Aging biomarkers in multimorbidity patients

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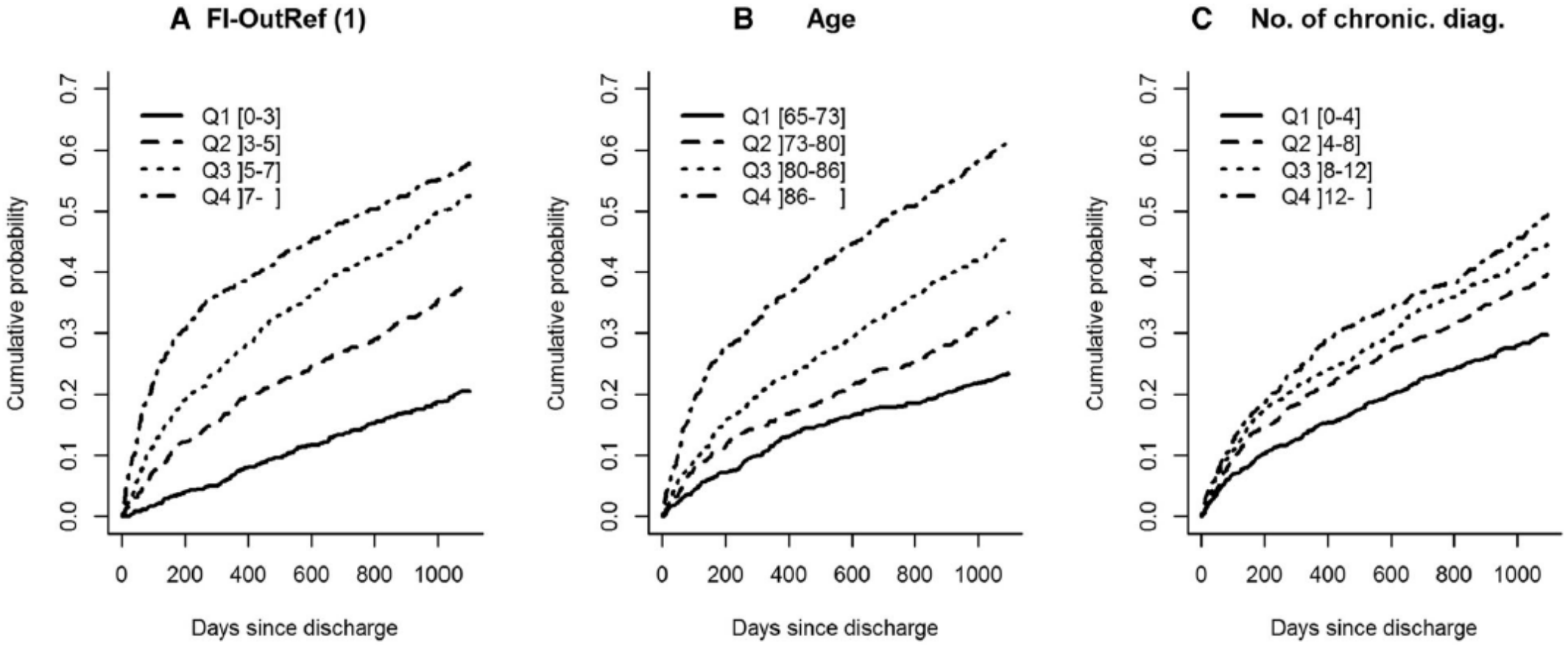
AIM

Introduce and implement prognostic biomarkers in routine clinical practice such as in emergency departments to predict short- or long-term all-cause mortality among acutely admitted patients

Cumulative incidence plots of mortality within 3 years post-discharge for 3172 patients without cancer diagnoses

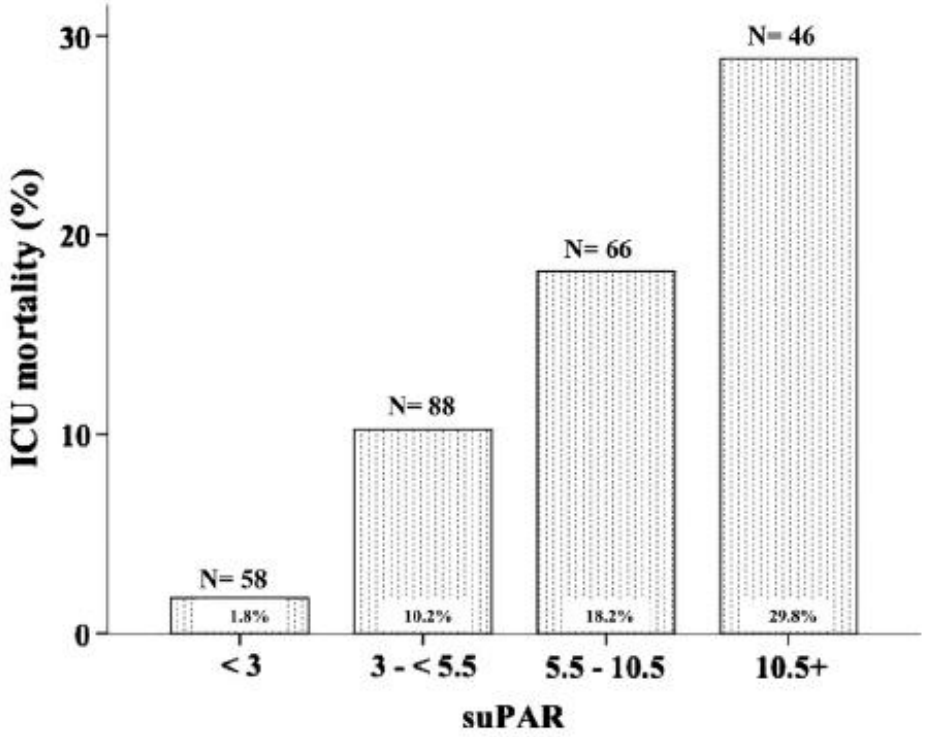


Klausen, HH et al. BMC Geriatrics (2017) 17:62

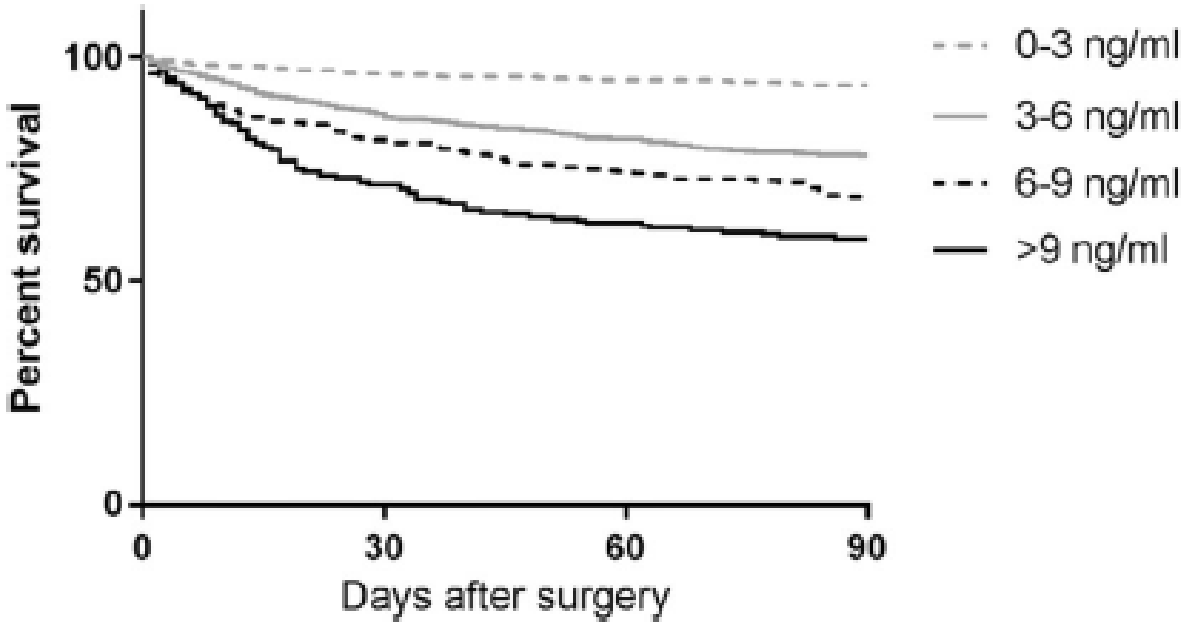
For acutely admitted patients, the emergency department performs a standard panel of laboratory tests

- C-reactive protein, leukocytes
- Differential blood count
- Hemoglobin
- Mean corpuscular hemoglobin concentration
- Mean corpuscular volume (MCV)
- Thrombocytes
- Creatinine
- Blood urea nitrogen (BUN)
- Sodium
- Potassium
- Albumin
- Alanine
- Aminotransferase
- Alkaline phosphatase
- Lactate dehydrogenase,
- Bilirubin
- Coagulation factors II, VII, and X
- (sUPAR)*

Intensive care unit mortality rates according to the 4 ranges of suPAR concentrations

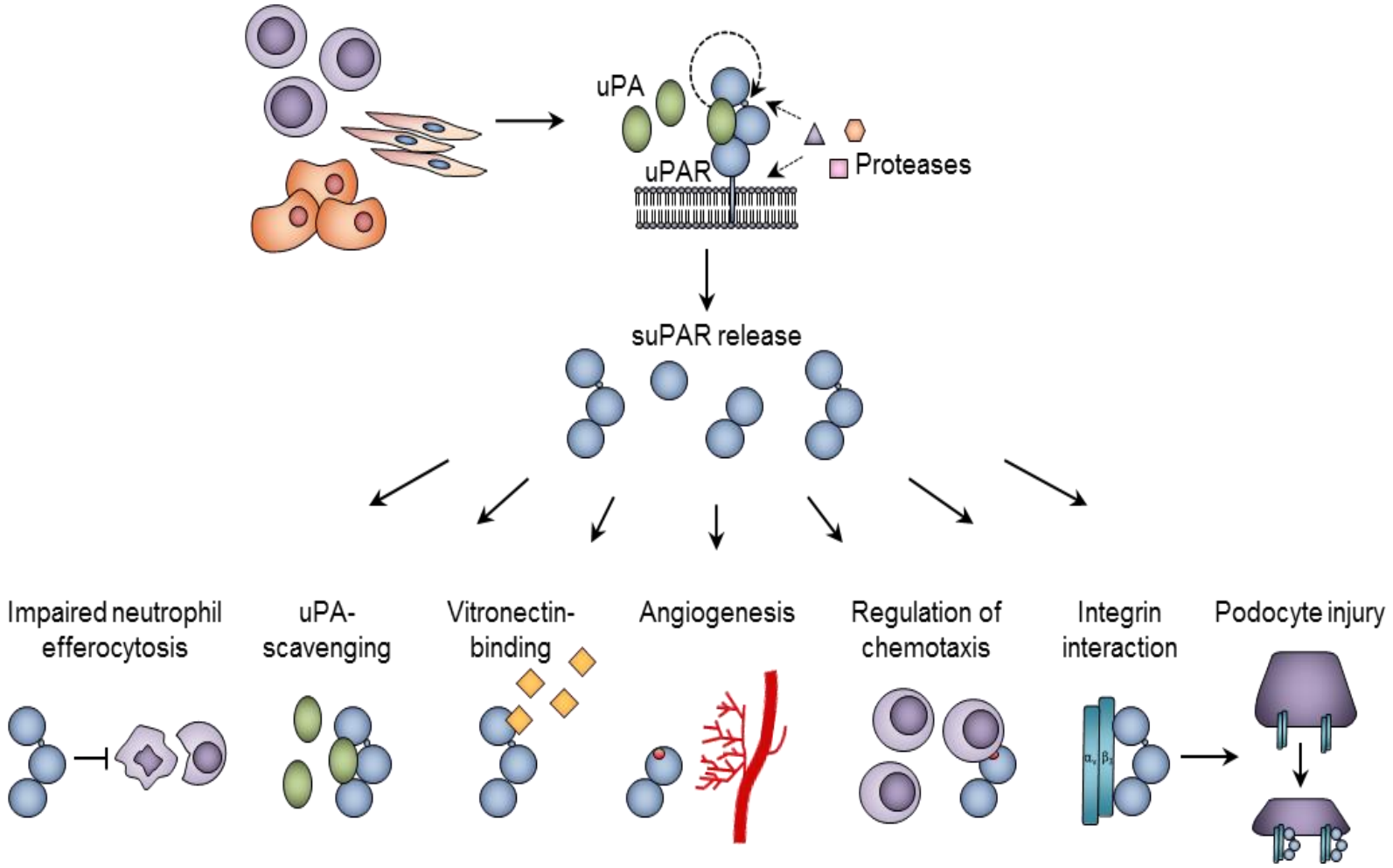


90-day post-operative mortality in acutely operated patients, stratified on suPAR concentrations

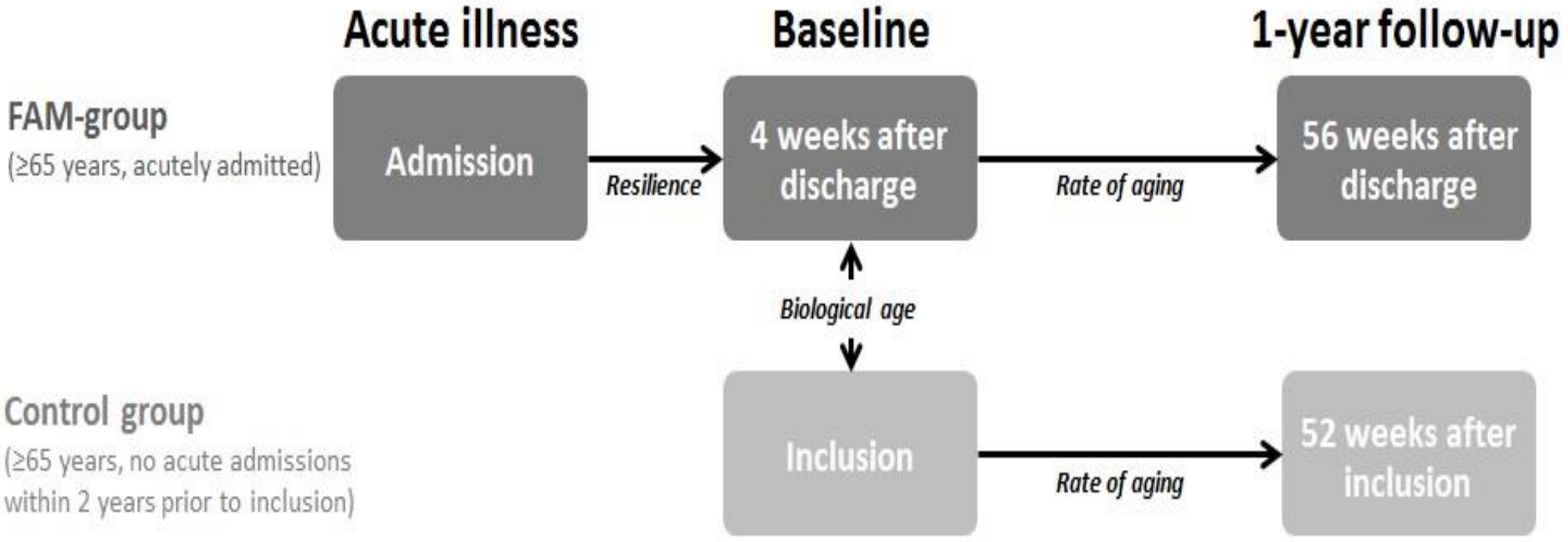


Donadello et al. (2014) Journal of Critical Care 29:144–149
Meyer, J. et al. (2018) Scand J Trauma Resusc Emerg Med 26:11

Targets of suPAR

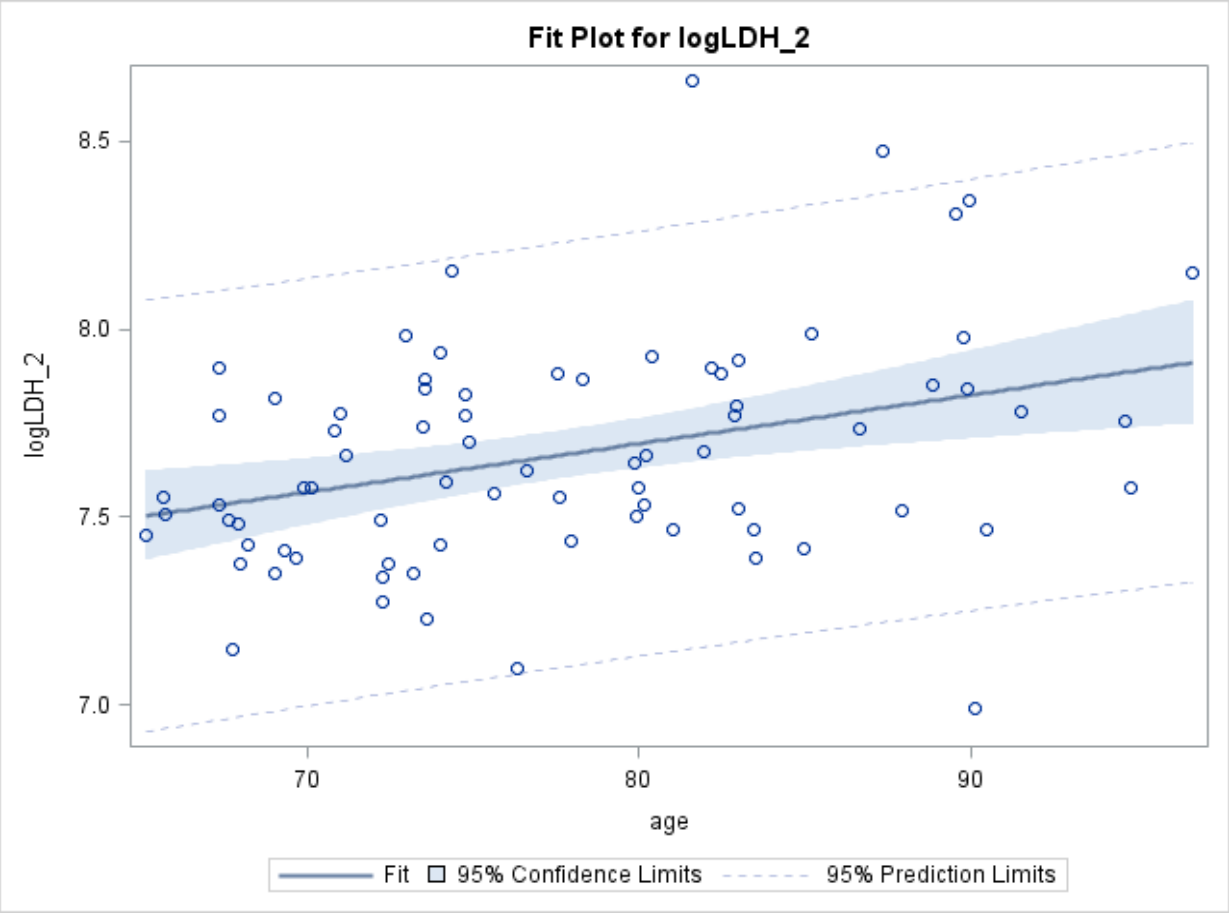


Design of the FAM-CPH cohort



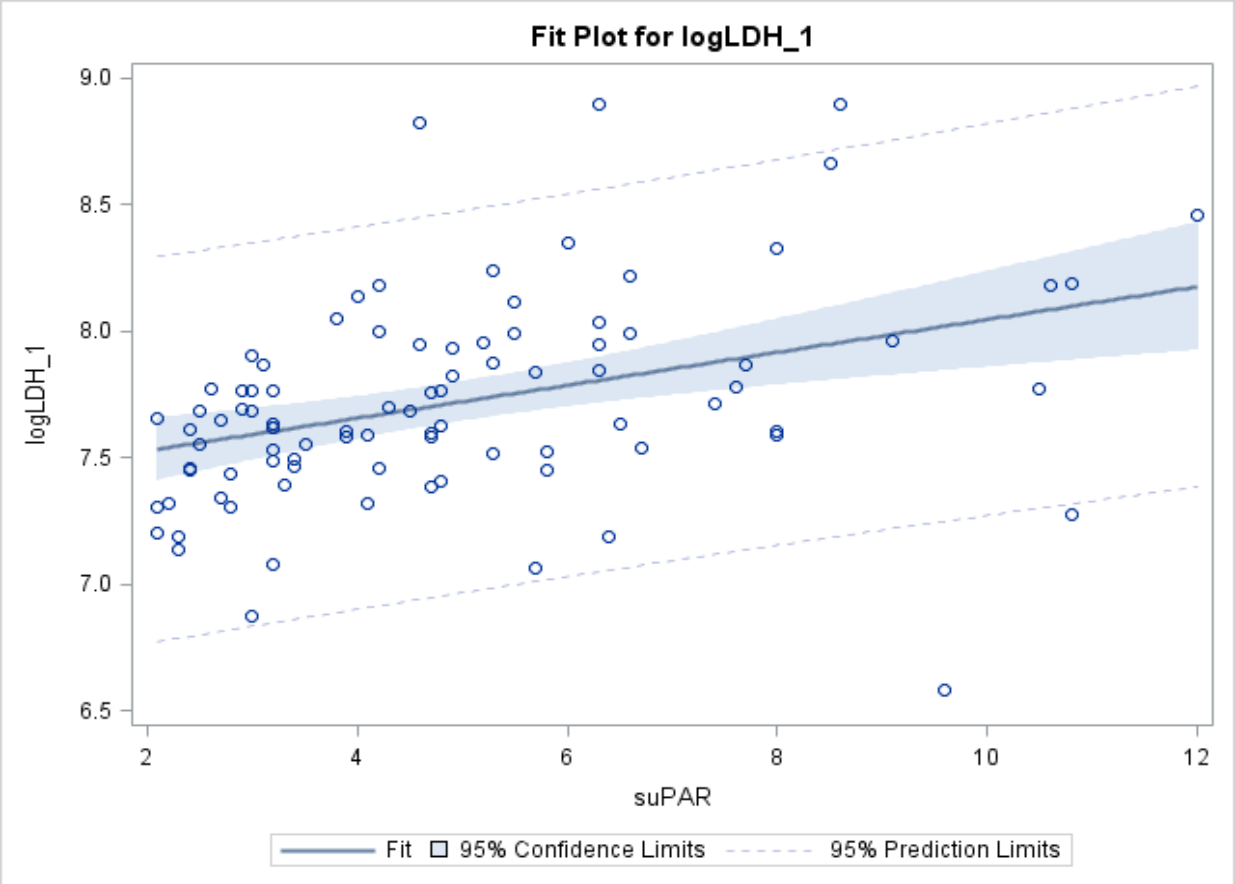
In dark grey, the FAM-group consists of acutely ill patients aged 65 and over (n=128). In light grey, the Control group consists of citizen matched 1:1 on age, sex and municipality with patients from the FAM-group, but with no recent acute hospital admission (n=54). In italics and arrows, the aspects of biological aging that we will investigate: resilience as the development in aging markers between acute illness and baseline, the biological age as the difference in aging markers between the groups at baseline, the rate of aging as the development in aging markers between baseline and the 1-year follow-up.

Plasma LDH levels 4 weeks after discharge and age



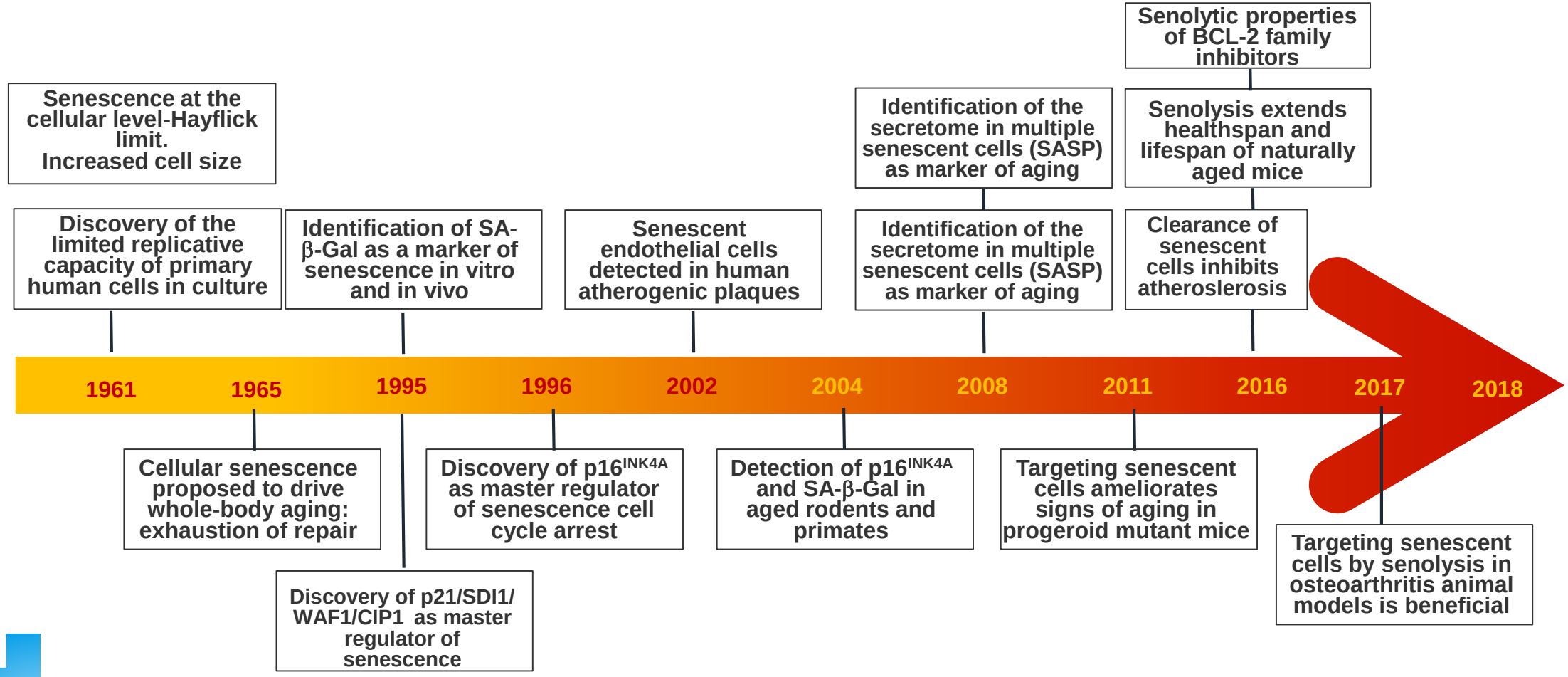
Plasma LDH at 4-weeks after discharge and age, n= 77 – significant: p = 0.0019

Plasma LDH and suPAR levels at admission



Plasma LDH and suPAR levels at admission, n = 91 – significant: p = 0.0003. The tissue-breakdown marker LDH (lactate dehydrogenase 1) [15] and the mortality marker suPAR (soluble urokinase-type plasminogen activator receptor) [16] are predictors of frailty and mortality.

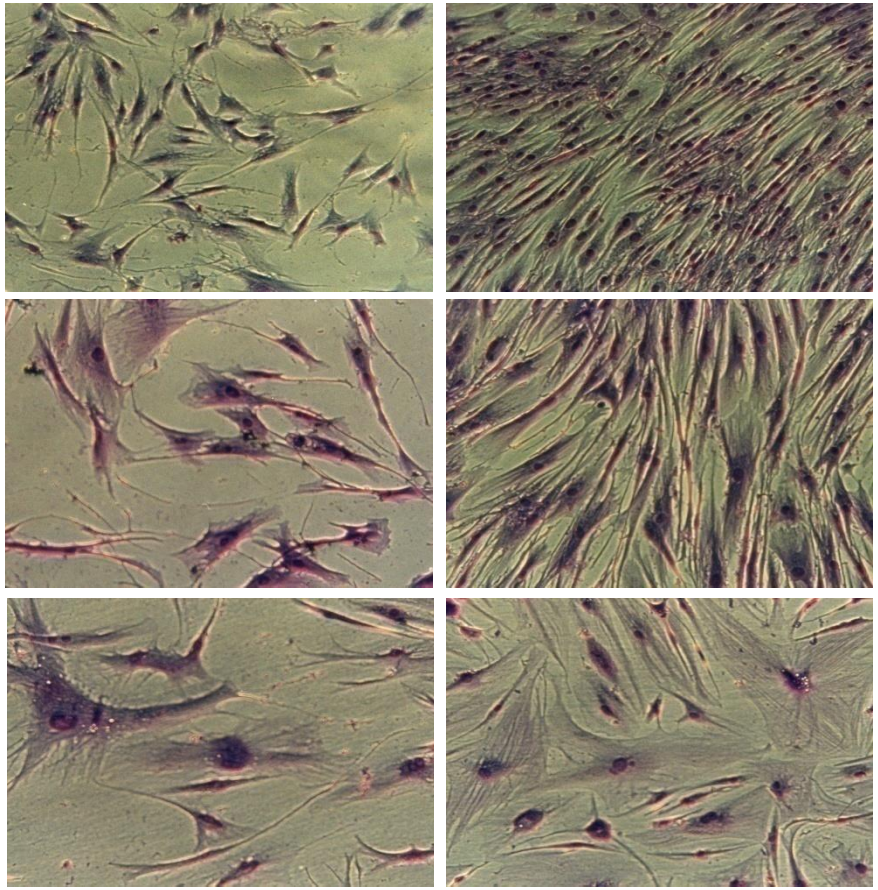
Timeline of milestones in aging biomarkers research



Continuous proliferation leads to aging of cells in culture

Replicative senescence

Increased cell size as biomarker of aging



Human skin fibroblasts

young: less than 30% lifespan completed

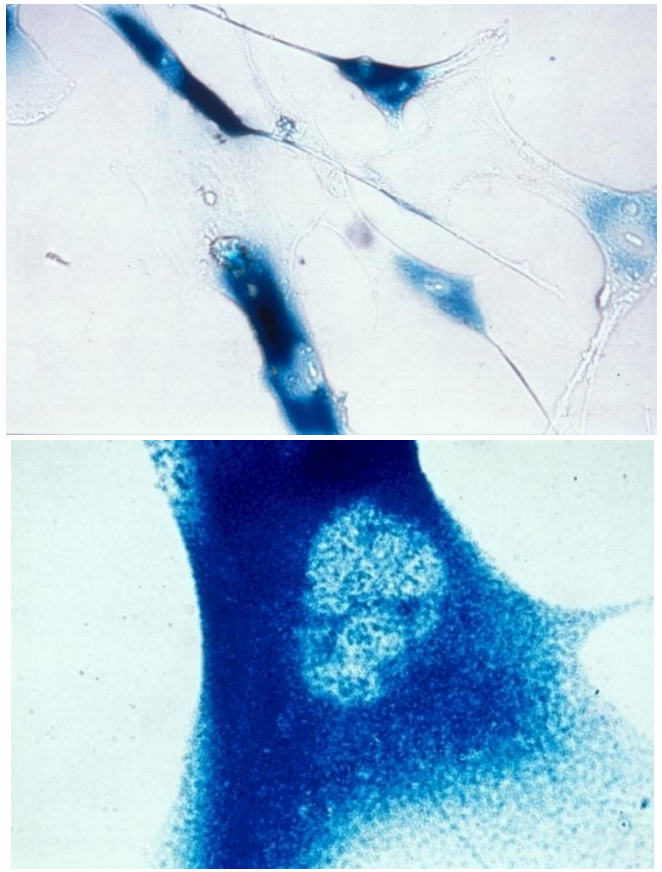
middle aged: between 60 and 80% lifespan completed

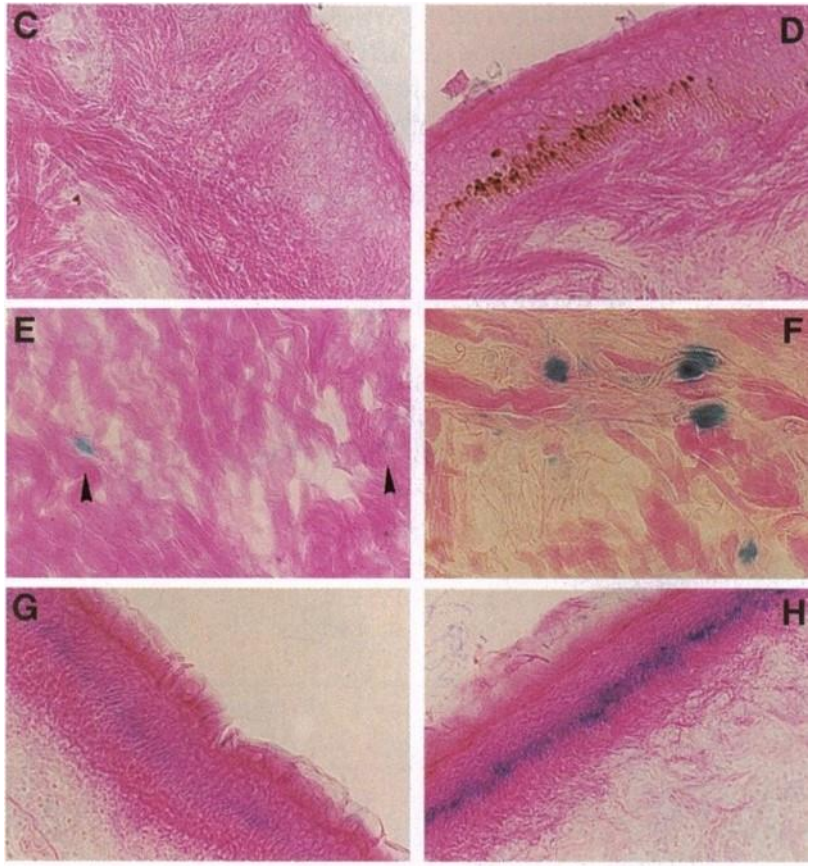
old: more than 95% lifespan completed

Senescence is the gradual and progressive slowing of cellular activity, including cell division, that occurs with aging. Cells lose the ability to divide over time

Hayflick, L. & Moorhead, P. S. Exp. Cell Res. 25, 585–621 (1961)

SA-βGal: First commonly used senescence biomarker



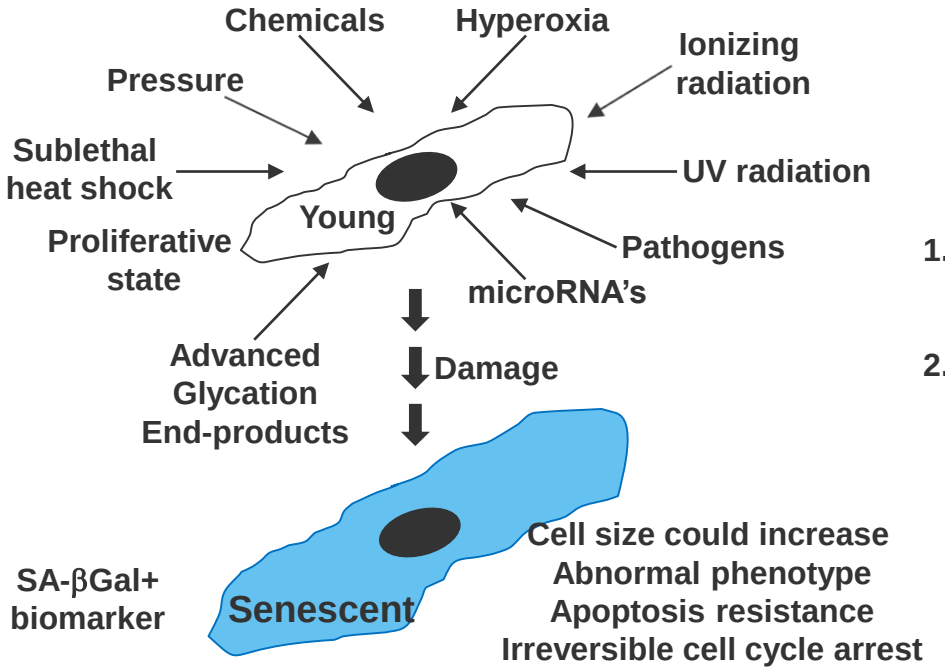


SA-βGal: Senescence-associated beta-galactosidase, a lysosomal marker that is specific for a pH 6.0 b-galactosidase

Dimri, GP et al (1995) Proc. Natl. Acad. Sci. USA 26;92(20):9363-7
Childs, BG et al (2017) Nat Rev Drugs Disc 16:718-

Causes of aging of cells in cell culture

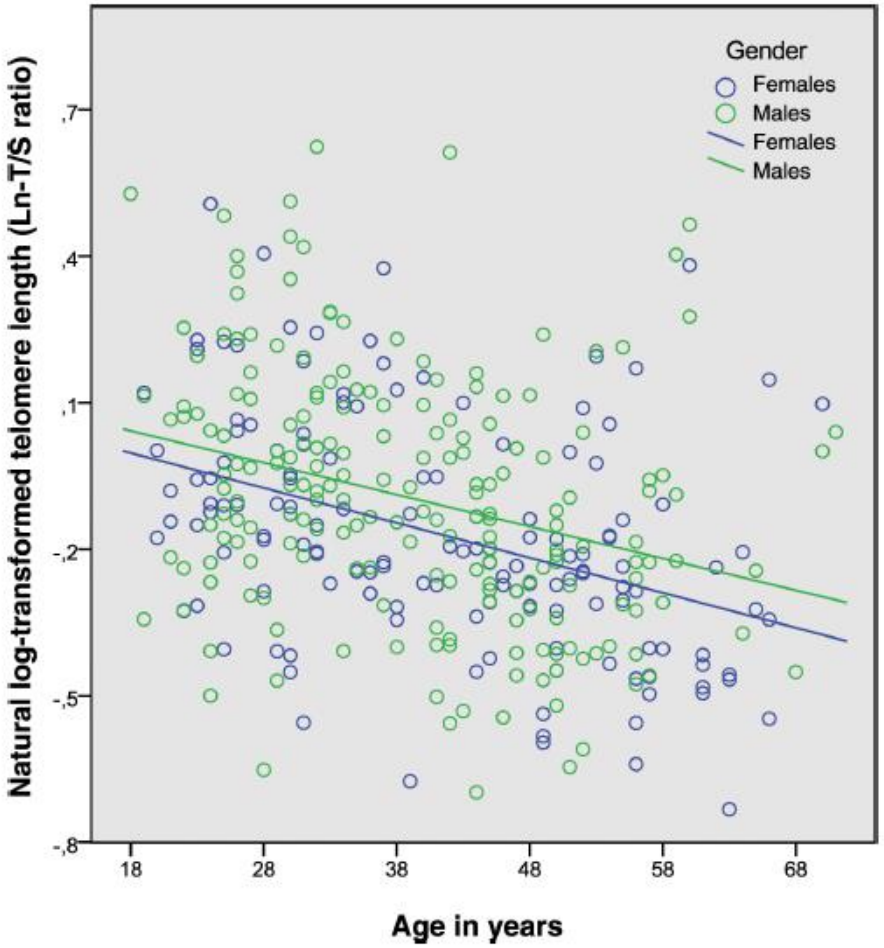
1. Replicative senescence
2. Stress-induced premature senescence (SIPS)
3. Oncogene-induced senescence (OIS)
4. Other….



1. The effects depend on dose and time of exposure
2. The SIPS cellular expression profiles are different from those of replicatively senescent cells

Hayflick, L & Moorhead, PS (1961) Exp Cell Res. 25:585-621.
Shay, J. (2000) Nat Rev Mol Cell Biol. 1(1):72-6.

Association of telomere length of peripheral blood leukocytes with age

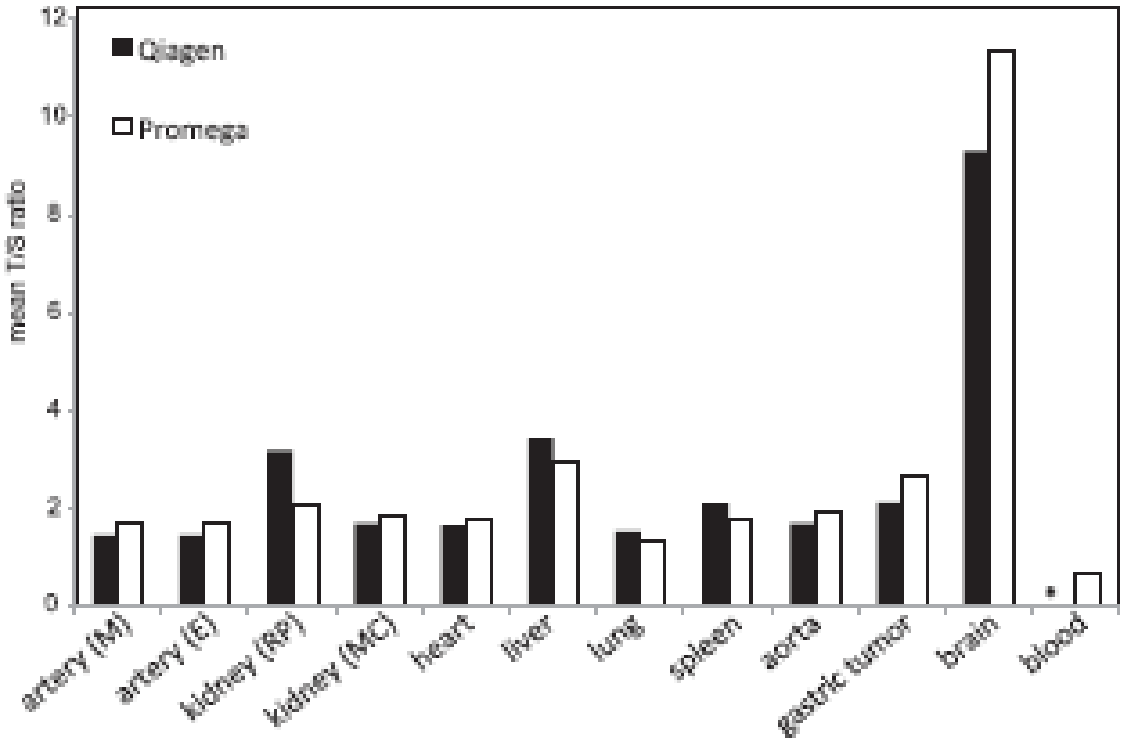


Telomere length reduction in comparison with chronological age

Age and natural log-transformed telomere length, males and females, n = 343. Regression lines for men (blue circles) and women (green circles)

Neuner, B et al. (2015) PLoS One. 10(10):e0139308 + many other refs.

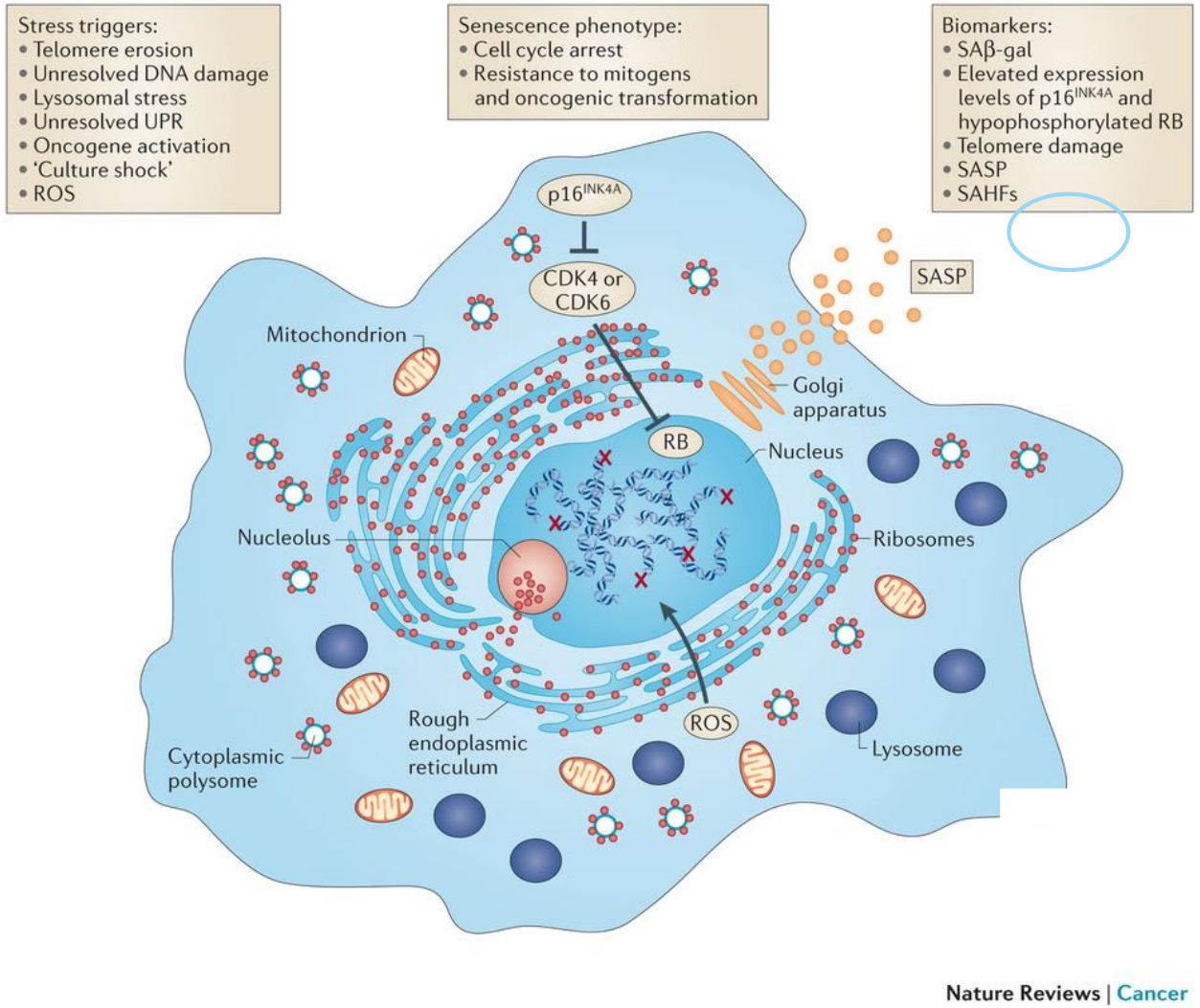
Mean telomere length of 115 year old tissues



- Telomere lengths of healthy white blood cells were significantly shorter than in cells from other tissues
- The finite lifespan of hematopoietic stem cells may lead to hematopoietic clonal evolution at extreme ages.

Holstege, H. et al. (2014) Genome Research 24, 733-742.

Senescence-associated secretory phenotype: SASP



The Senescence-associated Secretory phenotype (SASP) consists of several secreted cytokines and chemokines that are involved in promoting a pro-inflammatory state

Childs, BG et al (2015) Nat Med. 21(12):1424-35; Serrano, M (2015) Trends Cell Biol. 25(11):634-6; Munoz-Espin, D & Serrano, M (2014) Nat Rev Mol Cell Biol. 15(7):482-96; Zhu, Y et al. (2015) Aging Cell. 14(4):644-58; Sharpless, NE & Sherr, CJ (2015) Nat Rev Cancer. 15(7):397-408

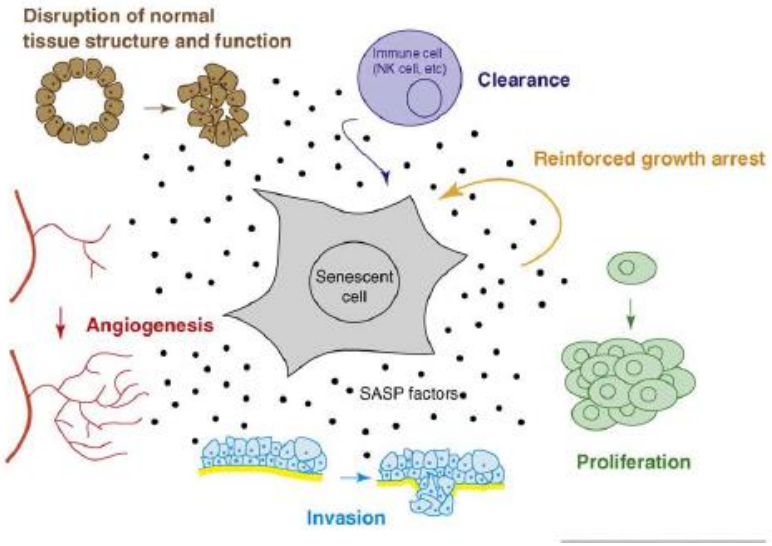
Secretome from senescent cells (SASP)

High increase (4+ fold)

Factor	Senescence inducer		Cell type	Reference
GM-CSF	OIS (RAS, MEK), DDIS (XRA), REP	+	IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3	Acosta, Coppe
GROα	OIS (RAS, MEK), DDIS (XRA, BLEO), REP	+	IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3, prostate fibroblasts (PSC27, PSC31, and PSC32)	Acosta, Coppe, Bavik
GROα,β,γ	OIS (RAS, MEK), DDIS (XRA), REP	+	IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3	Acosta, Rodier, Coppe
IGFBP-7	OIS (BRAF)	+	melanocytes	Wajapeyee
IL-1α	OIS (RAS, MEK), DDIS (XRA, BLEO)	+	IMR-90, HCA2, PrECs, BPH1, RWPE1, PC3	Acosta, Coppe, Liu
IL-6	OIS (RAS, MEK), DDIS (XRA), REP	+	IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3	Acosta, Rodier, Coppe
IL-7	OIS (RAS), DDIS (XRA), REP	+	IMR-90, HCA2, WI-38, BJ	Coppe
IL-8	OIS (RAS, MEK), DDIS (XRA, BLEO), REP	+	IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3, prostate fibroblasts (PSC27, PSC31, and PSC32)	Acosta, Rodier, Coppe, Bavik
MCP-1	OIS (RAS, MEK), DDIS (XRA), REP	+	IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3	Acosta, Rodier, Coppe, Liu
MCP-2	OIS (RAS), DDIS (XRA), REP	+	IMR-90, HCA2, WI-38, BJ	Coppe
MIP-1α	OIS (RAS), DDIS (XRA), REP	+	IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3	Coppe
MMP-1	OIS (RAS), DDIS (XRA, BLEO), REP		IMR-90, HCA2, WI-38, BJ	Coppe, Liu
MMP-10	OIS (RAS), DDIS (XRA, BLEO, ETOP), REP		IMR-90, HCA2, WI-38, BJ, hepatic myofibroblasts	Coppe, Krizhanovsky
MMP-3	OIS (RAS), DDIS (XRA, BLEO), REP		IMR-90, HCA2, WI-38, BJ	Coppe, Liu, Parrinello

Intermediate increase (2-4 fold)

Factor	Senescence inducer		Cell type	Reference
Amphiregulin	OIS (RAS), DDIS (XRA, BLEO)		PrECs, BPH1, RWPE1, PC3, prostate fibroblasts (PSC27, PSC31, and PSC32)	Coppe, Bavik
ENA-78	OIS (RAS, MEK), DDIS (XRA)	+	IMR-90, PrECs, BPH1, PC3	Acosta, Coppe
Eotaxin-3	OIS (RAS), DDIS (XRA), REP	+	IMR-90, HCA2	Coppe
GCP-2	OIS (RAS), DDIS (XRA)	+	HCA2, PrECs, RWPE1, PC3	Coppe
GITR	OIS (RAS), DDIS (XRA)		HCA2, PrECs, BPH1, RWPE1, PC3	Coppe
HGF	OIS (RAS), DDIS (XRA, BLEO), REP		IMR-90, HCA2, WI-38, BJ, prostate fibroblasts (PSC27, PSC31, and PSC32)	Coppe, Bavik, Liu
ICAM-1	OIS (RAS), DDIS (XRA), REP		IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3	Rodier, Coppe
IGFBP-2	DDIS (XRA, BLEO), REP	+	IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3, prostate fibroblasts (PSC27, PSC31, and PSC32)	Rodier, Coppe, Bavik
IGFBP-4	OIS (RAS), DDIS (XRA), REP	+	IMR-90, HCA2, WI-38, BJ	Coppe
IGFBP-5	DDIS (BLEO), REP	+	prostate fibroblasts (PSC27, PSC31, and PSC32)	Bavik
IGFBP-6	OIS (RAS), DDIS (XRA), REP	+	IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3	Coppe
IL-13	OIS (RAS), DDIS (XRA), REP	+	IMR-90, WI-38	Coppe
IL-1β	OIS (RAS), DDIS (XRA, BLEO), REP	+	IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3	Coppe, Liu
MCP-4	OIS (RAS, MEK), DDIS (XRA), REP	+	IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3	Acosta, Coppe
MIF	OIS (RAS), DDIS (XRA), REP		IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3	Coppe
MIP-3α	OIS (RAS, MEK), DDIS (XRA), REP	+	IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3	Acosta, Coppe
MMP-12	DDIS (XRA, ETOP)		IMR-90, HCA2, WI-38, BJ, hepatic myofibroblasts	Coppe, Krizhanovsky
MMP-13	DDIS (XRA, ETOP)		IMR-90, HCA2, WI-38, BJ, hepatic myofibroblasts	Coppe, Krizhanovsky
MMP-14	DDIS (XRA)		IMR-90, HCA2, WI-38, BJ	Coppe
NAP2	OIS (MEK)		IMR-90	Acosta
Oncostatin M	OIS (MEK), DDIS (XRA)		IMR-90, WI-38	Acosta, Coppe
Osteoprotegerin	OIS (RAS), DDIS (XRA), REP		IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3	Rodier, Coppe
PIGF	OIS (RAS), DDIS (XRA), REP		IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3	Coppe
RANTES	DDIS (BLEO)		HCA2	Liu
sgp130	OIS (RAS), DDIS (XRA), REP		IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3	Rodier, Coppe
TIMP-2	OIS (RAS), DDIS (XRA), REP		IMR-90, HCA2, WI-38, BJ, PrECs, BPH1, RWPE1, PC3	Rodier, Coppe
TRAIL-R3	DDIS (XRA), REP		IMR-90, HCA2, WI-38, BJ	Rodier, Coppe



Senescence-messaging secretome (SMS)
Senescence-associated secretory phenotype (SASP)

Kuilman, T & Peeper, DS (2009) Nat Rev Cancer. ;9(2):81-94
Freund, A et al (2010) Trends Mol Med. 16(5):238-46.

In vitro cellular senescence biomarkers

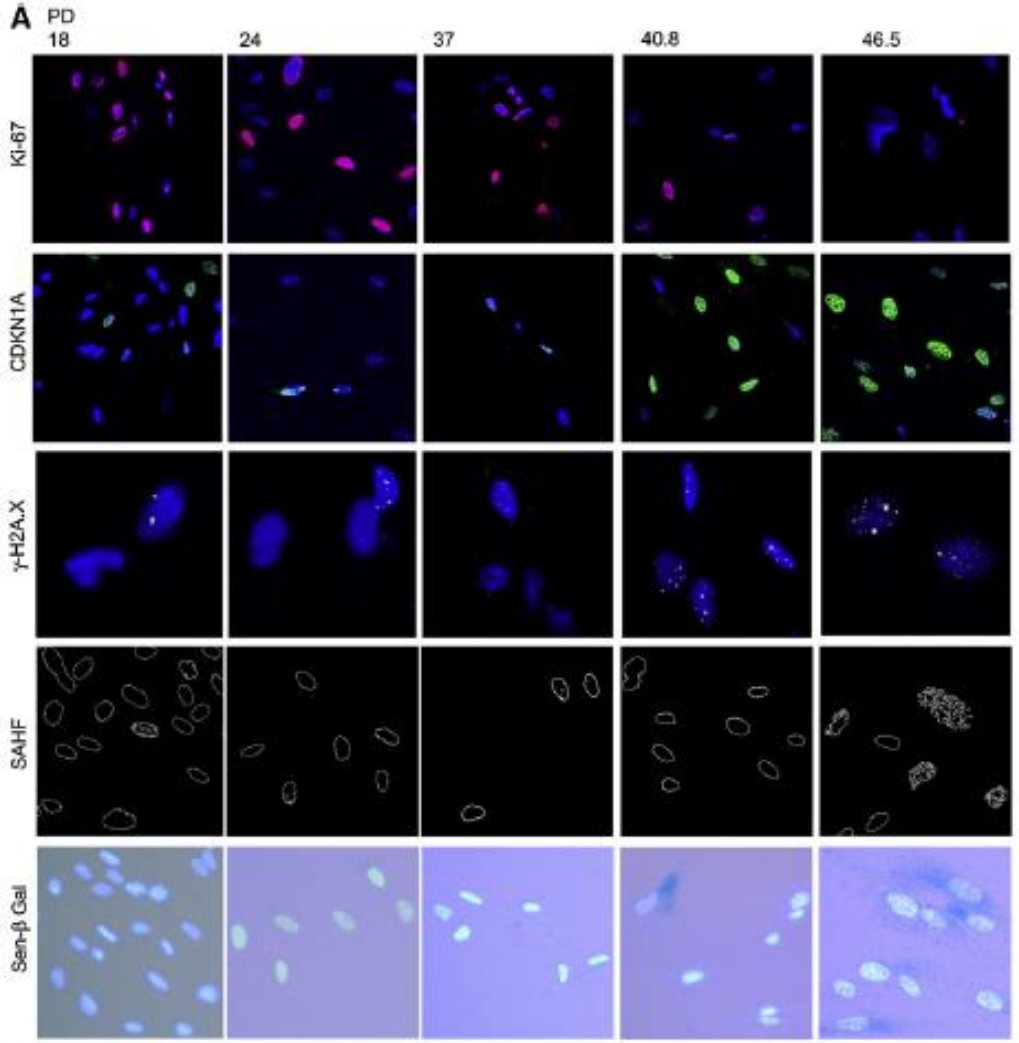
- SA-β-Gal
- p53
- p21
- $p16^{INK4a}$
- $p14^{ARF}$
- $p15^{INK4b}$
- DPP4
- Lipofuscin
- H2AX (SAHF)
- DEC1/DEC2
- DCR2
- H1/macroH2A/H3.3/H3metLys9
- Asf1a/HIRA
- HP1/HMGA
- SASP/SMS *(IL6/IL8)*
- Telomere-associated DNA damage foci(TAF)/DDR

DDR: DNA-damage response; SA-β-Gal: senescence-associated β-Galactosidase; SAHF: senescence-associated heterochromatin foci; SASP: senescence-associated secretory phenotype; SMS: senescence-messaging secretome.

Collado, M & Serrano, M (2006) Nat Rev Cancer. 6(6):472-6; Bernardes de Jesus, B & Blasco, M (2012) Circ Res. 111(1):97-109; Jurk, D. et al (2014) Nat Commun. 2:4172
Nehlin, J (2016) *Biomarkers of replicative senescence revisited. In: Cellular Ageing and Replicative Senescence*. Springer, ISBN 978-3-319-26237-6

Intracellular senescence biomarkers

Staining examples
(DAPI: counterstain)



Ki-67
Proliferation marker

p21

H2AX

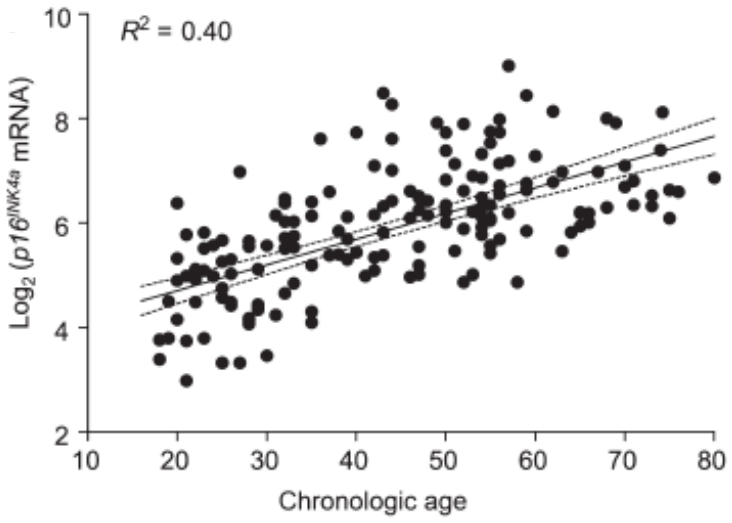
SAHF

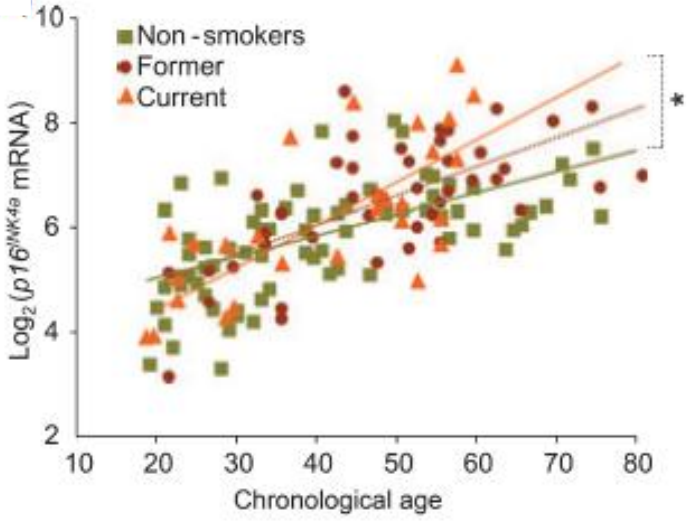
SAβG

Lawless, C et al (2010) Exp Gerontol. 45(10):772-8

Expression of p16^{INK4a} in peripheral blood T lymphocytes is a biomarker of human aging

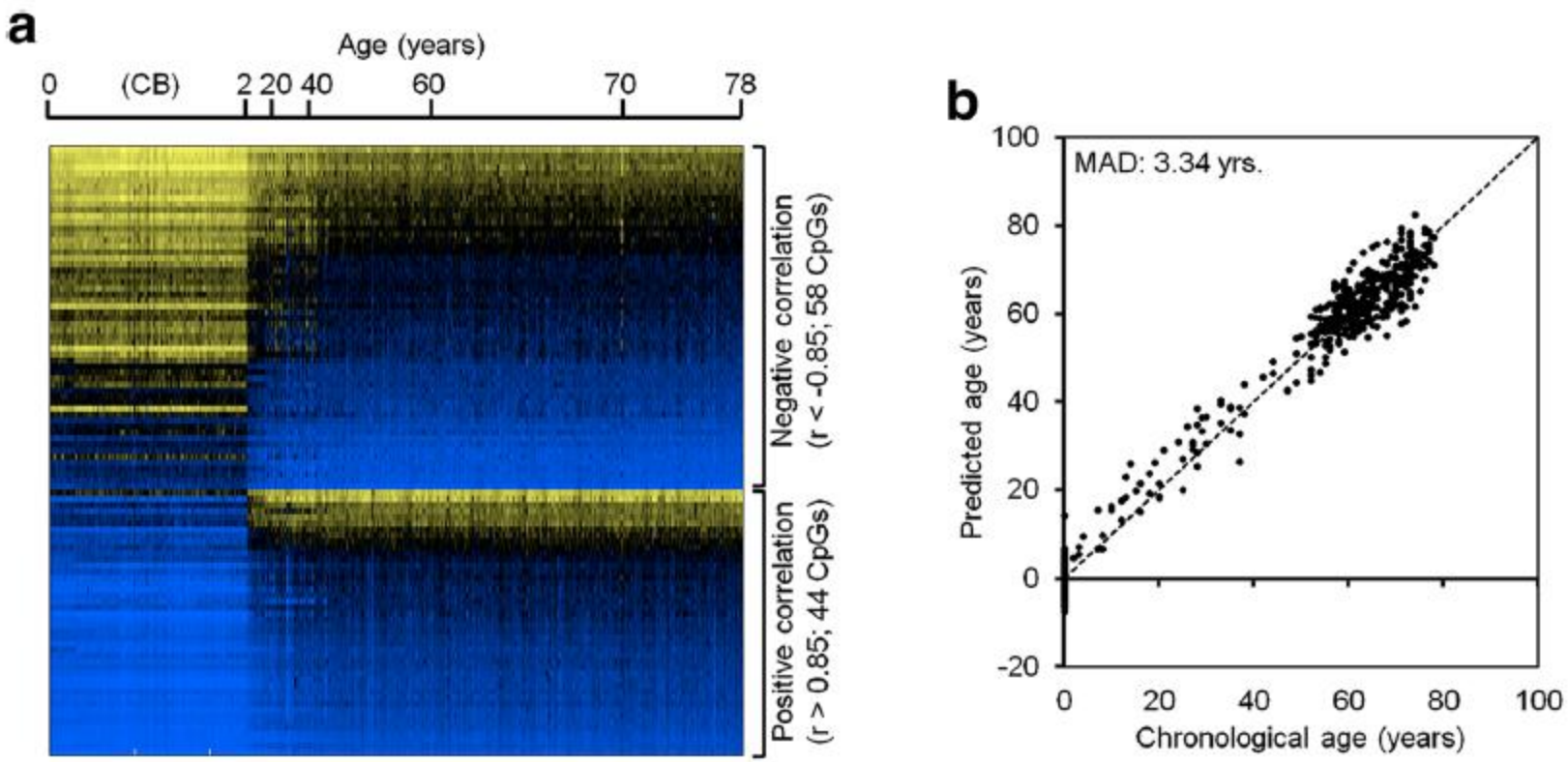
- Analyses of p16^{INK4a} expression from human whole blood showed the highest expression in peripheral blood T lymphocytes (PBTL)
- Expression of p16^{INK4a}, but not other INK4/ARF transcripts, appeared to exponentially increase with donor chronologic age (170 donors). Importantly, p16^{INK4a} expression did not independently correlate with gender or body mass index, but was significantly associated with tobacco use and physical inactivity.
- p16^{INK4a} expression was associated with plasma interleukin-6 concentration, a marker of human frailty.
- p16^{INK4a} expression in PBTL is an easily measured, peripheral blood biomarker of molecular age.





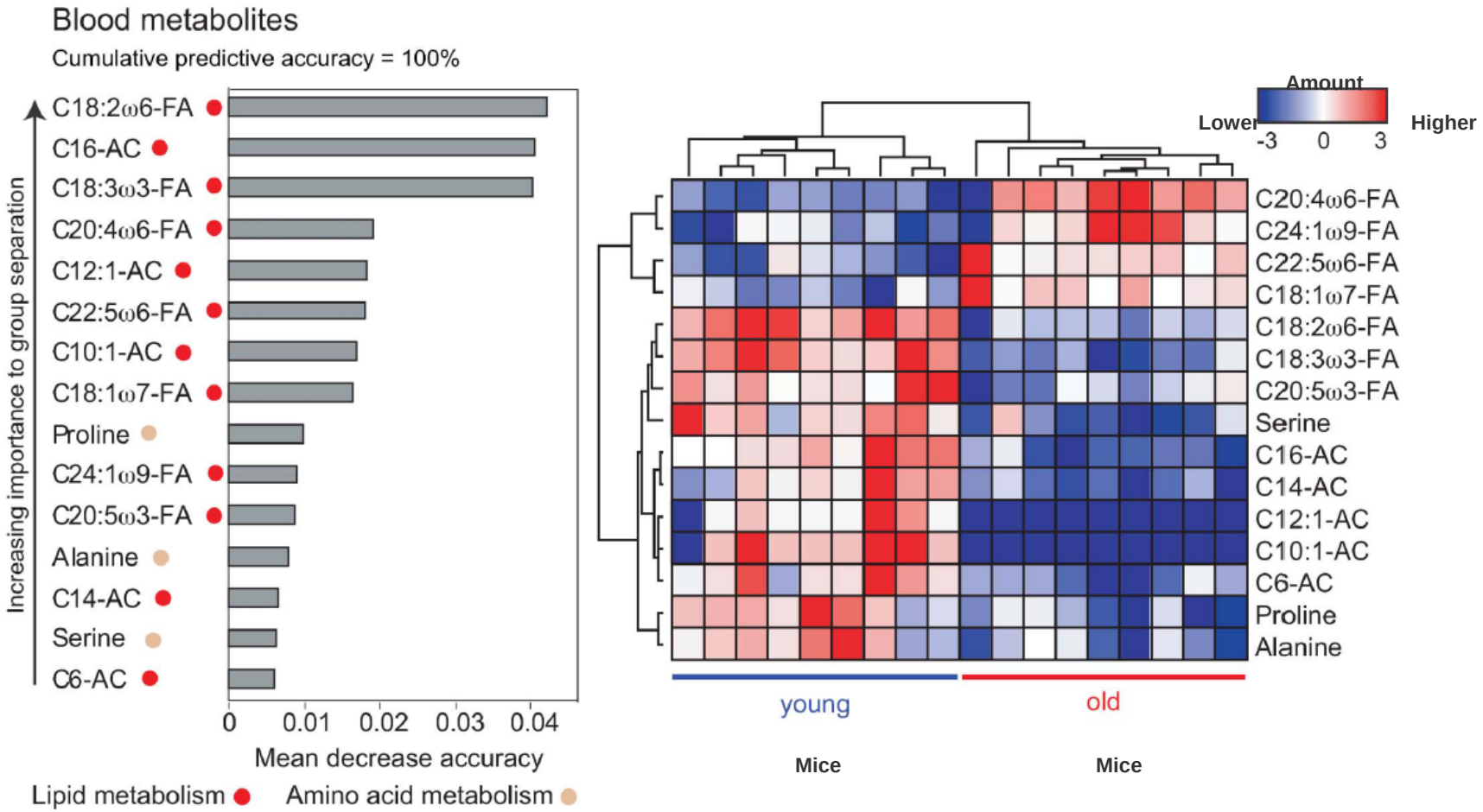
Liu, Y et al (2009) Aging Cell. 8(4):439-48.

DNA methylation is an epigenetic marker of age that is a reliable predictor of biological aging



Garagnani , P et al. (2012) Aging Cell. 11(6):1132-4
Weidner , CI et al. (2014) Genome Biol. 15(2):R24.
Lin, Q et al (2016) Aging (Albany NY). 8(2):394-401.

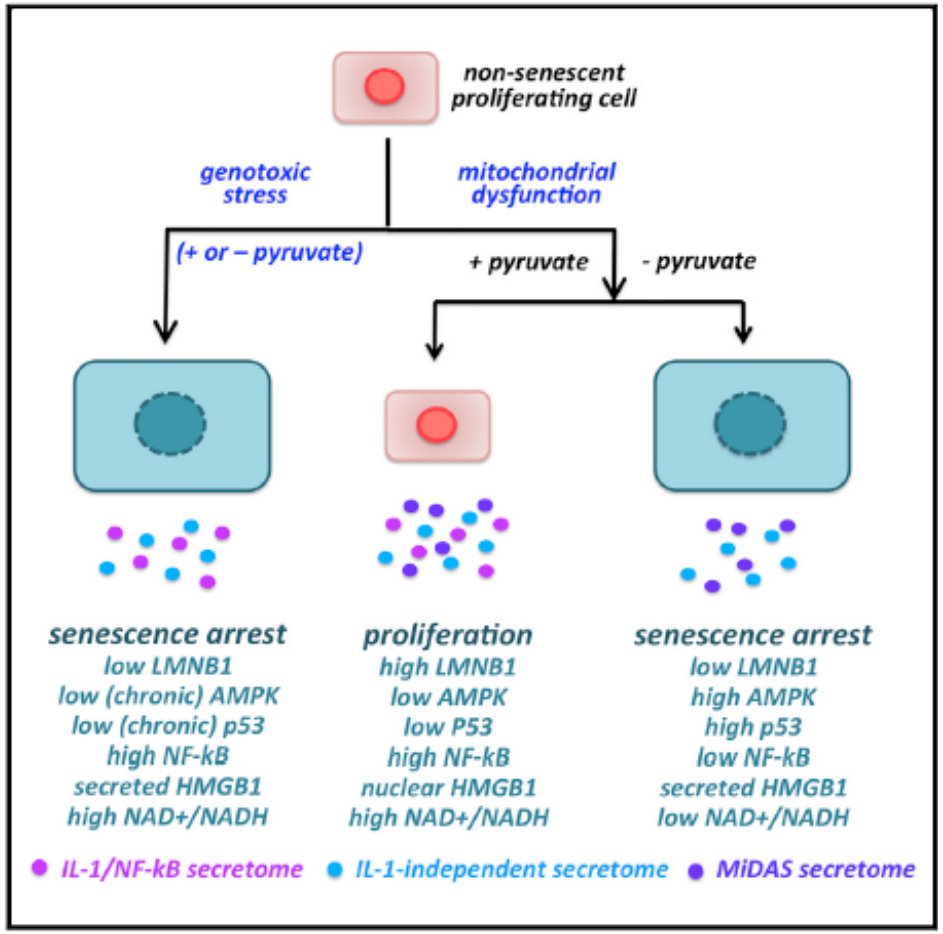
Aging metabolome: Long-chain fatty acids as blood biomarkers of aging



Metabolite and pathway enrichment of targeted metabolomics and microarrays

Houtkooper, RH et al. (2011) Sci Rep. 1:134

Mitochondrial Dysfunction Induces Senescence with a Distinct Secretory Phenotype (mitSASP)

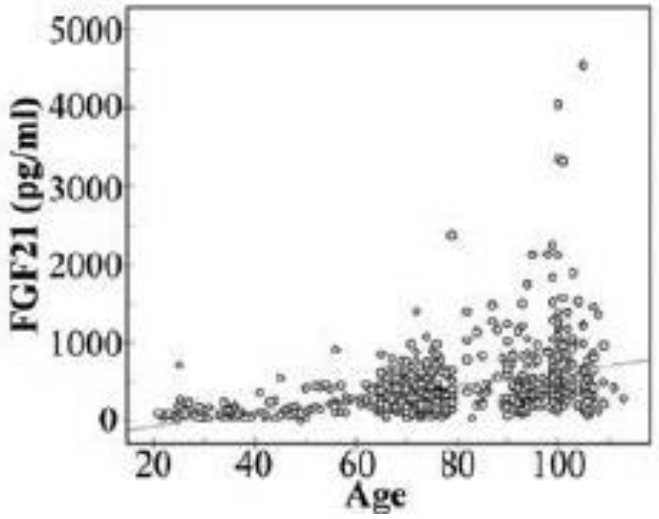


- **Dysfunctional mitochondria cause cell senescence and a distinct secretory phenotype (MiDAS: Mitochondria Dysfunction Associated Senescence)**
- **This secretory phenotype can influence the differentiation of certain cell types**
- **An NAD-AMPK-p53 pathway controls the secretory and mitotic arrest phenotypes**
- **Mice with dysfunctional mitochondria and premature aging accumulate senescent cells**

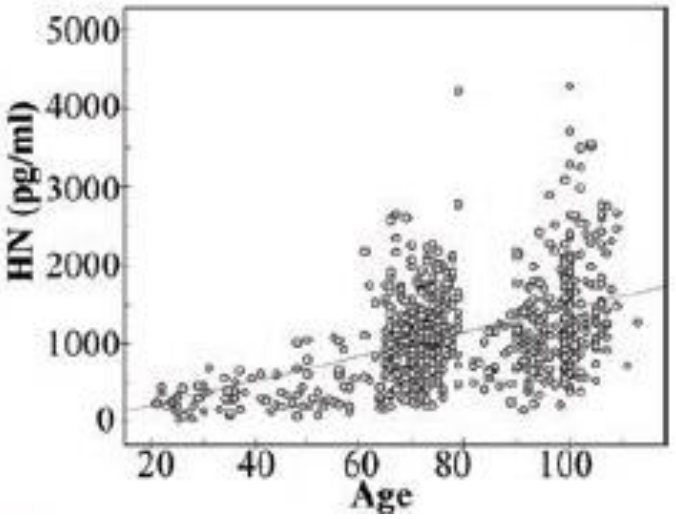
Wiley, CD et al (2016) Cell Metab. 23(2):303-14

Human aging and longevity are characterized by high levels of mitokines

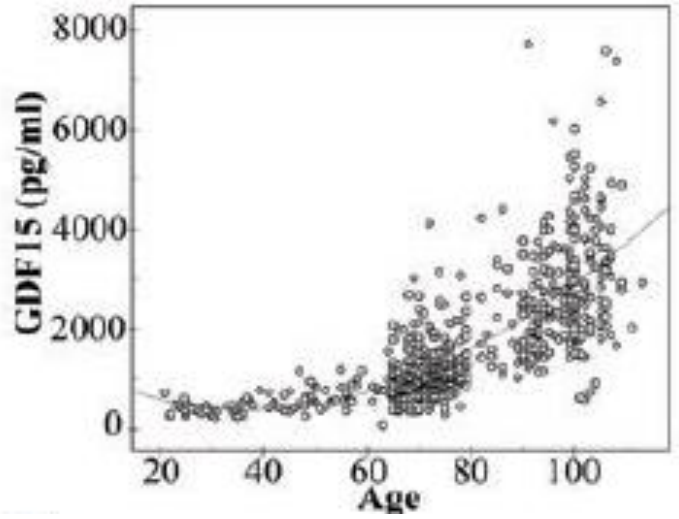
FGF-21



Humanin



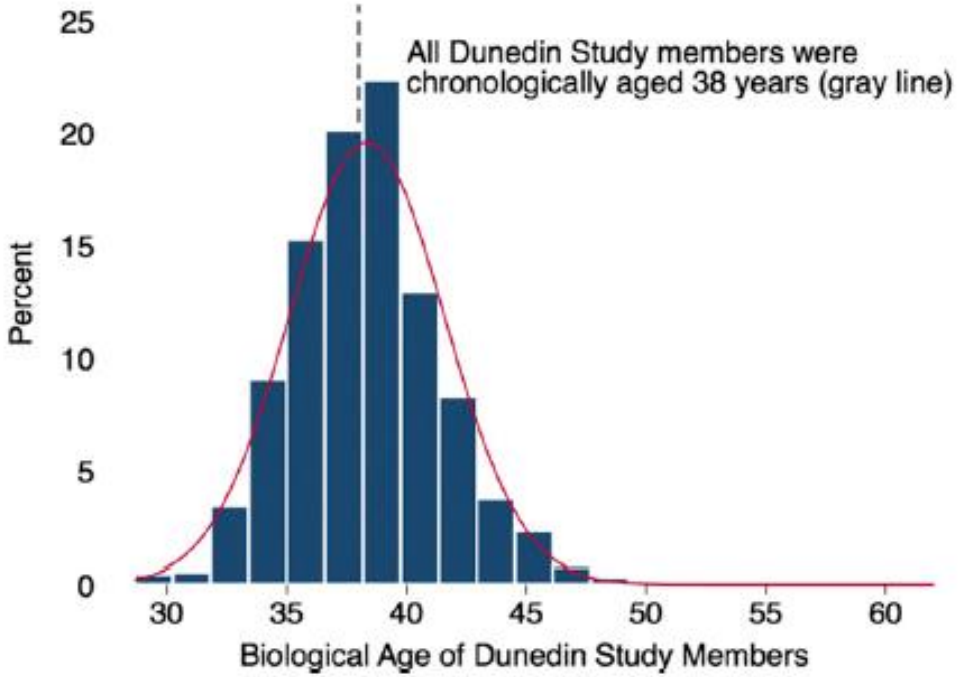
GDF-15

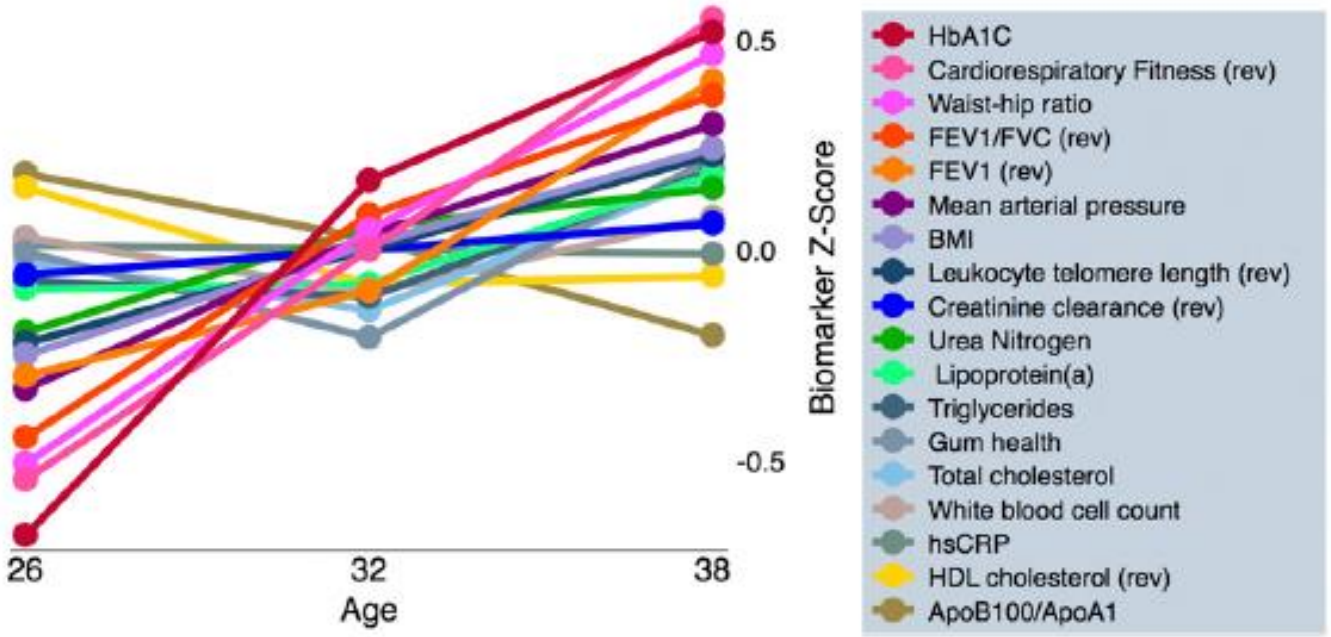


The mitokines are secreted in response to mitochondrial stress and are associated with worsened parameters (such as handgrip strength, insulin sensitivity, triglycerides), particularly in 70-year-old persons, and their levels are inversely correlated with survival in the oldest subjects.

Conte, M. et al. (2018) *Gerontol A Biol Sci Med Sci*, 2018, Vol. XX, No. XX, 1–8

Quantification of biological aging in young adults

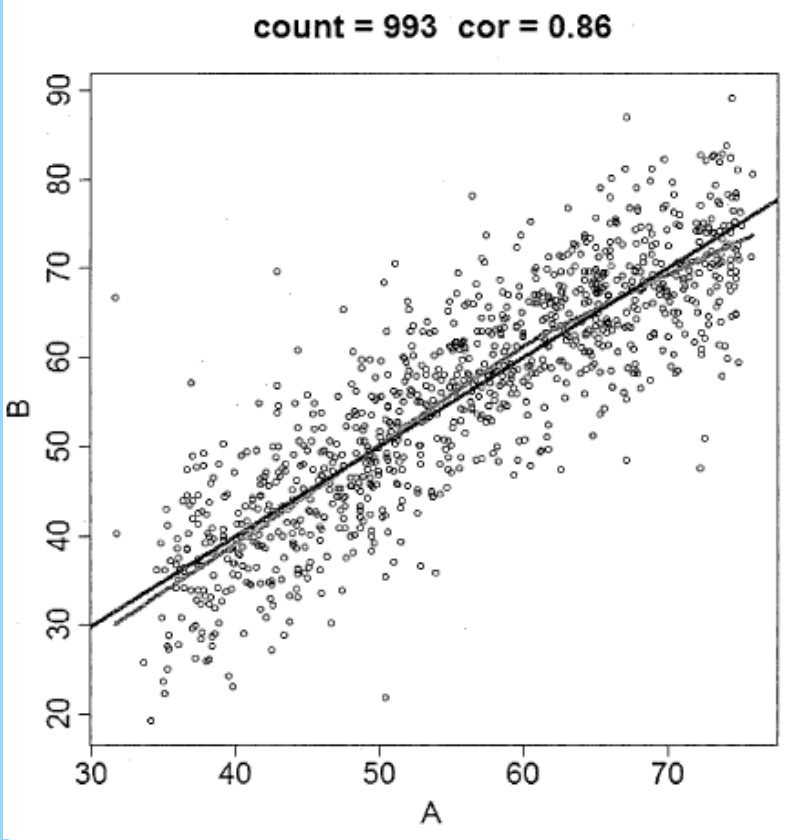




The science of healthspan extension may be focused on the wrong end of the lifespan; rather than only studying old humans, geroscience should also study the young

Various proposed approaches to quantifying biological aging may not measure the same aspects of the aging process.

Belsky, DW et al (2015) Proc Natl Acad Sci U S A. 112(30):E4104-10
Belsky, DW et al (2018) Am J Epidemiol. 187(6):1220-1230.



BioAge: Method for the determination of biological age in humans

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MARK-AGE biomarkers of ageing

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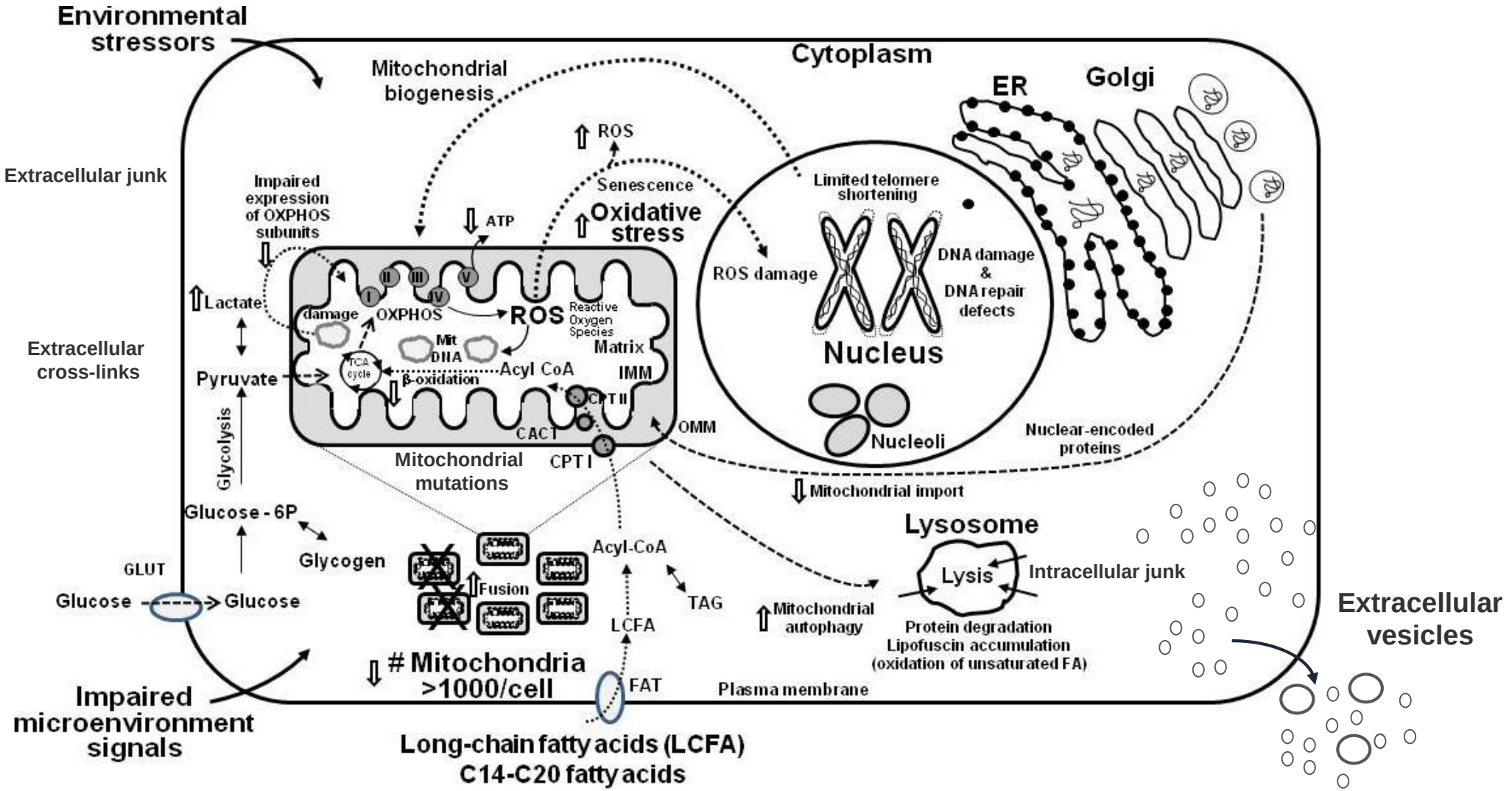
Causes of senescence and biomarker validation



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Thank you!