Cellular Senescence, Senolytics, and Organ Regeneration and Transplantation

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Understanding the Role of the Immune System in Improving Tissue Regeneration: A Workshop
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Unitary Theory of Fundamental Aging Mechanisms

Fundamental Aging Mechanisms

Inflammation (chronic, low-grade, sterile), Fibrosis

Macromolecular/Organelle Dysfunction (DNA, protein aggregates, autophagy, AGEs, lipotoxicity, mitochondria)

Stem Cell and Progenitor Dysfunction

Cellular Senescence

Phenotypes

Geriatric Syndromes:
Sarcopenia
Frailty
Immobility
MCI

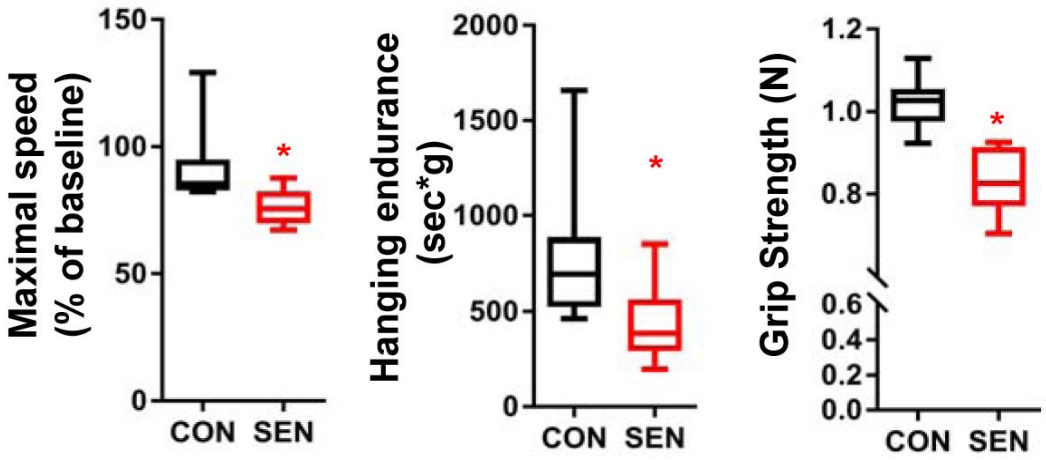
Chronic Diseases:
Dementias
Cancers
Atherosclerosis
Diabetes
Osteoporosis
Osteoarthritis
Renal dysfunction
Blindness
Chronic lung disease

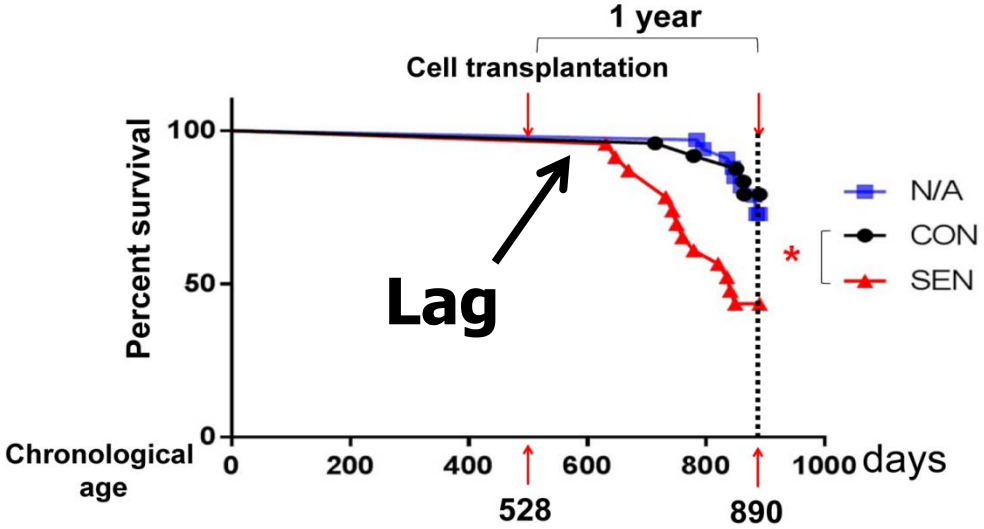
Decreased Resilience:
Infections
Delirium
Delayed wound healing
Slow rehabilitation
Chemotherapy toxicity
ICU Care

Acute, "Beneficial" *vs.* Persistent Deleterious Senescent Cells

- Newly formed senescent cells may recruit immune system components and produce factors that ↑ wound healing, ↑ defense against/ resolution of infection, ↓ or eliminate cancers, and enable parturition
- → Do not interfere with capacity to generate senescent cells
- Persistent, SASP-expressing senescent cells appear to ↑ the other "pillars of aging": ↑inflammation, ↑fibrosis, stem/ progenitor cell dysfunction, ↓NAD^+, ↑CD38, ↑mTOR, ↓SIRT-1, ↓α-Klotho, ↑deleterious microbiome, cancers, multiple chronic diseases, and spread of senescence

Transplanting Senescent Cells Causes Physical Dysfunction and Decreases Survival

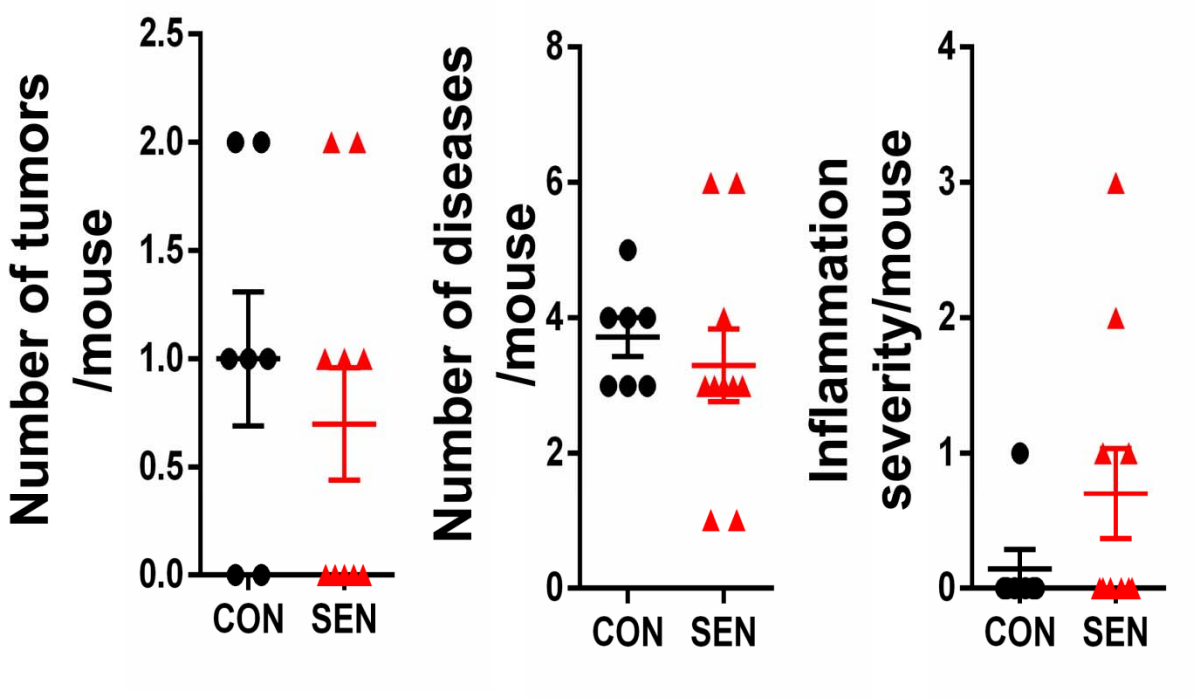


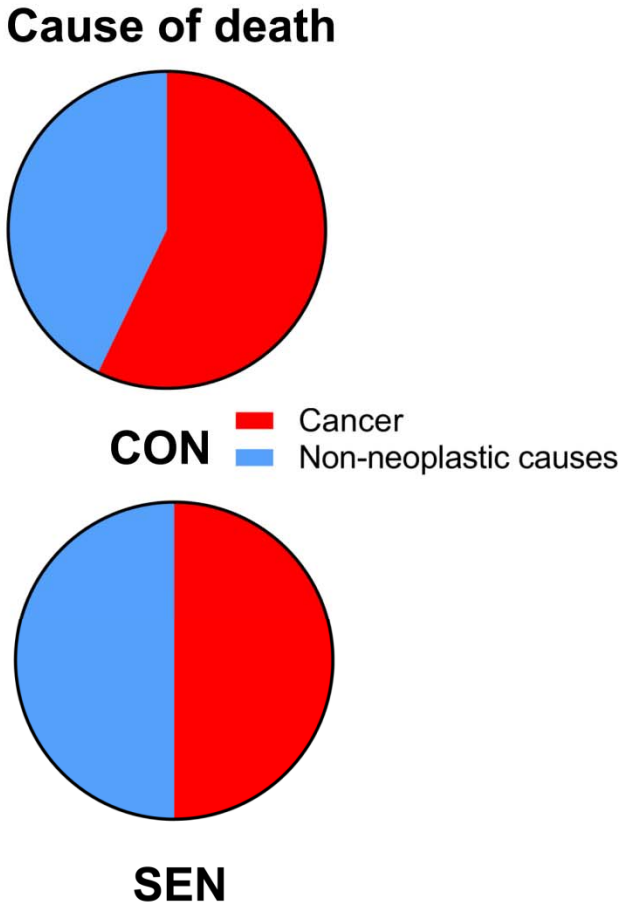


Senescence induced by 10 Gy radiation

Xu *et al.*, Nature Medicine, 2018

Transplanting Senescent Cells Accelerates Death From All Causes

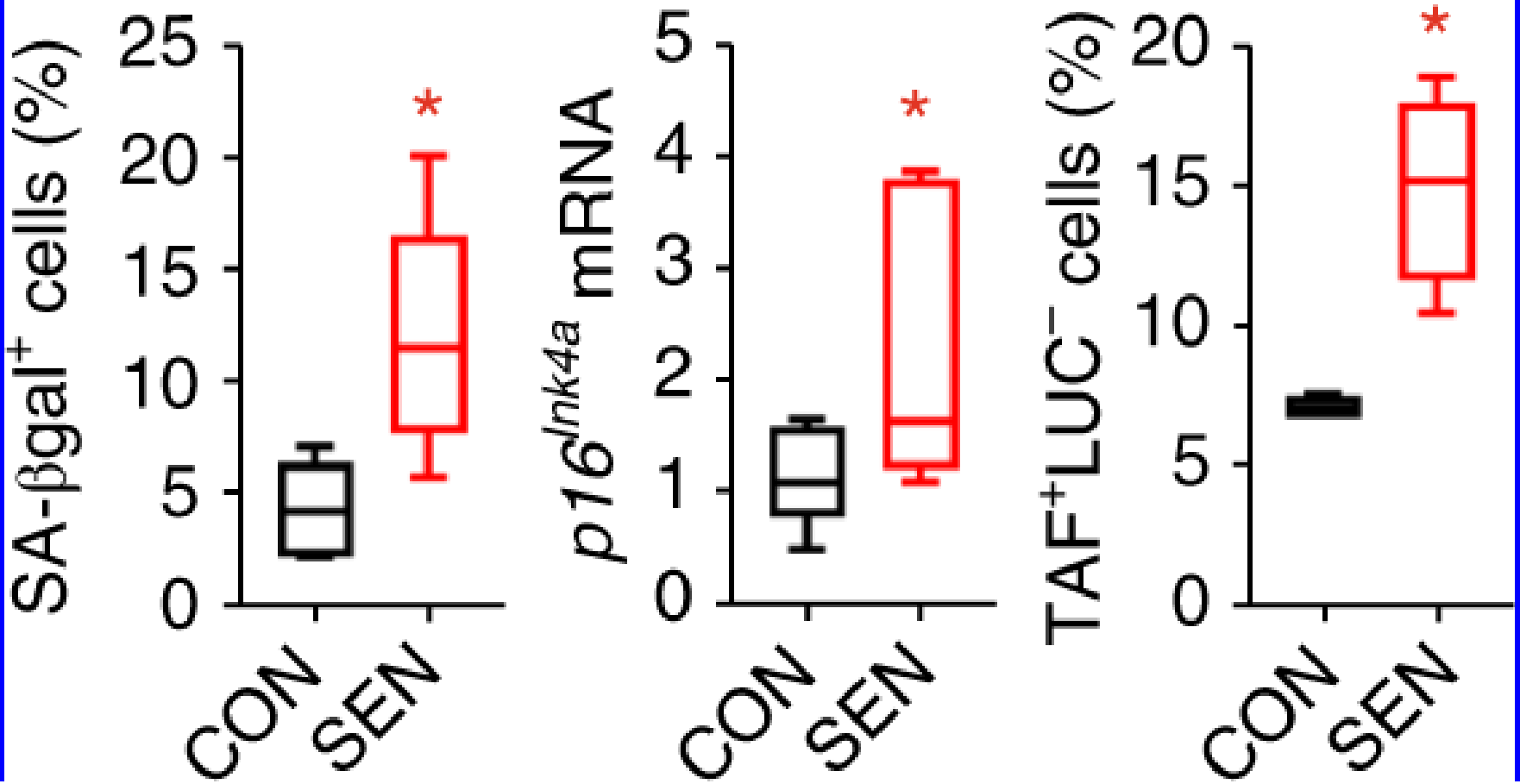




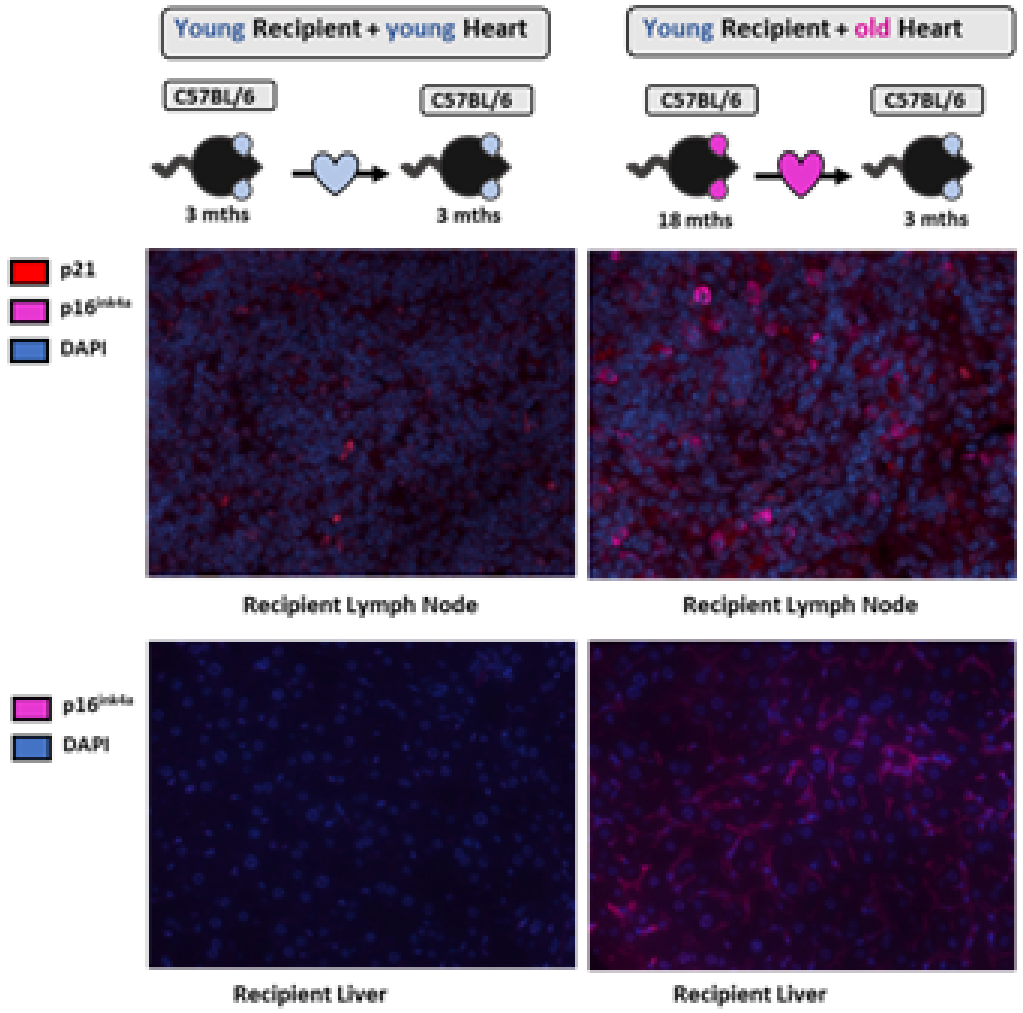
Xu *et al.*, Nature Medicine, 2018

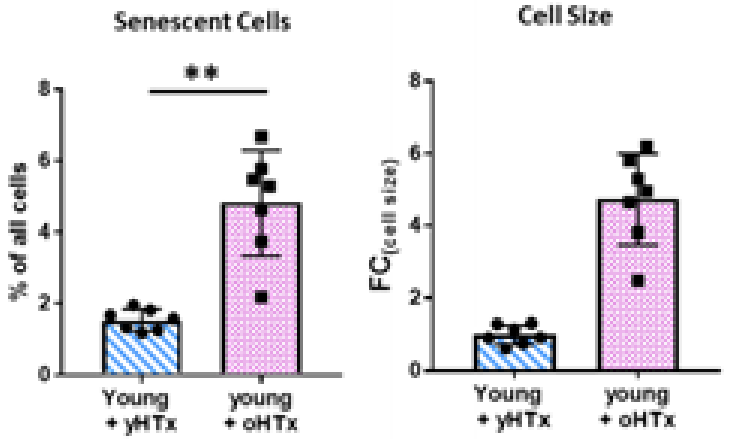
Transplanted Senescent Cells Spread Senescence to Host Cells

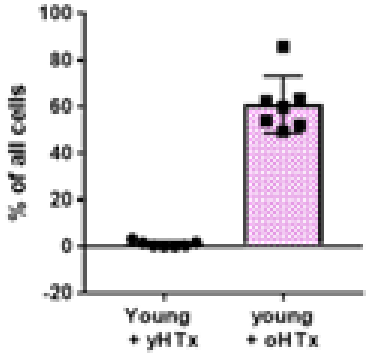
Xu *et al.*, Nature Medicine, 2018



Transplantation of Old Cardiac Allografts Induces Cellular Senescence in Young Recipient Organs



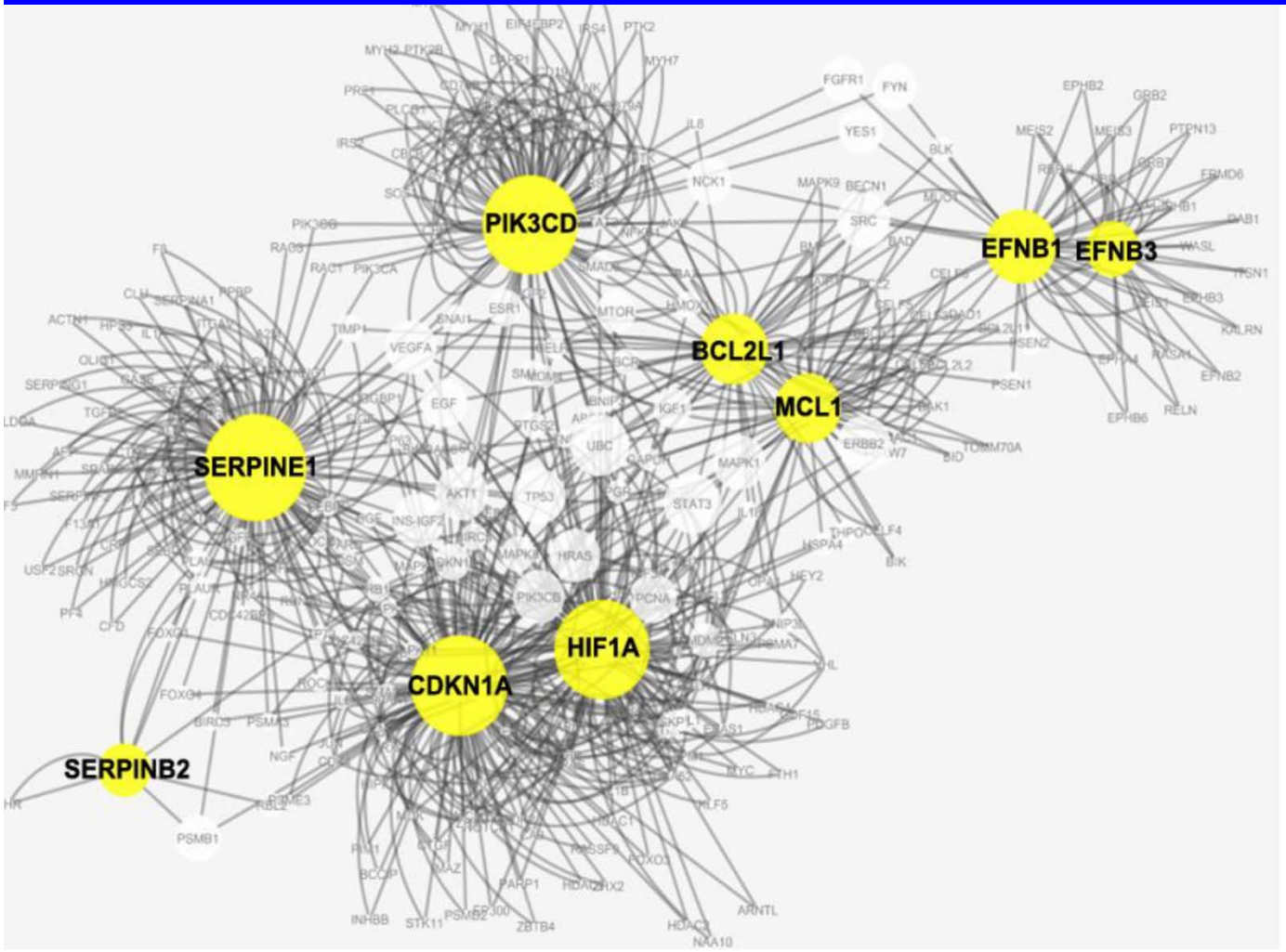




Nature Commun., 2020

Hearts from either young or old C57BL/6 (2 and 18 months) were transplanted to young syngeneic recipients. Liver and draining lymph nodes were collected from donors 30 days after engraftment, cut into slides, and co-stained for $p16^{Ink4a}$, $p21^{Cip1}$, and DAPI

Hypothesis-Driven Senolytic Drug Development: Networks of Anti-Apoptotic Regulators Confer Resistance to Apoptosis in Senescent Cells

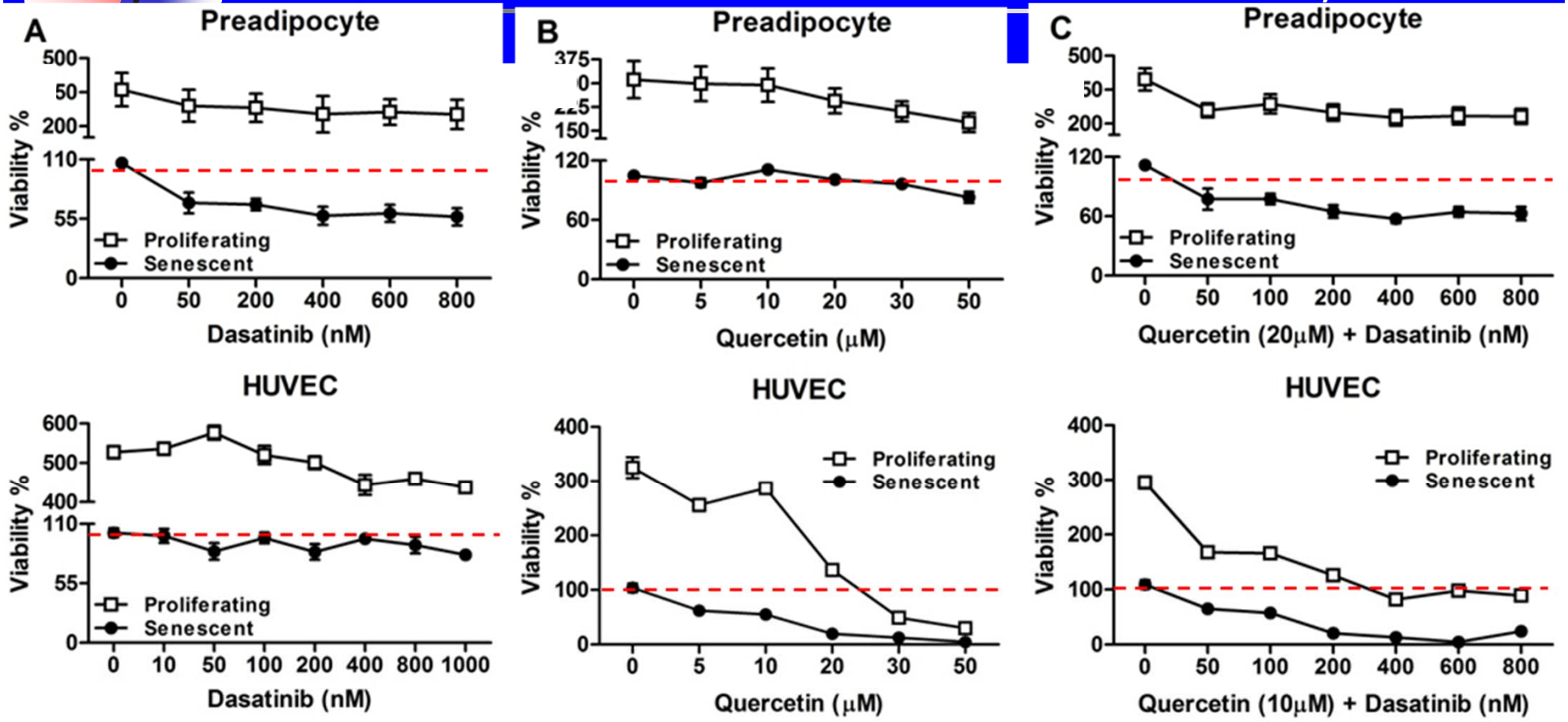


Pathways: Ephrins/ dependence receptors; PI3Kδ/ Akt/ metabolic; Bcl-2 (Bcl-xl, Bcl-2, Bcl-w); p53/ FOXO4/ p21/ serpine (PAI-1&2); HIF-1α; HSP90

Discovered in May 2013; Aging Cell, March, 2015; Nature Commun, Sept., 2017

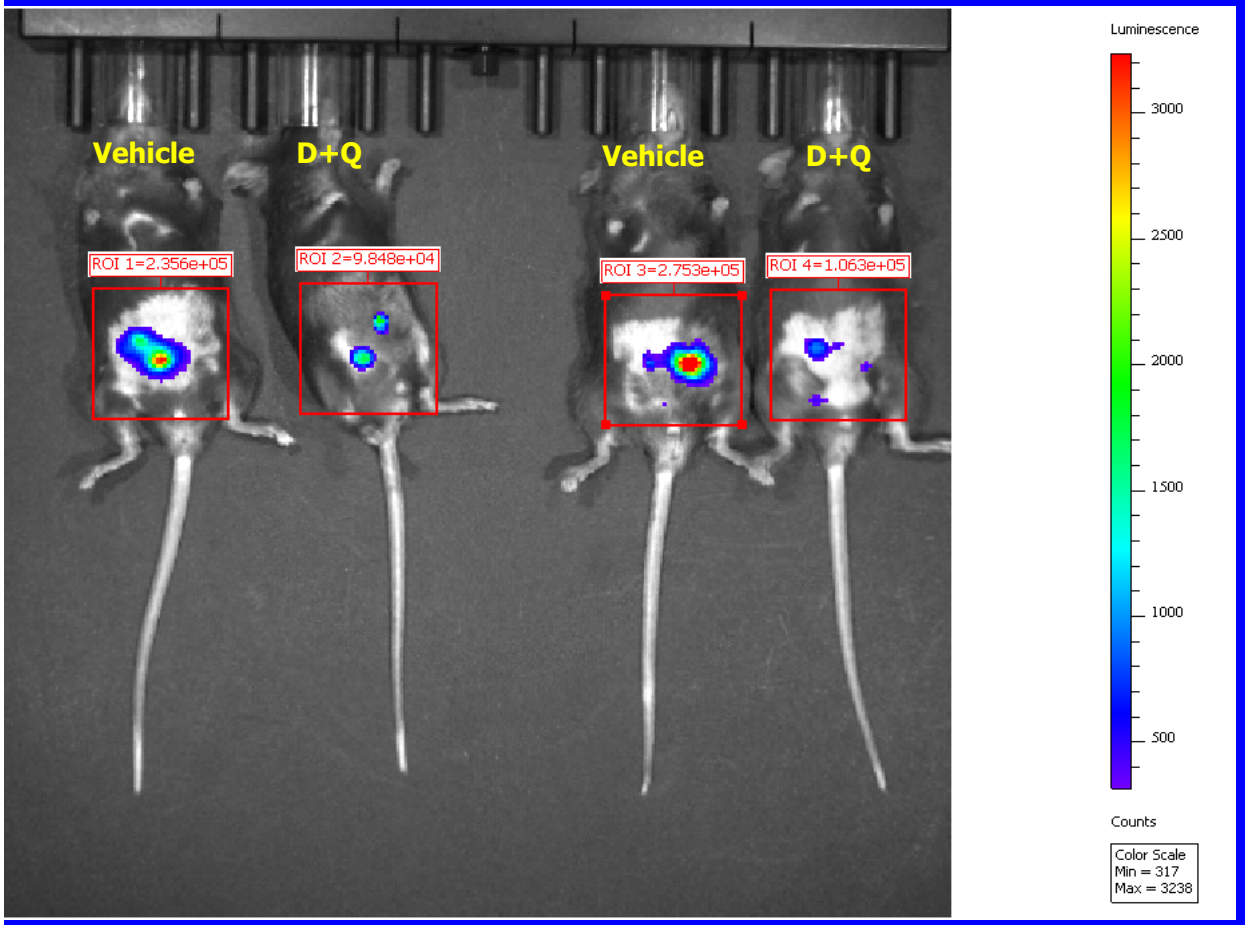
D Targets Senescent Human Preadipocytes, Q Targets Senescent HUVECs

Zhu *et al.*, Aging Cell, March, 2015



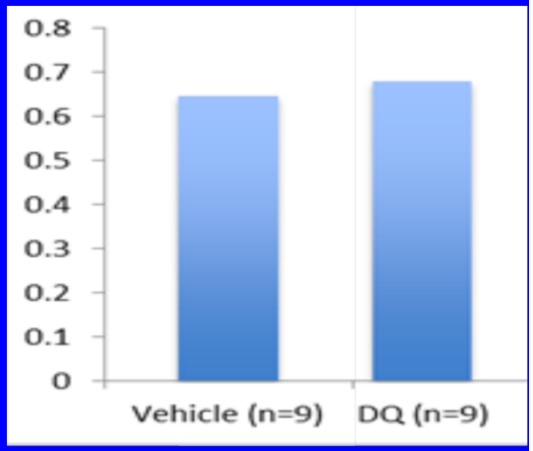
ATP Lite; validated by crystal violet; abdominal subcutaneous preadipocytes from 4 healthy kidney transplant donors; for HUVEC's N=5 replicates.

D+Q Clears Transplanted Luciferase-Expressing Senescent Preadipocytes

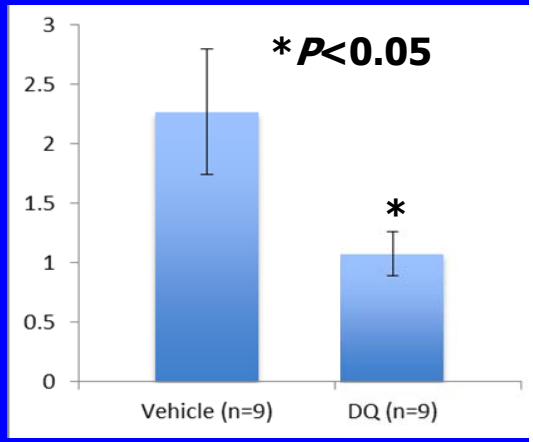


SFFV Promoter-Luciferase; 10^5 Cells Transplanted/ Mouse

Xu *et al.*, Nature Medicine, 2018



Non-senescent cell-transplanted



Senescent cell-transplanted

Routes to Discovering Senolytics

1st Generation
Mechanism-Based

Discovered by Identifying SCAPs and Then Selecting Drugs with Known SCAP Targets

Dasatinib
Quercetin
Fisetin
Luteolin
Enzastaurin
Navitoclax (ABT263)
A1331852
A1155463
Piperlongumine
FOXO4-Related Peptide
Cardiac Glycosides
Geldanamycin
Tanespimycin
Alvespimycin
More being developed

2nd Generation
Randomly Identified

Identified by Chance or with High-Throughput Compound Library Screens

Many being developed

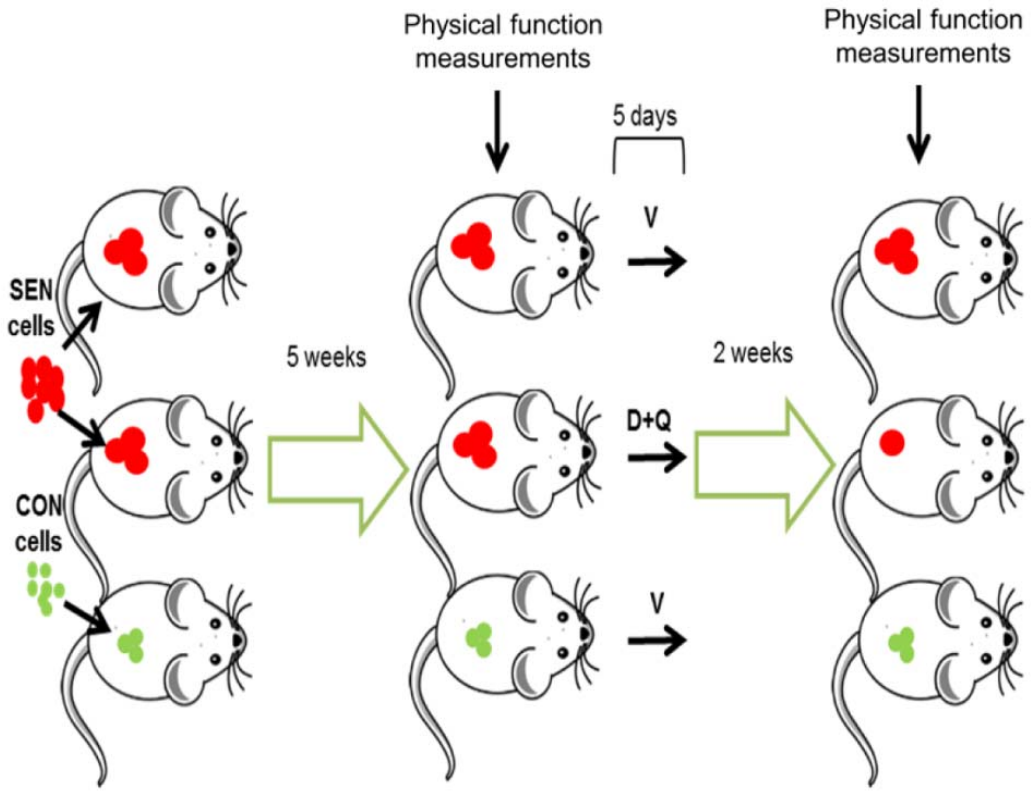
Other approaches:
Immunomodulators
CAR-T
Vaccines
SA β-gal-activated toxins
Nanoparticle Toxins
Others

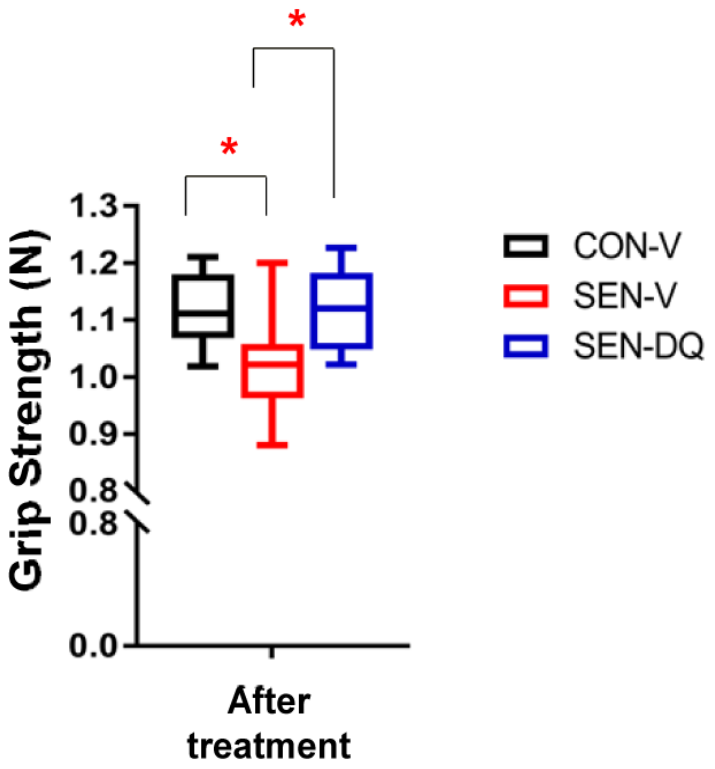
The first senolytics were discovered based on their mechanisms of action and targets. The next generation is being identified using random high-throughput approaches such as drug library screens

Emerging Evidence for Benefits of Senolytics On:

Diabetes/ Obesity
Age-Related Lipodystrophy
Cardiac Dysfunction
Vascular Hyporeactivity/ Calcification/ AV Fistulae
Frailty/ Sarcopenia
Response to Chemotherapy/ Response to Radiation
Cancer
Sequellae of Bone Marrow Transplantation
Sequellae of Organ Transplantation
Myeloma/ MGUS
Cognition/ Alzheimer's/ Parkinson's/ ALS/ Anxiety
Renal Dysfunction
Osteoporosis/ Osteoarthritis/ Rheumatoid Arthritis/ Degenerated Discs
COPD/ Idiopathic Pulmonary Fibrosis/ Tobacco/ Hyperoxic Lung Damage
Hepatic Steatosis/ Liver Cirrhosis/ Primary Biliary Cirrhosis
Progerias
Critical Illness Myopathy
Pre-eclampsia/ Uterine Fibrosis/ Ovarian Involution
Cataracts/ Macular Degeneration/ Glaucoma
Prostatic Hypertrophy
Skin Disorders
Stem Cell Activation/ Progenitor Dysfunction
Lifespan
COVID-19

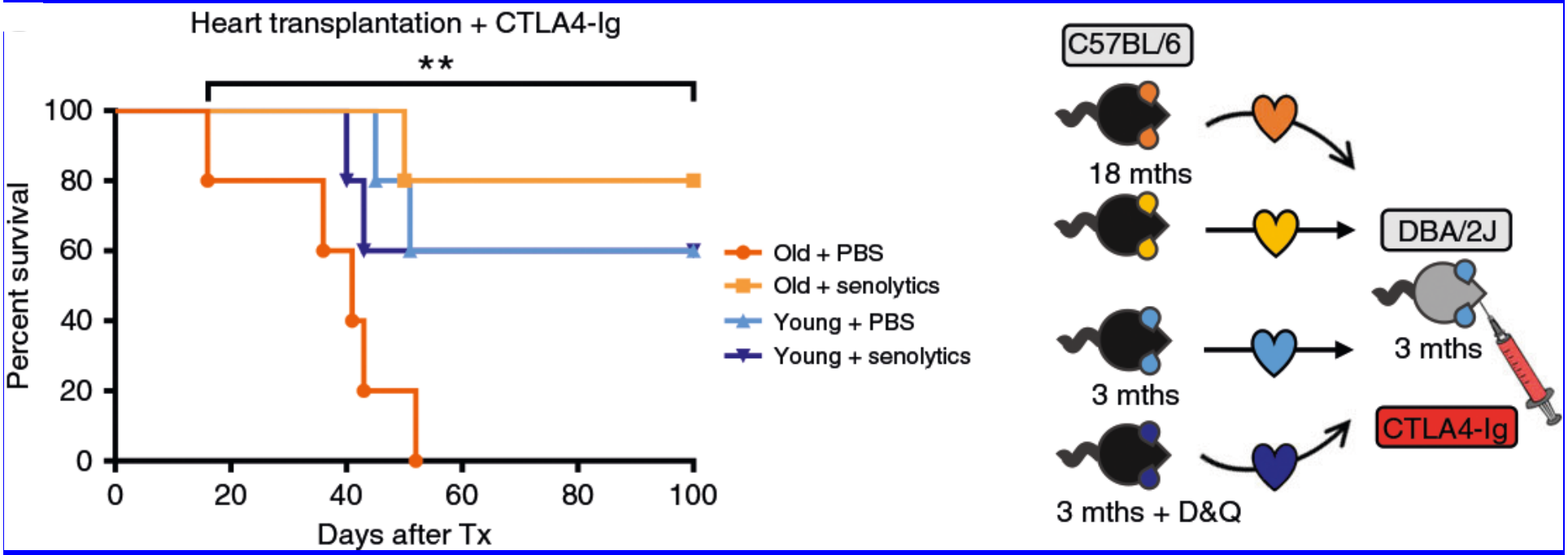
Senolytics Prevent and Alleviate Dysfunction Caused by Transplanting Senescent Cells into Middle-Aged Mice





Xu *et al.*, Nature Medicine, 2018

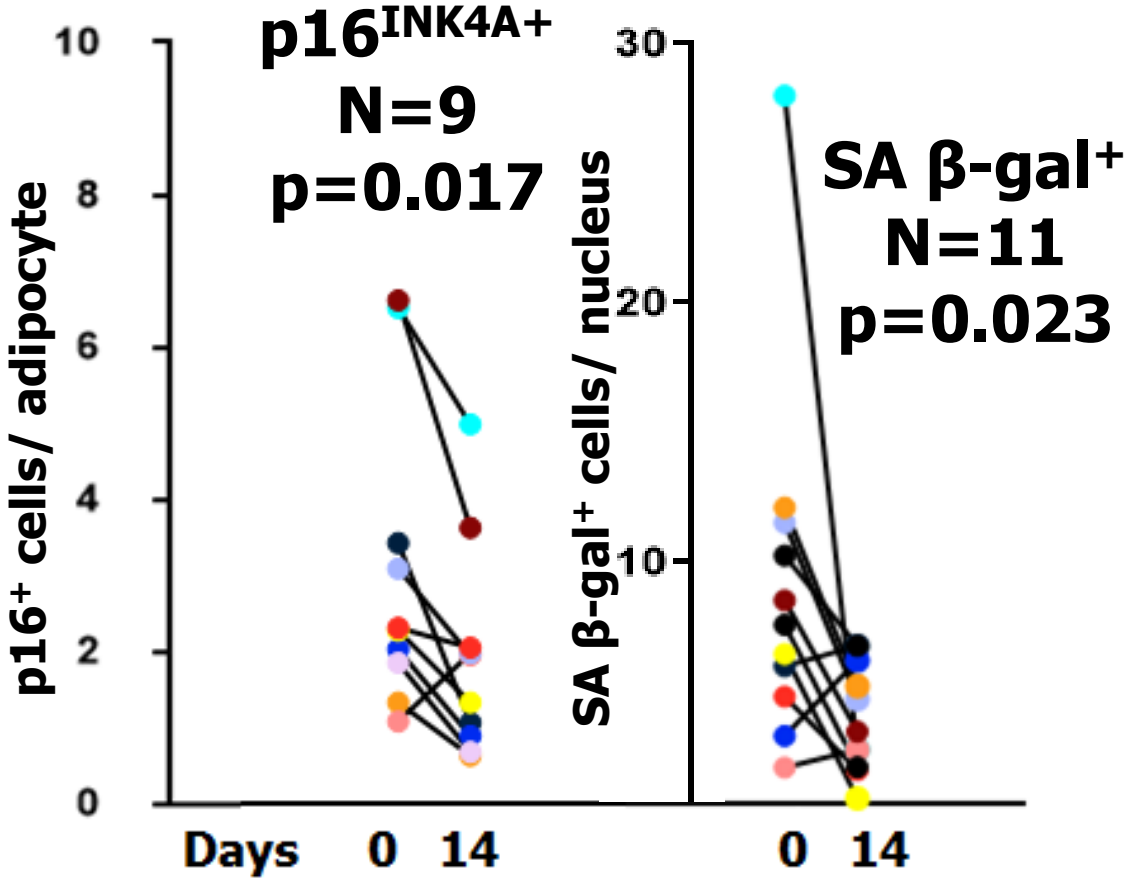
Senolytics Decrease Senescent Cells and cf-mt-DNA Levels, ↓Systemic Hyper-Inflammatory Immune Responses, and Prolong Cardiac Allograft Survival



C57BL/6 mice were treated with D+Q or PBS prior to fully mismatched cardiac transplantation. Recipients were treated weekly with CTLA4-IG, a fusion protein of CLTA-4 and IgG that blocks the interaction of CD80/86 with CD28 on naive T cells. Allograft survival was monitored by daily palpation. N=at least 3 independent expts.

Iske *et al.*, Nature Commun, 2020

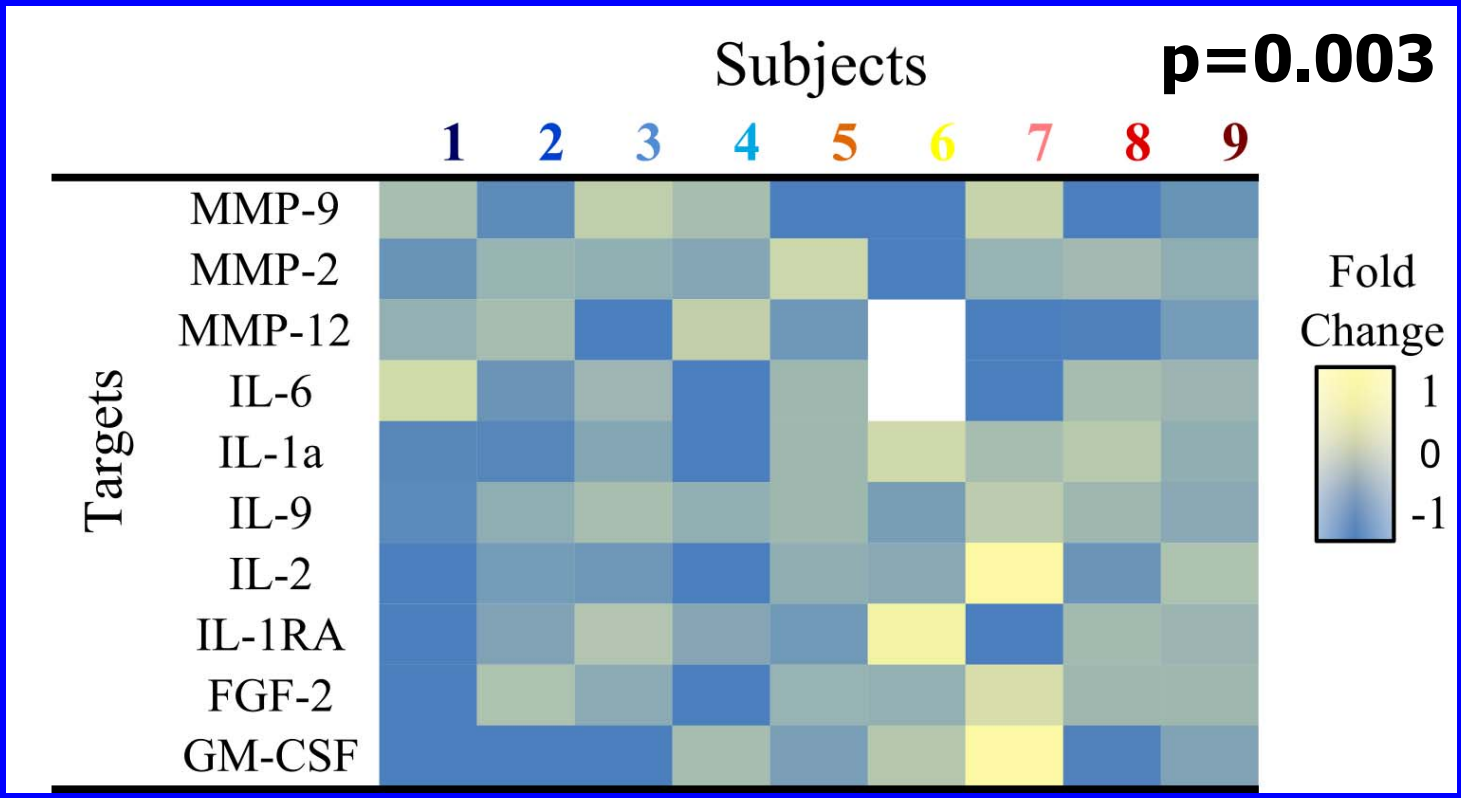
D+Q Clears Senescent Cells From Diabetic Subjects' Adipose Tissue



Abdominal subcutaneous adipose biopsies at baseline (BL) and 11 days after the last dose (PT) of a 3 day course of D+Q; N=9 subjects (paired T test)

ClinicalTrials.gov identifier: NCT02848131

D+Q Decreases Plasma SASP Factors in Patients with Diabetic Kidney Disease



Plasma SASP factors were assayed at baseline (Day 0) and after treatment (Day 14). Colors indicate fold change for each individual between Days 0 and 14 (post-treatment/ baseline value; N=9; p=0.003, composite score of differences (after-before) in z-scores of log-transformed values)

Selected Current and Planned Translational Geroscience Network Clinical Trials of Senolytics

Trial	Senolytic Agent	Notes	Status	Funding Agency	Site(s)	ClinicalTrials.gov Identifier (NCT)
AFFIRM: Alleviation by Fisetin of Frailty, Inflammation, and Related Measures in Older Women	Fisetin	Phase 2 Double-Blind, Placebo-Controlled. Gait Speed <0.6 M/sec	Recruiting	Benefactor	Mayo	03430037
AFFIRM-LITE: Alleviation by Fisetin of Frailty, Inflammation, and Related Measures in Older Adults	Fisetin	Phase 2 Double-Blind, Placebo-Controlled. Gait Speed ≥0.6 M/sec	Recruiting	Benefactor	Mayo	03675724
ALSENLITE: An Open-Label, Pilot Study of Senolytics for Alzheimer Disease	D+Q	Target Engagement; Double-Blind, Placebo-Controlled	Active, Not yet recruiting	Alzheimer's Association	Mayo	04785300
Pilot Study to Investigate the Safety and Feasibility of Senolytic Therapy to Modulate Progression of Alzheimer's Disease (SToMP-AD)	D+Q	Open Label Pilot Phase	Recruiting	UTHSCSA Internal Funding	UTHSCSA	04063124
Senolytic Therapy to Modulate the Progression of Alzheimer's Disease (SToMP-AD)	D+Q	Cognitive Function; Double-Blind, Placebo-Controlled	Active, Not yet recruiting	Alzheimer's Drug Discovery Foundation	Mayo, UTHSCSA, Wake Forest	04685590
Senescence in Chronic Kidney Disease	D+Q	Open Label	Recruiting; Pilot Study Published[89,90]	Benefactor	Mayo	02848131
Inflammation and Stem Cells in Diabetic and Chronic Kidney Disease	Fisetin	Double-Blind, Placebo-Controlled	Recruiting	Benefactor	Mayo	03325322
Hematopoietic Stem Cell Transplant Survivors Study (HTSS)	D+Q	Randomized; Parallel Assignment; Open-Label	Recruiting	Benefactor	Mayo	02652052
Senolytics to Improve Cognition and Mobility in Older Adults at Risk of Alzheimer's Disease	D+Q	Single Arm, Open Label, Pre-Post Pilot Study	Not yet recruiting	NIH	Harvard (Hebrew Rehab. Center)	Pending

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SENSURV: An Open-Label Intervention Trial to Reduce Senescence and Improve Frailty in Adult Survivors of Childhood Cancer	Fisetin; D+Q	Randomized, Open-Label	Active, Not yet recruiting	NIH	St. Jude (1° Site); Mayo (Assays)	04733534
IPF: Trial of Senolytics in Idiopathic Pulmonary Fibrosis	D+Q	Double-Blind, Placebo-Controlled	Planned (Open-Label Pilot Study Published[60]	Pending	UTHSCSA; Wake Forest; Mayo	02874989 (Pilot Phase)
Targeting Cellular Senescence with Senolytics to Improve Skeletal Health in Older Humans	Fisetin; D+Q	Randomized; Parallel Assignment; Open Label	Recruiting	NIH	Mayo	04313634
Senolytic Drugs Attenuate Osteoarthritis-Related Articular Cartilage Degeneration: A Clinical Trial	Fisetin	Double-Blind, Placebo-Controlled	Recruiting	Office of Naval Research	Steadman Clinic (1° Site); Mayo (Assays)	04210986
COVID-FIS, A Study of Fisetin for Skilled Nursing Facility Residents with COVID-19	Fisetin	Double-Blind, Placebo-Controlled	Active, Not yet recruiting	NIH	Mayo	04537299
COVID-FISETIN: Pilot in SARS-CoV-2 of Fisetin to Alleviate Dysfunction and Inflammation	Fisetin	Double-Blind, Placebo-Controlled	Enrolling by Invitation	Benefactor	Mayo	04476953
COVFIS-HOME: COVID-19 Pilot Study of Fisetin to Alleviate Dysfunction and Disease Complications	Fisetin	Double-Blind, Placebo-Controlled	Active, Not yet recruiting	Benefactor	Mayo	04771611

Conclusions

- Persistent senescent cells cause inflammation, fibrosis, progenitor cell dysfunction, spread of senescence, and multiple disease- and age-related disorders
- The target of senolytics is senescent cells, not a single molecule or pathway
- Senolytics attenuate tissue inflammation and fibrosis, improve function, and reduce rejection after transplanting organs from old individuals
- "Hit and run" intermittent senolytic treatment may be effective
- Senolytics delay or alleviate multiple chronic diseases, improve tissue regeneration, and enhance healthspan in mice
- These agents could lead to interventions for humans that delay, prevent, or alleviate senescence- and age-related conditions – if clinical trials continue to demonstrate effectiveness and low toxicity

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