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The Clinical Potential of Senolytic Drugs

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Abstract

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 pre-clinical studies are frailty, cardiac dysfunction, vascular hyporeactivity and calcification, diabetes, liver steatosis, osteoporosis, vertebral disk degeneration, pulmonary fibrosis, and radiation-induced damage. Senolytic agents are at the point of being tested in proof-of-concept clinical trials. To do so, new clinical trials paradigms for testing senolytics and other agents that target fundamental aging mechanisms are being developed, since use of long-term endpoints such as life- or healthspan is not feasible. These strategies include testing effects on multi-morbidity, 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.

Introduction

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, cardiovascular disease, most cancers, dementias, other neurodegenerative diseases, arthritis, osteoporosis, blindness, and many others[1]. Aging also predisposes to geriatric syndromes, including frailty, weakness, reduced mobility, mild cognitive impairment, and incontinence,

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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 "multi-morbidity"[3]. This supports the concept that fundamental aging processes not only cause aging phenotypes, but also predispose to chronic diseases and the geriatric syndromes. Thus, therapeutically targeting these processes are predicted to 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 also active at the nidus of most chronic diseases include: 1) chronic, low-grade, "sterile" (absence of known pathogens) inflammation; 2) macromolecular and organelle dysfunction (*e.g.*, changes in DNA [telomere erosion, unrepaired damage, mutations, polyploidy, *etc.*], proteins [aggregation, misfolding, autophagy, *etc.*], carbohydrates, lipids, or mitochondria); 3) stem and progenitor cell dysfunction; and 4) increased burden of senescent cells. These four processes are linked, *i.e.*, in general, interventions that target one process also attenuate the others. For example, increased DNA damage causes increased senescent cell burden and mitochondrial and stem/progenitor cell dysfunction[4-6]. Conversely, reducing senescent cell burden can lead to reduced inflammation, decreased macromolecular dysregulation, and enhanced function of stem and progenitor cells[7-9].

Cellular Senescence and the Senescence-Associated Secretory Phenotype (SASP)

Senescence is a cell fate that involves loss of proliferative potential of normally replication-competent cells, resistance to apoptosis, increased metabolic activity, and frequently the development of a senescence-associated secretory phenotype (SASP). The SASP entails release of pro-inflammatory cytokines and chemokines, tissue-damaging proteases, factors that can impact 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 osteoporosis-like condition resembling the non-injury-related osteoarthritis common in elderly humans[10]. Senescent cells also undergo metabolic shifts, including reduced fatty acid utilization, increased glycolysis, and increased reactive oxygen species (ROS) 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 regulator, $p16^{INK4A}$, $p21^{CIP1}$, and SASP factors (*e.g.*, IL-6, IL-8, monocyte chemoattractant protein-1, plasminogen-activated inhibitor-1, and many others), increased cellular senescence-associated β-galactosidase (SA-βgal) activity, and appearance of senescent-associated distension of satellites (SADS) and telomere-associated DNA damage foci (TAFs), among others. None of these markers are fully sensitive or specific, so

combinations of them are needed to draw conclusions about effects of diseases or interventions on senescent cell numbers.

Eliminating Senescent Cells from Transgenic "Suicide Gene"-Expressing Mice

Senescent cells increase 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 us to devise a strategy 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. AP20187 activates a "suicide" protein encoded by the transgene, which is only present in $p16^{Ink4a}$-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]. However, 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, the effects of removing potentially pathogenic classically senescent cells with an active SASP may not be recapitulated by models dependent upon $p16^{INK4A}$ expression. Third, tumor cells can have high $p16^{INK4A}$ expression[20], confounding interpretation of life- or healthspan studies in INK-ATTAC mice since the vast majority of mice die of/with cancer.

Senescent Cell Anti-Apoptotic Pathways (SCAPs): Exploiting the Achilles' Heels of Senescent Cells

To remove senescent cells pharmacologically from non-genetically modified individuals, "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 remarkably 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 bioinformatics approaches based on the RNA and protein expression profiles of senescent cells, five Senescent-Cell Anti-Apoptotic Pathways (SCAPs) were identified (Table 1). That SCAPs are indeed required for senescent cell viability was verified by RNA interference studies, in which key proteins in these pathways were reduced. Through this approach, survival proteins were identified as the "Achilles' heels" of senescent cells. Knocking-down expression of these proteins causes death of senescent but not non-senescent cells. Since the discovery of the first five SCAPs, another was identified (Table 1)[24]. This approach and the SCAPs discovered were subsequently used by others and us to identify putative senolytic targets[22, 24, 25, 26, 27].

We tested if agents known to interfere with the activity of SCAP pathways are senolytic. The first senolytic agents discovered using this hypothesis-driven approach were dasatinib and quercetin[22]. Ten months later, another group and ours simultaneously reported the third senolytic drug, navitoclax, a BCL-2 pro-survival pathway inhibitor[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). More senolytics are currently in development and additional potential SCAP's are being identified.

The SCAPs required for senescent cell resistance to apoptosis vary among cell types. The Achilles' heels, for example of senescent human primary adipose progenitors differ from those in a senescent human endothelial cell strain, implying that agents targeting a single SCAP may not eliminate all types of senescent cells. So far, 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 very effective against senescent primary human fat cell progenitors[27]. Even within a particular cell type, human lung fibroblasts, nativoclax is senolytic in the culture-acclimated IMR-90 lung fibroblast-like cell strain, while it is less so in primary human lung fibroblasts isolated from patients[19, 27]. Without extensive testing across 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, while neither dasatinib nor quercetin was significantly senolytic in mouse embryonic fibroblasts *in vitro*, the combination of dasatinib and quercetin was 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 that are 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 (SA-βgal) activity, $p16^{Ink4a+}$ cells, $p16^{Ink4a}$, $p21^{Cip1}$, and SASP factor mRNAs, telomere-associated foci, and other senescent cell indicators[18, 19, 22, 24-26]. Among the effects of senolytics in mice so far are: 1) improved cardiac ejection fraction and fractional shortening in old mice[22]; 2) enhanced vascular reactivity in old mice[18]; 3) decreased vascular calcification and increased vascular reactivity in hypercholesterolemic, high fat fed $ApoE^{-/-}$ mice[18]; 4) reduced senescent cell-like, intimal foam cell/macrophages in vascular plaques in high fat fed $LdlR^{-/-}$ mice[28]; 5) decreased frailty, osteoporosis, loss of intervertebral disc glycosaminoglycans, and spondylosis in progeroid $Ercc1^{-/\Delta}$ mice[22]; 6) decreased gait disturbance in mice following radiation damage to a leg[22] and hematological dysfunction caused by whole body radiation[25]; 7) increased coat density[26], and 8) 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 yet been reported to our knowledge. 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 accumulation, which probably varies among conditions that induce cellular senescence. For example, continued high fat feeding or exposure to genotoxic cancer therapies likely causes more rapid accumulation of senescent cells than chronological aging. Advantages of intermittent administration include reduced opportunity to develop side-effects, the feasibility of administering senolytic drugs during periods of relatively good health, and decreased risk for off-target effects caused by continuous exposure to drugs. Another advantage of senolytics is that cell division-dependent drug resistance is unlikely to occur, since senescent cells do not divide and therefore cannot acquire advantageous mutations, unlike the situation in treating cancers or infectious agents.

Clinical Trials Strategies

New clinical trials strategies will be needed in order to test senolytics or other agents that target fundamental aging processes. Obviously, outcomes such as effects on median or maximum lifespan cannot be tested feasibly in humans. According to the geroscience hypothesis, if a candidate drug actually targets fundamental aging processes, such an agent should affect a range of chronic diseases, geriatric syndromes, and age-related loss of physiological resiliencies[1, 2, 30-33]. Thus, potential clinical trials scenarios include the following:

1. *Simultaneous alleviation of multiple co-morbidities*. In patients with multi-morbidity, which is common in older patients[3], candidate senolytics should alleviate more than one existing pathology, such as glucose intolerance, mild cognitive impairment, joint pain due to osteoarthritis, systolic hypertension, or decreased carotid flow. Alternatively, these drugs should delay the onset of a second age-related disorder in patients who already have one disorder, similar to the design of the TAME 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, among others[19, 34]. Senolytic agents hold promise as potential 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, aerosol, or topically, respectively. This 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, HIV infection, obesity, or genetic progeroid syndromes[1, 30, 31]. Short term trials examining outcomes such as reduction of multi-morbidity, 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]. Decreased resilience also underlies such conditions as reduced immune response to influenza vaccination or decreased 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, scheduled medical stress paradigms or acute injury might be useful for testing interventions targeting fundamental processes of aging. Indeed, senolytics reduce adverse consequences of bleomycin-induced pulmonary injury[19] or radiation-induced injury in mice[22]. A drug related to rapamycin, an agent that inhibits the SASP, increases 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 subjects with a moderate degree of frailty to determine if strength, gait, body weight, or other relevant parameters improve.

The 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 the key 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]. Furthermore, they could delay or treat the geriatric syndromes, including sarcopenia, frailty, immobility, and cognitive impairment among others, 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 very rapidly in the last few years toward clinical intervention. There is currently a severe shortage of geriatricians with sufficient understanding of basic biology and translational science to lead early proof-of-concept clinical trials to determine if 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 application.

Senolytics might prove to be interventions that can prevent or delay chronic diseases as a group, instead of one-at-a-time in pre-symptomatic or at-risk patients. Furthermore, if what can be achieved in pre-clinical aging animal models can be achieved in humans, it may be feasible to alleviate dysfunction even in frail individuals with multiple co-morbidities, a group that until recently was felt to be beyond the point of treatment other than palliative and supportive measures. Although considerable caution must be emphasized, 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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References

1. Kirkland JL. Translating the science of aging into therapeuticiInterventions. Cold Spring Harb Perspect Med. 2016; 6:a025908. [PubMed: 26931808]
2. Kirkland JL, Stout MB, Sierra F. Resilience in aging mice. J Gerontol Series A, Biological Sci Med Sci. 2016; 71:1407–1414.
3. St Sauver JL, Boyd CM, Grossardt BR, et al. Risk of developing multimorbidity across all ages in an historical cohort study: differences by sex and ethnicity. BMJ Open. 2015; 5:e006413.
4. Fang EF, Scheibye-Knudsen M, Brace LE, et al. Defective mitophagy in XPA via PARP-1 hyperactivation and NAD(+)/SIRT1 reduction. Cell. 2014; 157:882–896. [PubMed: 24813611]
5. Lavasani M, Robinson AR, Lu A, et al. Muscle-derived stem/progenitor cell dysfunction limits healthspan and lifespan in a murine progeria model. Nature Commun. 2012; 3:608. [PubMed: 22215083]
6. Armstrong GT, Kawashima T, Leisenring W, et al. Aging and risk of severe, disabling, life-threatening, and fatal events in the childhood cancer survivor study. J Clin Oncology. 2014; 32:1218–1227.
7. Tchkonia T, Zhu Y, van Deursen J, Campisi J, Kirkland JL. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. J Clin Invest. 2013; 123:966–972. [PubMed: 23454759]
8. Xu M, Palmer AK, Ding H, et al. Targeting senescent cells enhances adipogenesis and metabolic function in old age. eLife. 2015; doi: 10.7554/eLife.12997
9. Zhu Y, Armstrong JL, Tchkonia T, Kirkland JL. Cellular senescence and the senescent secretory phenotype in age-related chronic diseases. Current Opin Cinical Nutr Metab Care. 2014; 17:324–328.
10. Xu M, Bradley EW, Weivoda MM, et al. Transplanted senescent cells induce an osteoarthritis-like condition in mice. J Gerontol Series A, Biological Sci Med Sci. 2016
11. Kim YM, Seo YH, Park CB, Yoon SH, Yoon G. Roles of GSK3 in metabolic shift toward abnormal anabolism in cell senescence. Ann N Y Acad Sci. 2010; 1201:65–71. [PubMed: 20649541]
12. Krishnamurthy J, Torrice C, Ramsey MR, et al. Ink4a/Arf expression is a biomarker of aging. J Clin Invest. 2004; 114:1299–1307. [PubMed: 15520862]

13. Tchkonia T, Morbeck DE, von Zglinicki T, et al. Fat tissue, aging, and cellular senescence. Aging Cell. 2010; 9:667–684. [PubMed: 20701600]
14. Jeyapalan JC, Ferreira M, Sedivy JM, Herbig U. Accumulation of senescent cells in mitotic tissue of aging primates. Mech Ageing Devel. 2007; 128:36–44. [PubMed: 17116315]
15. Waaijer ME, Parish WE, Strongitharm BH, et al. The number of p16INK4a positive cells in human skin reflects biological age. Aging Cell. 2012; 11:722–725. [PubMed: 22612594]
16. Stout MB, Tchkonia T, Pirtskhalava T, et al. Growth hormone action predicts age-related white adipose tissue dysfunction and senescent cell burden in mice. Aging (Milano). 2014; 6:575–586.
17. Baker DJ, Wijshake T, Tchkonia T, et al. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature. 2011; 479:232–236. [PubMed: 22048312]
18. Roos CM, Zhang B, Palmer AK, et al. Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice. Aging Cell. 2016 Feb 10. [Epub ahead of print]. doi: 10.1111/acel.12458
19. Schafer MJ, White TA, Iijima K, et al. Cellular senescence mediates fibrotic pulmonary disease. Nature Commun. 2017; 8:14532. [PubMed: 28230051]
20. Hall BM, Balan V, Gleiberman AS, et al. Aging of mice is associated with p16(Ink4a)- and beta-galactosidase-positive macrophage accumulation that can be induced in young mice by senescent cells. Aging (Milano). 2016; 8:1294–1315.
21. Kirkland JL, Tchkonia T. Clinical strategies and animal models for developing senolytic agents. Exp Gerontol. 2014
22. Zhu Y, Tchkonia T, Pirtskhalava T, et al. The Achilles' heel of senescent cells: From transcriptome to senolytic drugs. Aging Cell. 2015; 14:644–658. [PubMed: 25754370]
23. Wang E. Senescent human fibroblasts resist programmed cell death, and failure to suppress bcl2 is involved. Cancer Res. 1995; 55:2284–2292. [PubMed: 7757977]
24. Fuhrmann-Stroissnigg H, Ling YY, Zhao J, et al. Identification of HSP90 inhibitors as senolytics for extending healthspan. Nature Commun. 2017 In press.
25. Chang J, Wang Y, Shao L, et al. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. Nature Med. 2016; 22:78–83. [PubMed: 26657143]
26. Baar MP, Brandt RM, Putavet DA, et al. Targeted apoptosis of senescent cells restores tissue homeostasis in response to chemotoxicity and aging. Cell. 2017; 169:132–147 e116. [PubMed: 28340339]
27. Zhu Y, Tchkonia T, Fuhrmann-Stroissnigg H, et al. Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. Aging Cell. 2015 Dec 29. [Epub ahead of print]. doi: 10.1111/acel.12445
28. Childs BG, Baker DJ, Wijshake T, Conover CA, Campisi J, van Deursen JM. Senescent intimal foam cells are deleterious at all stages of atherosclerosis. Science. 2016; 354:472–477. [PubMed: 27789842]
29. Demaria M, Ohtani N, Youssef SA, et al. An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. Dev Cell. 2014; 31:722–733. [PubMed: 25499914]
30. Newman JC, Milman S, Hashmi SK, et al. Strategies and Challenges in Clinical Trials Targeting Human Aging. J Gerontol Series A, Biological Sci Med Sci. 2016; 71:1424–1434.
31. Justice J, Miller JD, Newman JC, et al. Frameworks for Proof-of-Concept Clinical Trials of Interventions That Target Fundamental Aging Processes. J Gerontol Series A, Biological Sci Med Sci. 2016; 71:1415–1423.
32. Huffman DM, Justice JN, Stout MB, Kirkland JL, Barzilai N, Austad SN. Evaluating health span in preclinical models of aging and disease: guidelines, challenges, and opportunities for geroscience. J Gerontol Series A, Biological Sci Med Sci. 2016; 71:1395–1406.
33. Burd CE, Gill MS, Niedernhofer LJ, et al. Barriers to the Preclinical Development of Therapeutics that Target Aging Mechanisms. J Gerontol Series A, Biological Sci Med Sci. 2016; 71:1388–1394.
34. Tabibian JH, O'Hara SP, Splinter PL, Trussoni CE, LaRusso NF. Cholangiocyte senescence by way of N-ras activation is a characteristic of primary sclerosing cholangitis. Hepatology. 2014; 59:2263–2275. [PubMed: 24390753]

35. Oubaha M, Miloudi K, Dejda A, et al. Senescence-associated secretory phenotype contributes to pathological angiogenesis in retinopathy. Sci Trans Med. 2016; 8:362ra144.

36. Mannick JB, Del Giudice G, Lattanzi M, et al. mTOR inhibition improves immune function in the elderly. Sci Trans Med. 2014; 6:268ra179.

37. Xu M, Tchkonia T, Ding H, et al. JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age. Proc Natl Acad Sci (USA). 2015; 112:E6301–E6310. [PubMed: 26578790]

38. Yosef R, Pilpel N, Tokarsky-Amiel R, et al. Directed elimination of senescent cells by inhibition of BCL-W and BCL-XL. Nature Commun. 2016; 7:11190. [PubMed: 27048913]

39. Pal HC, Sharma S, Elmets CA, Athar M, Afaq F. Fisetin inhibits growth, induces G(2) /M arrest and apoptosis of human epidermoid carcinoma A431 cells: role of mitochondrial membrane potential disruption and consequent caspases activation. Exp Dermatol. 2013; 22:470–475. [PubMed: 23800058]

40. Zhu Y, Doornebal EJ, Pirtskhalava T, et al. New agents that target senescent cells: the flavone, fisetin, and the BCL-XL inhibitors, A1331852 and A1155463. Aging (Milano). 2017

41. Zhang XJ, Jia SS. Fisetin inhibits laryngeal carcinoma through regulation of AKT/NF-kappaB/mTOR and ERK1/2 signaling pathways. Biomed Pharmacother. 2016; 83:1164–1174. [PubMed: 27551764]

42. Makhov P, Golovine K, Teper E, et al. Piperlongumine promotes autophagy via inhibition of Akt/mTOR signalling and mediates cancer cell death. Br J Cancer. 2014; 110:899–907. [PubMed: 24434432]

43. Wang F, Mao Y, You Q, Hua D, Cai D. Piperlongumine induces apoptosis and autophagy in human lung cancer cells through inhibition of PI3K/Akt/mTOR pathway. Int J Immunopathol Pharmacol. 2015; 28:362–373. [PubMed: 26246196]

44. Li J, Cheng Y, Qu W, et al. Fisetin, a dietary flavonoid, induces cell cycle arrest and apoptosis through activation of p53 and inhibition of NF-kappa B pathways in bladder cancer cells. Basic Clin Pharmacol Toxicol. 2011; 108:84–93. [PubMed: 21054790]

45. Golovine KV, Makhov PB, Teper E, et al. Piperlongumine induces rapid depletion of the androgen receptor in human prostate cancer cells. Prostate. 2013; 73:23–30. [PubMed: 22592999]

46. Triantafyllou A, Mylonis I, Simos G, Bonanou S, Tsakalof A. Flavonoids induce HIF-1alpha but impair its nuclear accumulation and activity. Free Radic Biol Med. 2008; 44:657–670. [PubMed: 18061585]

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	√	√
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