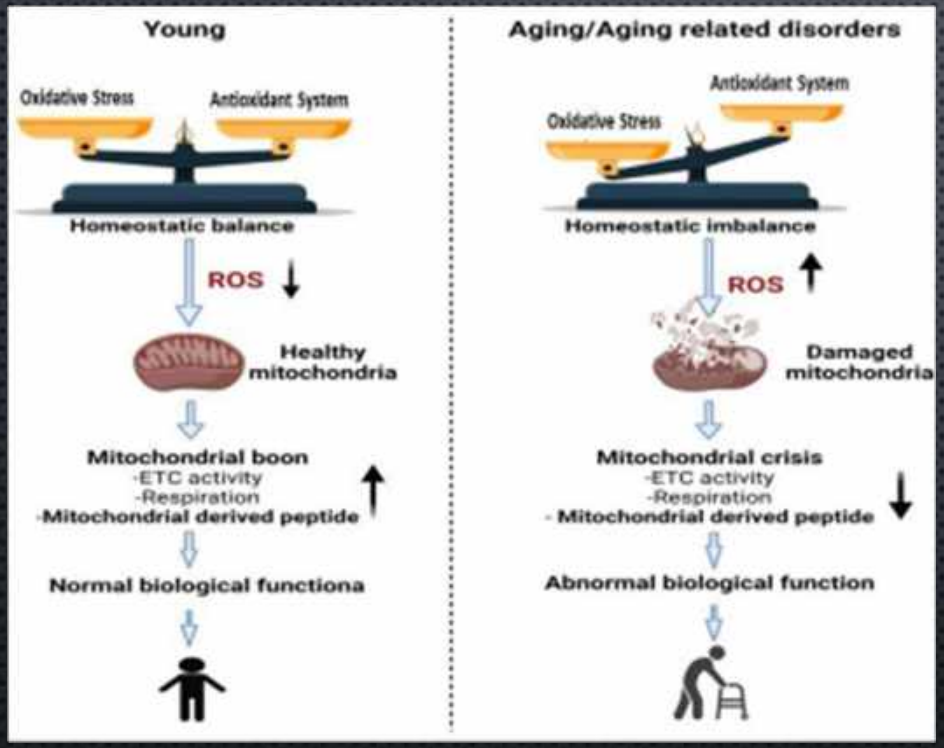
Humanin • MOTS-c • SS-31

PURPOSE OF MITOCHONDRIAL SECTION

- Unified mechanistic model of three mitochondrial peptides:
  - Humanin: anti-apoptotic, STAT3, Bax/Bid blockade
  - MOTS-c: AMPK activation, metabolic adaptation
  - SS-31: cardiolipin binding, ETC stabilization – (electron transport) ATP production

MITOCHONDRIAL PEPTIDES HUMANIN, MOTS-C AND SS31

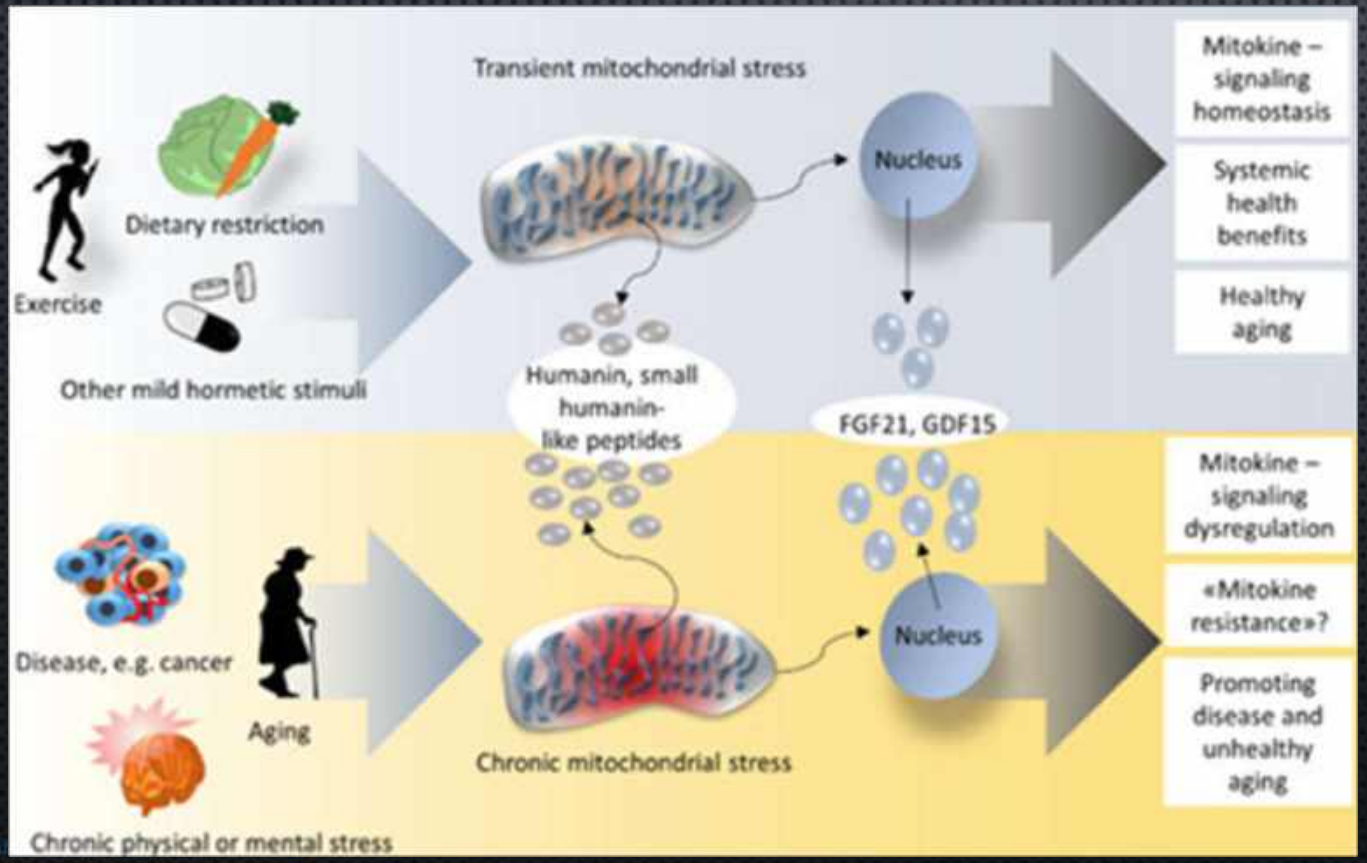
- **Short small bio active peptides** encoded by short opening reading frames (sORF) in mitochondrial DNA
- 8 MDPs currently identified
- **metabolic signal transducers** communicating mitochondrial status with the cell and distal tissues
  - **Modulating adaptive responses to metabolic stress**
  - **Cytoprotective and metaboprotective properties**
- Obesity, diabetes and aging are associated with lower circulating MDPs
- They all can enhance insulin sensitivity and important protection against age associated metabolic disorders



MITOCHONDRIAL PEPTIDES AND AGING

- ROS INCREASES THROUGHOUT LIFE IF NOT MANAGED EFFECTIVELY
- BY AGE 70 MITOCHONDRIA AND MITOCHONDRIAL PEPTIDE LEVELS ARE REDUCED BY 70% ON AVG

MITOCHONDRIAL PEPTIDES AND AGING



- HORMETIC EFFECT ON MITOCHONDRIA
- MITOKINES EXPRESSED
  - HUMANIN
  - SS 31
  - MOTSC
  - FGF21
  - GDF15

HUMANIN – CORE ACTIONS

- Blocks Bax/Bid-mediated apoptosis
  - Activates STAT3 via CNTFR/gp130/WSX-1
  - Reduces ROS
  - Supports ESCs (Embryonic stem cell) and germline progenitors under stress

MOTS-C – CORE ACTIONS

- Activates AMPK and metabolic stress-response pathways
  - Enhances mitochondrial resilience
  - Improves fatty acid oxidation
  - Supports metabolic microenvironment for stem cells

SS-31 – CORE ACTIONS

- Binds cardiolipin and stabilizes cristae
  - Reduces proton leak and increases ATP
  - Lowers ROS and NOS nitrosative stress
  - Rejuvenates aged MSCs and boosts osteogenesis

MITOCHONDRIAL CONVERGENCE HUB HUMANIN SS 31 MOTSC

- Shared mechanisms:
  - Cristae stability (SS-31 strongest)
  - ATP restoration and metabolic flexibility
  - ROS reduction and apoptosis blockade
  - Enhanced mitophagy/autophagy

SUPPORTS NORMAL STEM CELL OUTCOMES

- IMPROVED SURVIVAL AND STRESS RESISTANCE
  - REDUCED SENESCENCE (↓ P16/P21)
  - ENHANCED PROLIFERATION AND MIGRATION
  - IMPROVED DIFFERENTIATION AND EV (EXTRACELLULAR VESICLES) MITOCHONDRIAL TRANSFER

ESC & GERMLINE PROGENITOR DATA HUMANIN

- HUMANIN/HNG PROTECT ESCS FROM UV/OXIDATIVE DAMAGE
  - ↓ CASPASE-3
  - GERM CELLS PROTECTED FROM CHEMO-INDUCED APOPTOSIS
  - STAT3 PATHWAY DOMINANT FOR SURVIVAL

MSC OUTCOMES (SS-31 FOCUS)

- Reverses aged MSC mitochondrial dysfunction
  - ↓ ROS, ↓ NOS2, ↑ ATP
  - ↑ Osteogenic differentiation
  - Improved survival in diabetic/inflammatory niches

MOTS-C AND STEM CELLS – INDIRECT SUPPORT

- AMPK → PGC-1A → MITOCHONDRIAL BIOGENESIS
  - IMPROVES STEM-CELL NICHE ENERGETICS
  - REDUCES CHRONIC METABOLIC STRESS ON PROGENITORS
  - POTENTIAL SYNERGY WITH SS-31

INTEGRATED MECHANISM ARCHITECTURE

- THREE PEPTIDE LANES (HUMANIN, MOTS-C, SS-31)
  - ALL CONVERGE AT MITOCHONDRIAL FUNCTIONAL HUB
  - OUTPUTS DIVERGE INTO:
  - NORMAL STEM-CELL ENHANCEMENT
  - CANCER STEM-CELL RISKS (HUMANIN)

CANCER STEM CELLS – HUMANIN RISK

- Integrin aV binding → cytoskeletal remodeling
  - TGF-b release → migration & invasion ↑
  - Potential support of malignant stem-like cells
  - Requires caution in oncology

SYNERGY BETWEEN MITOCHONDRIAL PEPTIDES

- SS-31 IMPROVES MITOCHONDRIAL HARDWARE
  - MOTS-C OPTIMIZES METABOLIC PROGRAMMING
  - HUMANIN PROTECTS APOPTOSIS-SENSITIVE PROGENITORS
  - THREE TOGETHER → POTENTIALLY MAXIMAL STEM-CELL RESILIENCE

SUMMARY – KEY TAKEAWAYS

- All three peptides enhance mitochondrial health
  - SS-31 → structural & energetic optimization
  - MOTS-c → metabolic stress response
  - Humanin → apoptosis blockade
  - Net effect: stronger, more resilient stem-cell systems

HUMANIN/MOTS-C/ SS-31COMPARISON

•COMPARATIVE TABLE: HUMANIN VS MOTS-C VS SS-31 (STEM-CELL SUPPORT FOCUS)

Feature / Axis	Humanin (HN/HNG)	MOTS-c	SS-31 (Elamipretide)
Origin / Type	Mitochondria-derived peptide (MDP), encoded in MT-RNR2 (16S rRNA)	Mitochondria-derived peptide, encoded in MT-RNR1 (12S rRNA)	Synthetic mitochondria-targeted tetrapeptide (D-Arg–Dmt–Lys–Phe-NH_2)
Primary Targets	Bax/Bid, IGFBP-3 (intracellular); CNTFRa/gp130/WSX-1, FPRL1/2, integrins (cell surface)	AMPK activation, folate cycle, one-carbon metabolism; nuclear gene expression shift toward stress resistance	Cardiolipin on inner mitochondrial membrane; ANT (ADP/ATP translocator); ETC complexes
Core Mitochondrial Effect	Blocks mitochondrial apoptosis (Bax/Bid → cytochrome c), ↓ ROS, preserves ΔΨm	Increases fatty acid oxidation, improves metabolic flexibility and mitochondrial stress resilience via AMPK and ROS hormesis	Stabilizes cristae and ETC supercomplexes, ↑ ATP, ↓ proton leak, ↓ mtROS, protects cardiolipin
Key Signaling Pathways	JAK2/STAT3, ERK, PI3K/Akt, modulation of IGF-1/IGFBP-3 axis	AMPK → downstream PGC-1a, SIRT pathways; metabolic stress signaling	Primarily biophysical/ETC; secondarily reduces ROS-driven NF-κB/TGF-β and improves autophagy/mitophagy
ESC Support	Direct data: HNG protects ESCs from UV/oxidative stress, ↓ caspase-3, preserves colonies	Limited direct ESC data (most work is myocytes, metabolic tissues); conceptual support via stress-resilience	Not much ESC-specific data; main data are organoids and differentiated tissues
Germline Stem / Germ Cell Support	Strong: protects testicular germ cells from chemo/toxicants, preserves spermatogonial/progenitor pool via STAT3 & mitochondrial protection	No clear direct germline stem data; systemic metabolic benefits might be supportive	No specific germline data; benefits inferred from mitochondrial protection in other tissues
Adult MSC Support	Direct MSC data limited; mechanisms (anti-apoptotic, ROS control) are stem-cell–friendly but mostly inferred	Growing interest; data on MOTS-c improving muscle satellite cell function and metabolic milieu, but MSC-specific work is still emerging	Direct: rejuvenates aged BM-MSCs (↑ ATP, ↓ ROS, ↓ NOS2, ↑ osteogenic differentiation); scaffold studies show better BMSC recruitment and bone regeneration
Neural / Progenitor Context	Strong neuroprotection; likely protective for neural progenitors, but limited direct NSC data; caution in brain tumors	Neuroprotective in some models; also improves systemic metabolism which indirectly supports NSC niches	Evidence largely in cardiomyocytes, renal cells, organoids; NSC data limited but mechanisms are favorable
Stem Cell Fate Effects (Net)	**Preservation** of stem/progenitor pools under stress (ESCs, germ cells); less apoptosis, possibly less senescence. No strong evidence of expansion/mobilization.	**Stress-adaptive metabolic reprogramming**: likely improves stem-cell function indirectly (better metabolic environment, AMPK signaling). Some early data for progenitor resilience.	**Functional rejuvenation** of aged MSCs and better differentiation (esp. osteogenic); improved survival and function of resident and transplanted stem/progenitors in hostile environments
Systemic Aging / Longevity Link	MDP family member; levels decline with age; linked to healthspan and GH/IGF-1 axis modulation	Strongly framed as an "exercise-mimetic" MDP; enhances metabolic fitness and stress resistance, relevant to stem-cell aging	Approved for mitochondrial cardiomyopathy; preclinical work suggests mitigation of age-related mitochondrial dysfunction, indirectly preserving stem-cell niches
Cancer / Cancer Stem Cell Risk	Significant concern: can support tumor survival; in glioblastoma stem cells, humanin via integrins/TGF-β increases invasiveness	More nuanced; can be anti-tumor in some metabolic contexts but long-term effects on cancer stem cells not fully characterized	Mostly protective in ischemia/degeneration; some concern that strong mitochondrial rescue could aid malignant cells, but data so far are less alarming than with humanin
Best-Defined Stem-Cell Role (Today)	**Bodyguard for ESCs and germline stem/progenitors**under stress; powerful pro-survival signal that can be dangerous in cancer	**Metabolic trainer** that likely improves stem-cell fitness via AMPK and systemic metabolic health	**Mitochondrial rejuvenator** for aged or stressed MSCs and niche environments, with concrete data on differentiation and tissue regeneration

HUMANIN – REGULATOR OF HEALTHSPAN AND LIFESPAN

- HN is a 24-amino acid cell-permeable antioxidant polypeptide encoded by the mitochondrial 16s rRNA gene
- Expressed in heart, brain, liver, colon, skeletal muscle, kidneys, testes, and vascular wall.
- humanin levels generally decline with age,
- Levels are surprisingly stable in the naked mole-rat, a model of negligible senescence.
- Children of centenarians levels are much greater than age-matched control subjects.
- Levels are decreased in human diseases esp Alzheimer's disease

Aging (Albany NY). 2020 Jun 30; 12(12): 11185–11199. Published online 2020 Jun 23. doi: The mitochondrial derived peptide humanin is a regulator of lifespan and healthspan Kelvin Yen,corresponding author1

Biology (Basel). 2023 Apr; 12(4): 558. Humanin and Its Pathophysiological Roles in Aging: A Systematic Review Donatella Coradduzza,

Human Aging and Longevity Are Characterized by High Levels of MitokinesMaria Conte, PhD,1Journals of Gerontology: Biological Sciences J Gerontol A Biol Sci Med Sci, 2018, Vol. XX, No. XX, 1–8
doi:10.1093/gerona/gly153 Advance Access publication June 27, 2018

HUMANIN – REGULATOR OF HEALTHSPAN AND LIFESPAN POTENTIAL CLINICAL APPLICATIONS

- ALZHEIMERS
- HEART DISEASE
- DIABETES
- CHRONIC FATIGUE SYNDROME
- LONGEVITY

Aging (Albany NY). 2020 Jun 30; 12(12): 11185–11199. Published online 2020 Jun 23. doi: The mitochondrial derived peptide humanin is a regulator of lifespan and healthspan Kelvin Yen,corresponding author

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HUMANIN – REGULATOR OF HEALTHSPAN AND LIFESPAN

- AUTOPHAGY REGULATOR
- Inhibits apoptosis that promotes cell survival – BAX blocker
- HN exerts anti-apoptotic effects binds to cell surface receptors, upregulate the phosphoinositide 3-kinase/AKT signaling pathway, mediator of cell survival and growth.

Aging (Albany NY). 2020 Jun 30; 12(12): 11185–11199. Published online 2020 Jun 23. doi: The mitochondrial derived peptide humanin is a regulator of lifespan and healthspan Kelvin Yen,corresponding author1

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HUMANIN – REGULATOR OF HEALTHSPAN AND LIFESPAN

- ENHANCE MITOCHONDRIAL RESPIRATION AND BIOGENESIS,
- SUPPRESS CALCIUM OVERLOAD, INHIBIT THE JUN N-TERMINAL KINASE AND P38 MITOGEN-ACTIVATED PROTEIN KINASE PATHWAYS
- REDUCE THE GENERATION OF MITOCHONDRIAL ROS, PROTECT MITOCHONDRIAL STRUCTURE, AND ALLEVIATE MITOCHONDRIAL DYSFUNCTION.
- SCAVENGE MITOCHONDRIAL ROS

Aging (Albany NY). 2020 Jun 30; 12(12): 11185–11199. Published online 2020 Jun 23. doi: The mitochondrial derived peptide humanin is a regulator of lifespan and healthspan Kelvin Yen,corresponding author1

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HUMANIN – ROLES IN AGING – HN IS A SENOLYTIC REGULATING AUTOPHAGY

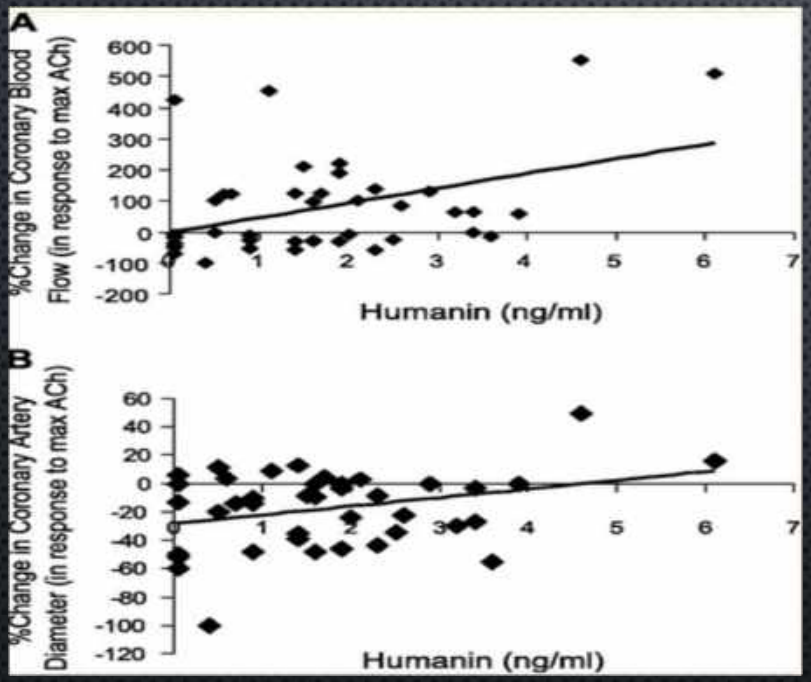
Neuroprotective effect of Humanin, N/A is not applicable.

Article	Study Design	Population	Outcome Measures
Zárate, S.C. et al. (2019) [50]	In vivo study	Rat	Neuroprotective effect of humanin and relationship with ovarian hormones
Yen, K. et al. (2018) [38]	In vitro and in vivo study	SH-SY5Y cells, Mouse	Neuroprotective effect of humanin
Yen, K. et al. (2020) [40]	In vivo study	*C. elegans*, Mouse, Human	Circulating levels of humanin and their relation to diseases of aging and lifespan
Gong, Z. et al. (2022) [49]	Review	Mouse, Porcine	Protective effect of humanin in myocardial ischemia-reperfusion.
Kim, S.J. et al. (2021) [37]	Review	N/A	Humanin in age-related disease
Gong, Z. et al. (2014) [51]	Review	N/A	Role of humanin in age-related disease
Caso, V.M. et al. (2021) [42]	In vitro study	N/A	Neuroprotective effects of humanin and its homologs (HNG) from Aβ
Zacharias, D.G. et al. (2012) [43]	In vivo study	Mouse	Humanin reduced plaque accumulation in Alzheimer's disease and has a cytoprotective action in stroke
Park, T. et al. (2013) [55]	In vivo study	Middle-aged APPswe/PS1dE9 mice	Treatment with HNG significantly improves spatial learning and memory deficits, reduces Aβ plaque accumulation and insoluble Aβ concentration; decreases neuro-inflammatory responses

- NEUROPROTECTIVE
- IMPROVED INSULIN SENSITIVITY –
- PROTECTION AGAINST CYTOKINE-INDUCED APOPTOSIS
- MITOCHONDRIAL HEALTH
- OBESITY LINKED TO LOWER LEVELS OF HN
- CV – REDUCES CARDIAC FIBROSIS, LINKED TO LOWER RISK OF CHF, REDUCED CARDIAC INFARCT SIZE (MICE)

Biology (Basel). 2023 Apr; 12(4): 558.Published online 2023 Apr 6. doi: 10.3390/biology12040558
Humanin and Its Pathophysiological Roles in Aging: A Systematic Review

HUMANIN – ENDOTHELIAL FUNCTION



- REDUCE THE GENERATION OF MITOCHONDRIAL ROS, PROTECT MITOCHONDRIAL STRUCTURE, AND ALLEVIATE MITOCHONDRIAL DYSFUNCTION.
- SCAVENGE MITOCHONDRIAL ROS
- HUMAN PLASMA HUMANIN LEVELS, SAMPLES WERE COLLECTED FROM 40 VOLUNTEERS UNDERGOING ANGIOGRAPHY.
- CIRCULATING ENDOGENOUS HUMANIN LEVELS ARE ASSOCIATED WITH PRESERVED CORONARY ENDOTHELIAL FUNCTION.

Am J Physiol Heart Circ Physiol. 2013 Feb 1; 304(3): H393–H397. Circulating humanin levels are associated with preserved coronary endothelial function R. J. Widmer

HUMANIN - DOSING

- 2 TO 5 MG 2 TO 3 TIMES A WEEK

Reduced skeletal muscle expression of mitochondrial-derived peptides humanin and MOTS-C and Nrf2 in chronic kidney disease Chang Liu,Eva-Karin Gidlund,Anna Witasp,Abdul Rashid Qureshi,Magnus Söderberg 16 OCT 2019https://doi.org/10.1152/ajprenal.00202.2019

SS-31 → MITOCHONDRIA → STEM CELL FATE

MECHANISM PATHWAY • PARADIGM LONGEVITY 365

SS-31 OVERVIEW

- Tetrapeptide: D-Arg–Dmt–Lys–Phe-NH_2
  - Selectively targets mitochondria
  - Binds cardiolipin on inner mitochondrial membrane
  - First FDA-approved mitochondria-targeted therapeutic

WHY MITOCHONDRIA DICTATE STEM CELL FATE

- STEM CELLS REQUIRE TIGHT METABOLIC CONTROL
  - LOW ROS + INTACT CRISTAE = SELF-RENEWAL
  - AGING STEM CELLS DEVELOP MITOCHONDRIAL DYSFUNCTION

SS-31: CARDIOLIPIN BINDING

- ANCHORS TO CARDIOLIPIN ON IMM (INNER MITO MEMEBRANE)
  - RESTORES CRISTAE CURVATURE
  - REORGANIZES ETC SUPERCOMPLEXES

ETC EFFICIENCY RESTORATION ELECTRON TRANSPORT

- ↑ Complex I & IV activity
  - ↓ Electron leakage / ROS
  - Restores mitochondrial membrane potential

ATP RESTORATION & PROTON LEAK REDUCTION

- TIGHTENS IMM STRUCTURE
  - ↓ PROTON LEAK
  - ↑ ATP GENERATION FOR STEM-CELL ACTIVATION

ANT NORMALIZATION ADP ATP TRANSLOCASE

- Enhances ADP/ATP translocator function
  - Improves ATP turnover
  - Supports metabolic flexibility

SS-31 OXIDATIVE/NITROSATIVE STRESS SUPPRESSION

- ↓ ROS / LIPID PEROXIDATION
  - ↓ iNOS / NOS2
  - PROTECTS MITOCHONDRIAL DNA AND STEM-CELL VIABILITY

MPTP STABILIZATION & ANTI-APOPTOTIC EFFECTS

- Prevents cytochrome-c peroxidation
  - Blocks mPTP opening
  - Improves stem-cell survival under stress

STEM-CELL METABOLIC REPROGRAMMING

- RESTORES GLYCOLYSIS ↔ OXPHOS SWITCHING
  - SUPPORTS ACTIVATION AND DIFFERENTIATION
  - COUNTERACTS METABOLIC AGING

SS 31 ANTI-SENESCENCE EFFECTS

- ↓ p16 / p21
  - Enhances mitophagy/autophagy
  - Rejuvenates stem-cell phenotype

ENHANCED STEM-CELL FUNCTIONS

- ↑ SELF-RENEWAL
  - ↑ PROLIFERATION
  - ↑ MIGRATION/HOMING
  - ↑ DIFFERENTIATION CAPACITY
  - ↓ SENESCENCE

SECRETOME / EV UPGRADING

- SS-31 increases MEV (mitochondria-rich EV) output
  - Improves mitochondrial transfer to damaged cells
  - Enhances systemic regeneration

INTEGRATED MECHANISM PATHWAY

- SS-31 → CARDIOLIPIN → CRISTAE → ETC → ATP
  - ↓ ROS → ↑ SURVIVAL → OPTIMAL STEM-CELL FATE

CLINICAL TRANSLATION CONSIDERATIONS

- SS-31: HUMAN APPROVAL FOR MITOCHONDRIAL CARDIOMYOPATHY
  - MOTS-C: NO CLINICAL APPROVAL YET
  - HUMANIN: EXPERIMENTAL ONLY
  - ONCOLOGY CAUTION WITH HUMANIN

CLINICAL IMPLICATIONS

- STEM CELL PRE-CONDITIONING
  - IN VIVO MITOCHONDRIAL REJUVENATION
  - SYNERGY: MOTS-C, BPC-157, GHK-CU, FOXO4-DRI
  - APPLICATIONS: METABOLIC, INFLAMMATORY, AGING TISSUES

SS 31 DOSING

- 50MG/ML – 5 ML
- .2 ML SQ DAILY
- CYCLE 1 MONTH ON AND ONE MONTH OFF (WHEN USED STAND ALONE THERAPY)

MOTS-C: RESEARCH, MECHANISMS, BENEFITS

MITOCHONDRIAL-DERIVED PEPTIDE • LONGEVITY • METABOLISM • EXERCISE BIOLOGY

MOTS-C OVERVIEW

- *Mitochondrial Open Reading Frame of the 12S rRNA type-c*
- **Length:** 16 amino acids
- **Origin:** Encoded by mitochondrial DNA (mtDNA, 12S rRNA region)
- **Function:** Acts as a *mitochondrial-derived peptide* that regulates metabolism, stress responses, and cellular resilience via the **AMPK, NRF2, and FOXO pathways**.

MOTSC – STEM CELL SUPPORT MECHANISMS

Mechanism	Pathways	Stem-Cell Benefit
Mitochondrial optimization	AMPK ↑ PGC-1a ↑	Improves bioenergetics & viability
Antioxidant defense	NRF2 ↑ SOD2 ↑ HO-1 ↑	Protects from ROS, senescence
Longevity signaling	FOXO3a ↑ SIRT1 ↑	Maintains quiescence, DNA repair
Anti-inflammatory	NF-κB ↓ IL-6 ↓ TNF-a ↓	Preserves niche & HSC pool
Differentiation cues	Runx2 ↑ mTOR mod	Osteo/myogenic maturation
Mobilization & repair	VEGF ↑ HIF-1a ↑	Improved vascular & tissue regeneration

MOTSC

MOTS-c supports stem-cell health primarily by:

- **Restoring mitochondrial function**
- **Activating AMPK/NRF2/FOXO3** pathways
- **Reducing oxidative and inflammatory stress**
- **Enhancing differentiation and regeneration potential**

It acts as a **master mitochondrial stress peptide**, linking energy metabolism with stem-cell maintenance and tissue repair — making it one of the most promising mitochondrial peptides for **longevity and regenerative medicine**.

MOTS-C DOSING

- 5 TO 10 MG SQ 2 TO 3 TIMES A WEEK

What BPC-157 Is

- **BPC-157** = a 15–amino acid fragment (Gly-Glu-Pro-Pro-Pro-Gly-Lys-Pro-Ala-Asp-Asp-Ala-Gly-Leu-Val) of the larger **Body Protection Compound (BPC)** found in gastric juice.

- Orally and parenterally active in animal models; very high safety margins reported in preclinical work.

- Not FDA-approved; all clinical use is off-label / experimental.

These are exactly the pathways you care about for **stem cell homing, survival, and niche repair**.

Core Mechanistic Themes

Across hundreds of animal studies, BPC-157 consistently shows:

1. **Angiogenic and microvascular repair**
   - Upregulates **VEGF** signaling, improves endothelial integrity, and accelerates capillary regeneration in tendon, ligament, muscle, GI and skin injury models.
   - Restores microcirculation after ischemia/reperfusion, crush, or transection injuries.
2. **Cytoprotection + anti-inflammatory effects**
   - Protects gastric mucosa, liver, pancreas, brain, and heart from toxins and ischemia.
   - Downregulates **pro-inflammatory cytokines** (TNF-α, IL-6) and COX-2 in many models.
3. **Modulation of NO / eNOS**
   - Normalizes nitric oxide signaling (up in deficiency, down in excess), improving endothelial function and wound bed perfusion.
4. **Activation of growth- and repair-related pathways**
   - In vitro and in vivo evidence of upregulation of:
     - **FAK–paxillin** (focal adhesion and cell migration),
     - **AKT–mTOR**,
     - **ERK1/2**,
     - **VEGF receptors**,
     - and modulation of **EGFR** and related repair cascades.

BPC 157

BPC-157 and Stem Cells / Progenitors

D**irect BPC-157 stem-cell activation data are limited**, but there is good *indirect* support across multiple tissues.

3.1 Mesenchymal and tendon/ligament progenitors

•BPC-157 accelerates healing

Interpretation: BPC-157 likely enhances **local progenitor proliferation and differentiation** (tenocytes, fibroblasts, resident MSC-like cells) by:

•Improving microvascular supply

•Activating **FAK/AKT/ERK** signaling

•Reducing inflammatory inhibition in the niche

BPC 157

3.3 Muscle regeneration and myogenic progenitors

•In skeletal muscle trauma, crush, or transection, BPC-157 speeds regeneration with:

- •less fibrosis and necrosis
- •more centrally nucleated regenerating fibers
- •better functional recovery

•These changes depend on **satellite cell activation / myoblast proliferation + differentiation**, again suggesting **pro-regenerative niche remodeling** and possible direct effects on myogenic progenitors.

3.4 Neural and glial progenitors (indirect)

•In CNS injury (TBI, spinal cord, stroke), BPC-157 reduces neuronal loss, edema, and functional deficits, and improves microvasculature.

•There are hints of enhanced **neurogenesis and glial remodeling**, but direct neural stem cell proliferation data are sparse.

•Mechanisms likely involve **VEGF, eNOS/NO, ERK, AKT**, and reduced excitotoxicity/inflammation—conditions that support NSC survival and differentiation.

BPC 157

Mechanistic Links to Stem Cell Biology

You can comfortably say BPC-157 supports **stem cell–mediated repair** by acting on:

1.Niche vasculature

- Angiogenesis + endothelial protection → better oxygen and nutrient supply → improved stem cell survival and engraftment.

2.Inflammatory tone

- SASP-like and acute inflammatory cytokines drop → less hostile microenvironment for MSCs, HSCs, satellite cells, and neural progenitors.

3.FAK/AKT/ERK signaling

- These pathways are central to **stem cell adhesion, migration, cytoskeletal remodeling, survival, and proliferation**.
- BPC-157's activation of FAK–paxillin complexes is a mechanistic bridge from peptide to actual stem cell behavior.

4.NO/eNOS modulation

- Proper NO tone supports endothelial progenitor and MSC functions; BPC-157 normalizes this axis in both deficiency and excess.

So even though papers seldom say "BPC-157 increases MSC count," the **mechanistic environment** it creates is very clearly **pro-stem-cell**.

BPC 157 DOSING

250–500 µg once or twice daily (SC, PO, SL)

EPITALON (AEDG) RESEARCH DECK

MECHANISMS • IN VITRO • IN VIVO • HUMAN STUDIES

WHAT IS EPITALON?

- • Synthetic tetrapeptide: Ala–Glu–Asp–Gly (AEDG)
- • Derived from pineal peptide Epithalamin
- • Studied for telomere, endocrine, antioxidant and immune effects

MECHANISMS OF ACTION

- • Telomerase activation → telomere elongation
  - • Chromatin remodeling (heterochromatin decondensation)
  - • Pineal/endocrine axis normalization (melatonin/cortisol)
  - • Antioxidant enzyme activation (↑SOD, ↑GPx)
  - • Immune enhancement (↑IL-2)

IN VITRO FINDINGS

- • Human fibroblasts: telomere elongation + lifespan extension
  - • Lymphocytes: telomerase activation
  - • Skin fibroblasts: ↓MMP-9, ECM normalization
  - • Retinal cells: anti-oxidative and anti-fibrotic

ANIMAL RESEARCH

- • Rodents: ↑antioxidant enzymes, ↓DNA damage
  - • ↓ spontaneous tumors and metastasis
  - • Lifespan extension in several models

PRIMATE RESEARCH

- • Restored melatonin rhythm
  - • Normalized cortisol cycles
  - • Improved glucose tolerance

HUMAN STUDIES

- • Increased telomere length in leukocytes
  - • Improved nocturnal melatonin output
  - • Reduced long-term mortality (Epithalamin cohorts)
  - • Retinitis pigmentosa: 90% functional improvement

ONCOSTATIC EFFECTS

- • ↓ Spontaneous tumor rates in mice
  - • Mechanism: IL-2 enhancement + DNA protection
  - • Not directly cytotoxic

SAFETY & CAVEATS

- • Very well tolerated in long-term human use
  - • No major adverse events reported
  - • Few Western studies
  - • Telomerase activation theoretical risk

SUMMARY

- • Telomerase / telomere support
  - • Endocrine & circadian normalization
  - • Antioxidant & anti-fibrotic
  - • Immune rejuvenation
  - • Strong geroprotective signals

EPITALON DOSING

- 10 MG SQ DAILY FOR 5 DAYS

EPITALON REFERENCES

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•Khavinson, V.Kh., “Effect of epitalon on the lifespan increase in Drosophila.” (Exp Gerontol) 2000.

SENOLYTICS - MANAGING SENESCENT CELLS WITH SENOLYTICS

- FOXO 4 DRI
- NATURAL KILLER CELLS AND EXOSOMES
- STEM CELLS
- KLOTHO
- SS 31
- HUMANIN

FOXO 4-DRI

FOXO4-DRI (also called **Pro-Senolytic FOXO4-DRI**, **FOXO4-p53 interfering peptide**, or simply **DRI peptide**) is a **synthetic D-retro-inverso peptide** designed to disrupt the interaction between:

- **FOXO4 → a nuclear forkhead transcription factor enriched in senescent cells**
- **p53 → the major cell-cycle arrest / apoptosis mediator**

In normal senescence, FOXO4 prevents p53 from triggering apoptosis, forcing the cell into a **non-apoptotic senescent state**. FOXO4-DRI breaks this interaction, causing **p53 nuclear export → mitochondrial translocation → caspase-dependent apoptosis → selective clearance of senescent cells**.

It was originally developed by **Peter de Keizer (Cell, 2017)**.

FOXO 4-DRI

Mechanism of Action

FOXO4 in senescent cells

Senescent cells accumulate high FOXO4 expression due to:

- Oxidative stress
- DNA damage
- Telomere dysfunction
- Oncogene activation

FOXO4 binds directly to p53 in the nucleus and prevents:

- p53-mediated apoptosis
- Clearance of damaged cells
- Restoration of tissue homeostasis

FOXO 4-DRI

2.2 How the D-retro-inverso peptide works
The D-retro-inverso design:

- Uses **D-amino acids**
- Peptide sequence reversed

FOXO4-DRI competes with FOXO4 at the **FOXO4–p53 interface**, thereby:

1. **Releasing p53 from FOXO4**
2. p53 **relocates to mitochondria**
3. Activates **BAX, BAK, Cytochrome C release**
4. **Caspase-9 → caspase-3 activation**
5. **Apoptosis of senescent cells**

FOXO 4 DRI

Why FOXO4-DRI selectively kills senescent cells:

- Senescent cells have extremely high FOXO4 levels
- Non-senescent cells rely less on FOXO4-mediated p53 retention
- Thus FOXO4-DRI disrupts a **senescence-specific survival pathway**

This reduces **off-target apoptosis**.

FOXO 4-DRI

Proven Benefits (Animal Studies)

3.1 Tissue regeneration and reversal of frailty (mouse studies)
(CELL 2017; Aging Cell 2018)

FOXO4-DRI treatment produced:

- **Improved fur density (alopecia reversal)**
- **Improved physical function**
- **Increased kidney function**
- **Reversal of hyperinsulinemia**
- **Better wound healing**
- **Extended median lifespan in treated aged mice**

FOXO 4 - DRI

Stem cell niche restoration
Clearing senescent MSCs and fibroblasts:

- Restores **hematopoietic stem cell (HSC)** function
- Improves **muscle regeneration**
- Reduces inflammatory SASP in niches

Reversal of chemo-induced toxicity
In doxorubicin-damaged mice:

- FOXO4-DRI removed chemotherapy-induced senescent cells
- Prevented **frailty syndrome**
- Regenerated **thymus**, **liver**, and **kidney** structure

Effects on SASP (Senescence-Associated Secretory Phenotype)
FOXO4-DRI reduces the SASP phenotype:

- ↓ IL-6
- ↓ TNF-α
- ↓ IL-1β
- ↓ MMPs and metalloproteinases
- ↓ ROS
- ↓ NF-κB pathway activation

This leads to less systemic inflammation.

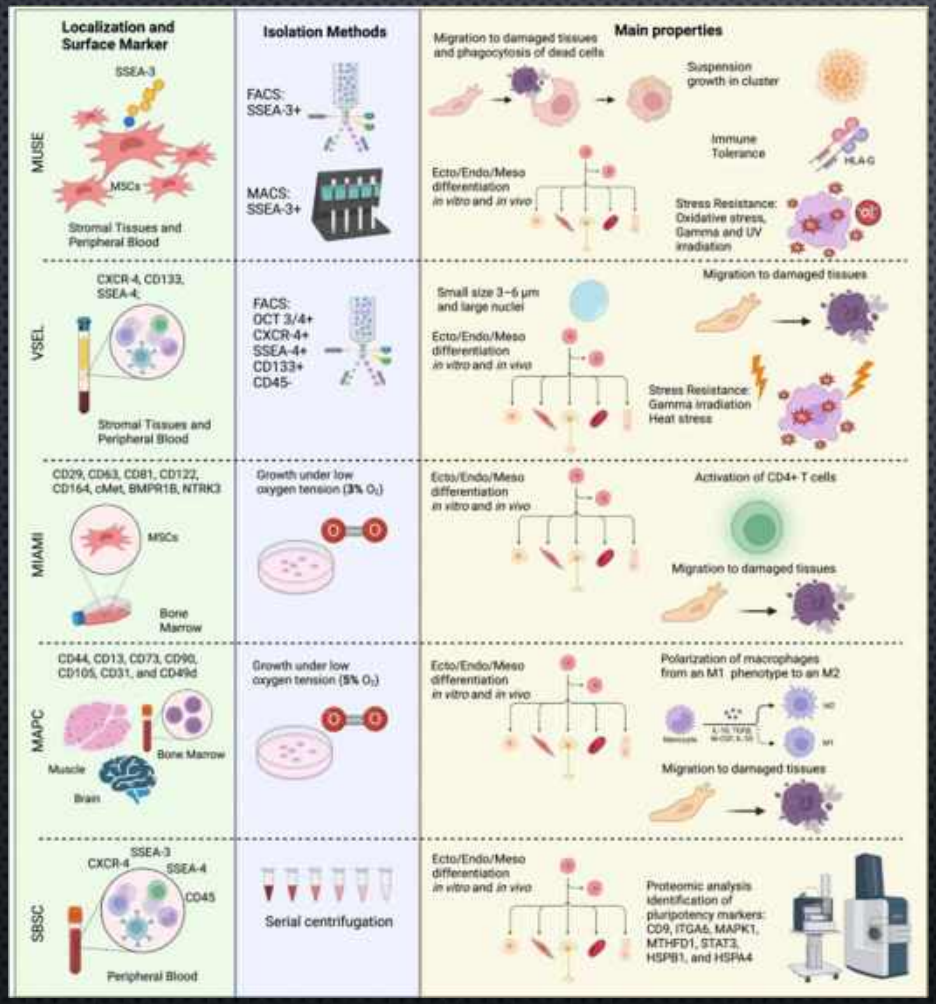
FOXO 4 DRI DOSING – EMPLOY FIRST IN SENOLYTIC PULSE PROGRAM

Human-Equivalent Dose (HED)
Using standard FDA mouse-to-human scaling:
Mouse 5 mg/kg → Human ~0.4–0.6 mg/kg
(depending on metabolic factor)
For a 70-kg human:
≈ 30–45 mg per administration
This is the **scientifically derived, weight-normalized translation**

Clinics & Experimental Longevity Programs Use (Off-Label)
Over the past 5 years, private research clinics & longevity physicians have converged on:
Typical Human Experimental Dose (Anecdotal Consensus)
- **10–30 mg per dose**
- **Subcutaneous (SQ) or IV**
- **1–2× weekly**
- **3–6 weeks per senolytic cycle**

NATURAL KILLER CELL EXOSOMES A NATURAL SENOLYTIC

- SPECIAL LINE OF NATURAL KILLER CELLS STIMULATED TO PRODUCE PORINS AND GRANZYME A AND B AND ADDITIONAL CYTOKINES NK CELLS KNOWN TO PRODUCE
- PROPERTIES INCLUDE
  - ANTI INFLAMMATORY
  - ANTI VIRAL
  - ANTI CANCER
  - SENOLYTIC

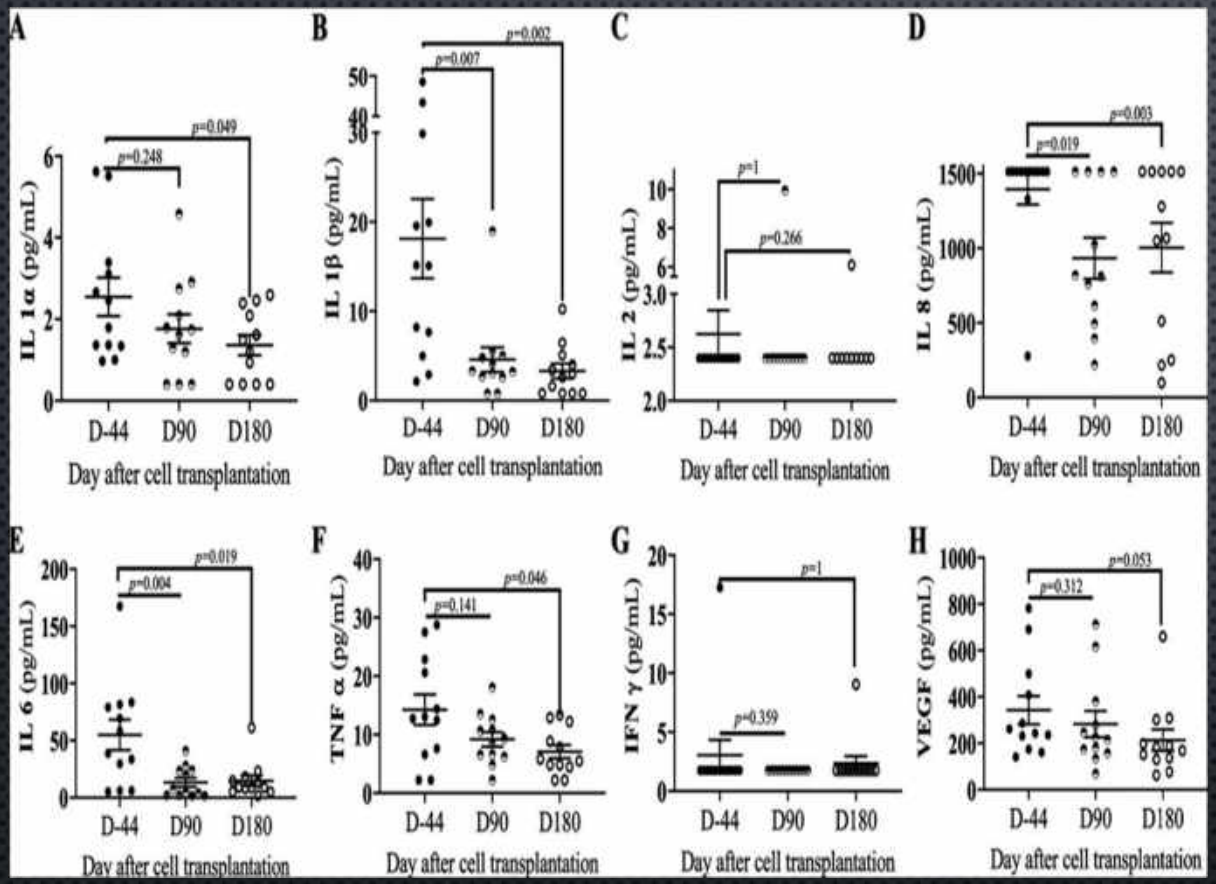


- TELOMERASE
- SENOLYTICS
- ANTI-INFLAMMATORY
- TISSUE REPAIR
- M1 TO M2 MACROPHAGES
- IMMUNE MODULATION TH1-TH2
- ACTIVATE CD4 CELLS
- EPIGENETIC AGE REVERSAL?

LONGEVITY BENEFITS TO STEM CELLS

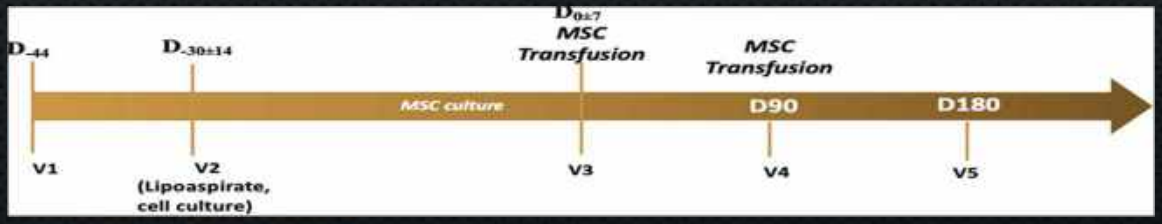
1.nature
Published: 20 July 2022
Ageing and rejuvenation of tissue stem cells and their niches
•Anne Brunet

STEM CELLS AND HEALTHY AGING Stem cells and healthy aging
MARGARET A. GOODELL AND THOMAS A. RANDO Authors Info & Affiliations
SCIENCE 4 Dec 2015 Vol 350, Issue 6265 pp. 1199-1204



- PHASE ONE OPEN LABEL
- 12 PARTICIPANTS AGES 40 TO 64
- DM, OBESITY, DYSLIPIDEMIA
- AUTOLOGOUS ADSC 100 MILLION
- INFUSION AT 30 AND 90 DAYS
- CYTOKINES AT 90 AND 180 DAYS

LONGEVITY BENEFITS - INFLAMMAGING TO STEM CELLS



Trials. 2024; 25: 309.
Published online 2024 May 8.
Safety and efficacy of autologous adipose tissue-derived stem cell transplantation in aging-related low-grade inflammation patients: a single-group, open-label, phase I clinical trial

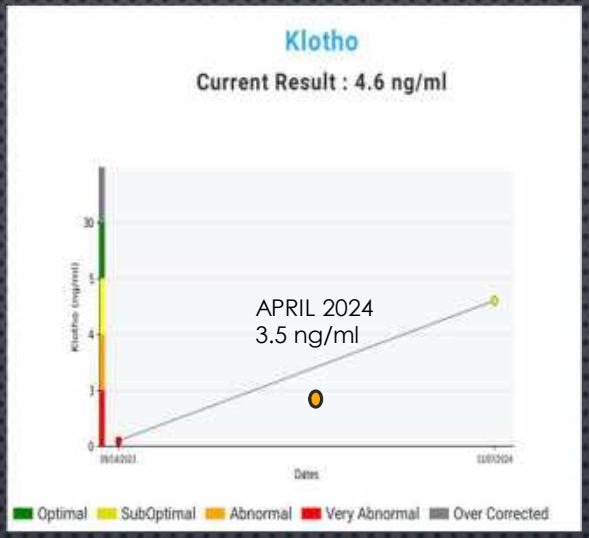
STEM CELL NATURAL THERAPY IV INFUSION SENOLYTIC PULSE/PEPTIDE STACK – PREP

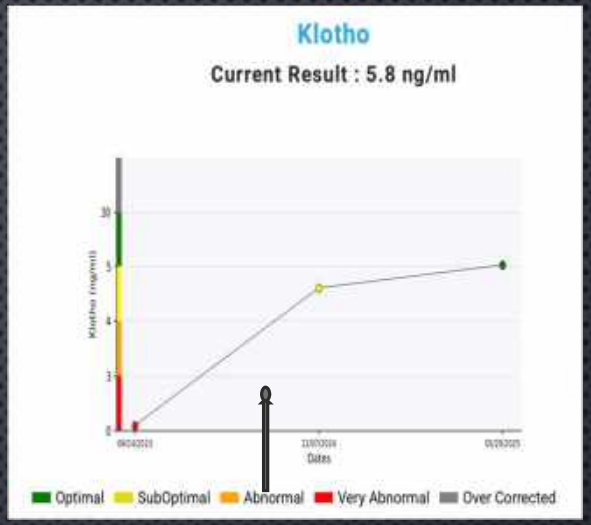
- STEP ONE: SENOLYTIC PULSE
  - FISETIN, NK CELL EXOSOMES, FOXO 4 DRI
- STEP TWO: MITOCHONDRIAL PULSE
  - HUMANIN, SS 31 MOTSC
  - BPC 157 – STEM CELL ACTIVATOR, ANTI- INFLAMMATORY,
  - GHKCU – STEM CELL ACTIVATOR, DNA REPAIR
  - EPITALON
  - TA1- IMMUNOMODULATOR
- STEP THREE: STEM CELL PULSE
  - NATURAL HEALING - STEM CELLS/BIOLOGICS
  - RPM: AUTOLOGOUS GROWTH FACTORS
  - OTHER: SEA BUCKTHORN, FUCOIDAN, NO ACTIVATOR, CURCUMIN, OMEGA THREES

CASE 1

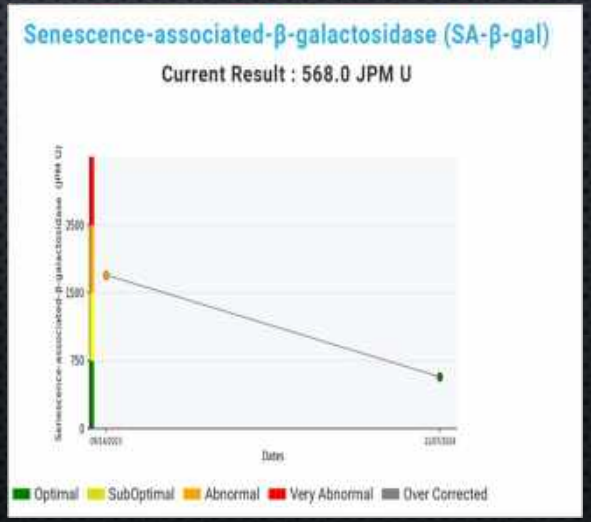
- 68 YO MALE
- HISTORY OF PROSTATE CANCER 2020 GLEASON 6 3 +3
- SUCESSFUL ROBOTIC SURGERY/FUNCTIONAL TX
- LONGEVITY PEPTIDE PROTOCOL
- KLOTHAMAX PROTOTYPE TESTING – CORDYCEPS, FISETIN, RHEIN, VIT D, CURCUMIN PRO, OTHERS
- PSA CONSISTENTLY 0.04
- 09/2023 KLOTHO 0 .1 NG/ML
- 11/24 KLOTHO 4.6 NG/ML
- 06/25 KLOTHO 5.8 NG/ML
- 09/23 SA-BETA GAL - 1750 JPM U
- 11/24 SA-BETA-GAL- 568 JPM U
- SASP MARKERS

- RECEIVED TWO ROUNDS OF LONGEVITY PULSE THERAPY





STEP ONE: SENOLYTIC PULSE
FISETIN, NK CELL EXOSOMES, FOXO 4 DRI
STEP TWO: MITOCHONDRIAL PULSE
HUMANIN, SS 31 MOTSC
BPC 157 – stem cell activator, anti-Inflammatory,
Ghkcu – stem cell activator, DNA repair
EPITALON
TA1- immunomodulator
STEP THREE: STEM CELL PULSE
NATURAL HEALING - STEM CELLS/BIOLOGICS
RPM: AUTOLOGOUS GROWTH FACTORS
OTHER: SEA BUCKTHORN, FUCOIDAN, NO activator, Curcumin, omega threes



CASE 1

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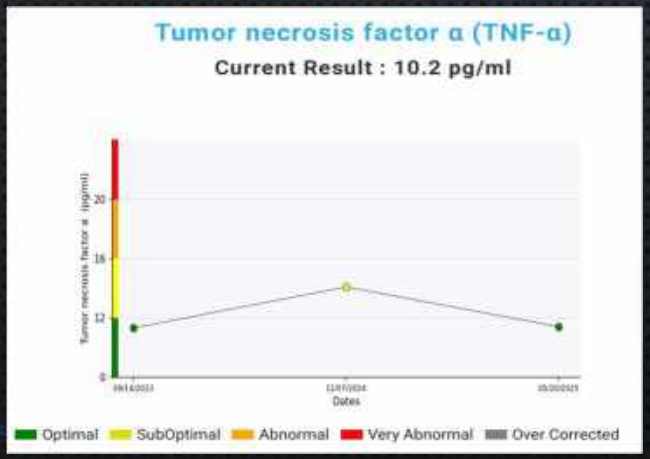
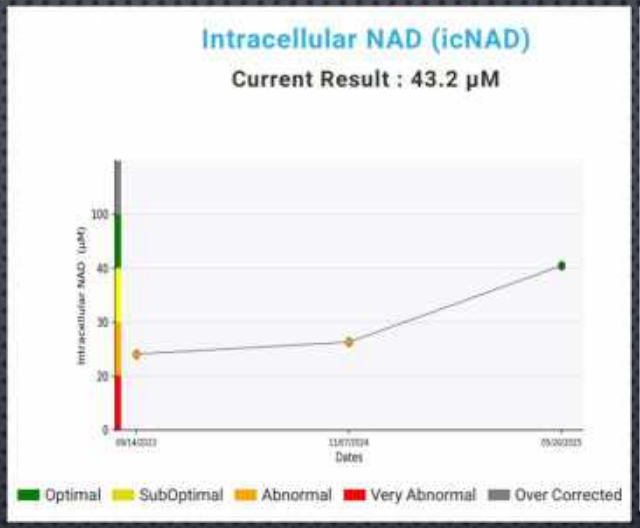
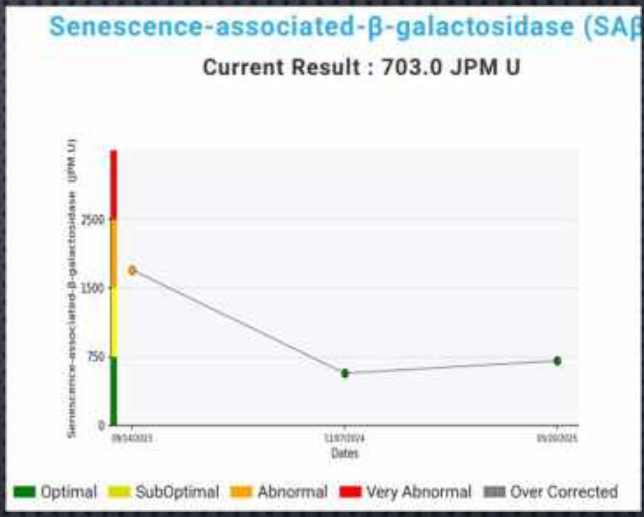
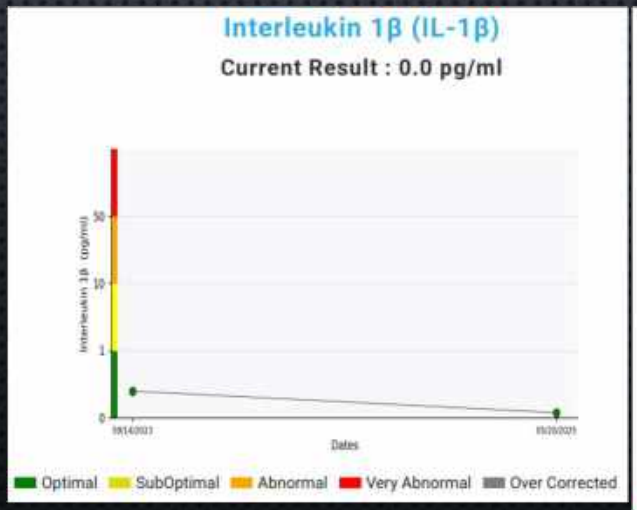
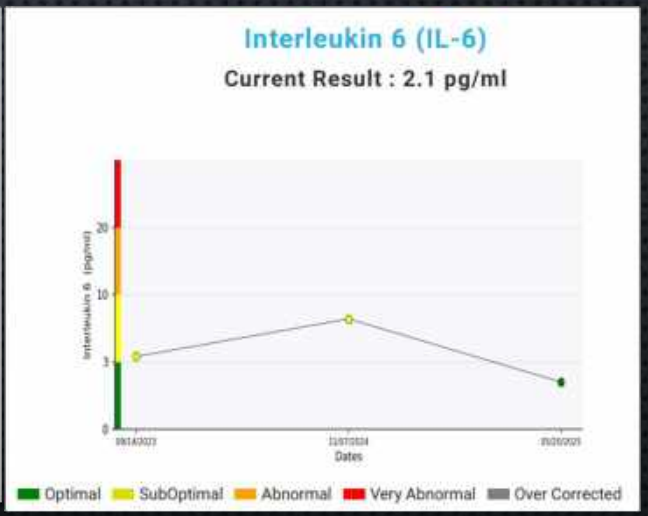
- SASP MARKERS









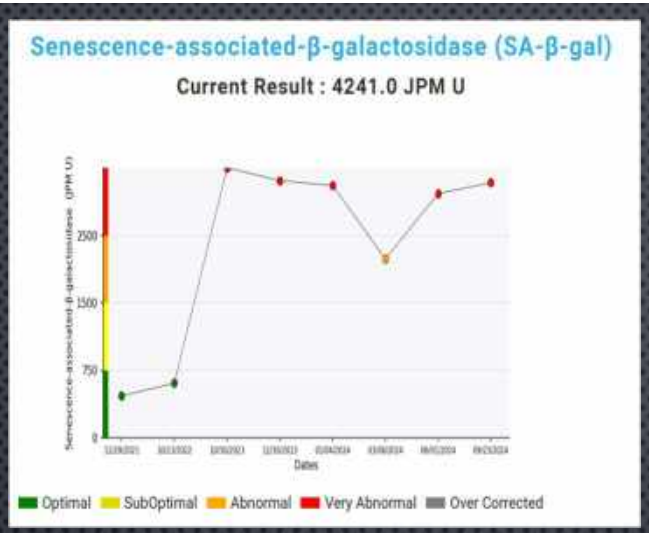


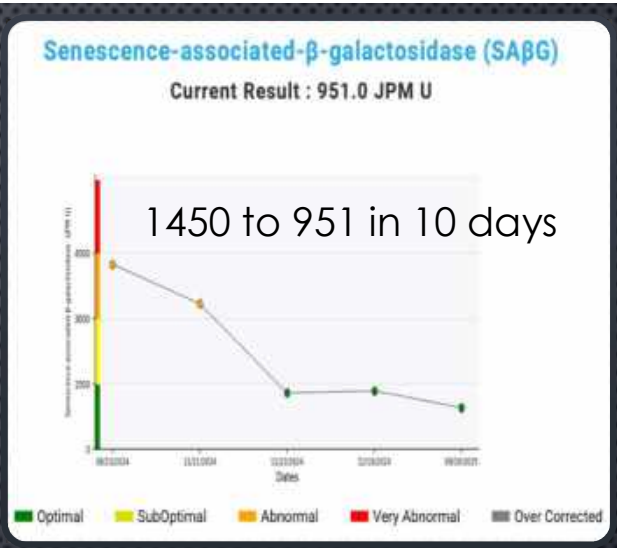
CASE TWO

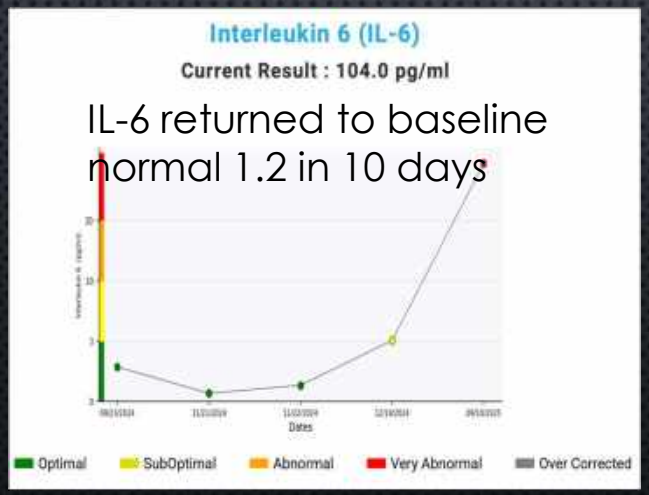
- 51 YEAR OLD MALE
- NO SIGNIFICANT PAST MEDICAL HISTORY
- SUCCESSFUL OVER ACHIEVER
- ATHLETIC BIOHACKER IN HIGH STRESS PROFESSION
- MEDS: SIROLIMUS 4 MG WEEKLY, KLOTHO SUPPORT, LONGEVITY PULSE PROTOCOL QUARTERLY, NMN

CASE TWO

- NK EXOSOMES FOR SENOLYTIC
- BETA GAL DECREASED FROM 1450 TO 951 IN 10 DAYS
- PREVIOUS BETA GAL IN 2022 WAS 4700 AND 4241 IN 2024







CASE TWO

- NK CELLS 572/UL GIVEN AS SENOLYTIC AND ANTIVIRAL
- EBC CHRONIC ACTIVATION
- EBV EARLY ANTIGEN AB 130
- DECREASED TO 100 IN 10 DAYS

T- and B-Lymphocyte/Nat Killer

	Test	Current Result and Flag		Previous Result and Date		Units	Reference Interval
	Abs.CD19+ Lymphs	110		59	08/08/2025	/uL	12-645
	% CD19+ Lymphs	13.7		5.4	08/08/2025	%	3.3-25.4
▼	**Absolute CD 3**	**434**	**Low**	**428**	08/08/2025	/uL	622-2402
▼	**% CD 3 Pos. Lymph.**	**54.2**	**Low**	**38.9**	08/08/2025	%	57.5-86.2
▼	**Absolute CD 4 Helper**	**353**	**Low**	**309**	08/08/2025	/uL	359-1519
	% CD 4 Pos. Lymph.	44.1		**28.1**	08/08/2025	%	30.8-58.5
▼	**Abs. CD 8 Suppressor**	**85**	**Low**	124	08/08/2025	/uL	109-897
▼	**% CD 8 Pos. Lymph.**	**10.6**	**Low**	**11.3**	08/08/2025	%	12.0-35.5
▲	**CD4/CD8 Ratio**	**4.16**	**High**	2.49	08/08/2025		0.92-3.72
	Ab NK (CD56/16)	251		**572**	08/08/2025	/uL	24-406
▲	**% NK (CD56/16)**	**31.4**	**High**	**52.0**	08/08/2025	%	1.4-19.4
	WBC	4.0		5.2	08/08/2025	x10E3/uL	3.4-10.8
	RBC	5.30		5.42	08/08/2025	x10E6/uL	4.14-5.80
	Hemoglobin	17.3		17.2	08/08/2025	g/dL	13.0-17.7
▲	**Hematocrit**	**51.2**	**High**	**52.3**	08/08/2025	%	37.5-51.0
	MCV	97		97	08/08/2025	fL	79-97
	MCH	32.6		31.7	08/08/2025	pg	26.6-33.0
	MCHC	33.8		32.9	08/08/2025	g/dL	31.5-35.7
	RDW	13.4		12.6	08/08/2025	%	11.6-15.4
	Platelets	223		224	08/08/2025	x10E3/uL	150-450
	Neutrophils	67		59	08/08/2025	%	Not Estab.
	Lymphs	21		21	08/08/2025	%	Not Estab.
	Monocytes	9		17	08/08/2025	%	Not Estab.
	Eos	2		2	08/08/2025	%	Not Estab.
	Basos	1		1	08/08/2025	%	Not Estab.
	Neutrophils (Absolute)	2.6		3.0	08/08/2025	x10E3/uL	1.4-7.0
	Lymphs (Absolute)	0.8		1.1	08/08/2025	x10E3/uL	0.7-3.1
	Monocytes(Absolute)	0.4		0.9	08/08/2025	x10E3/uL	0.1-0.9
	Eos (Absolute)	0.1		0.1	08/08/2025	x10E3/uL	0.0-0.4
	Baso (Absolute)	0.0		0.0	08/08/2025	x10E3/uL	0.0-0.2
	Immature Granulocytes	0		0	08/08/2025	%	Not Estab.
	Immature Grans (Abs)	0.0		0.0	08/08/2025	x10E3/uL	0.0-0.1

Comp. Metabolic Panel (14)

Andre Maurois

French author 1885

"OLD AGE IS FAR MORE THAN WHITE HAIR, WRINKLES, THE FEELING THAT IT IS TOO LATE AND THE GAME FINISHED, THAT THE STAGE BELONGS TO THE RISING GENERATIONS.

THE TRUE EVIL IS NOT THE WEAKENING OF THE BODY…BUT THE INDIFFERENCE OF THE SOUL."

JOSEPH CLEAVER MD
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- WEBSITE - WWW.BOUTIQUEWELLNESSNC.COM

THYMIC PEPTIDES REFERENCES

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full text links

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N S Lin'kova, V O Poliakova, I M Kvetnoĭ, A V Trofimov, N N Sevost'ianova

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Clinical Trial Neuro Endocrinol Lett 2003 Jun-Aug;24(3-4):233-40.
Peptides of pineal gland and thymus prolong human life
Vladimir Kh Khavinson 1 , Vyacheslav G Morozov

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Published online 2011 Nov 28. doi: 10.4061/2011/517137
Peptides Regulate Cortical Thymocytes Differentiation, Proliferation, and Apoptosis
V. Kh. Khavinson, 1, 2 , V. O. Polyakova, 1 N. S. Linkova, 1 ,* A. V. Dudkov, 1 and I. M. Kvetnoy 1

THYMOSIN STUDIES

- Guo et al[36] 2015 The anti-tumor effect of thymosin alpha 1 was studied on human cancer cell lines. The study concluded that thymosin alpha 1 can decrease proliferation and induce apoptosis in human leukemia, non-small cell lung cancer, melanoma, and other cancers. The study concluded that thymosin alpha 1 could be an approach to breast cancer treatment
- Clinical studies
- Sherman et al[29] 2010 Thymosin alpha 1 was tested as monotherapy and in combination with interferon-alpha for the treatment of chronic hepatitis B. It was also shown to stimulate IL-2 receptor expression and IL-2 internalization and to enhance immune response in patients with immunodeficiency
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