The Clinical Potential of Senolytic Drugs

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Senolytic drugs are agents that selectively induce apoptosis of senescent cells. These cells accumulate in many tissues with aging and at sites of pathology in multiple chronic diseases. In studies in animals, targeting senescent cells using genetic or pharmacological approaches delays, prevents, or alleviates multiple age-related phenotypes, chronic diseases, geriatric syndromes, and loss of physiological resilience. Among the chronic conditions successfully treated by depleting senescent cells in preclinical studies are frailty, cardiac dysfunction, vascular hyporeactivity and calcification, diabetes mellitus, liver steatosis, osteoporosis, vertebral disk degeneration, pulmonary fibrosis, and radiation-induced damage. Senolytic agents are being tested in proof-of-concept clinical trials. To do so, new clinical trial paradigms for testing senolytics and other agents that target fundamental aging mechanisms are being developed, because use of long-term endpoints such as lifespan or healthspan is not feasible. These strategies include testing effects on multimorbidity, accelerated aging-like conditions, diseases with localized accumulation of senescent cells, potentially fatal diseases associated with senescent cell accumulation, age-related loss of physiological resilience, and frailty. If senolytics or other interventions that target fundamental aging processes prove to be effective and safe in clinical trials, they could transform geriatric medicine by enabling prevention or treatment of multiple diseases and functional deficits in parallel, instead of one at a time. **J Am Geriatr Soc 2017.**

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Chronological aging is the leading predictor of the major chronic diseases that account for the bulk of morbidity, mortality, and health costs worldwide. These include diabetes mellitus, cardiovascular disease, most cancers, dementia, other neurodegenerative diseases, arthritis, osteoporosis, and blindness.[1] Aging also predisposes to geriatric syndromes, including frailty, weakness, lack of mobility, mild cognitive impairment, and incontinence, as well as loss of physiological resilience.[2] This loss of resilience leads to prolonged recovery after illnesses such as pneumonia or myocardial infarction, impaired ability to withstand interventions such as chemotherapy or surgery, and an attenuated response to vaccination. Furthermore, age-related chronic diseases, geriatric syndromes, and disabilities tend to cluster within individuals, leading to multimorbidity.[3] These observations support the concept that fundamental aging processes not only cause aging phenotypes, but also predispose to chronic diseases and the geriatric syndromes. Thus, it has been predicted that therapeutically targeting these processes can delay, prevent, or alleviate age-related chronic diseases and disabilities as a group, instead of one at a time—the "geroscience hypothesis."

The biological processes that underlie aging phenotypes and are active at the nidus of most chronic diseases include chronic, low-grade, "sterile" (absence of known pathogens) inflammation; macromolecular and organelle dysfunction (e.g., changes in deoxyribonucleic acid (DNA), such as telomere erosion, unrepaired damage, mutations, polyploidy, proteins (e.g., aggregation, misfolding, autophagy), carbohydrates, lipids, or mitochondria); stem and progenitor cell dysfunction; and accumulation of senescent cells. These four processes are linked; that is, in general, interventions that target one process also attenuate the others. For example, DNA damage causes increased senescent cell burden and mitochondrial and stem or progenitor cell dysfunction.[4–6] Conversely, reducing senescent cell burden can lead to less inflammation, less macromolecular dysregulation, and enhanced function of stem and progenitor cells.[7–9]

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CELLULAR SENESCENCE AND THE SENESCENCE-ASSOCIATED SECRETORY PHENOTYPE

Senescence is a process whereby a cell loses the proliferative potential of normally replication-competent cells and becomes resistant to apoptosis, with an increase in metabolic activity and frequently development of a senescence-associated secretory phenotype (SASP). The SASP entails release of proinflammatory cytokines and chemokines, tissue-damaging proteases, factors that can affect stem and progenitor cell function, hemostatic factors, and growth factors, among others.[7] Senescent cells that express the SASP can have substantial local and systemic pathogenic effects. For example, transplanting small numbers of senescent cells around the knee joints of mice induces an osteoarthritis-like condition resembling the non-injury-related osteoarthritis common in elderly humans.[10] Senescent cells also undergo metabolic shifts, including a reduction in fatty acid usage, increases in glycolysis, and reactive oxygen species generation, which can affect other cells and even spread senescence to nearby cells.[11]

Markers of senescent cells include increases in cell size; lipofuscin accumulation; high expression of the cell cycle regulators, $p16^{INK4A}$ and $p21^{CIP1}$, as well as SASP factors (e.g., interleukin-6, interleukin-8, monocyte chemoattractant protein-1, plasminogen-activated inhibitor-1, and many others); an increase in cellular senescence–associated β-galactosidase activity; and appearance of senescent-associated distension of satellites and telomere-associated DNA damage foci, among others. None of these markers are fully sensitive or specific, so testing more than one marker is needed to draw conclusions about effects of diseases or interventions on senescent cell numbers.

ELIMINATING SENESCENT CELLS FROM TRANSGENIC "SUICIDE GENE"–EXPRESSING MICE

The number of senescent cells increases with aging in mice, monkeys, and humans,[12–15] and interventions that increase lifespan, including caloric restriction or mutations in the growth hormone axis, are associated with decreased senescent cell abundance.[12,16] These observations led the authors of the current study to devise a strategy and propose a collaboration for making transgenic INK-ATTAC mice, from which senescent cells can be eliminated using a drug (AP20187). This drug does not affect wild-type mice; it activates a "suicide" protein encoded by the transgene, which is present in only $p16^{INK4A}$-expressing cells in INK-ATTAC mice. Eliminating senescent cells using this genetic approach alleviates a number of age-, progeria-, and hypercholesterolemia-related conditions, consistent with the geroscience hypothesis,[8,17–19] but this approach has limitations. First and most importantly, it would be difficult to translate an approach involving insertion of a transgene into humans. Second, not all cells with increased $p16^{INK4A}$ are senescent, and not every senescent cell has increased expression of $p16^{INK4A}$. Therefore, models dependent upon $p16^{INK4A}$ expression may not recapitulate the effects of removing potentially pathogenic classically senescent cells with an active SASP. Third, tumor cells can have high $p16^{INK4A}$ expression,[20] confounding interpretation of lifespan or healthspan studies in INK-ATTAC mice because the vast majority of mice die of or with cancer.

SENESCENT CELL ANTI-APOPTOTIC PATHWAYS: EXPLOITING THE ACHILLES' HEEL OF SENESCENT CELLS

To remove senescent cells pharmacologically from non-genetically modified animals, "senolytic" agents, including small molecules, peptides, and antibodies, are being developed.[21] Since the article describing the first senolytic agents was published in March 2015,[22] progress in identifying additional senolytic agents and their effects has been rapid. In that first article, a hypothesis-driven senolytic agent discovery paradigm was implemented. Senescent cells are resistant to apoptosis, despite the SASP factors they release, which should trigger apoptosis. Indeed, pro-apoptotic pathways are up-regulated in senescent cells,[22] yet these cells resist apoptosis.[23] The hypothesis was therefore tested that senescent cells depend on pro-survival pathways to defend against their own pro-apoptotic SASP. Using bioinformatic approaches based on the ribonucleic acid (RNA) and protein expression profiles of senescent cells, five senescent-cell anti-apoptotic pathways (SCAPs) were identified (Table 1). That SCAPs are required for senescent cell viability was verified in RNA interference studies, in which levels of key proteins in these pathways were reduced. Through this approach, survival proteins were identified as the Achilles' heel of senescent cells. Knocking down expression of these proteins causes death of senescent but not nonsenescent cells. Since the discovery of the first five SCAPs, another has been identified (Table 1).[24] This approach and the SCAPs discovered so far have been used to identify putative senolytic targets.[24–27]

Whether agents known to interfere with the activity of SCAP pathways are senolytic was tested. The first senolytic agents discovered using this hypothesis-driven approach were dasatinib and quercetin.[22] Ten months later, the third senolytic drug, navitoclax, a BCL-2 pro-survival pathway inhibitor, was reported.[25,27] Since then, a growing number of senolytics, including natural products, synthetic small molecules, and peptides, which target the original SCAPs, and another involving the HSP-90 SCAP have been reported (Table 1). Yet more senolytics are in development, and additional potential SCAPs are being identified.

The SCAPs required for senescent cell resistance to apoptosis vary according to cell type. The Achilles' heels, for example, of senescent human primary adipose progenitors differ from those of a senescent human endothelial cell strain, indicating that agents targeting a single SCAP may not eliminate all types of senescent cells. The senolytics that have been tested across a wide range of senescent cell types have all exhibited a degree of cell type specificity. For example, navitoclax is senolytic in a cell culture–acclimated human umbilical vein endothelial cell strain but is not effective against senescent primary human fat cell progenitors.[27] Even within a particular cell type, human lung fibroblasts, navitoclax is senolytic in the culture-acclimated IMR-90 lung fibroblast-like cell strain but is less so in primary human lung fibroblasts isolated from individuals.[19,27]

Table 1. Senescent Cell Anti-Apoptotic Pathways (SCAPs)

SCAP	Original Description	Agents Targeting SCAP	Effective *in vitro*	Effective *in vivo*
1. BCL-2, BCL-X_L family	[22]	Navitoclax (ABT-263)[25,27,38]	√	√
		Fisetin[39,40]	√	
		A1331852[40]	√	
		A1155463[40]	√	
2. PI3Kδ, AKT, ROS-protective, metabolic*,§	[22]	Quercetin[22]	√	√
		Fisetin[40,41]	√	
		Piperlongumine[42,43]	√	
3. MDM2, p53, p21, serpine (PAI-1&2)*	[22]	Quercetin[22]	√	√
		Fisetin[40,44]	√	
		FOXO4-related peptide	√	√
		Dasatinib (FOXO-p53 interaction)		
4. Ephrins, dependence receptors, tyrosine kinases	[22]	Dasatinib (ephrin receptors)[22]	√	√
		Piperlongumine (androgen receptors)[45]	√	
5. HIF-1α	[22]	Quercetin[46]	√	√
		Fisetin[46]	√	
6. HSP-90§	[24]	17-AAG (tanespimycin)	√	
		Geldanamycin	√	
		17-DMAG (alvespimycin)	√	√

*Closely-interconnected SCAPs.
§Closely-interconnected SCAPs.

Without extensive testing of a range of truly primary cells, as opposed to cell lines or culture-acclimated cell strains, it is difficult to contend that any particular candidate senolytic drug is universally effective for all types of senescent cells. Furthermore, senolytics can act synergistically in some cell types. For example, although neither dasatinib nor quercetin was significantly senolytic in mouse embryonic fibroblasts in vitro, in combination, they were senolytic.[22] Thus, different senolytics may prove to be optimal for different indications, and combinations of senolytics can be used to broaden the range of senescent cell types targeted.

Several senolytics, including dasatinib plus quercetin, navitoclax, 17-DMAG, and a peptide that targets the BCL-2- and p53-related SCAPs, have been demonstrated to be effective in reducing senescent cell burden in mice, with decreases in cellular senescence–associated β-galactosidase activity; $p16^{Ink4a+}$ cells; $p16^{Ink4a}$, $p21^{Cip1}$, and SASP factor messenger RNA; telomere-associated foci; and other senescent cell indicators.[18,19,22,24–26] Among the effects of senolytics in mice so far are improved cardiac ejection fraction and fractional shortening in old mice[22]; enhanced vascular reactivity in old mice[18]; decreased vascular calcification and increased vascular reactivity in hypercholesterolemic, high fat–fed apolipoprotein $E^{-/-}$ mice[18]; reduced senescent cell-like, intimal foam cells and macrophages in vascular plaques in high fat–fed low-density lipoprotein $receptor^{-/-}$ mice[28]; decreased frailty, osteoporosis, loss of intervertebral disc glycosaminoglycans, and spondylosis in progeroid $Ercc1^{-/\Delta}$ mice[22]; decreased gait disturbance in mice after radiation damage to a leg[22] and hematological dysfunction caused by whole body radiation[25]; increased coat density[26]; and improved pulmonary function and reduced pulmonary fibrosis in mice with bleomycin-induced lung damage, a model of idiopathic pulmonary fibrosis.[19] Senolytics also had beneficial effects in mouse models of several other human chronic diseases and geriatric syndromes, which are about to be published. Information about whether senolytics affect lifespan has not been reported to the knowledge of the authors of the current study. In addition, more needs to be learned about the potential side effects of using senolytic drugs. For example, genetic clearance of senescent cells delays wound healing.[29]

Senolytics do not have to be continuously present to exert their effect. Brief disruption of pro-survival pathways is adequate to kill senescent cells. Thus, senolytics can be effective when administered intermittently.[22] For example, dasatinib and quercetin have an elimination half-life of a few hours, yet a single short course alleviates effects of leg radiation for at least 7 months. The frequency of senolytic treatment will depend on rates of senescent cell re-accumulation, which probably varies according to conditions that induce cellular senescence. For example, it is likely that continued high-fat feeding or exposure to genotoxic cancer therapies causes more-rapid accumulation of senescent cells than chronological aging. Advantages of intermittent administration include less opportunity to develop side effects, the feasibility of administering senolytic drugs during periods of relatively good health, and less risk of off-target effects caused by continuous exposure to drugs. Another advantage of senolytics is that cell division–dependent drug resistance is unlikely to occur, because senescent cells do not divide and therefore cannot acquire advantageous mutations, unlike the situation in treating cancers or infectious agents.

CLINICAL TRIAL STRATEGIES

New clinical trial strategies will be needed to test senolytics or other agents that target fundamental aging processes. Outcomes such as effects on median or maximum lifespan cannot be tested feasibly in humans. According to the geroscience hypothesis, if a candidate drug targets fundamental aging processes, it should affect a range of chronic

diseases, geriatric syndromes, and age-related loss of physiological resiliencies.[1,2,30–33] Thus, potential clinical trial scenarios include the following.

1. Simultaneous alleviation of multiple comorbidities. In individuals with multimorbidity, which is common in older adults,[3] candidate senolytics should alleviate more than one pathology, such as glucose intolerance, mild cognitive impairment, joint pain due to osteoarthritis, systolic hypertension, or low carotid flow. Alternatively, these drugs should delay the onset of a second age-related disorder in individuals who already have one disorder, similar to the design of the Targeting Aging with Metformin trial for testing the effect of metformin on fundamental aging processes.[1,30,32]

2. Alleviation of potentially fatal diseases. A number of diseases for which there is no effective treatment are related to accumulation of senescent cells. These include idiopathic pulmonary fibrosis and primary sclerosing cholangitis.[19,34] Senolytic agents hold promise as treatments for these conditions. In these examples, the potential benefits of treatment are likely to outweigh the risk of side effects.

3. Treatment of conditions with localized senescent cell accumulation. Several disorders, including osteoarthritis,[10] idiopathic pulmonary fibrosis,[19] and retinopathies,[35] are associated with localized accumulation of senescent cells. This offers the opportunity to administer senolytics by injection or aerosol, which will reduce the risk of side effects.

4. Treatment of accelerated aging-like states. Senolytics or other agents that target basic aging processes may be effective in treating conditions associated with accelerated aging-like phenotypes, including those induced by chemotherapy related to bone marrow transplantation or treatment of childhood cancers, human immunodeficiency infection, obesity, or genetic progeroid syndromes.[1,30,31] Short-term trials examining outcomes such as reduction of multimorbidity, frailty, or rate of functional decline may hold promise.

5. Augmenting physiological resilience. Resilience, or capacity to recover after a stress such as surgery, chemotherapy, radiation, pneumonia, or a myocardial infarction, declines with aging.[2] Lack of resilience also underlies such conditions as poor immune response to influenza vaccination or deceased ability to exercise with aging. Loss of resilience occurs before the onset of frailty and other conditions that are visible even in the absence of stress. Thus, testing if drugs that target fundamental aging processes enhance recovery following stressful medical interventions or acute injury might be an informative clinical trial strategy. For example, such trials could be based on the observations that senolytics reduce adverse consequences of bleomycin-induced pulmonary injury[19] and radiation-induced injury in mice.[22] A drug related to rapamycin, an agent that inhibits the SASP, increased immune responses to influenza vaccination in elderly community-living subjects.[36]

6. Alleviation of frailty. Targeting senescent cells, even in late life in rodents, appears to reduce immobility, weakness, fat tissue loss, and other parameters associated with frailty.[17,22,37] Senolytics may be tested in short-term clinical trials that include older adults with a moderate degree of frailty to determine whether strength, gait, body weight, or other relevant parameters improve.

POTENTIAL OF SENOLYTICS TO TRANSFORM GERIATRIC MEDICINE

The introduction of effective senolytics or other agents that target fundamental aging processes into clinical practice could be transformative. These drugs may be critical to increasing healthspan and delaying, preventing, or alleviating the multiple chronic diseases that account for the bulk of morbidity, mortality, and health costs in developed and developing societies.[1] They could also delay or treat the geriatric syndromes, including sarcopenia, frailty, immobility, and cognitive impairment, as well as age-related loss of physiological resilience, in a way not imaginable until recently. These agents could transform geriatric medicine from being a discipline focused mainly on tertiary or quaternary prevention into one with important primary preventive options centered on a solid science foundation equivalent to, or even better than, that of other medical specialties.

The basic biology of aging has moved rapidly in the last few years toward clinical intervention. There is a severe shortage of geriatricians with sufficient understanding of basic biology and translational science to lead early proof-of-concept clinical trials to determine whether these emerging interventions will have clinical utility. Such investigators are needed now. Until they are trained, clinical geriatricians, scientists trained in the basic biology of aging, and investigators with experience in early-phase clinical trials and drug regulatory systems could work in teams to translate senolytics and other drugs that target basic aging processes into clinical interventions.

Senolytics might prevent or delay chronic diseases as a group, instead of one at a time in presymptomatic or at-risk individuals. Furthermore, if what can be achieved in preclinical aging animal models can be achieved in humans, it may be feasible to alleviate dysfunction even in frail individuals with multiple comorbidities, a group that until recently was felt to be beyond the point of treatment other than palliative and supportive measures. Although considerable care must be taken, particularly until clinical trials are completed and the potential adverse effects of senolytic drugs are understood fully, it is conceivable that the rapidly emerging repertoire of senolytic agents might transform medicine as we know it.

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