The Clinical Potential of Senolytic Drugs

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Disclosures

Mayo Clinic and the JLK lab hold patents on senolytics and have earned proceeds from Unity Biotechnology.

JLK has no current consulting or advising position with Unity. JLK holds shares in Unity. Unity did not support the research presented and Unity products will not be discussed.

JLK is a scientific advisor for other organizations developing interventions targeting basic aging processes.
Consequences of Fundamental Aging Processes: Geroscience Hypothesis

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

Fundamental Aging Mechanisms

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

Macromolecular/ Organelle Dysfunction (DNA, protein aggregation, autophagy, AGE’s, lipotoxicity, mitochondria)

Stem Cell and Progenitor Dysfunction

Cellular Senescence
DNA Damage (telomere shortening, mutations, alkylating agents, radiation)
Oncogenes (e.g., Ras, Myc)
Reactive Metabolites (ROS, ceramides, fatty acids, leukotrienes, high glucose)
Mitogens/IGF-1
Proteotoxic Stress (protein aggregation, unfolded protein response, mTOR)
Mechanical Stress, Shear Stress, Hypoxic or Hyperoxic Stress
Inflammation, Damage-Associated Molecular Pattern Proteins (DAMPs), Pathogen-Associated Molecular Pattern Proteins (PAMPs)

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

Apoptosis Resistance
Senescent Cell Accumulation

p16/Rb
p53/p21
SELENCE

IL-1α
IL-6
C/EBPβ

DNA Damage Response/LINE1 & Other Transposons
GATA4/TGFβ/NFκB
ROS/Mitochondrial Dysfunction

Senescence-Associated Secretory Phenotype (SASP), ↑mTOR
Tissue Dysfunction

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

Xu, et al., PNAS, 2015
Transplanting Senescent Cells Around Knees Causes Osteoarthritis-Like Joint Destruction

Xu, et al., J. Gerontol., 2017
Transplanting Senescent Cells Causes Physical Dysfunction and Decreases Survival

Xu et al., Nature Medicine, 2018
Transplanting Senescent Cells Accelerates Death From All Causes

Xu et al., Nature Medicine, 2018
Transplanting Senescent Cells Spreads Senescence to Host Cells

Xu et al., Nature Medicine, 2018
Development of Senolytics

Developing senolytics began before and independently from making or studying INK-ATTAC mice.
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
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

Aging Cell, March, 2015; Nature Commun, Sept., 2017
siRNA’s Against Anti-Apoptotic Regulators Selectively Decrease Senescent Cell Viability

Selected from 39 pro-survival transcripts targeted, 17 of which affected senescent cell viability

Zhu et al., Aging Cell, March, 2015
D Targets Senescent Human Preadipocytes, Q Targets Senescent HUVECs

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

Zhu et al., Aging Cell, March, 2015
D+Q Impacts the Anti-Apoptotic Regulators That Confer Resistance to Apoptosis in Senescent Cells

Aging Cell, March, 2015; Nature Commun, Sept., 2017
D+Q Impacts the Anti-Apoptotic Regulators That Confer Resistance to Apoptosis in Senescent Cells

D: Ephrin/dependence receptors; CDKN1A
Q: PIK3CD; HIF1α; SERPINE1
D+Q: SERPINEB2; BCL2L1

Aging Cell, March, 2015; Nature Commun, Sept., 2017
## Routes to Discovering Senolytics

<table>
<thead>
<tr>
<th>1st Generation Mechanism-Based</th>
<th>2nd Generation Randomly Identified</th>
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</thead>
<tbody>
<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>
</tr>
<tr>
<td>Dasatinib</td>
<td>Geldanamycin</td>
</tr>
<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>
</tr>
<tr>
<td>Enzastaurin</td>
<td></td>
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<tr>
<td>Navitoclax (ABT263)</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>
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<tr>
<td>FOXO4-Related Peptide</td>
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</tr>
<tr>
<td>Nutlin-3a</td>
<td></td>
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<tr>
<td>Cardiac Glycosides</td>
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</tbody>
</table>

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 include:

- Immunomodulators
- Vaccines
- SA β-gal-activated toxins
- Nanoparticle Toxins
- Others
Navitoclax (ABT263), A BCL-2 Family Inhibitor, Is Senolytic In Some, But Not All Cell Types

Navitoclax is less specific than some other senolytics: it causes off-target killing of neutrophils and platelets.

Aging Cell Dec., 2015
The Target is Senescent Cells, Not a Molecule, Receptor, or Biochemical Pathway

Single Molecular Target
↓
Same Target Present in Many Cell Types
↓
Off-Target Apoptosis of Non-Senescent Cell Types
↓
More Side-Effects
↓
“Panolytic”; Less Senolytic

\[e.g., \text{A1331852, navitoclax, nutlins}\]

Network of Molecular Impacts
↓
Networks Are More Likely to be Cell Type-Specific
↓
More Specifically Senolytic
↓
Fewer Side-Effects
↓
More Truly Senolytic

\[e.g., D+Q, fisetin\]
D+Q Clears Transplanted Luciferase-Expressing Senescent Preadipocytes

Vehicle

D+Q

Vehicle

D+Q

Non-senescent cell-transplanted

*P<0.05

SFFV Promoter-Luciferase; 10^5 Cells Transplanted/ Mouse Xu et al., Nature Medicine, 2018
D+Q is Senolytic in Freshly-Isolated Human Adipose Tissue

Xu et al., Nature Medicine, 2018
D+Q is Senolytic in Freshly-Isolated Human Adipose Tissue

Xu et al., Nature Medicine, 2018
D+Q is Senolytic in Freshly-Isolated Human Adipose Tissue

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

N=6-9 mice/group; *P<0.05; **P<0.001

Zhu et al., Aging Cell March, 2015
D+Q Increase Cortical and Trabecular Femoral Bone Mass in Old Mice

Farr et al., Nature Medicine, 2017
Senolytics Prevent Age-Related Bone Loss in Old Mice

Farr et al., Nature Medicine, 2017
Insulin Resistance is Alleviated in Obese Mice by Senolytics

Lean
Obese
Obese D+Q

ipGTT

Palmer et al., Aging Cell, 2019
D+Q Do Not Directly Target Human Adipose Tissue p16$^{\text{INK4A}+}$ Macrophages

Xu et al., Nature Medicine, 2018

Adipose tissue explants freshly isolated from abdominal subcutaneous depots of obese subjects (age 42±4 years; BMI 50±6; N=4)
Decreased Homing of Transplanted Luciferase\(^+\) Monocytes to Adipose Tissue of \textit{db/db} Mice After D+Q

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N/A</th>
<th>Vehicle</th>
<th>D+Q</th>
</tr>
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<tbody>
<tr>
<td>Monocytes</td>
<td>N/A</td>
<td>10(^6)</td>
<td>10(^6)</td>
</tr>
</tbody>
</table>

Normalized to vehicle-treated mice, \(n=5/\text{group}\)

Palmer \textit{et al.}, Aging Cell, 2019
Senolytics Prevent and Alleviate Dysfunction Caused by Transplanting Senescent Cells into Young Mice

Xu et al., Nature Medicine, 2018
Senolytics Alleviate Physical Dysfunction in Old Mice

Xu et al., Nature Medicine, 2018
D+Q Extends Lifespan in Old Mice by 36%

Physical function tests monthly

24-27-month-old

Vehicle

Natural death

D+Q

Bi-weekly

Days

Post-treatment

Male + Female

Percent survival

100

50

Days after 1st treatment

0 100 200 300 400 500

Days

140 d 191 d

* V DQ

Xu et al., Nature Medicine, 2018
D+Q Delays Death From All Causes

Xu et al., Nature Medicine, 2018
D+Q Reduces Burden of Senescent Adipocyte-Like Periventricular Ependymal Cells in db/db Mice

Ogrodnik et al., Cell Metabolism, 2019
D+Q Alleviates Anxiety in Obesity

Ogrodnik et al., Cell Metabolism, 2019
Senolytics Alleviate Alzheimer’s-Like Changes in Tau+ Mice

D+Q every 2 weeks for 3 months reduced neurofibrillary tangles, neuroinflammation, gliosis, small vessel hypoperfusion, and ventricular enlargement and increased cortical volume and neurogenesis in 23 month old tau-NFT-Mapt0/0 mice

Musi et al., Aging Cell, 2018
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
- 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
Strategies for Developing Agents Targeting Aging Processes

- Risk
- Immediate Benefit

Serious, life-threatening conditions with no good treatment options
Multiple small, parallel trials

Prevention in healthy individuals
Fewer, larger trials
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 Macrophages and Crown-Like Structures in 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)
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
Senolytic Clinical Trials
Underway or Planned

Phase I/II:
Senolytics:
- Childhood cancer survivors
- Idiopathic pulmonary fibrosis
- Age-related osteoporosis
- Osteoarthritis
- Frailty
- Bone marrow transplant survivors
- Alzheimer’s
- Diabetic chronic kidney disease
- Improving outcomes after transplanting organs from old donors
- Many others
Conclusions

• The target of senolytics is senescent cells, not a single molecule or pathway – targeting networks yields more truly senolytic, less panolytic, treatments

• Senolytics alleviate progenitor dysfunction

• Senolytics attenuate tissue inflammation

• Intermittent treatment may be effective

• Senolytics delay or alleviate multiple chronic diseases and enhance healthspan and lifespan 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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