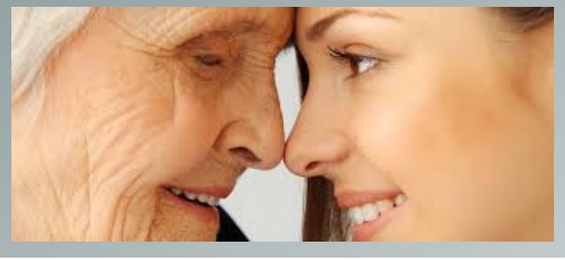
Interventional Strategies for Healthy Aging & Delay Age-Related Disorders



Johnny Huard, PhD

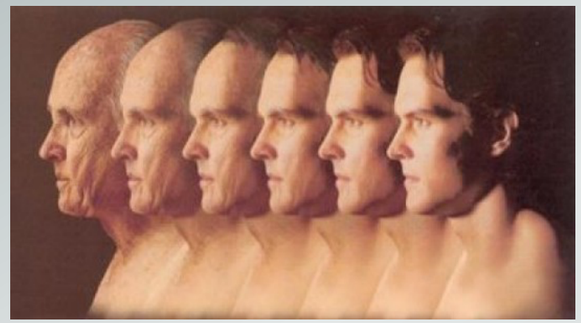
Chief Scientific Officer

Director, Center of Regenerative Sports Medicine

The Steadman Clinic

Steadman Philippon Research Institute

March 21st, 2019



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Disclosures

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National Institute Musculoskeletal NATIONAL INSTI

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ARMED FORCES INSTITUTE OF REGENERATIVE MEDICINE AFIRM

DOD W81XWH-15-2-0003

Thank you

Herb & Barbara Shear

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S 1R21 AR073509
S 1R01 AR065445

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Industry and University Research Collaboration

ProofPoint Biologics

THE STEADMAN CLINIC

COOK MYOSITE

Philanthropy

Linda/Mitch Hart

Steven/Mary Read

Mary-Sue/Mike Shannon

Eric Borgen family

Shear family

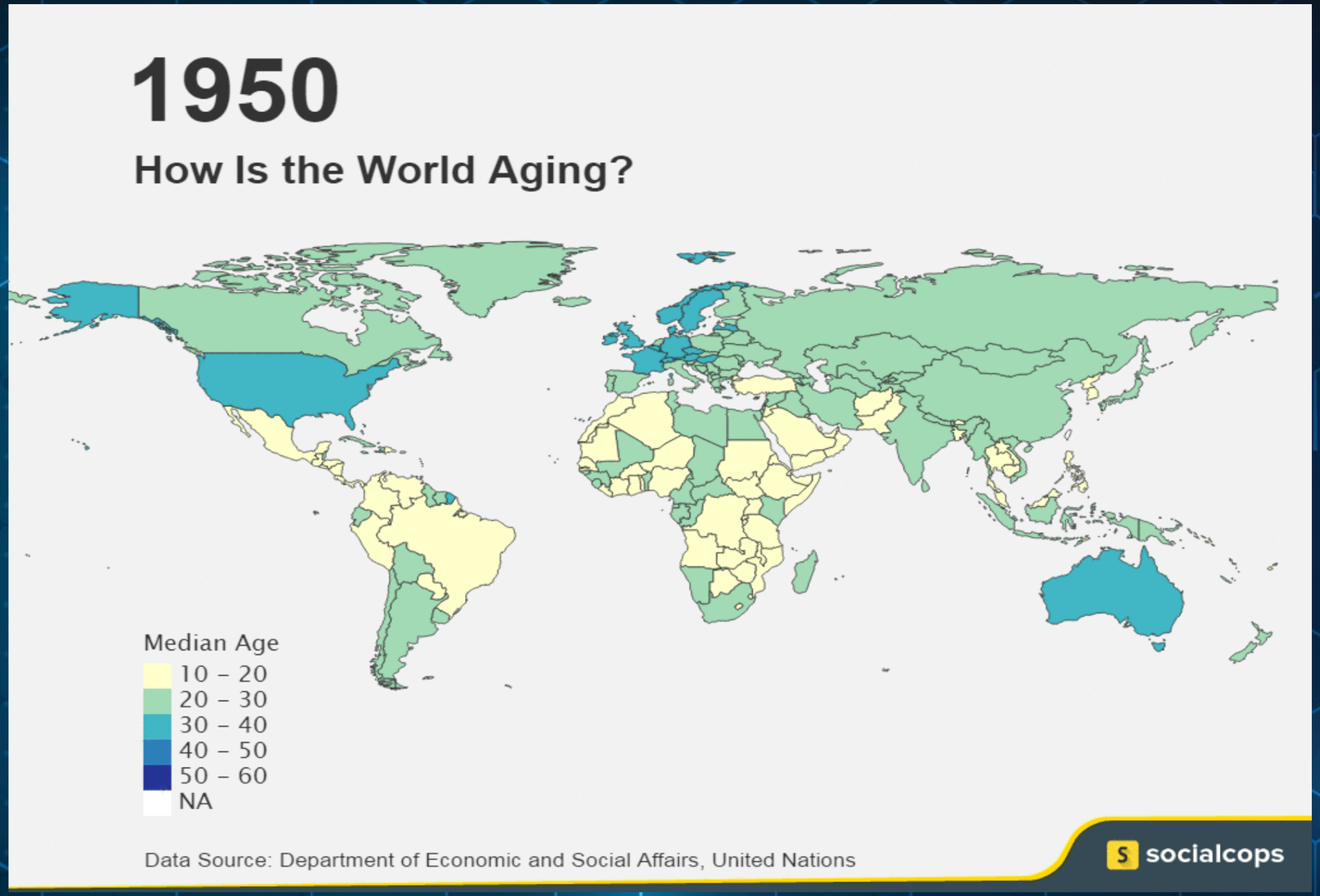
Kenneth Griffin

Daniel/Monica Porter

Ann Smead & Michael Byram

We are living longer on a global scale

Why working on healthy-aging research?



The US population ages 90 & older will quadruple between between 2010 & 2050

“The idea is to die young
as late as possible.”
—Ashley Montagu
anthropologist

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Healthy Aging

Disease Free-High Physical/Cognitive Level-Socially Engaged- Productive

Regenerative Medicine

Stem Cell

Banking

Stem Cell

Therapy

PRP

Exosomes

Drugs

Metformin

Rapamycin

Senolytics

Telomerase activity

Personalized Medicine

Diet

Caloric Restriction

Intermittent Fasting

Ketogenic Diet

Lifestyle

Aerobic Exercise

Angiogenesis

Meditation

Yoga

Evidence Based Medicine / Patient Centered Care

Changing Lives by Keeping People Healthy/Active

Why do we age?
The Hallmarks of Aging

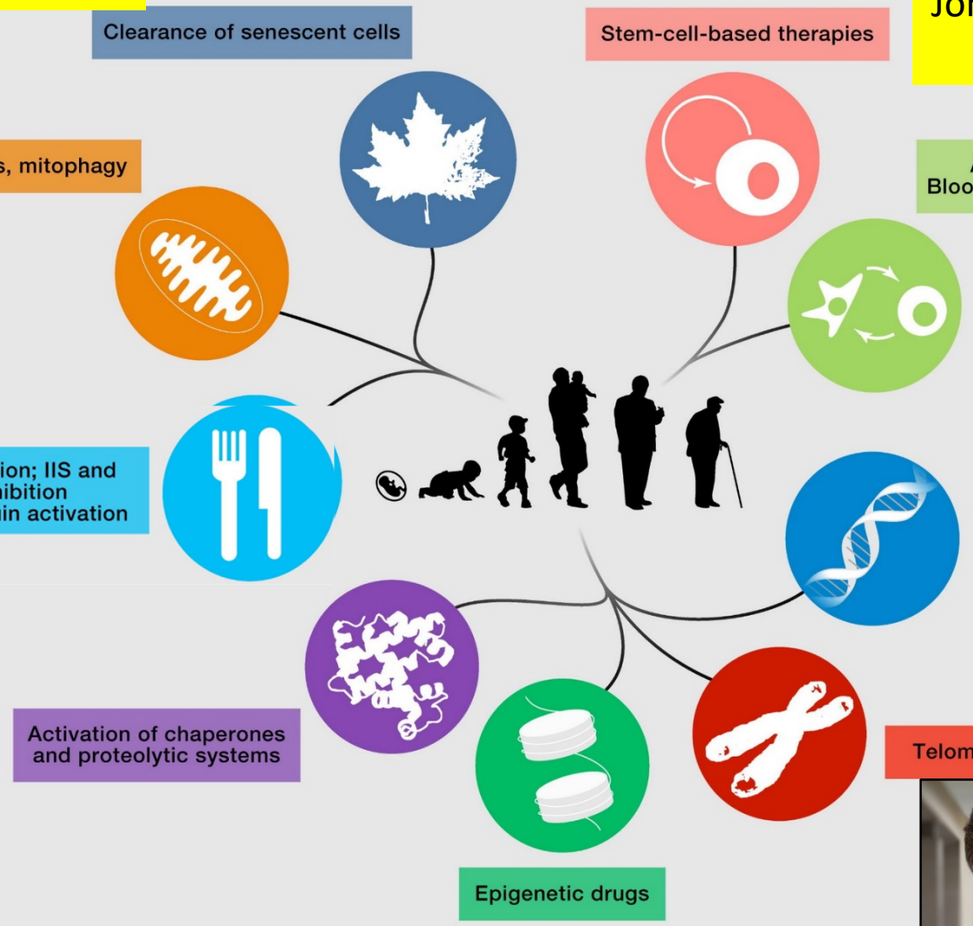
James Kirkland's lab
Mayo Clinic

Johnny Huard's/ Marc Philippon
SPRI

Laura Niedernhofer's lab
U. Minnesota

Paul Robbins's lab
U. Minnesota

Johnny Huard's
SPRI



Johnny Huard's lab

James Kirkland's lab

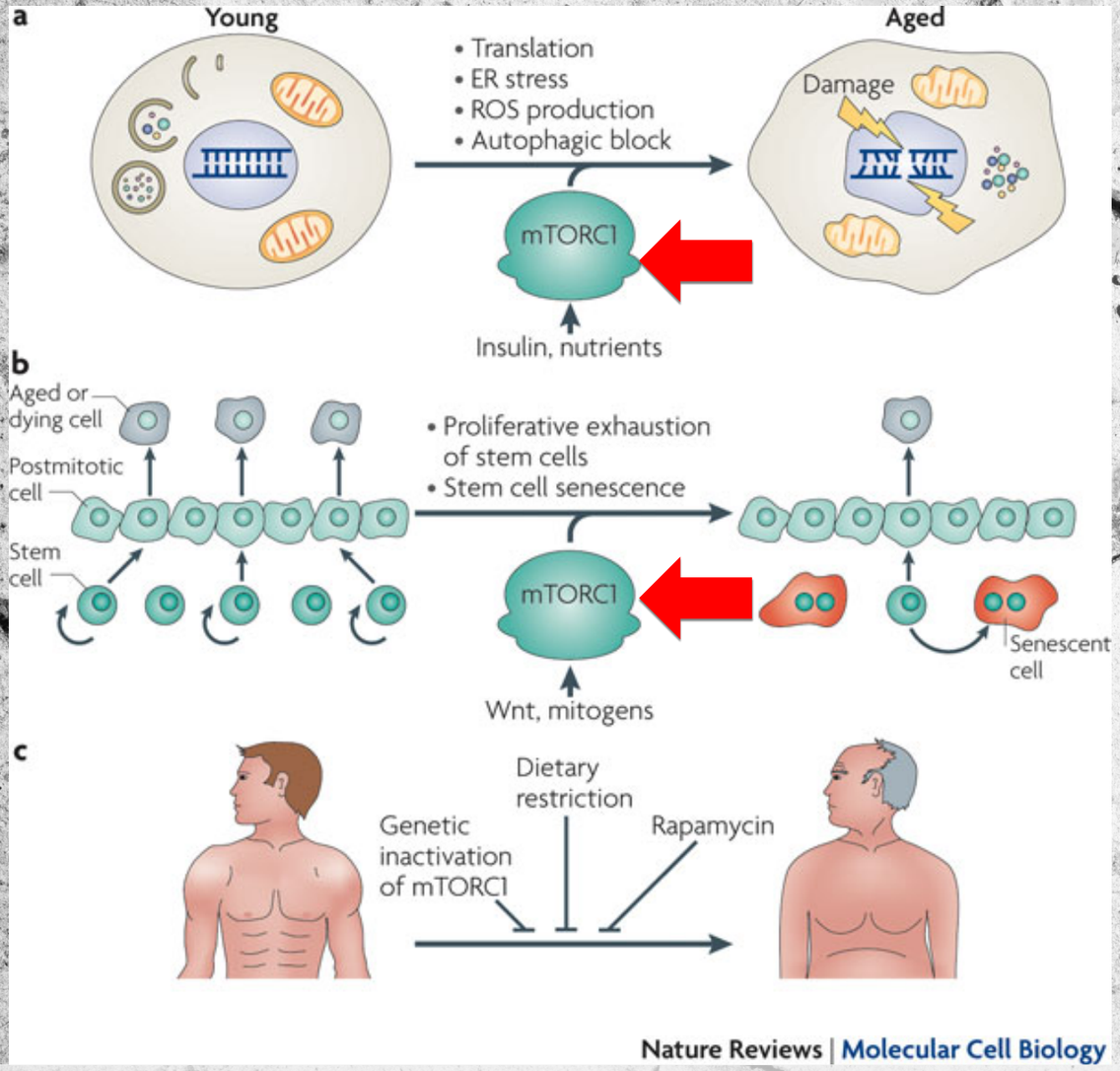
John Cooke's lab
Methodist Inst. Houston

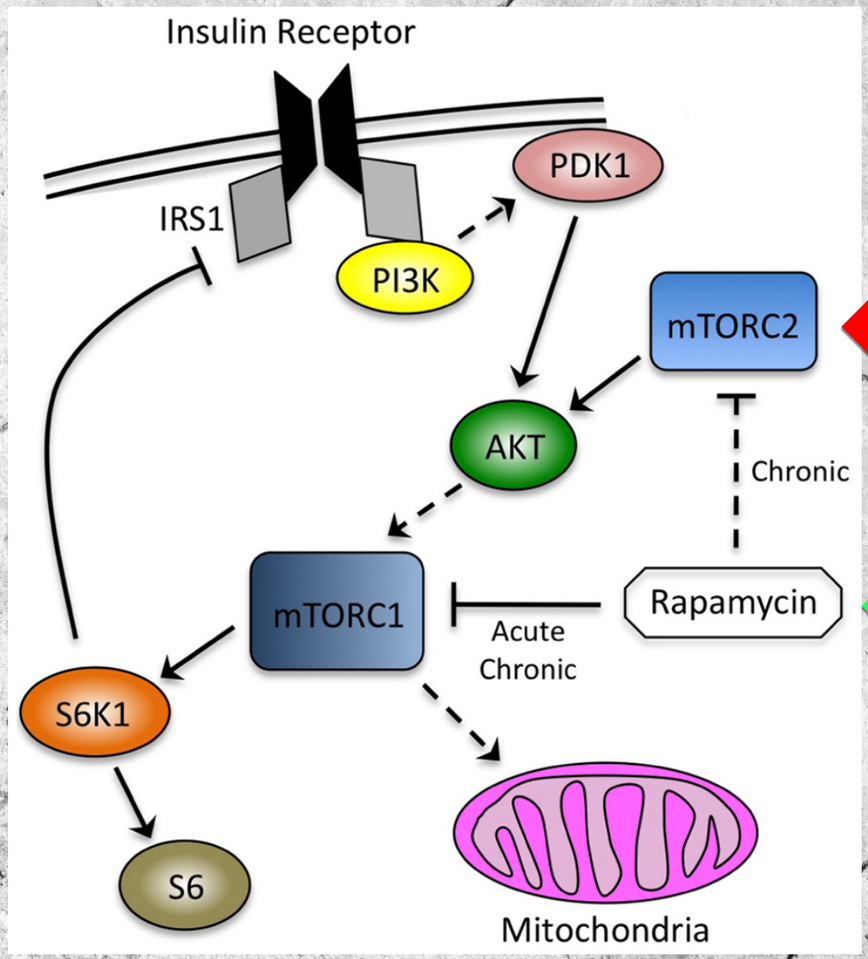
Lopez-Otin et al. Cell 2013

Good Stem cells become dysfunctional (tired) during the aging process

Why stem cells become dysfunctional during the aging process?

Why Stem Cells Become Defective with Aging?





J Orthop Res. 2017 Jul;35(7):1375-1382

mTOR signaling plays a critical role in the defects observed in muscle-derived stem/progenitor cells isolated from a murine model of accelerated aging. Takayama K[1,2,3], Fu FH[1], Robbins PD[6], Niedernhofer LJ[6], Huard J

Rapamycin (m-TOR inhibitor) Treatment to Delay Aging

- Transient rapamycin treatment can increase lifespan and healthspan in middle-aged mice. Elife. 2016 Aug 23;5. pii: e16351. Bitto A1 et al.
- Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature. 2009 Jul 16;460(7253):392-5. Harrison DE et al.

The following article was published in one of Britain's largest newspapers called *The Telegraph* on November 29, 2015:

November 29, 2015

The Telegraph

World's First Anti-Ageing Drug Could See Humans Live to 120

BY SARAH KNAPTON

The world's first anti-ageing drug will be tested on humans next year in trials which could see diseases like Alzheimer's and Parkinson's consigned to distant memory.

Scientists now believe that it is possible to actually stop people growing old as quickly and help them live in good health well into their 110s and 120s.

Although it might seem like science fiction, researchers have already proven that the diabetes drug metformin extends the life of animals, and the Food and Drug Administration in the US has now given the go ahead for a trial to see if the same effects can be replicated in humans.

If successful it will mean that a person in their 70s would be as biologically healthy as a 50 year old. It could usher in a new era of 'geroscience' where doctors would no longer fight individual conditions like cancer, diabetes and dementia, but instead treat the underlying mechanism – ageing.

Scottish ageing expert Professor Gordon Lithgow of the Buck Institute for Research on Ageing in California, is one of the study advisors.

"If you target an ageing process and you slow down ageing then you slow down all the diseases and pathology of ageing as well," he said. "That's revolutionary. That's never happened before.

"I have been doing research into ageing for 25 years and the idea that we would be talking about a clinical trial in humans for an anti-ageing drug would have been though inconceivable.

"But there is every reason to believe it's possible. The future is taking the biology that we've now developed and applying it to humans. 20 years ago ageing was a biological mystery. Now we are starting to understand what is going on."

Ageing is not an inevitable part of life because all cells contain a DNA blueprint which could keep a body functioning correctly forever. Some marine creatures do not age at all.

However over our lifetime billions of cell divisions must occur to keep our bodies functioning

70 | LIFE EXTENSION | MARCH 2016

TheScientist
EXPLORING LIFE, INSPIRING INNOVATION

NEWS & OPINION MAGAZINE SU

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Could Rapamycin Help Humans Live Longer?

From extending lifespan to bolstering the immune system, the drug's effects are only just beginning to be understood.

Mar 1, 2018
ANNE N. CONNOR

102 60

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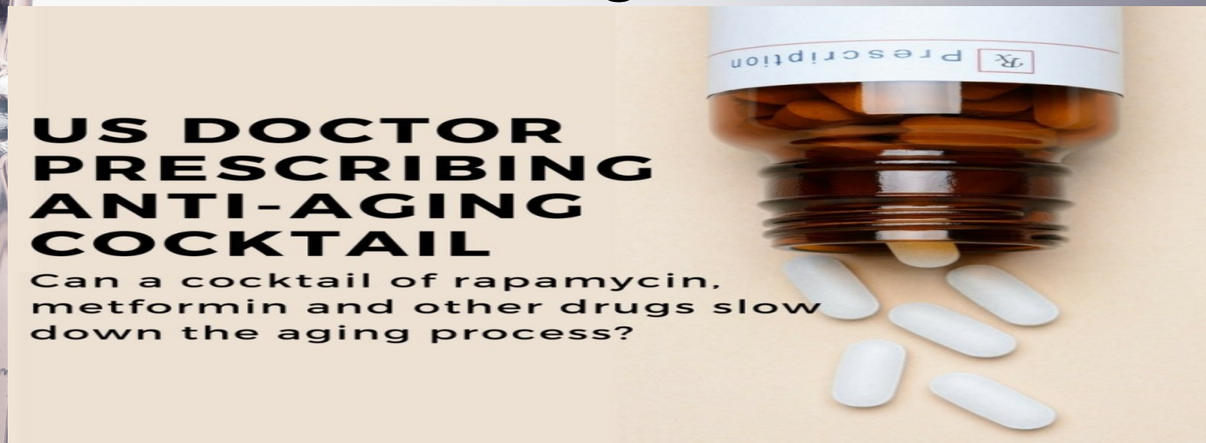
In the 1990s, pharmacologist Dave Sharp of the University of Texas's Barshop Institute for Longevity and Aging Studies in San Antonio was studying mice with pituitary dwarfism—a condition in which the pituitary gland fails to make enough growth hormone for normal development. The puzzle, Sharp explains, was that research had shown that these hormone-deficient dwarf mice lived longer than normal mice. "I wondered, why is being small connected with longer life?" he says.

FDA-approved drug Metformin (M-Tor inhibitor) to delay multiple age-related conditions

A diabetes medication that costs 6 cents a pill could be a key to living longer

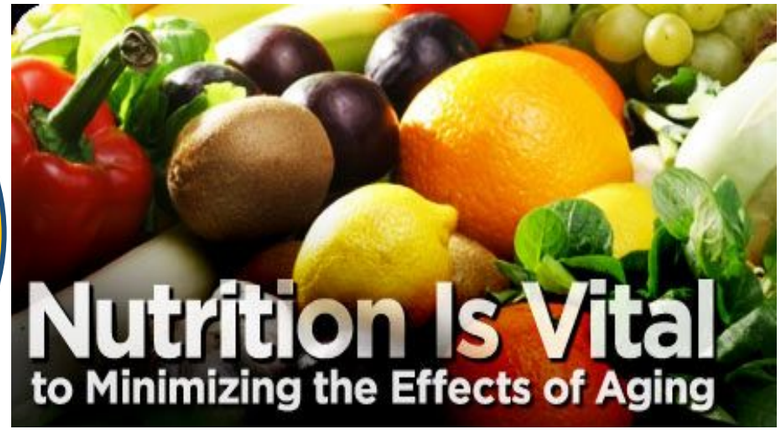


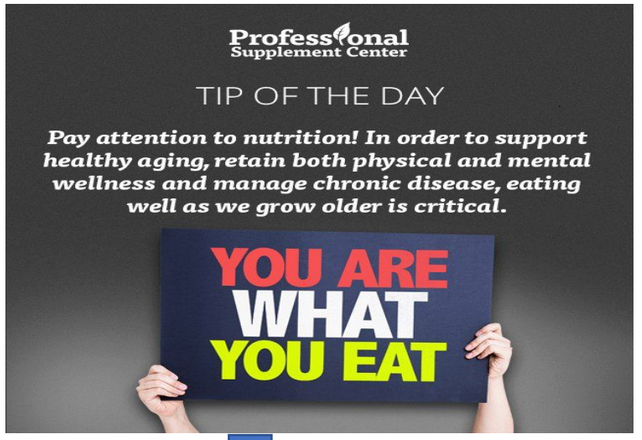
US DOCTOR PRESCRIBING ANTI-AGING COCKTAIL

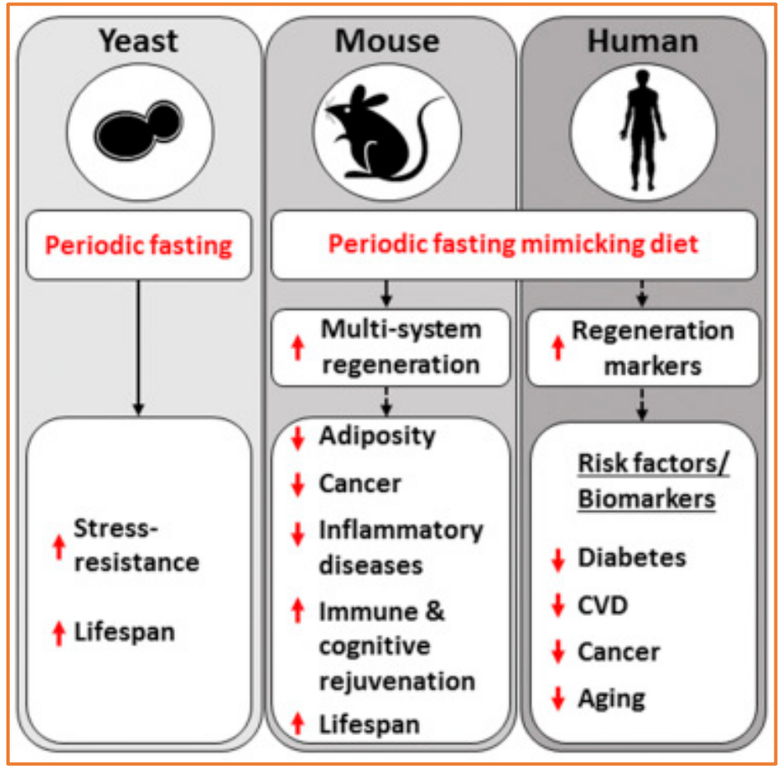
Can a cocktail of rapamycin, metformin and other drugs slow down the aging process?

Diet

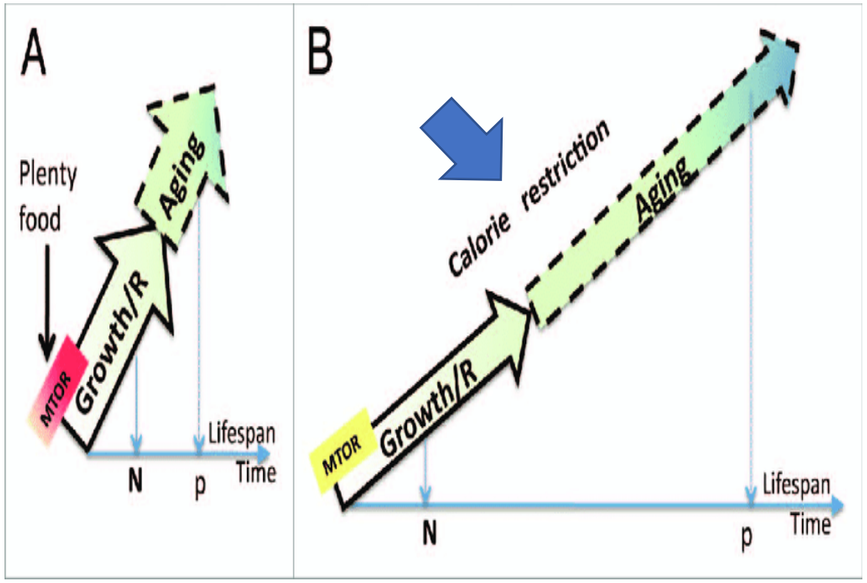
Caloric Restriction
Intermittent Fasting
Ketogenic Diet

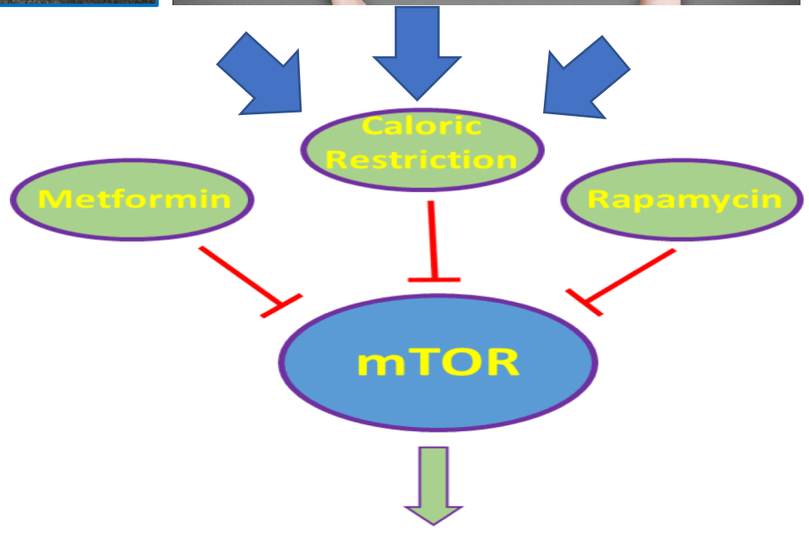






Longo et al. Cell Met. 2015

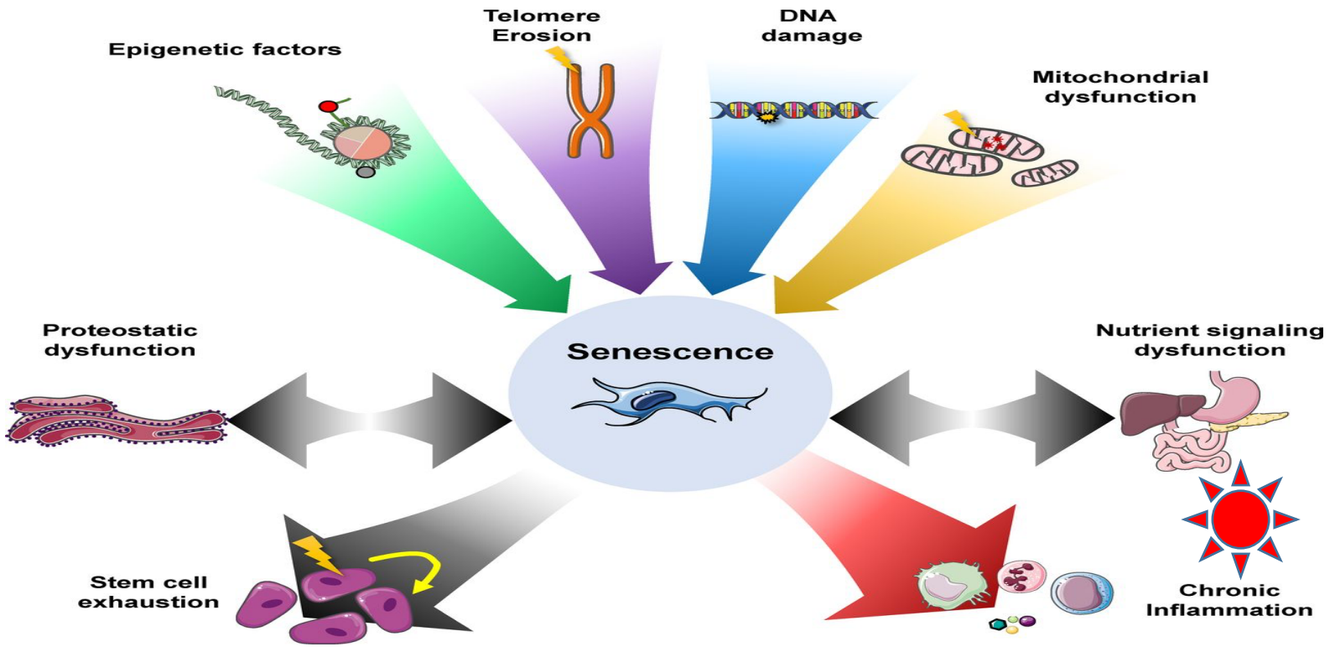


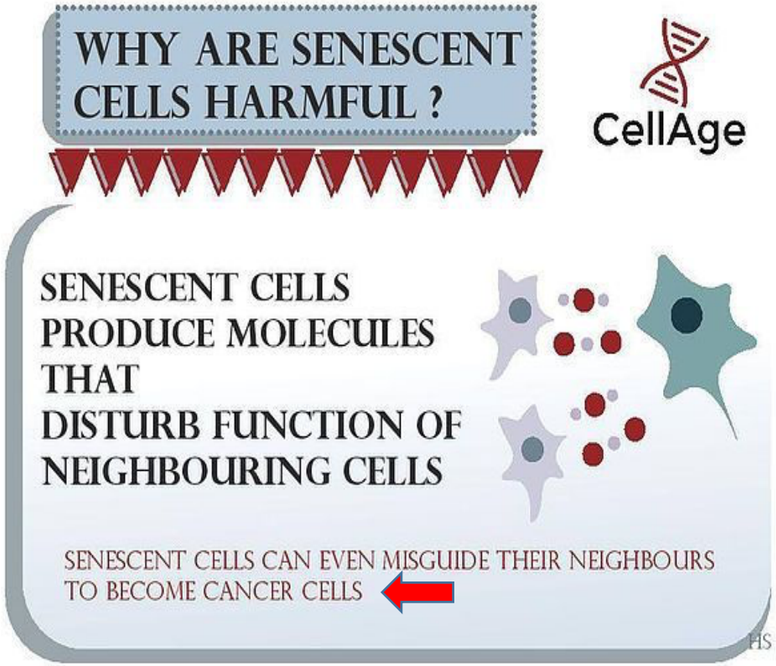


Cell Cycle 12:12, 1842–1847; June 15, 2013; © 2013 Landes Bioscience

James Kirkland's lab
Mayo Clinic

Killing/ Clearing Senescent Cells for Healthy Aging



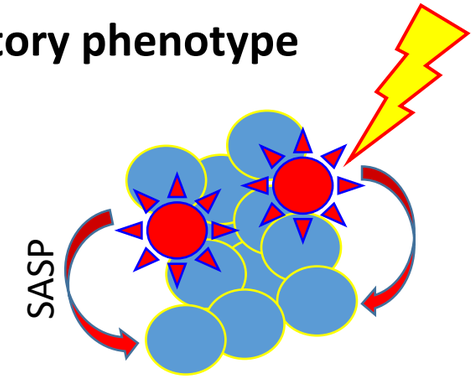


Killing senescent cells to delay aging

Senescent cells

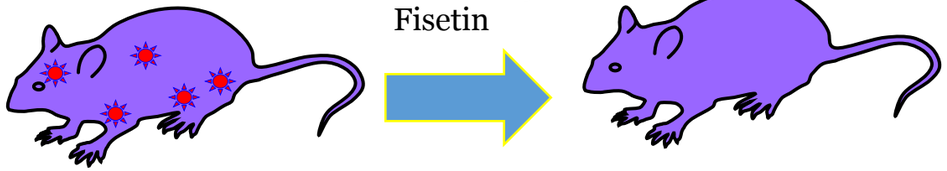
Senescence associated secretory phenotype (SASP)

Stem Cells

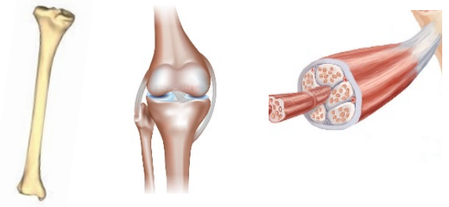


Dasatinib (chemotherapy medication)
Quercetin (Reduce free radicals)
Alvespimycin (heat shock protein (HSP) 90 inhibitor)
Fisetin (antioxidant & mitochondrial function)

Various drugs that specifically kill/clear senescent cells to delay aging



Improved musculoskeletal health, strength, and function. Increased lifespan.



Aging (Albany NY). 2017 Mar 8;9(3):955-963. **New agents that target senescent cells: the flavone, fisetin, and the BCL-X_L inhibitors, A1331852 and A1155463.** Zhu Y[1], Doornebal EJ[1,2], Pirtskhalava T[1], Giorgadze N[1], Wentworth M[3], Fuhrmann-Stroissnigg H[4], Niedernhofer LJ[4], Robbins PD[4], Tchkonia T[1], Kirkland JL[1]

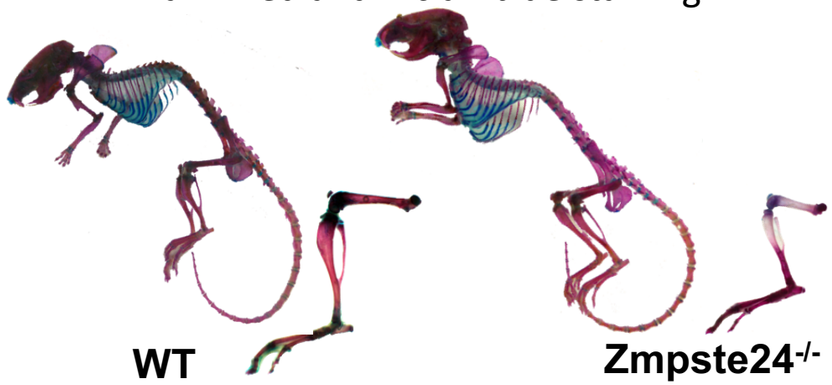
Nat Commun. 2017 Sep 4;8(1):422. **Identification of HSP90 inhibitors as a novel class of senolytics.** Fuhrmann-Stroissnigg H[1], Ling YY[1], Zhao J[1], McGowan SJ[1], Zhu Y[2], Brooks RW[1], Grassi D[1], Gregg SQ[3], Stripay JL[3], Dorronsoro A[1], Corbo L[1], Tang P[1], Bukata C[1], Ring N[4], Giacca M[4], Li X[1], Tchkonia T[2], Kirkland JL[2], Niedernhofer LJ[1], Robbins PD[5]

Articular Cartilage abnormalities (OA) in Zmpste24 progeria mice

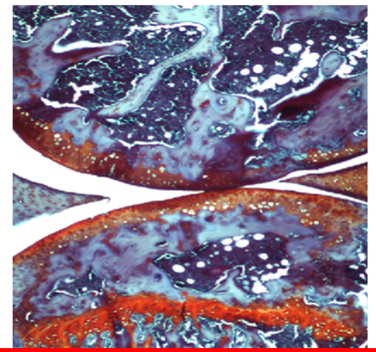
- HGPS is an autosomal dominant disease that involves premature aging (lamin A deficiency) causing early death in childhood, rapid sclerotic skin, joint contractures, bone abnormalities and growth impairment are observed.

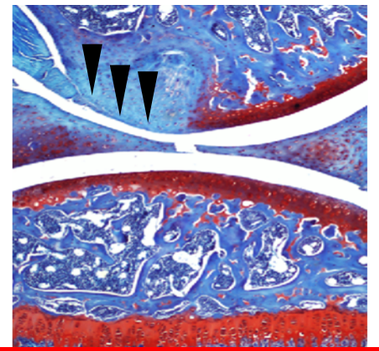
Zmpste24-/- (Z24-/-) mice are reliable model of human Hutchinson-Gilford progeria (HGPS). They are incapable of producing lamin A, an essential component of the nuclear

Alizarin Red and Alcian blue staining



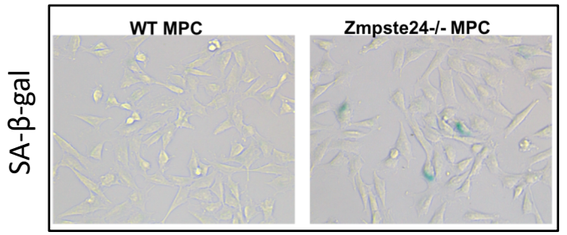
Adult mice

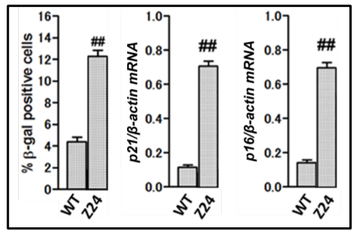


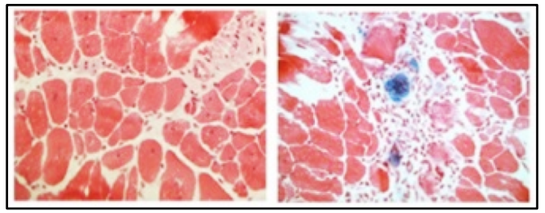


Premature senescence in the *Zmpste24*$^{-/-}$ model

Beta-gal cells (blue)= senescent stem cells







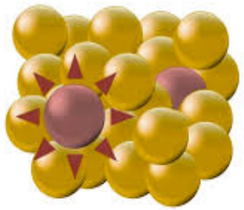
Can we delay OA in progeria mice by killing senescent cells with senolytic agent?

Proof Of Concept

Proof Of Concept

Effects of Senolytic Drugs on osteoarthritis (OA) in *Z24$^{-/-}$* mice

Zmpste24-/- (Z24-/-) mice
Progeria mice



Articular cartilage analyses
For OA progression

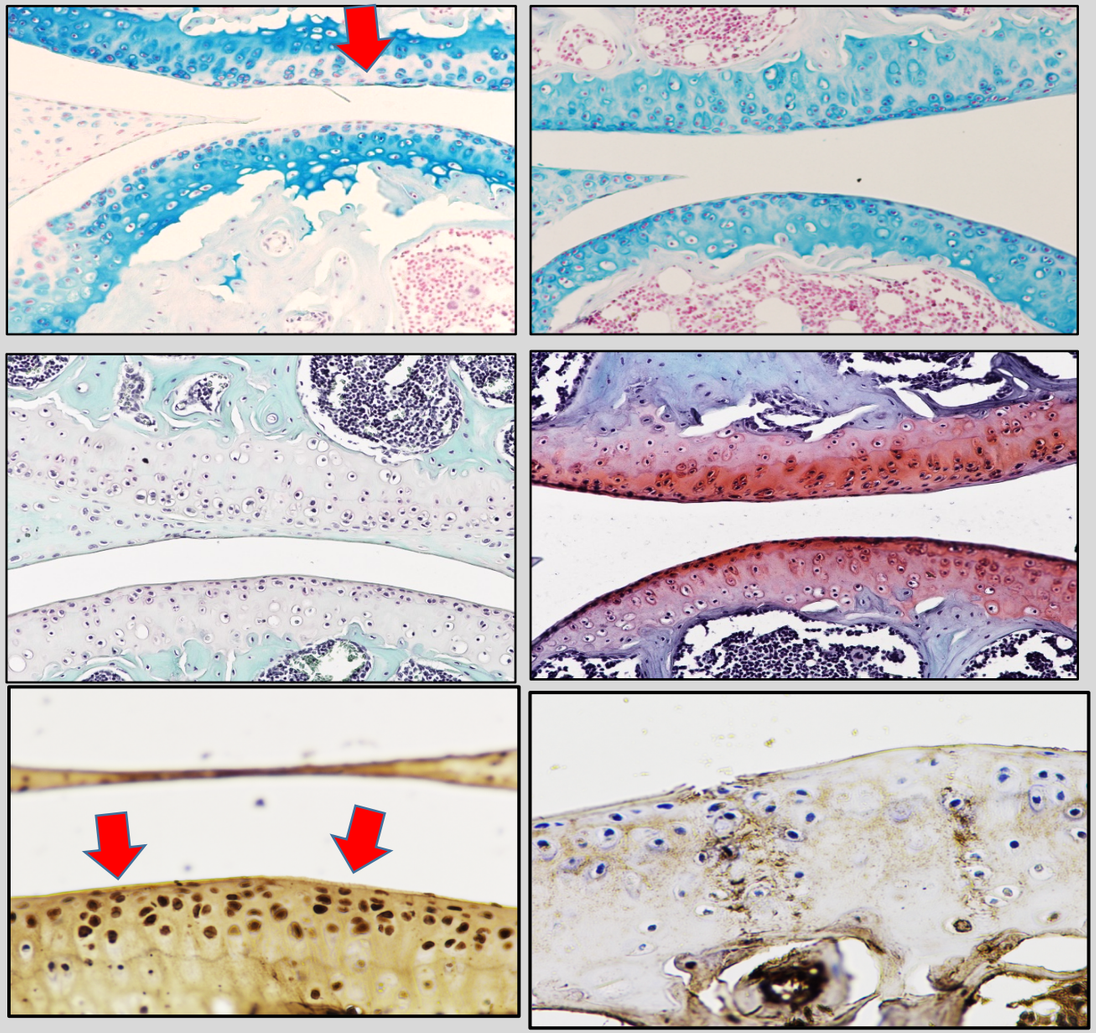
Progeria-Z24$^{-/-}$

Progeria-Z24$^{-/-}$ + senolytic drugs

Alcian Blue

Safranin O

p16



p16: marker for senescent cells

Killing senescent cells with senolytic drugs delay OA

Quercetin is a plant pigment (flavonoid).

Reduce free radicals

GNC LIVE WELL

Fisetin:
a plant polyphenol from the flavonoid group.
Antioxidant

amazon

Clinical trial of senolytic agents to delay Osteoarthritis (OA) in 2019

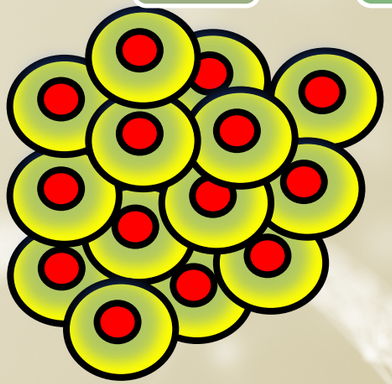
- Dr. James Kirkland (Mayo Clinic)
- Dr. Marc Philippon/Johnny Huard (SPRI)
- 60 patients with moderate OA will need to be recruited
  - 20 patients on Quercitin (250mg)(2 days on and 13 days off) for a period of 3 months
  - 20 patients on Fisetin (20 mg/KG daily, 100mg capsule).. 2 days on and 13 days off) for a period of 3 months
  - 20 patients on placebo
- Investigational New Drugs (INDs) already approved by FDA
- IRB already approved at Mayo Clinic (for Alzheimer) and will need to be transferred to SPRI/Vail Health for OA
- NIH funded grant at Mayo Clinic to identify biomarkers that can be used to determine improvement of OA
- OA patients will be monitored at 4-8 and 12 months post treatment
- Biomotion and Imaging will allow to monitor the progression of OA
- The goal is to delay OA progression so we can post-pone the first joint replacement
  - Keep the people active with limited OA
  - Delay the first joint replacement
  - Eliminate joint replacement revision

Quercetin is a plant pigment (flavonoid).

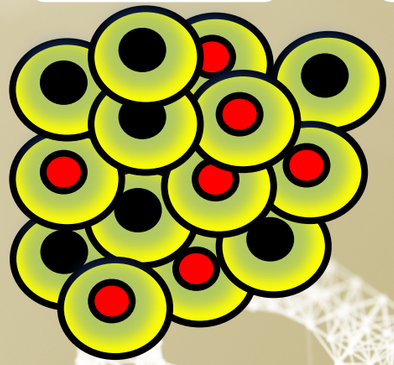
Reduce free radicals

Fisetin:
a plant polyphenol from the flavonoid group.
Antioxidant

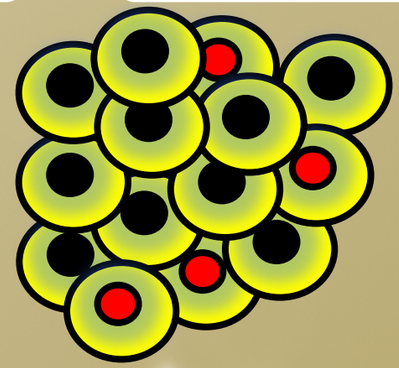
Stem cells depletion during the aging process!



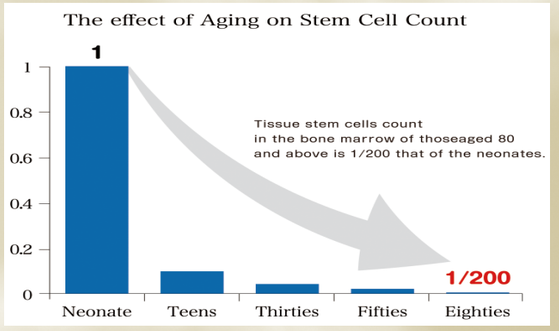
At birth 100% of cells are good with rejuvenation/ regenerative potential

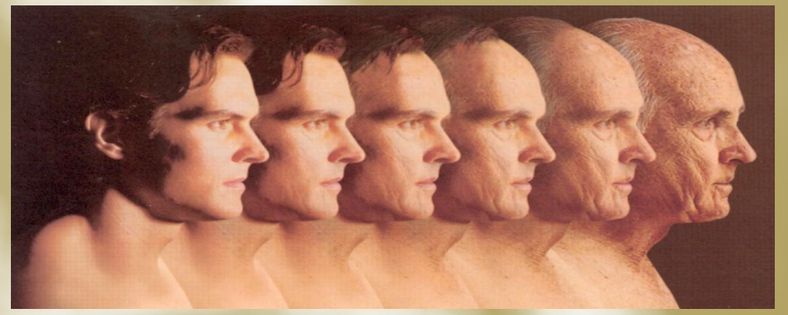


In adults 50% of cells are good with rejuvenation/ regenerative potential



At older age, 75% of cells become senescent and lose rejuvenation potential





Kuhn H.G. Neuroscience (1996) ; Molofsky A.V. . Nature (2006); De Barros S. Mol. Ther. (2013); Huard J. Nature Commun. 2012

Paul Robbins's lab
U. Minnesota

Stem cell defects in Progeria *ERCC* deficient mice

Laura Niedernhofer's lab. U. Minnesota

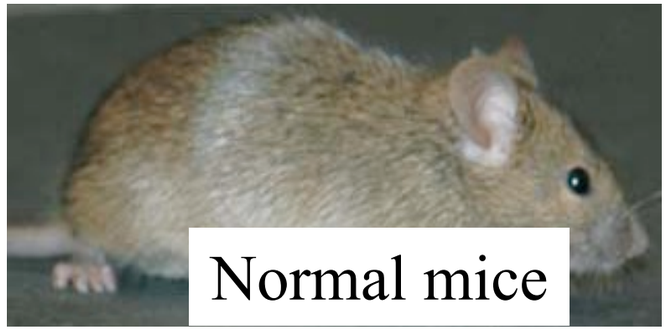
Spontaneous accelerated aging in progeria mice

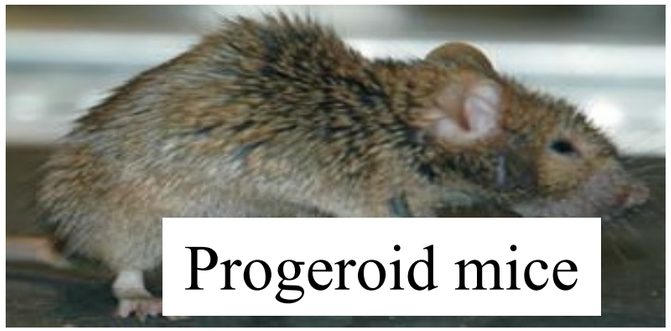
Niedernhofer LJ et al. Nature (2006) 444; 1038-43

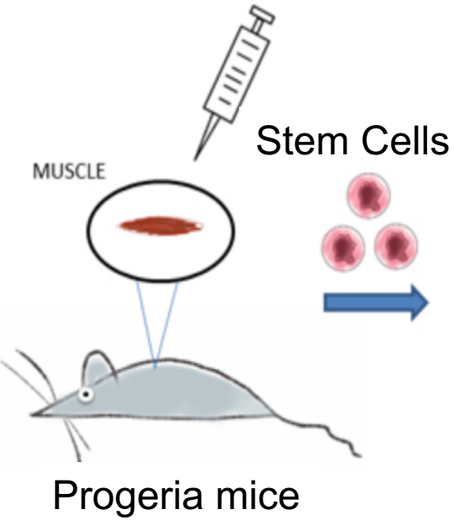
Symptoms:

cachexia & ***sarcopenia***, osteoporosis, bone marrow hypoplasia, epidermal atrophy, renal & liver function, loss of vision and hearing, Neurodegeneration, osteoporosis, osteoarthritis (OA)

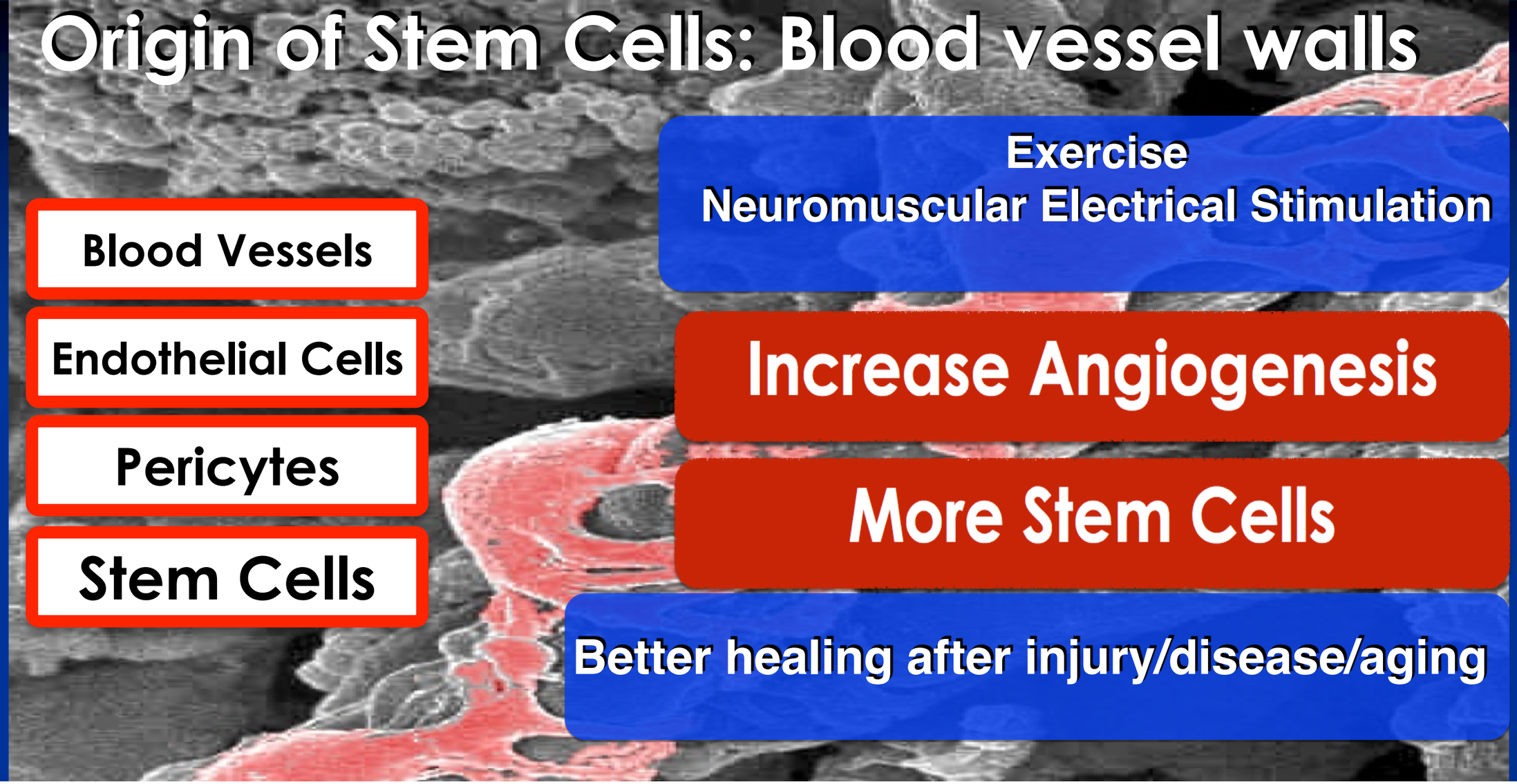
Hypothesis: ***Defects exist in the stem cells compartment of ERCC-XPF-deficient progeroid mice***







Origin of Stem Cells: Blood vessel walls



- Blood Vessels
- Endothelial Cells
- Pericytes
- Stem Cells

Exercise
Neuromuscular Electrical Stimulation

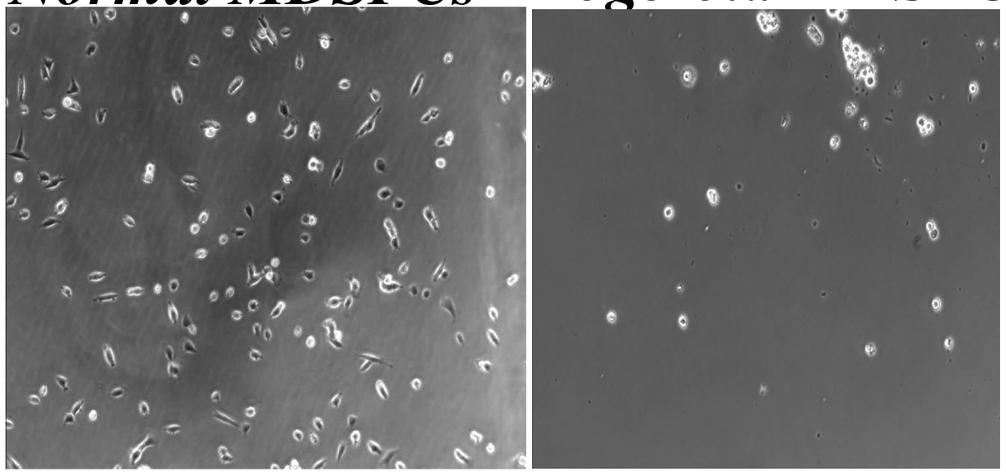
Increase Angiogenesis

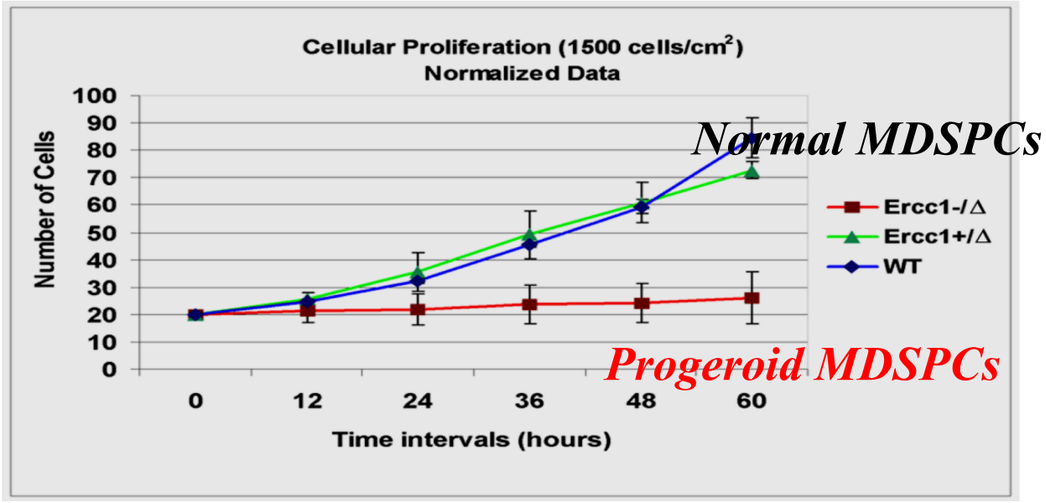
More Stem Cells

Better healing after injury/disease/aging

The synergistic effect of treadmill running on stem cell transplantation to heal injured skeletal muscle. **Tissue Eng Part A. 2010 Mar;16(3):839-49. Neuromuscular** electrical stimulation as a method to maximize the beneficial effect of muscle stem cell transplanted into dystrophic muscle. **PloS One 2013, 8(3):e54922**

Normal MDSPCs ***Progeroid MDSPCs***

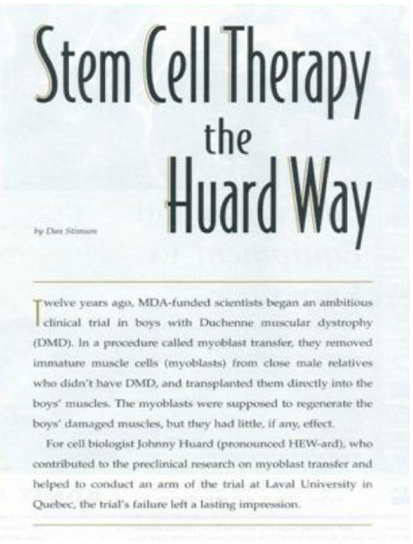




Stem Cells from Progeroid mice display limited proliferation & differentiation potentials (myogenic, osteogenic, and chondrogenic)

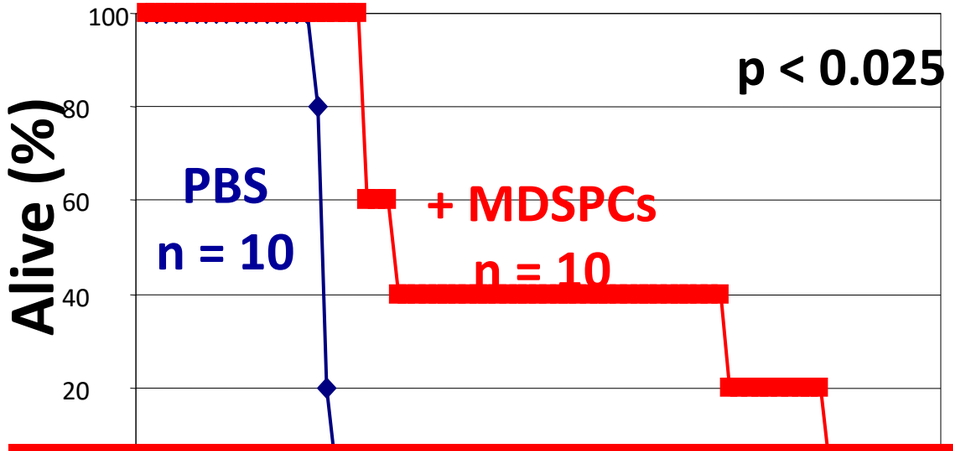
"Tired Stem Cells"

Injection of young muscle stem cells extends the lifespan of *progeroid* mice!

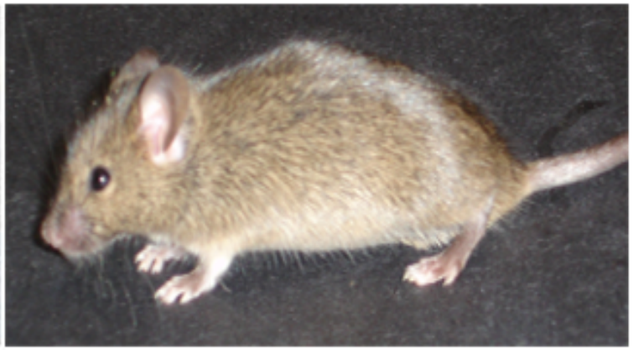


progeroid mouse

5X10^5 young stem cells injected into 17 day old progeroid mice …



21 days
untreated



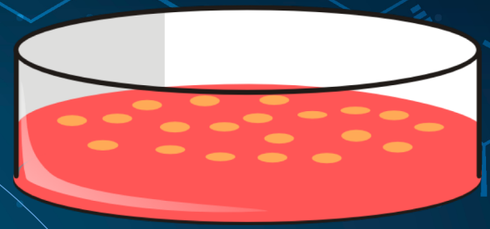
45 days
post-transplantation

The progeroid mice injected with young stem cells showed a 3-fold increase in their lifespan and had a significantly increased healthspan, when compared to non-injected mice.

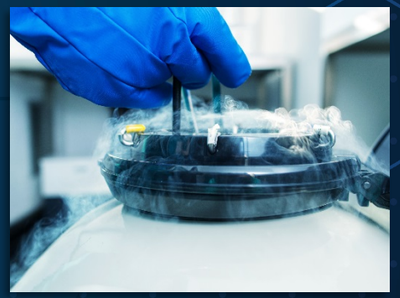
Clinical Translation: The Future of Stem Cell Therapy

- Muscle biopsy and MDSC isolation
- Umbilical cord cells
- Other sources of stem cells

Stem Cell isolation, expansion at a GMP facility



Cell coding and cataloging for long-term storage in liquid nitrogen



The same approach can be used for soldiers prior to deployment, NASA prior to Space Shuttle launches and patients before disease progression

Based on FDA approved protocols for clinical trials, clinicians request that patients' cells are prepared for injections.

Cells are thawed and expanded

The requested number of cells are shipped frozen to the operating room and cell therapy is initiated

The goal is no to make you look younger; it is to make you age better.

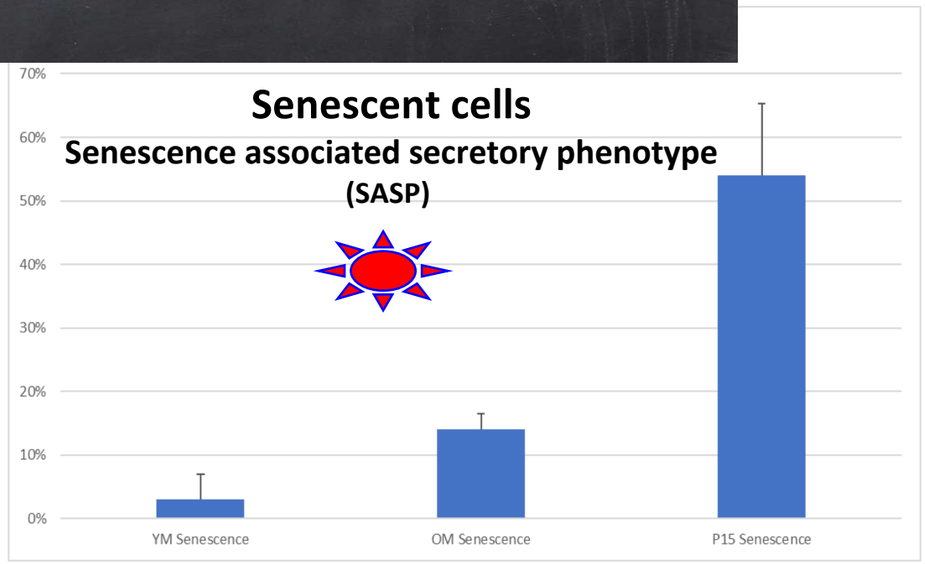
Aging process

Clinical Translation: Current Status of Stem Cell Banking

Sten
Bo

reservation of
red stem cells

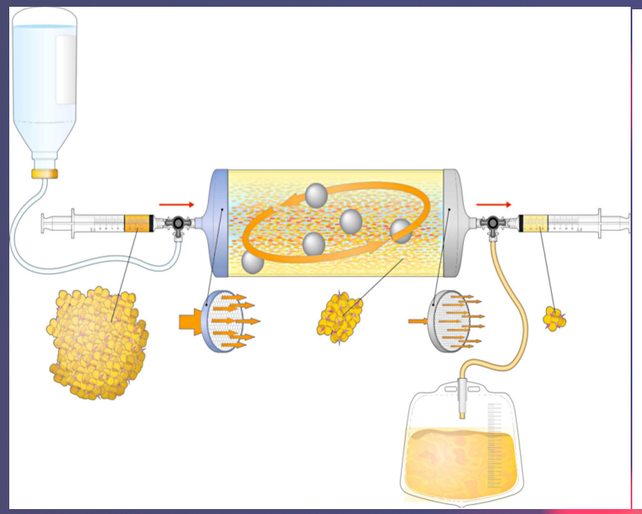
xpansion of
-gal) staining

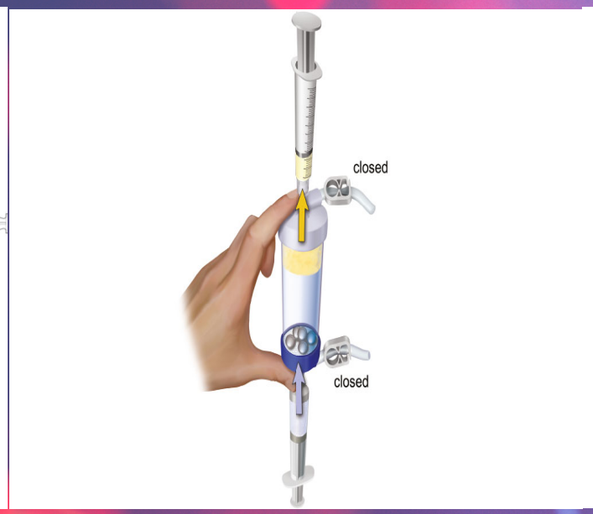


FDA U.S. FOOD & DRUG ADMINISTRATION

New Direction in 2019

Method to Isolate Adult Stem Cells without Compromising their potency and regenerative potential!



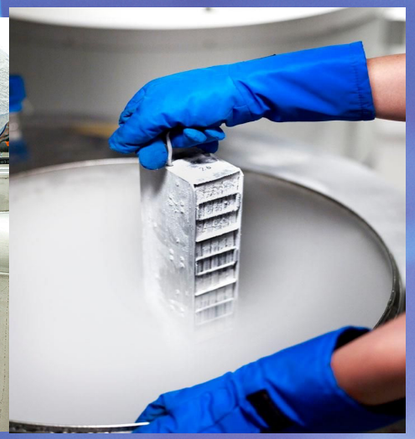


No cell expansion in vitro! FDA Compliant

Banking of stem cells at the Steadman Clinic

Transplantation in the USA (no need to go overseas)

FDA U.S. FOOD & DRUG ADMINISTRATION



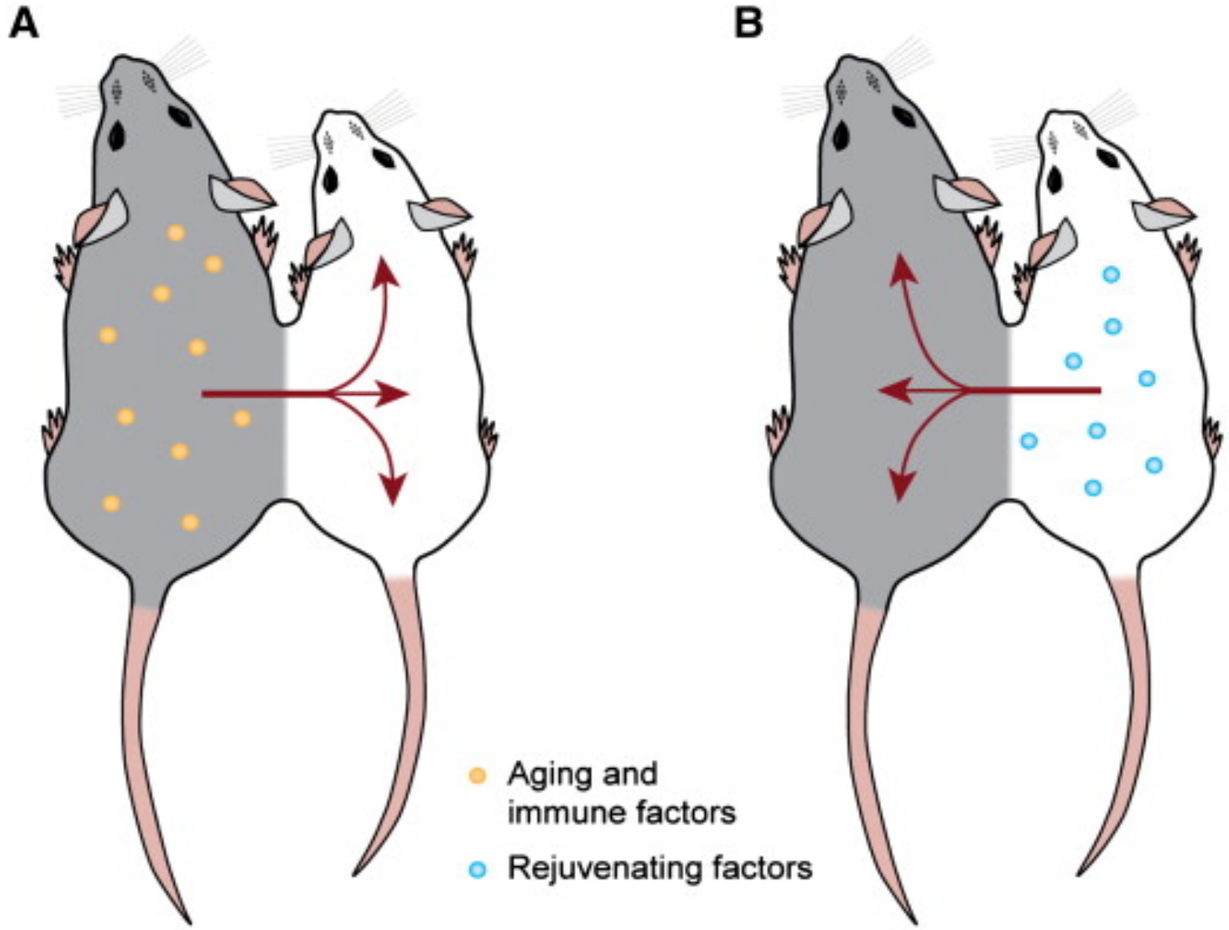
Young blood can rejuvenate aged animals

Young Blood

Injections

Could Fight

Alzheimer's

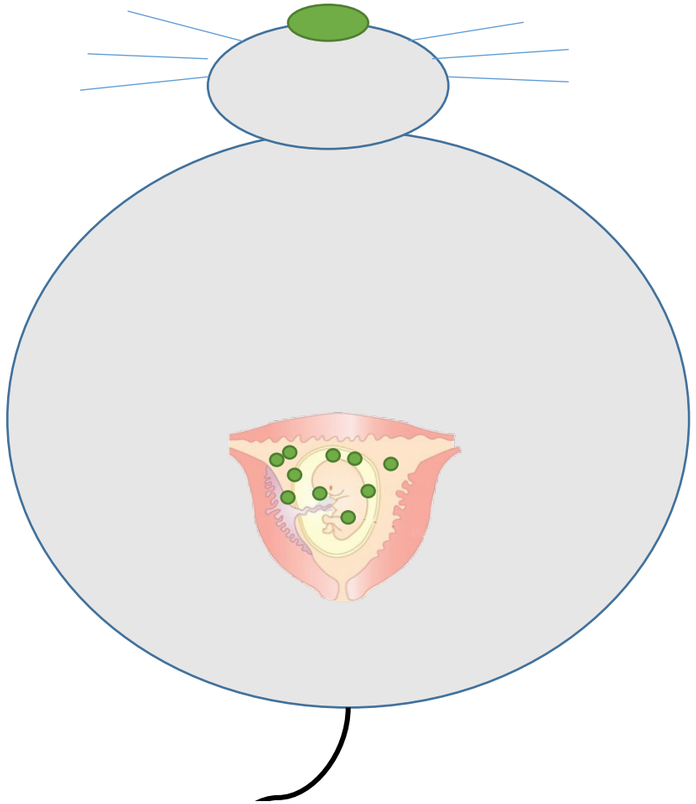


In addition of banking our stem cells, perhaps we need to bank our blood when we are younger as well…..

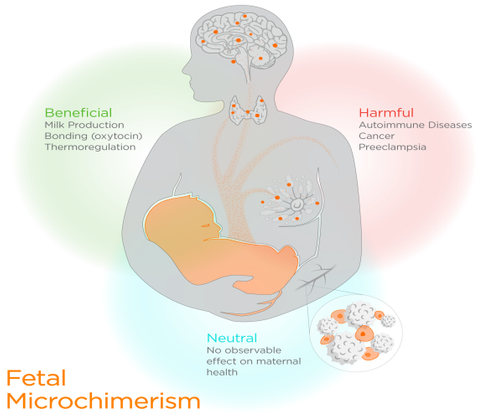
Women have longer life span than men…..

Testosterone, has been linked to violence and risk taking!

Mother & Fetus interaction during Pregnancy
Natural exchange of blood/stem cells between fetus & mother



Can the fetus release rejuvenating factors or stem cells that help tissue repair in mother?



Late-in-life pregnancy could predict longevity, Health Jun 26, 2014 5:35 PM EDT

”Women who become pregnant (40-50 years old) age slower & achieve extreme longevity”.

Nature **389**, 133 1997.

Pregnancy is a good example that youthful factors and young stem cells can extend lifespan!

NIH Funded Project

- Title: “**Effects of Circulating Factors and Progenitors on Wound Healing during Pregnancy**”
  - Principal Investigator: **Dr. Johnny Huard (SPRI)**
  - Co-investigator: **Dr. Aiping Lu (UT Health)**
- Funded by the NIH. National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
- Funding period: 06/01/18 – 05/31/20

Adult stem cells come from the blood vessels!

Bruno Peault

Blood vessel cells ,endothelial cells (CD34, CD144) & pericytes (CD146) are likely the source of muscle stem cells!

Zheng B et al. Nature Biotech. 25, 9: 1025-1034, 2007

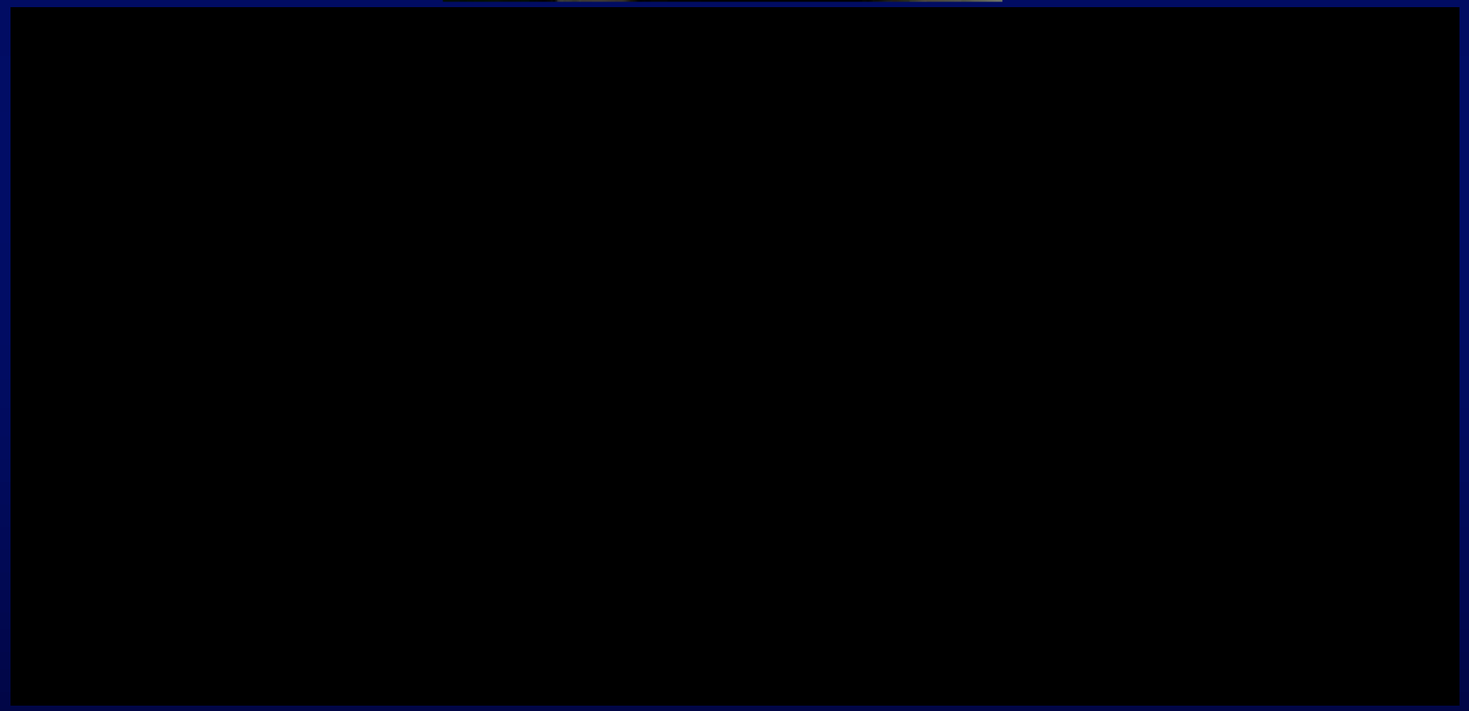
The blood vessel wall represents a potential source of stem cells in other post-natal tissues (Fat, Bone marrow) !

Crisan M et al. Cell Stem Cell. 2008 Sep 11;3(3):301-13

Angiogenesis (creation of blood vessels) and vascularity of a given tissue can be manipulated: Exercise (training); Neuromuscular electrical stimulation (NES)

modified from http://www.geocities.co.jp/HeartLand-Suzuran/9389/kekkan

Experiments to demonstrate that exercise is good for you: Mice on treadmill..



Exercise delays aging!

Delay aging

Moderate and i … the effects

Exercise-induce … ddle-aged rats. These resu … earch for strategies to dela … CX et al.

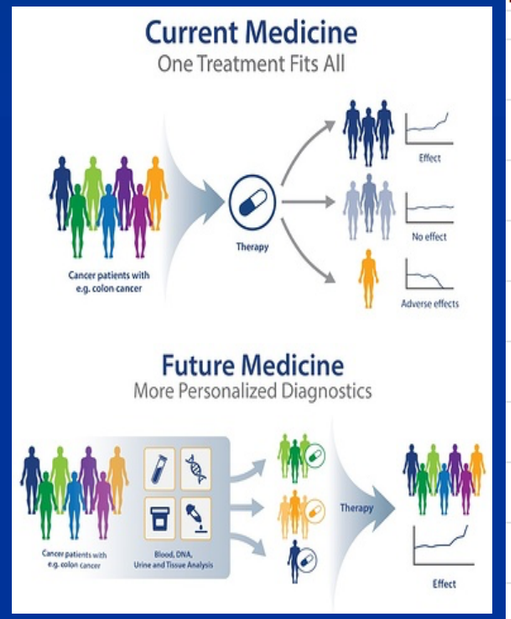
Mental and physi … ersons with

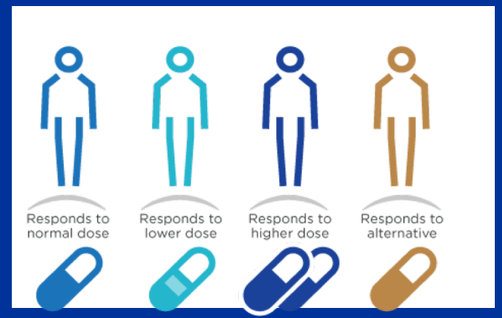
Bottom line:

"Exercise is good for you"

(Lifestyle important for healthy aging)

Personalized medicine for Healthy Aging!



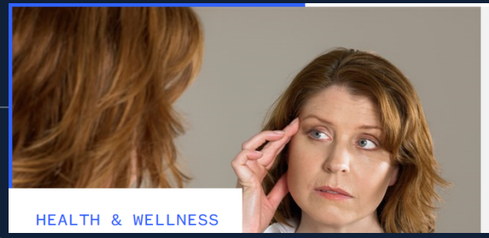


ReCODE Tests

	Target Values
GENETICS	
ApoE	Negative for ApoE4
BLOOD TESTS	
Inflamation vs cellular protection	
CRP	< .9 mg/dL
Homocysteine	< 7 mcmol/L
Vitamin B6	60-100 mcg/L
Vitamin B12	500-1500 pg/ml
Folate	10-25 ng/ml
Vitamin C	1.3-2.5 mg/dL
Vitamin D3	50-80 ng/ml
Vitamin E	12-20 mcg/ml
Omega-6 to 3 ratio	.5-3.0
A/G ratio (albumin:globulin)	>=1.8
Albumin	> 4.5 g/dL
Fasting insulin	<=4.5 microIU/ml
Fasting glucose	70-90 mg/dL
HgbA1c	< 5.6%
Body Mass Index	18-25
LDL-p	700-1000 nmol/L
sdLDL	< 20 mg/dL
oxidized LDL	< 60 U/l
Cholesterol	> 150 mg/dL
HDL	> 50 mg/dL
Triglycerides	< 150 mg/dL
Glutathione	5.0-5.5 micromolar
Serum thiamine	20-30 nmol/l
RBC thiamine pyrophosphate	100-150 ng/ml
Leaky gut	Cyrex Array 2 - Negative
Leaky blood-brain barrier	Cyrex Array 20 - Negative
Gluten sensitivity	Tissue transglutaminase antibodies - negative OR Cyrex Array3 and Cyrex Array 4 -
Autoantibodies	Cyrex Array 5 - negative
Trophic Support	
Vitamin D3	50-80 ng/ml
Estradiol	50-250 pg/ml
Progesterone	1-20 ng/ml
Estradiol : progesterone ratio	10-100
Pregnenolone	50-100 ng/dL
Cortisol	10-18 mcg/dL
DHEA sulfate (f)	350-430 mcg/dL
Total testosterone	500-1000 ng/dL
Free testosterone	6.5-15 ng/dL
Free T3	3.2-4.2 pg/ml
Free T4	1.3-1.8 ng/dL
Reverse T3	< 20 ng/dL
TSH	< 2.0 microIU/ml
Free T3x100 : Rev T3 ratio	> 20
Toxin Related	
Mercury	< 5 mcg/L
Lead	< 2 mcg/dL
Arsenic	< 7 mcg/L
Cadmium	< 2.5 mcg/L
Copper : zinc ratio	0.8-1.2
C4a	< 2830 ng/ml
TGF-beta 1	< 2380 pg/ml
MSH	35-81 pg/ml
HLA-DR/DQ	Benign
Metals	
RBC-magnesium	5.2-6.5 mg/dL
Copper	90-110 mcg/dL
Zinc	90-110 mcg/dL
Serum Selenium	110-150 ng/ml
Potassium	4.5-5.5 mmol/L
Calcium	8.5-10.5 mg/dL
URINE TESTS	
Trichothecenes	Negative
Ochratoxin A	Negative
Aflatoxin	Negative
Gliotoxin derivative	Negative
COGNITIVE PERFORMANCE	
CNS vital signs, Brain HQ or equivalent	> 50th percentile for age, improving with practice
IMAGING	
MRI with volumetrics	Hippocampal, cortical volume percentiles steady (or increasing)

NEWS

POLITICS U.S. NEWS BUSINESS WORLD TECH & MEDIA THINK SPORTS



What works, what doesn't: The truth about popular anti-aging products

Opinion | Research suggets the key to a longer life is simpler than we think

HEALTH NEWS

A pill to reverse aging? Enthusiasm outpaces reality

"There is financial incentive and inducement to overpromise before all the research is in."

"

If you say you're a terrific scientist and you have a treatment for aging, it gets a lot of attention.

"

They said I was 58, and then one or two blood tests later they said I was 31.4.

A combinatorial approach to provide personalized anti-aging therapy

THE STEADMAN CLINIC
STEADMAN PHILIPPON
RESEARCH INSTITUTE

U.S. OLYMPIC
NATIONAL
MEDICAL CENTER

Healthy Aging

Evidence Based Medicine / Patient Centered Care

Regenerative Medicine

Stem Cell

Banking

Stem Cell

Therapy

PRP

Exosomes

Drugs

Metformin

Rapamycin

Senolytics

Telomerase activity

Personalized Medicine

PERSONALIZED MEDICINE

PRECISIONMEDICINE ANEWERA

Diet

Caloric

Restriction

Intermittent Fasting

Ketogenic Diet

Lifestyle

Aerobic Exercise

Angiogenesis

Meditation

Yoga

Changing Lives by Keeping People Healthy/Active

Personal trainer for Healthy Aging

THE STEADMAN CLINIC AND STEADMAN PHILIPPON RESEARCH INSTITUTE

THE STEADMAN CLINIC AND STEADMAN PHILIPPON RESEARCH INSTITUTE

Center for Regenerative Sports Medicine
SPRI

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David Kuppersmith
Joel Matta

Marc Philippon

Dan Drawbaugh, Marc Philippon
Richard Steadman, Johnny Huard
In 2015

FINAL TIPS!

Thank you to my family for their support!

Why Happy Marriages And Relationships Are Key To Healthy And Positive Aging

02/28/2017 05:01 pm ET | **Updated** May 16, 2017

Happy Life-Happy Marriage-Healthy Aging