

SENOLYTIC DRUGS FOR AGE RELATED DISEASES

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ABSTRACT:

Aging is the greatest risk factor for the development of chronic diseases, including cardiovascular disorders, neurodegenerative diseases, diabetes, osteoarthritis, chronic kidney disease, pulmonary fibrosis, and certain cancers. Cellular senescence, characterized by irreversible cell-cycle arrest and the secretion of pro-inflammatory factors collectively known as the senescence-associated secretory phenotype (SASP), plays a pivotal role in driving chronic inflammation, tissue dysfunction, and age-related pathologies. Senolytic drugs are a novel class of therapeutics that selectively eliminate senescent cells, thereby reducing SASP-mediated damage and restoring tissue homeostasis. Several senolytic agents, including dasatinib, quercetin, fisetin, navitoclax, and emerging BCL-2 family inhibitors, have demonstrated promising results in preclinical studies by improving physical function, attenuating disease progression, and extending healthspan. Early clinical trials have also shown encouraging therapeutic potential in conditions such as idiopathic pulmonary fibrosis, diabetic kidney disease, and frailty, although further evidence is needed to confirm their long-term safety and efficacy. This review summarizes the molecular mechanisms of cellular senescence and the therapeutic rationale for senolytic interventions. It discusses the major classes of senolytic drugs, their mechanisms of action, recent preclinical and clinical advances, and their potential applications in age-related diseases. Furthermore, the review highlights current challenges, including target specificity, adverse effects, and translational barriers, while exploring emerging strategies to improve senolytic efficacy. Overall, senolytic therapy represents a promising geroscience-based approach that targets fundamental mechanisms of aging, with the potential to delay or prevent multiple age-related diseases and enhance healthy lifespan.

KEY WORDS:

Senotherapeutics, Dasatinib, Quercetin, Fisetin, Navitoclax, BCL-2 inhibitors, Inflammaging, Healthspan.

1.INTRODUCTION:

Ageing is strongly linked to a progressive increase in the risk of developing numerous chronic diseases, geriatric syndromes, reduced physical resilience, and higher mortality rates^[1-4]. These risks rise exponentially during the final 25% of the human lifespan^[5]. Chronological age is recognized as one of the most significant risk factors for a wide range of disorders, including congestive heart failure, myocardial infarction, dementia, stroke, most forms of cancer, diabetes and other metabolic disorders, renal impairment, chronic respiratory diseases, osteoporosis, arthritis, blindness, and several additional age-related conditions^[6-8]. Geriatric syndromes commonly associated with ageing include frailty, sarcopenia, falls, urinary incontinence, and mild cognitive impairment. A decline in physiological resilience is often reflected by delayed recovery following events such as myocardial infarction, stroke, trauma, or surgical procedures, as well as increased susceptibility to severe infections, including influenza and coronavirus infections, and a

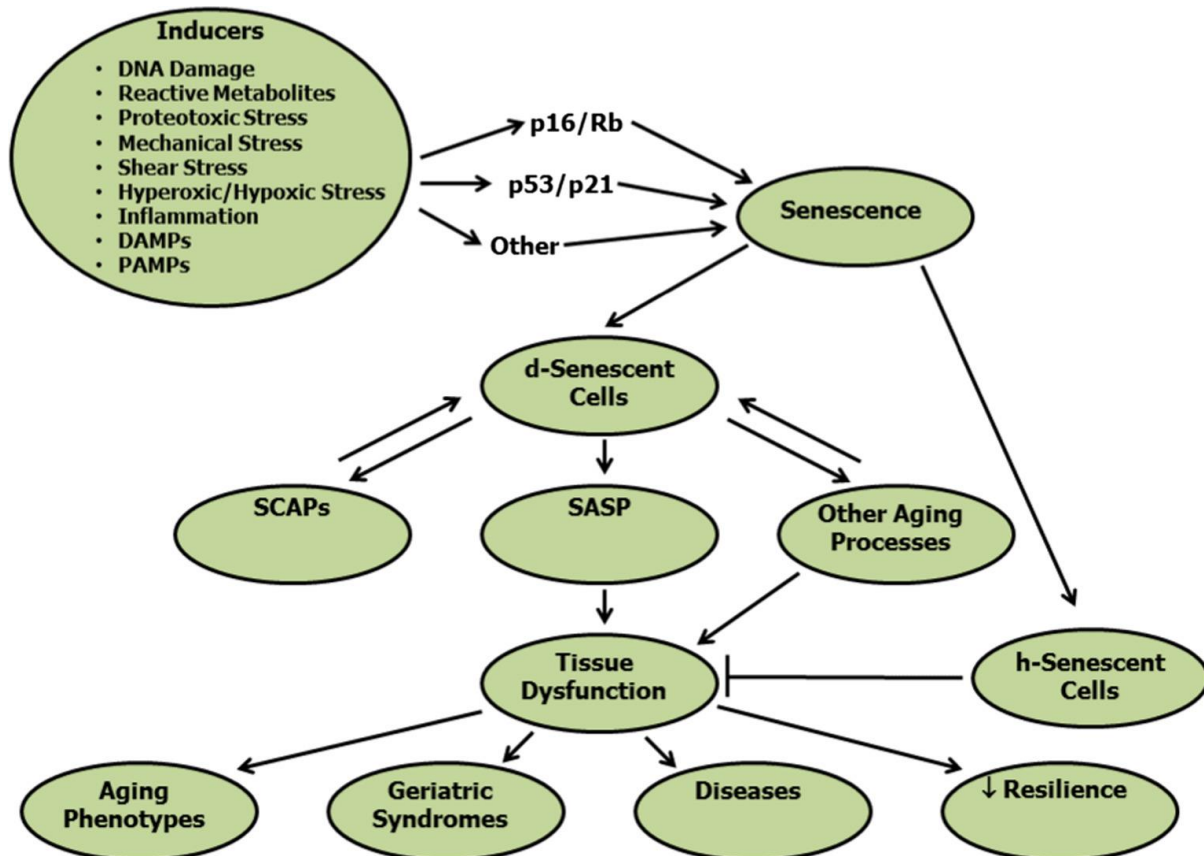
reduced antibody response following vaccination^[3]. In older adults, multiple chronic diseases, geriatric syndromes, and impairments in resilience frequently coexist. The prevalence of multimorbidity, defined as the simultaneous presence of three or more health conditions, increases exponentially from adulthood onward^[5]. Comparable age-related increases in disease burden have also been observed across a variety of species.

The Geroscience Hypothesis proposes that the fundamental biological mechanisms of ageing act as the primary drivers of the increasing incidence of chronic diseases and disorders associated with advancing age, which account for a substantial proportion of morbidity, mortality, and healthcare expenditure worldwide^[9]. These core ageing mechanisms include: persistent low-grade sterile inflammation, occurring in the absence of infectious agents such as bacteria or fungi, and frequently accompanied by fibrosis; macromolecular and organelle dysfunction, characterized by DNA damage, telomere uncapping, protein misfolding and aggregation, impaired autophagy, accumulation of advanced glycation end-products (AGEs), lipotoxicity, and the buildup of bioactive lipids, together with organelle abnormalities such as altered nuclear membrane integrity resulting from lamin B deficiency, mitochondrial dysfunction leading to reduced fatty acid oxidation, increased glucose utilization, depletion of NAD⁺, and enhanced production of reactive oxygen species (ROS); dysfunction of stem cells, progenitor cells, and immune cells, including reduced proliferative capacity, abnormal differentiation into mature functional cells, and decreased levels of geroprotective factors such as α -Klotho, which further contribute to impaired stem and progenitor cell function; and (cellular senescence. The Unitary Theory of Fundamental Aging Processes suggests that these biological mechanisms are interconnected rather than acting independently. According to this theory, genetic or pharmacological modulation of any single fundamental ageing process, such as cellular senescence, is expected to influence many, or potentially all, of the remaining ageing mechanisms^[10,11].

2. CELLULAR SENESENCE:

Cellular senescence, first described by Hayflick and Moorhead in 1961, represents a distinct cellular state characterized by a largely irreversible arrest of cell proliferation while maintaining cell viability, exhibiting resistance to apoptosis, and often displaying elevated metabolic activity^[6,8,12,14]. The transition of cells into a senescent state is triggered by a variety of intracellular and extracellular stimuli, primarily those associated with tissue injury, cellular stress, or the development of cancer. These stimuli include DNA damage, telomere uncapping or dysfunction, exposure to extracellular DNA, activation of oncogenes, replicative stress, excessive proliferative signals such as growth hormone and insulin-like growth factor-1 (IGF-1), accumulation of protein aggregates, the presence of misfolded proteins, and impaired protein degradation or clearance mechanisms.

These senescence-inducing stimuli activate one or more transcriptional pathways that promote the establishment of cellular senescence. Depending on the nature of the trigger, these pathways may involve the p16^{INK4a}-retinoblastoma protein (Rb) axis, the p53–p21^{CIP1} pathway, a combination of both, or alternative signaling mechanisms. Activation of these transcriptional networks leads to permanent cell-cycle arrest, extensive alterations in the expression of hundreds of genes, and widespread epigenetic modifications of DNA. Unlike other cellular outcomes such as proliferation, differentiation, apoptosis, or necrosis, the development of cellular senescence is a relatively slow process. In cell culture, the transition from the initial senescence-inducing stimulus to the establishment of a fully senescent phenotype typically requires approximately 10 days to 6 weeks, depending on the cell type and the specific stimulus responsible for inducing senescence.



The composition and intensity of the senescence-associated secretory phenotype (SASP) can be influenced by a variety of endogenous and exogenous factors, including hormones, pharmacological agents, pathogens, and other environmental stimuli. For instance, endogenous or therapeutically administered glucocorticoids have been shown to suppress the secretion of specific SASP components^[15]. Similarly, drugs such as rapamycin and metformin can reduce the production of selected SASP factors.^[16,17] In contrast, bacterial toxins, including lipopolysaccharide (LPS), as well as pro-inflammatory cytokines such as TNF- α , can amplify SASP activity^[18] (unpublished observations). The SASP is also a dynamic phenotype that evolves over time in response to both intracellular and extracellular influences. During prolonged senescence, transpositional events involving LINE-1 and other mobile genetic elements may occur after several months.^[19,20] These events can induce chromosomal rearrangements and activate the cyclic GMP–AMP synthase/stimulator of interferon genes (cGAS/STING) signaling pathway, which detects DNA damage and enhances the production of inflammatory SASP factors through interferon-1 signaling. Consequently, the characteristics of the SASP differ substantially according to the tissue in which deleterious senescent (D-senescent) cells reside, their cell of origin, the stimuli responsible for inducing senescence, the surrounding hormonal environment, exposure to therapeutic agents or pathogens, and the duration of the senescent state.

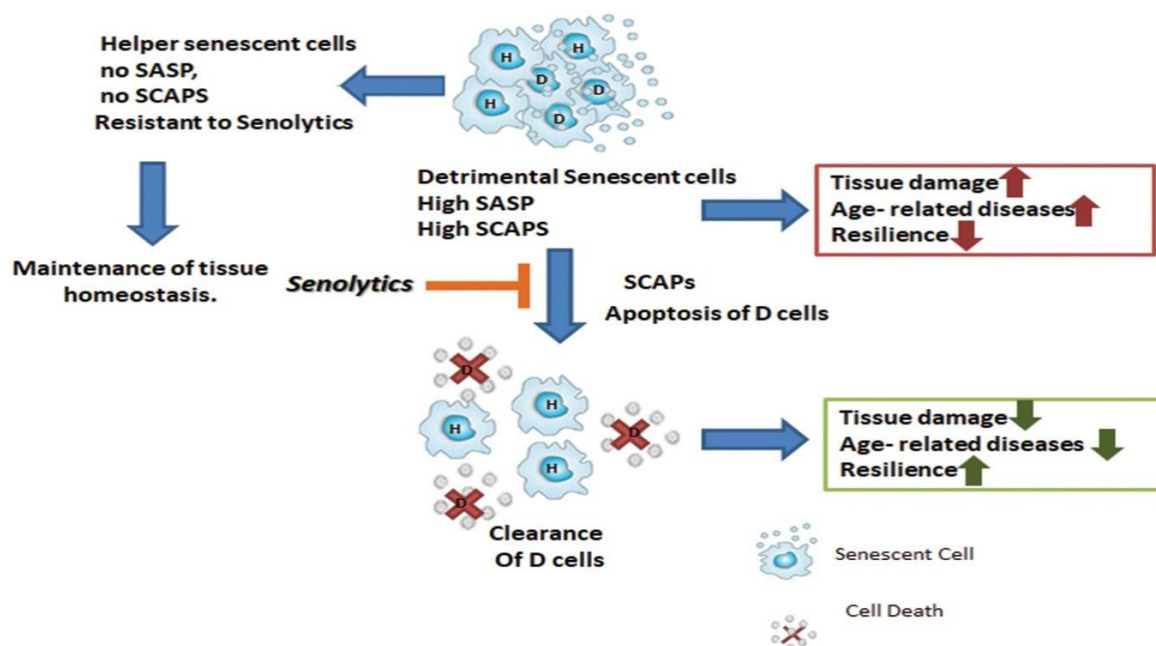
In contrast to deleterious senescent cells, another subset, referred to as helper (H-) senescent cells, appears to produce little or no inflammatory or pro-apoptotic secretory factors. These cells are thought to play beneficial roles by supporting the differentiation of stem and progenitor cells into appropriate functional lineages and facilitating tissue homeostasis^[21,22] (unpublished observations). It has been estimated that H-senescent cells may constitute approximately 30–70% of the total senescent cell population. It has also been proposed that helper (H-) and deleterious (D-) senescent cells may possess the ability to transition between these functional states through currently unidentified regulatory mechanisms; however, this possibility requires further investigation.

Currently, no single biomarker possesses complete sensitivity or specificity for the identification of senescent cells. In general, senescent cells exhibit a larger cellular size than their non-senescent counterparts. They commonly display multiple DNA damage foci that can be detected by immunostaining for γ -histone H2AX (γ H2AX); however, γ H2AX-positive foci are not unique to senescent cells and may also be present in cancer cells and various normal cell types.^[23] A more reliable indicator of cellular senescence is the presence of two or more γ H2AX foci localized within telomeric DNA, known as telomere-associated foci (TAFs), which provide greater sensitivity and specificity for senescence detection. Another characteristic feature is the development of senescence-associated distension of satellites (SADS), involving the expansion of pericentromeric satellite DNA, which can be identified using antibodies against centromere protein B (CENP-B). Senescent cells also frequently exhibit elevated lysosomal β -galactosidase activity, commonly referred to as senescence-associated β -galactosidase (SA- β -gal). Nevertheless, this marker lacks specificity because increased β -galactosidase activity is also observed in macrophages and several other cell types. Similarly, increased expression of p16^{INK4a} is detected in only a subset of senescent cells and is not exclusive to senescence, as activated macrophages and many other cell populations can also express high levels of this protein. Likewise, p21^{CIP1} is neither sufficiently sensitive nor specific to serve as a definitive marker of cellular senescence.^[24] In addition, certain senescent cell populations express components of the senescence-associated secretory phenotype (SASP) or damage-associated molecules such as cytosolic HMGB1, although these features are also not universally present in all senescent cells.

A proportion of senescent cells exhibit enlarged and fused mitochondria accompanied by significant alterations in mitochondrial function. These changes include a metabolic transition from fatty acid oxidation to increased glucose utilization for energy production, resembling the Warburg effect observed in cancer cells.^[25,26] Enhanced reliance on glycolysis is often associated with intracellular lipid accumulation, leading to the formation of lipid droplets and the development of lipotoxicity. Consequently, senescent mesenchymal cells may acquire an adipocyte-like phenotype, referred to as mesenchymal adipocyte-like default (MAD) cells, which are characterized by insulin resistance.^[27] A notable example is the accumulation of lipid-rich, perilipin-positive, pro-inflammatory senescent ependymal MAD cells surrounding the third ventricle in obesity.^[11] These cells have been implicated in promoting neuroinflammation, disrupting cerebral microcirculation, impairing neurogenesis, and contributing to neuropsychiatric abnormalities in obese, insulin-resistant mice.

Although senescent cells can arise at any stage of life in vertebrates, including as early as the 32-cell stage of human blastocyst development,^[28] their abundance increases progressively in numerous tissues during the later stages of the lifespan. For instance, in otherwise healthy individuals, the number of senescent cells in the skin rises markedly, typically between 60 and 80 years of age, although this observation remains a topic of debate. A similar age-related accumulation of senescent cells has been reported in adipose tissue obtained from healthy human kidney donors as well as in adipose depots of ageing mice.^[29,30]

Experimental studies further demonstrate the pathological consequences of senescent cell accumulation. Transplantation of 10^6 syngeneic adipose progenitor cells or autologous ear fibroblasts rendered senescent through irradiation, doxorubicin treatment, or repeated cell replication is sufficient to induce frailty and accelerate all-cause mortality in middle-aged mice, whereas transplantation of equivalent numbers of non-senescent cells does not produce these effects.^[31] Similarly, the introduction of 10^6 senescent human cells into immunodeficient mice results in the development of frailty. In another study, transplantation of senescent porcine renal scattered tubular-like cells into the aorta of young mice induced renal inflammation and fibrosis in recipient animals, whereas transplantation of non-senescent cells failed to produce comparable pathological changes.^[32]



A delay exists between the accumulation of senescent cells and the onset of measurable functional impairments. In middle-aged mice, signs of frailty became evident approximately one month after senescent cell transplantation, whereas an increase in all-cause mortality was observed only after about three months.^[31] These findings indicate that the presence of as few as one transplanted senescent cell per 10,000 recipient cells is sufficient to trigger an accelerated ageing-like phenotype. However, transplantation of 500,000 senescent cells into middle-aged mice failed to produce the same outcome, suggesting the existence of a critical senescent cell threshold required to induce pathological effects. Supporting this concept, transplantation of 500,000 senescent cells into aged mice, rather than middle-aged recipients, was sufficient to induce frailty. Likewise, diet-induced obese (DIO) middle-aged mice developed frailty following transplantation of 500,000 senescent cells, whereas age-matched lean mice required transplantation of 10^6 senescent cells to exhibit similar effects. These observations suggest that the existing senescent cell burden, combined with the accumulation of additional senescent cells, can exceed a pathological threshold and ultimately lead to clinically significant manifestations.

3.DISCOVERY OF SENOLYTIC DRUGS:

These findings suggest an inverse relationship between senescent cell burden and healthspan, with increased accumulation of senescent cells being associated with declining physiological function. In 2005, efforts were initiated to develop fusion proteins capable of selectively eliminating senescent cells by combining senescent cell-specific recognition domains with cytotoxic molecules. Simultaneously, high-throughput screening approaches were employed to identify compounds that could selectively induce the death of senescent cells while sparing non-senescent cells. However, neither strategy proved successful. A major limitation of the high-throughput screening approach was the selection of appropriate control cells. To prevent proliferation, non-senescent control cells had to be rendered quiescent through serum starvation or contact inhibition, but both conditions introduced experimental artefacts. Alternatively, proliferating non-senescent cells could be used as controls; however, comparisons between actively dividing cells and senescent cells, which are permanently growth-arrested by definition, created a significant confounding factor that complicated interpretation of the screening results.

Consequently, instead of relying on conventional random high-throughput drug screening or traditional drug discovery methods, a hypothesis-driven, mechanism-based approach was adopted to identify the first senolytic agents. This strategy was inspired by the observation made by E. Wang in 1995 that senescent cells

exhibit resistance to apoptosis. Notably, this resistance persists despite the secretion of pro-apoptotic factors as part of the senescence-associated secretory phenotype (SASP). Although the concept of the SASP had been discussed within the scientific community for several years, it was formally described only after its subsequent publication.^[33,34]

The ability of small interfering RNAs (siRNAs) targeting anti-apoptotic regulators to selectively reduce the viability of senescent cells was found to depend on the specific senescent cell type. For example, siRNAs directed against BCL-xL and other members of the BCL-2 protein family effectively induced apoptosis in senescent, but not non-senescent, primary human umbilical vein endothelial cells (HUVECs), a finding that was subsequently confirmed by independent studies.^[35] In contrast, the same siRNAs failed to selectively eliminate senescent human primary adipocyte progenitor cells. Furthermore, effective elimination of certain senescent cell populations required simultaneous targeting of multiple senescent cell anti-apoptotic pathways (SCAPs), indicating that resistance to apoptosis in these cells is maintained through redundant survival mechanisms.

Subsequently, bioinformatics-based analyses were employed to identify compounds capable of targeting the SCAP network components that are essential for senescent cell survival. This approach yielded 46 candidate senolytic agents. To facilitate rapid clinical translation, initial efforts focused on compounds that were already approved or commonly used in humans for other therapeutic purposes, many of which were established anticancer agents. Among the most promising candidates were the tyrosine kinase inhibitor Dasatinib (D), approved for clinical use in the United States since 2006, and Quercetin (Q), a naturally occurring flavonoid responsible for the characteristic bitter taste of apple peels. Unlike several other tyrosine kinase inhibitors, such as Imatinib, which do not exhibit senolytic activity, Dasatinib promotes apoptosis mediated by dependence receptors, including ephrin receptors, partly through inhibition of Src kinase. Another naturally occurring flavonoid with senolytic properties is Fisetin (F).^[36,37] The senolytic effects of these flavonoids are mediated, at least in part, through inhibition of BCL-2 family proteins such as BCL-xL, as well as suppression of HIF-1 α and other key components of the SCAP signaling network.

The primary objective of authentic senolytic agents is the selective elimination of senescent cells rather than inhibition of a single receptor, enzyme, or biochemical pathway. Strategies aimed at identifying compounds that simultaneously target multiple components of the senescent cell anti-apoptotic pathway (SCAP) network enhance selectivity toward senescent cells while minimizing unintended effects on non-senescent cells. Compared with agents directed against a single molecular target, such multi-target senolytics are expected to reduce off-target toxicity and improve overall safety profiles. Consistent with this concept, compounds such as Navitoclax (N)^[38,39] and Nutlin-3a,^[40] which primarily inhibit one or a limited number of molecular targets, exhibit considerable off-target apoptotic effects in non-senescent cells, including platelets and immune cells. Consequently, these agents may function as "panolytic" compounds rather than being truly senolytic. Moreover, Nutlin compounds have been reported to induce cellular senescence under certain conditions,^[41] while agents such as Navitoclax, which target only a few SCAP components, are capable of eliminating only a restricted subset of senescent cell populations. For example, Navitoclax and other BCL-2 family inhibitors, including A1331852 and A1155463, do not exhibit senolytic activity against human adipocyte progenitor cells, one of the most abundant senescent cell types found in older adults and in individuals with obesity or diabetes.^[37,38] Therefore, compounds that act on only one or a few SCAP-related targets generally display lower senescent cell specificity and greater panolytic activity, resulting in apoptosis or functional impairment of multiple non-senescent cell types. In contrast, agents or drug combinations that simultaneously inhibit multiple SCAP network nodes provide greater selectivity for senescent cells.

Based on their established molecular targets, it was hypothesized that Dasatinib (D) would selectively eliminate senescent human adipocyte progenitor cells, whereas Quercetin (Q) would be effective against senescent cultured human umbilical vein endothelial cells (HUVECs). Experimental findings confirmed

these predictions. Importantly, the combined administration of Dasatinib and Quercetin (D + Q) selectively induced apoptosis in both senescent human adipocyte progenitor cells and senescent HUVECs while sparing their non-senescent counterparts. Furthermore, owing to the redundancy of SCAP signaling pathways in certain cell types, such as mouse embryonic fibroblasts, neither Dasatinib nor Quercetin alone demonstrated senolytic activity, whereas their combination effectively eliminated senescent cells.^[42]

The effectiveness of small interfering RNAs (siRNAs) directed against anti-apoptotic regulators in selectively reducing senescent cell viability was found to vary according to the senescent cell type. For instance, siRNAs targeting BCL-xL and other members of the BCL-2 family efficiently induced apoptosis in senescent, but not non-senescent, primary human umbilical vein endothelial cells (HUVECs), a finding that was later validated by independent studies.^[42,43] However, these same siRNAs failed to selectively eliminate senescent human primary adipocyte progenitor cells. Moreover, successful eradication of certain senescent cell populations required the simultaneous inhibition of multiple senescent cell anti-apoptotic pathways (SCAPs), indicating that apoptosis resistance in these cells is maintained through redundant survival mechanisms.

To identify potential senolytic agents, bioinformatics-based analyses were subsequently employed to screen for compounds capable of targeting the SCAP network components that are essential for senescent cell survival. This strategy led to the identification of 46 candidate senolytic compounds. To facilitate rapid clinical translation, priority was given to drugs and naturally occurring compounds that were already in clinical use for other therapeutic indications, particularly anticancer agents. Among these were Dasatinib (D), a tyrosine kinase inhibitor approved for clinical use in the United States in 2006, and Quercetin (Q), a naturally occurring flavonoid responsible for the characteristic bitter taste of apple peels. Unlike tyrosine kinase inhibitors such as Imatinib, which lack senolytic activity, Dasatinib promotes apoptosis mediated by dependence receptors, including ephrin receptors, partly through inhibition of Src kinase.^[44] Another naturally occurring flavonoid with demonstrated senolytic activity is Fisetin (F).^[36,37] The senolytic effects of flavonoids such as Quercetin and Fisetin are mediated, at least in part, through inhibition of BCL-2 family proteins, including BCL-xL, as well as HIF-1 α and other critical components of the SCAP signaling network.

The primary objective of true senolytic agents is the selective elimination of senescent cells rather than targeting a single receptor, enzyme, or individual biochemical pathway. Consequently, the development of senolytics has focused on identifying compounds capable of simultaneously inhibiting multiple components of the senescent cell anti-apoptotic pathway (SCAP) network. Such multi-target approaches enhance specificity toward senescent cells, minimize off-target effects on non-senescent cells compared with agents acting on a single molecular target, and are expected to improve overall safety profiles. Supporting this concept, compounds that primarily inhibit one or only a few targets, such as Navitoclax (N)^[38,39] and Nutlin-3a,^[40] exhibit substantial off-target apoptotic effects in non-senescent cell populations, including platelets and immune cells,^[45,46] thereby functioning as potential "panolytic" agents rather than selective senolytics. Moreover, Nutlin compounds have been reported to induce cellular senescence under certain conditions,^[41] while Navitoclax and similar agents targeting only a limited number of SCAP components eliminate only specific subsets of senescent cells. For example, Navitoclax and other BCL-2 family inhibitors, including A1331852 and A1155463, fail to exhibit senolytic activity against human adipocyte progenitor cells, one of the most prevalent senescent cell populations in older individuals and patients with obesity or diabetes.^[37,38] Therefore, compounds acting on a narrow range of SCAP-associated targets generally possess lower senescent cell specificity and greater panolytic activity, resulting in apoptosis or dysfunction of multiple non-senescent cell types, whereas agents or drug combinations that inhibit multiple SCAP network nodes provide more selective senolytic effects.

Both the Dasatinib plus Quercetin (D + Q) combination and Fisetin (F) have been demonstrated to reduce senescent cell burden in vivo without causing significant elimination of non-senescent cells.^[31,36] In one study, 10⁵ radiation-induced syngeneic senescent adipocyte progenitor cells expressing luciferase under the constitutive SFFV promoter were transplanted intraperitoneally into middle-aged mice. Oral administration of D + Q for three consecutive days resulted in a marked reduction in luminescence two days after the final dose, indicating successful clearance of transplanted senescent cell.^[31] In contrast, no reduction in luminescence was observed in vehicle-treated mice receiving senescent cell transplants, nor in mice transplanted with non-senescent luciferase-expressing cells regardless of whether they received D + Q or vehicle treatment. Similarly, oral administration of Fisetin to progeroid *Ercc*^{-/Δ} mice expressing luciferase under the control of the p16^{Ink4a} promoter significantly reduced luminescence, whereas vehicle treatment produced no detectable effect. Collectively, these findings demonstrate that both D + Q and Fisetin exhibit senolytic activity in mice, eliminating approximately 30–70% of transplanted or endogenous senescent cells, respectively.

To evaluate the senolytic activity of Dasatinib plus Quercetin (D + Q) in human tissue, freshly excised abdominal subcutaneous adipose tissue samples obtained during surgery from patients with obesity and diabetes—conditions associated with substantial accumulation of senescent adipose cells^[47,48,49]—were immediately maintained in organ culture and treated with either D + Q or vehicle control.^[31] After two days of treatment, the number of senescent cells, assessed by the presence of telomere-associated foci (TAFs), was reduced by approximately 70%. This reduction was accompanied by increased levels of cleaved caspase-3, confirming that senescent cell clearance occurred through apoptosis. In addition, conditioned media collected from D + Q-treated organ cultures exhibited significantly lower concentrations of key senescence-associated secretory phenotype (SASP) factors, including IL-6, IL-8, MCP-1, PAI-1, and GM-CSF, compared with vehicle-treated controls. Similar senolytic effects were observed with Fisetin (F), which also decreased the abundance of senescent cells in adipose tissue organ cultures.^[36] Collectively, these findings demonstrate that both D + Q and Fisetin effectively induce apoptotic elimination of senescent cells in human adipose tissue, resulting in a marked reduction in senescent cell burden within two days.

4. SENOLYTICS ALLEVIATE MULTIPLE DISORDERS IN EXPERIMENTAL ANIMALS:

Senescent cells have been identified in the venous segment of surgically created arteriovenous (AV) fistulae used for haemodialysis, where they may contribute to thrombosis and eventual fistula failure. In murine models, treatment with Dasatinib plus Quercetin (D + Q) significantly reduced the accumulation of senescent cells within AV fistulae. In addition, D + Q decreased both senescent cell burden and medial vascular calcification in high-fat diet-fed *ApoE*^{-/-} mice, an established model of atherosclerosis.^[50] Similarly, Navitoclax (N) has demonstrated senolytic activity in the ageing heart.^[51] One month of Navitoclax treatment reduced cardiac fibrosis and produced approximately a 40% decrease in telomere-associated foci (TAF)-positive cardiomyocytes, suggesting that terminally differentiated, non-proliferating cells such as cardiomyocytes can acquire a senescence-like phenotype. Collectively, these findings indicate that senolytic therapies can attenuate age- and lipid-associated cardiovascular dysfunction in experimental animal models.

Intermittent oral administration of D + Q has also been shown to improve insulin sensitivity in both diet-induced obese (DIO) mice and genetically obese mouse models, as demonstrated by enhanced glucose tolerance and insulin responsiveness. These metabolic benefits are primarily attributed to a reduction in peripheral insulin resistance. Mechanistically, D + Q restores insulin-mediated AKT signaling in adipose tissue and improves the differentiation capacity of adipocyte progenitor cells, thereby promoting the formation of insulin-responsive adipocytes. Furthermore, intermittent D + Q treatment alleviates adipose tissue inflammation, a major contributor to the development of type 2 diabetes. This anti-inflammatory effect is achieved partly through reduced recruitment of circulating monocytes into adipose tissue, as evidenced by decreased migration of luciferase-labeled monocytes injected through the tail vein into the adipose tissue of

genetically obese mice treated with D + Q compared with vehicle-treated controls. Importantly, D + Q does not eliminate activated macrophages that accumulate in diabetic adipose tissue.^[31] Although these macrophages express elevated levels of p16^{INK4a}, they are not truly senescent. Consistent with this observation, senolytic agents also do not target atherosclerotic foam cells, which are activated macrophages. Instead, they selectively eliminate authentic senescent cells located deeper within atherosclerotic plaques and the vascular media, thereby contributing to improved vascular health.^[50]

Senolytic therapies have been shown to ameliorate several diabetes-associated complications in experimental mouse models. Hepatic steatosis, which can progress to cirrhosis and liver failure, is an increasingly prevalent consequence of obesity and diabetes.^[47,52] Treatment with Dasatinib plus Quercetin (D + Q) reduces the accumulation of senescent cells in the liver and attenuates hepatic steatosis in genetically obese mice. Renal dysfunction and proteinuria are also common complications of diabetes. Compared with lean controls, diet-induced obese (DIO) mice exhibit an increased burden of senescent renal tubular cells, characterized by elevated expression of p16^{Ink4a}, p53, and multiple senescence-associated secretory phenotype (SASP) factors, together with glomerular enlargement, impaired kidney function, and reduced renal cortical oxygenation. Administration of D + Q significantly decreases proteinuria in DIO mice.^[48] Likewise, intermittent oral treatment with Quercetin (Q) for five days every two weeks over a ten-week period reduces renal senescent cell abundance and SASP expression, limits renal fibrosis, improves cortical oxygenation, and lowers plasma creatinine concentrations compared with vehicle-treated animals.^[53]

Beyond metabolic disorders, senolytic agents have demonstrated beneficial effects in mouse models of Alzheimer's disease (AD) characterized by either Tau or β -amyloid ($A\beta$) pathology. Abnormal accumulation of Tau protein aggregates is a hallmark of numerous neurodegenerative disorders, including AD, progressive supranuclear palsy, traumatic brain injury, and several other tauopathies. Moreover, neurofibrillary tangles composed of Tau strongly correlate with neuronal loss and cognitive decline. Transcriptomic analyses of neurofibrillary tangle-containing neurons isolated from postmortem AD brains reveal molecular signatures consistent with cellular senescence. In both humans and mice, increased p16^{INK4a} expression is positively associated with brain atrophy and the burden of neurofibrillary tangles. In 23-month-old tauNFT-Mapt0/0 mice overexpressing Tau, intermittent D + Q treatment administered every two weeks for three months reduced neurofibrillary tangle density, neuronal degeneration, neuroinflammation, gliosis, cerebral microvascular hypoperfusion, and ventricular enlargement, while simultaneously increasing cortical volume and promoting neurogenesis despite the advanced age of the animals. Similarly, D + Q treatment in $A\beta$ -positive mouse models selectively eliminated senescent cells surrounding amyloid plaques, reduced neuroinflammation and amyloid burden, and improved cognitive performance, including memory and executive function.^[54] Collectively, these findings suggest that senolytic therapies represent promising mechanism-based interventions for Alzheimer's disease and other chronic neurodegenerative disorders, although confirmation of their clinical efficacy will depend on the outcomes of ongoing human trials.

Exposure of naïve airway smooth muscle (ASM) cells to conditioned media obtained from hyperoxia-induced senescent ASM cells resulted in increased collagen and fibronectin production together with enhanced cellular contractility, indicating that senescent cells promote tissue remodeling through paracrine signaling. Treatment with the senolytic combination Dasatinib plus Quercetin (D + Q) selectively eliminated hyperoxia-induced senescent ASM cells, highlighting the potential of senolytic therapy for the management of a variety of pulmonary disorders. Supporting this concept, the first clinical trial investigating senolytics demonstrated that D + Q improved physical function in a preliminary study involving patients with idiopathic pulmonary fibrosis (IPF)(see below). In addition, the senolytic flavonoid Fisetin (F) has been shown to reduce mortality in mice infected with murine β -coronavirus, while viral antigens from COVID-19 have been observed to amplify the senescence-associated secretory phenotype (SASP) in human senescent cells (unpublished observations). These findings provided the rationale for a clinical trial evaluating Fisetin

in older hospitalized patients with COVID-19 to prevent progression to cytokine storm and acute respiratory distress syndrome (ARDS), a study that received approval from the U.S. Food and Drug Administration (FDA) and was scheduled to commence.

Treatment with D + Q also increased femoral cortical thickness and improved bone strength. These beneficial effects were accompanied by reduced numbers of endocortical osteoclasts together with increased endocortical osteoblast populations, enhanced mineral apposition, and elevated bone formation rates. Consequently, senolytic therapy suppressed bone resorption while maintaining trabecular bone formation and enhancing cortical bone formation. This therapeutic profile differs from that of conventional antiresorptive therapies, which typically reduce both bone resorption and bone formation simultaneously. The beneficial skeletal effects of D + Q appear to result from decreased differentiation of bone-resorbing osteoclasts and enhanced generation of bone-forming osteoblasts from their respective stem and progenitor cell populations. Furthermore, intermittent administration of D + Q produced efficacy comparable to continuous treatment, supporting the concept that a "hit-and-run" dosing strategy is sufficient for senolytic therapy while potentially minimizing adverse effects. Based on these encouraging findings, a clinical trial investigating D + Q for age-related osteoporosis is currently underway (see below). Although Navitoclax (N) also reduces senescent cell burden in the bones of aged mice, it fails to improve age-related osteoporosis because, consistent with its broader panolytic activity, it exerts direct cytotoxic effects on bone-forming osteoblasts.^[55]

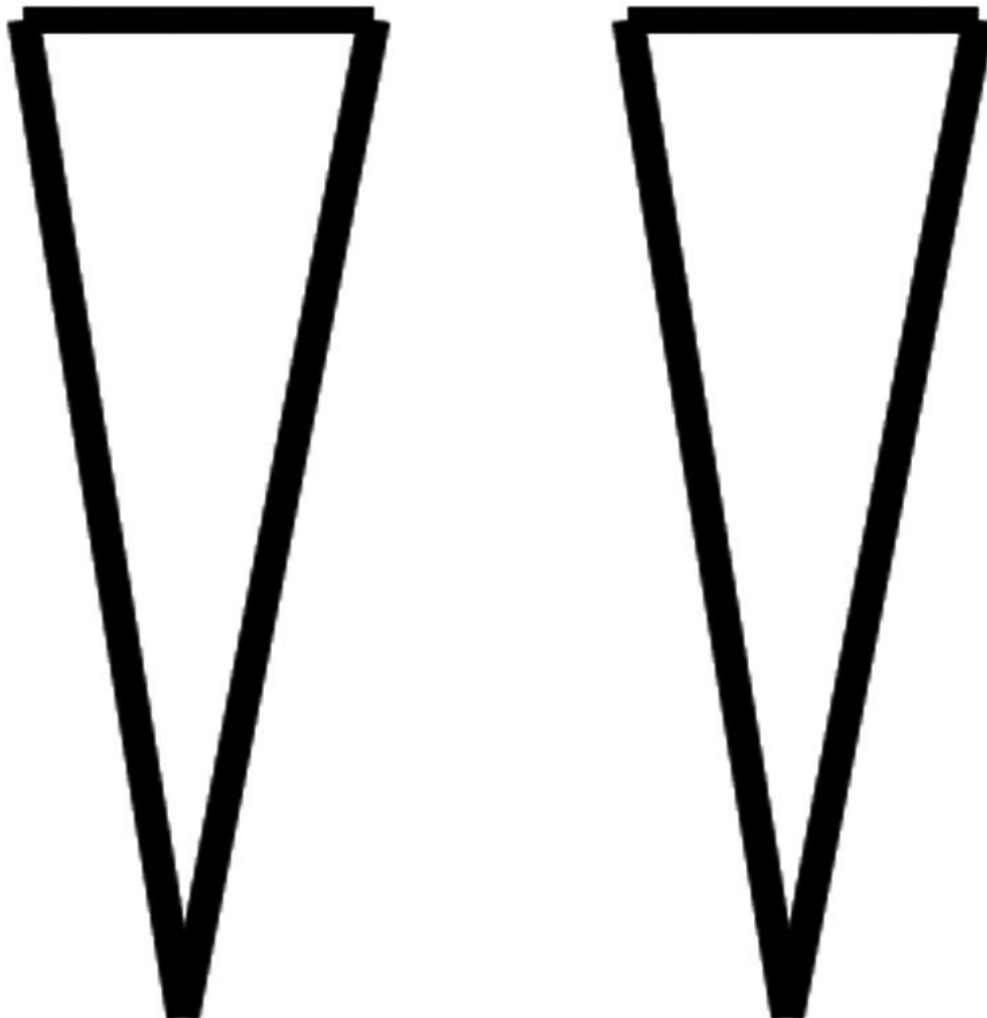
5. CLINICAL TRIALS:

Because the elimination of senescent cells using senolytic agents represents an entirely new therapeutic strategy, its clinical translation requires a carefully designed development pathway. An important consideration is the ethical balance between potential risks and anticipated benefits. Consequently, initial clinical evaluation of senolytic drugs should focus on severe disorders for which existing treatment options provide limited therapeutic benefit. Examples include idiopathic pulmonary fibrosis (IPF), advanced diabetic complications, heart failure with preserved ejection fraction (diastolic heart failure), severe osteoporosis, dementias, and COVID-19 infection in hospitalized older adults. To accelerate clinical progress, multiple small parallel clinical trials have been proposed rather than the traditional sequential approach of conducting one trial after another. If these early studies demonstrate acceptable safety profiles, good tolerability, evidence of target engagement through successful elimination of senescent cells, and meaningful clinical efficacy in serious diseases, senolytic therapies could subsequently be evaluated for less severe conditions associated with cellular senescence.

Following successful outcomes in these settings, the next stage of clinical development would involve investigating whether senolytic drugs can delay the onset or prevent the development of clinically apparent age-related diseases in individuals with detectable senescent cell burden. Identification of such individuals may be achieved using biomarkers measured in blood, saliva, urine, imaging techniques, or tissue biopsies. This preventive approach is supported by experimental findings demonstrating a temporal delay between senescent cell accumulation and the appearance of overt clinical disease. For example, in middle-aged mice, frailty developed only after a period of time following transplantation of senescent cells, indicating that a therapeutic window may exist during which senolytic intervention could prevent or delay disease progression.^[31]

Risk

**Immediate
Benefit**



STRATEGIESFOR TRANSLATING AGENTS TARGETING FUNDAMENTAL AGEING PROCESSES INTO TREATMENTS:

The initial clinical evaluation of senolytic therapies should preferably involve compounds with well-established safety profiles in humans and strong supporting evidence from preclinical studies for the diseases being investigated. An intermittent dosing strategy, rather than continuous administration, may help minimize off-target adverse effects while maintaining therapeutic efficacy. Clinical trials should be rigorously designed to assess not only safety and tolerability but also target engagement, reflected by successful elimination of senescent cells, and overall clinical effectiveness. To facilitate rapid clinical translation, multiple parallel trials across different age-related diseases may be more advantageous than conducting studies sequentially. If senolytic agents demonstrate favorable safety and efficacy in the treatment of severe diseases, they may subsequently be evaluated for less serious age-related conditions and, ultimately, for preventive use in individuals with an elevated senescent cell burden who are at increased risk of developing senescence-associated disorders. At present, however, the available evidence is insufficient to support routine prescription of senolytic drugs outside carefully monitored clinical trial settings.

Preliminary findings from an early open-label clinical study involving three days of oral administration of Dasatinib plus Quercetin (D + Q) in patients with diabetes complicated by renal dysfunction provided evidence of successful target engagement. Compared with adipose tissue biopsies obtained before treatment,

samples collected 11 days after the final D + Q dose—despite the drugs having an elimination half-life of approximately 11 hours—showed significant reductions in p16^{INK4a}-positive and SA-β-gal-positive senescent cells. In addition, D + Q treatment decreased adipose tissue inflammation, as demonstrated by reduced macrophage infiltration and fewer inflammation-associated crown-like structures. A composite index of circulating senescence-associated secretory phenotype (SASP) factors was also significantly reduced following treatment. These findings indicate that, consistent with observations in animal models, D + Q is capable of effectively reducing senescent cell burden in humans.

Numerous clinical studies investigating senolytic therapies are currently in progress or are being planned (Table 3). Ongoing or proposed Phase I and Phase II trials of the Dasatinib plus Quercetin (D + Q) combination are evaluating its therapeutic potential in idiopathic pulmonary fibrosis (IPF), age-related osteoporosis, age-related frailty, frailty among bone marrow transplant recipients with accelerated ageing-like syndromes, and childhood cancer survivors in their late thirties and forties who also exhibit features of accelerated biological ageing. Additional studies include the continuing clinical trial in patients with diabetic chronic kidney disease, investigations in symptomatic Tau-positive Alzheimer's disease (clinical dementia rating score of 1), and trials assessing whether senolytic therapy can improve outcomes following transplantation of organs obtained from older donors.

Similarly, Phase I and II clinical trials of Fisetin (F) are underway or in preparation for conditions including age-related frailty, osteoporosis, osteoarthritis, and the prevention or delay of cytokine storm and acute respiratory distress syndrome (ARDS) in older adults infected with COVID-19. Clinical evaluation of other senolytic candidates is also progressing, with Nutlin-3a being investigated as an injectable therapy for osteoarthritis and Navitoclax (N) undergoing assessment for retinal degenerative disorders. Beyond these studies, additional preclinical research and clinical trial planning are taking place internationally. In Sweden, for example, preclinical investigations at the Karolinska Institute are exploring senolytic therapy for Critical Illness Myopathy, while researchers at the University of Gothenburg are preparing clinical studies targeting hereditary type II diabetes.

CONCLUSIONS:

Senolytic therapies are designed to selectively eliminate senescent cells rather than inhibit a single molecular target or signaling pathway. Consequently, therapeutic strategies that simultaneously target multiple components of the senescent cell anti-apoptotic pathway (SCAP) network are expected to produce more selective senolytic agents while minimizing undesirable panolytic effects on healthy cells. Consistent with this principle, intermittent administration of Dasatinib plus Quercetin (D + Q) or Fisetin (F) has demonstrated efficacy comparable to continuous dosing despite the relatively short elimination half-lives of these compounds. Moreover, D + Q has fulfilled a modified set of Koch's postulates for several age-related disorders, while accumulating evidence suggests that both D + Q and F may satisfy these criteria across additional disease conditions. In contrast, definitive evidence establishing true senolytic activity for Navitoclax (N) and Nutlin-3a remains insufficient.

In accordance with the Unitary Theory of Fundamental Aging Processes, senolytic therapies appear to restore progenitor cell function, reduce chronic tissue inflammation, and improve metabolic abnormalities associated with ageing and disease across multiple tissues and cell types. Preclinical studies further indicate that senolytics can delay, prevent, or alleviate a broad spectrum of age-related disorders while extending both healthspan and lifespan in experimental animals. These findings highlight the potential of senolytic drugs as mechanism-based interventions for the prevention and treatment of senescence-associated diseases in humans, provided that ongoing and future clinical trials continue to demonstrate favorable efficacy and acceptable safety profiles. Nevertheless, until robust clinical evidence confirms their safety, tolerability, target engagement, and therapeutic effectiveness, senolytic agents should not be prescribed for routine

clinical use or self-administered by the general population. Their use should remain restricted to carefully designed and closely monitored clinical trials.

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