SENOLYTICS

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AFAR President
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
DNA Damage (telomere shortening, mutations, alkylating agents, radiation)
Oncogenes (*e.g.*, Ras, Myc)
Reactive Metabolites (ROS, ceramides, fatty acids, high glucose)
Mitogens/IGF-1
Proteotoxic Stress (protein aggregation, unfolded protein response, mTOR)
Shear Stress, Hypoxic Stress, Hyperoxic Stress
Inflammation, Damage-Associated Molecular Pattern Proteins (DAMPs)
Pathogen-Associated Molecular Pattern Proteins (PAMPs)
Drugs: Nutlin3a, Alkylating Agents, Anthracyclines

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SCAPs
BCL-2 Family
PI3K/Akt/Metabolic
p53/FOXO4/p21/Serpin
Dep. Receptor/Tyrosine Kinase
HIF-1α
HSP-90

Apoptosis Resistance
Senescent Cell Accumulation

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p16/Rb

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p53/p21

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SENECENCE

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IL-1α
IL-6
C/EBPβ

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DNA Damage Response/LINE1 Transpositions
GATA4/TGFβ/NFκB
ROS/Mitochondrial Dysfunction

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Senescence-Associated Secretory Phenotype (SASP)

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Tissue Dysfunction

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Aging Phenotypes
↓Resilience
Chronic Diseases
Senescent Cells Accumulate in Human Adipose Tissue With Aging

4 younger (31 ± 5 y) and 4 older (71 ± 2 y) healthy male volunteers. *p<0.05
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

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\textsuperscript{Ink4a}, p21\textsuperscript{Cip1}, and DAPI.
1) Senescent cells can resist apoptotic stimuli, implying increased pro-survival – anti-apoptotic defenses

2) In some respects, senescent cells are like cancer cells that do not divide, including apoptosis resistance and metabolic shifts
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-xI, 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
siRNA’s Against Anti-Apoptotic Regulators Selectively Decrease Senescent Cell Viability

From 39 pro-survival transcripts targeted, 17 of which specifically eliminated senescent cells, including Bcl-xL, leading to our discovery that Navitoclax is senolytic.

Radiation-induced senescent cells

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 Targets Senescent Human Preadipocytes, Q Targets Senescent HUVECs

Zhu et al., Aging Cell, March, 2015
Routes to Discovering Senolytics

<table>
<thead>
<tr>
<th>1st Generation</th>
<th>2nd Generation</th>
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<tbody>
<tr>
<td>Mechanism-Based</td>
<td>Randomly Identified</td>
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<tr>
<td>Discovered by Identifying SCAPs and Then Selecting Drugs with Known SCAP Targets</td>
<td>Identified by Chance or with High-Throughput Compound Library Screens</td>
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<tr>
<td>Dasatinib</td>
<td>Geldanamycin</td>
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<tr>
<td>Quercetin</td>
<td>Tanespimycin</td>
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<tr>
<td>Fisetin</td>
<td>Alvespimycin</td>
</tr>
<tr>
<td>Luteolin</td>
<td>More being developed</td>
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<tr>
<td>Enzastaurin</td>
<td></td>
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<tr>
<td>Navitoclax (ABT263)</td>
<td>Many being developed</td>
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<tr>
<td>A1331852</td>
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<tr>
<td>A1155463</td>
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<tr>
<td>Piperlongumine</td>
<td></td>
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<tr>
<td>FOXO4-Related Peptide</td>
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<tr>
<td>Cardiac Glycosides</td>
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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.

Other approaches:
- Immunomodulators
- CAR-T
- Vaccines
- SA β-gal-activated toxins
- Nanoparticle Toxins
- Others
D+Q Clears Transplanted Luciferase-Expressing Senescent Preadipocytes

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

Xu et al., Nature Medicine, 2018
A Single Dose of Senolytics Alleviates Radiation-Induced Gait Disturbance for 7 Months

Zhu et al., Aging Cell March, 2015

N=6-9 mice/group; *P<0.05; **P<0.001
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, Alleviate 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.
Targeting Senescent Cells Slows Death of Old Mice Infected with Mouse β-Coronavirus

Science 2021; PMID: 34103349
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
D+Q Clears Senescent Cells From Diabetic Subjects’ Adipose Tissue

p16$^{\text{INK4A}}$ $^+$ cells/ adipocyte  
N=9  
p=0.017

SA β-gal$^+$ cells/ nucleus  
N=11  
p=0.023

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).
First-in-Human Trial of Senolytics: D+Q for Idiopathic Pulmonary Fibrosis

No severe adverse events
9 doses/ 3 wks
Functional measures 5 days after last dose

Justice et al., eBioMed., 2019
<table>
<thead>
<tr>
<th>Trial</th>
<th>Senolytic Agent</th>
<th>Notes</th>
<th>Status</th>
<th>Funding Agency</th>
<th>Site(s)</th>
<th>ClinicalTrials.gov Identifier (NCT)</th>
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<tbody>
<tr>
<td>AFFIRM: Alleviation by Fisetin of Frailty, Inflammation, and Related Measures in Older Women</td>
<td>Fisetin</td>
<td>Phase 2 Double-Blind, Placebo-Controlled. Gait Speed &lt;0.6 M/sec</td>
<td>Recruiting</td>
<td>Benefactor</td>
<td>Mayo</td>
<td>03430037</td>
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<tr>
<td>AFFIRM-LITE: Alleviation by Fisetin of Frailty, Inflammation, and Related Measures in Older Adults</td>
<td>Fisetin</td>
<td>Phase 2 Double-Blind, Placebo-Controlled. Gait Speed ≥0.6 M/sec</td>
<td>Recruiting</td>
<td>Benefactor</td>
<td>Mayo</td>
<td>03675724</td>
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<tr>
<td>ALSENLITE: An Open-Label, Pilot Study of Senolytics for Alzheimer Disease</td>
<td>D+Q</td>
<td>Target Engagement; Double-Blind, Placebo-Controlled</td>
<td>Recruiting</td>
<td>Alzheimer’s Association</td>
<td>Mayo</td>
<td>04785300</td>
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<tr>
<td>Pilot Study to Investigate the Safety and Feasibility of Senolytic Therapy to Modulate Progression of Alzheimer’s Disease (SToMP-AD)</td>
<td>D+Q</td>
<td>Open Label Pilot Phase</td>
<td>Recruiting</td>
<td>UTHSCSA Internal Funding</td>
<td>UTHSCSA</td>
<td>04063124</td>
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<tr>
<td>Senolytic Therapy to Modulate the Progression of Alzheimer’s Disease (SToMP-AD)</td>
<td>D+Q</td>
<td>Cognitive Function; Double-Blind, Placebo-Controlled</td>
<td>Active, Not yet recruiting</td>
<td>Alzheimer’s Drug Discovery Foundation</td>
<td>Mayo, UTHSCSA, Wake Forest</td>
<td>04685590</td>
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<tr>
<td>Senescence in Chronic Kidney Disease</td>
<td>D+Q</td>
<td>Open Label</td>
<td>Recruiting; Pilot Study</td>
<td>Benefactor</td>
<td>Mayo</td>
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<tr>
<td>Inflammation and Stem Cells in Diabetic and Chronic Kidney Disease</td>
<td>Fisetin</td>
<td>Double-Blind, Placebo-Controlled</td>
<td>Recruiting</td>
<td>Benefactor</td>
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<td>Hematopoietic Stem Cell Transplant Survivors Study (HTSS)</td>
<td>D+Q</td>
<td>Randomized; Parallel Assignment; Open-Label</td>
<td>Recruiting</td>
<td>Benefactor</td>
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<td>Senolytics to Improve Cognition and Mobility in Older Adults at Risk of Alzheimer’s</td>
<td>D+Q</td>
<td>Single Arm, Open Label, Pre-Post Pilot Study</td>
<td>Recruiting</td>
<td>NIH</td>
<td>Harvard (Hebrew Rehab. Center)</td>
<td>Pending</td>
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## Selected Current and Planned Translational Geroscience Network Clinical Trials of Senolytics

<table>
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<tr>
<th>Trial</th>
<th>Senolytic Agent</th>
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<th>ClinicalTrials.gov Identifier (NCT)</th>
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<tr>
<td>SENSURV: An Open-Label Intervention Trial to Reduce Senescence and Improve Frailty in Adult Survivors of Childhood Cancer</td>
<td>Fisetin; D+Q</td>
<td>Randomized, Open-Label</td>
<td>Active, Not yet recruiting</td>
<td>NIH</td>
<td>St. Jude (1° Site); Mayo (Assays)</td>
<td>04733534</td>
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<tr>
<td>IPF: Trial of Senolytics in Idiopathic Pulmonary Fibrosis</td>
<td>D+Q</td>
<td>Double-Blind, Placebo-Controlled</td>
<td>Planned (Open-Label Pilot Study Published(^\text{60}))</td>
<td>Pending</td>
<td>UTHSCSA; Wake Forest; Mayo</td>
<td>02874989 (Pilot Phase)</td>
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<tr>
<td>Targeting Cellular Senescence with Senolytics to Improve Skeletal Health in Older Humans</td>
<td>Fisetin; D+Q</td>
<td>Randomized; Parallel Assignment; Open Label</td>
<td>Recruiting</td>
<td>NIH</td>
<td>Mayo</td>
<td>04313634</td>
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<tr>
<td>Senolytic Drugs Attenuate Osteoarthritis-Related Articular Cartilage Degeneration: A Clinical Trial</td>
<td>Fisetin</td>
<td>Double-Blind, Placebo-Controlled</td>
<td>Recruiting</td>
<td>Office of Naval Research</td>
<td>Steadman Clinic (1° Site); Mayo (Assays)</td>
<td>04210986</td>
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<td>COVID-FIS, A Study of Fisetin for Skilled Nursing Facility Residents with COVID-19</td>
<td>Fisetin</td>
<td>Double-Blind, Placebo-Controlled</td>
<td>Recruiting</td>
<td>NIH</td>
<td>Mayo</td>
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<td>COVID-FISETIN: Pilot in SARS-CoV-2 of Fisetin to Alleviate Dysfunction and Inflammation</td>
<td>Fisetin</td>
<td>Double-Blind, Placebo-Controlled</td>
<td>Recruiting</td>
<td>Benefactor</td>
<td>Mayo</td>
<td>04476953</td>
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<tr>
<td>COVFIS-HOME: COVID-19 Pilot Study of Fisetin to Alleviate Dysfunction and Disease Complications</td>
<td>Fisetin</td>
<td>Double-Blind, Placebo-Controlled</td>
<td>Recruiting</td>
<td>Benefactor</td>
<td>Mayo</td>
<td>04771611</td>
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</tbody>
</table>
Ax1 Mission Overview
~1 day of launch and transfer to ISS
~8 days on ISS
~1 day to return and re-enter Earth
Launch and land off the coast of Florida

• Project 1: Impact of Near Earth Low Gravity Space Travel on Fundamental Aging Processes. An Exploratory Human Minimal Risk Study

• Project 2: Impact of Near Earth Low Gravity Space Travel on Fundamental Aging Processes. Does the Low Gravity Environment Cause Cultured Human Cells to Undergo Senescence?
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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