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Cellular senescence and senolytics: the path to the clinic

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Interlinked and fundamental aging processes appear to be a root-cause contributor to many disorders and diseases. One such process is cellular senescence, which entails a state of cell cycle arrest in response to damaging stimuli. Senescent cells can arise throughout the lifespan and, if persistent, can have deleterious effects on tissue function due to the many proteins they secrete. In preclinical models, interventions targeting those senescent cells that are persistent and cause tissue damage have been shown to delay, prevent or alleviate multiple disorders. In line with this, the discovery of small-molecule senolytic drugs that selectively clear senescent cells has led to promising strategies for preventing or treating multiple diseases and age-related conditions in humans. In this Review, we outline the rationale for senescent cells as a therapeutic target for disorders across the lifespan and discuss the most promising strategies—including recent and ongoing clinical trials—for translating small-molecule senolytics and other senescence-targeting interventions into clinical use.

The aging population is steadily increasing. The World Health Organization (WHO) estimates that 1 in 6 people, or 2.1 billion, are expected to be over age 60 by 2030 (ref. [1]). Chronological age is the major predictor for most of the diseases that account for the bulk of morbidity, mortality and health costs across low-, middle- and high-income countries[2–7].

Aging progresses throughout the lifespan can be accentuated at etiologic sites of multiple acute and chronic diseases, including in children[6,8–12]. Indeed, fundamental aging processes can operate even before conception, for example, in the context of aged oocytes linked to Down syndrome[12,13]. A fundamental aging mechanism that has gained increasing attention is cellular senescence. Senescent cells accumulate during aging and at pathogenic sites of multiple disorders and diseases. After the first reports of senolytic drugs (agents that selectively eliminate senescent cells) in 2015 (ref. [14]), promising results from preclinical studies have facilitated progression to early-phase clinical trials evaluating the safety and efficacy of senolytics, some of which have now been published (Table 1).

Fundamental aging mechanisms can be grouped into so-called hallmarks or 'pillars' of aging; these include genomic instability, progenitor cell exhaustion/dysfunction, telomeric and epigenetic changes, dysregulated protein homeostasis, altered nutrient sensing, mitochondrial dysfunction, altered intercellular communication, chronic low-grade inflammation, fibrosis, microbiome dysregulation and cellular senescence[3,15]. The Geroscience Hypothesis holds that these pillars of aging, including cellular senescence, tend to progress in concert and may be root-cause contributors to the pathophysiology of multiple diseases, age-related dysfunction (including the geriatric syndromes such as frailty, immobility, sarcopenia/muscle wasting, mild cognitive impairment and incontinence) and loss of resilience (for example, decreased ability to recover from stresses such as injury, surgery, chemotherapy or infections or to mount an antibody response to immunizations)[3,15–18]. The Unitary Theory of Fundamental Aging Mechanisms builds on the Geroscience Hypothesis by positing that interventions targeting any one fundamental mechanism may target the others[6]. For example, interventions that target cellular senescence tend to attenuate other fundamental aging mechanisms leading to reduced inflammation, attenuated exhaustion of progenitors, decreased fibrosis, alleviated mitochondrial dysfunction and a partially restored microbiome in experimental animal models of aging and chronic diseases[6,7,18–36].

By understanding and targeting cellular senescence and the other pillars of aging, rather than targeting individual diseases that are downstream of fundamental aging processes, it is conceivable that multimorbidity could be reduced and healthspan increased, with realization of substantial societal and economic benefits[4,6]. In this Review, we consider the potential value of senescent cells as a therapeutic target, the current state of senolytic drug development and the path to bring preventive and therapeutic strategies targeting senescent cells to the clinic.

Cellular senescence: mechanisms and pathways

Cellular senescence was first reported in 1961 by Hayflick and Moorhead after serially subculturing human fibroblasts[37]. Senescent cells, which are in a state of essentially irreversible cell cycle arrest but remain viable, can accumulate with aging, especially in more frail individuals, and at pathogenic sites of multiple disorders and diseases in experimental animals and humans across the lifespan. The senescent cell fate can be triggered by a number of stressors including DNA damage, cancerous mutations or oncogene activation, mitochondrial dysfunction, reactive metabolites, hyperoxia or hypoxia, proteotoxic stress, extracellular signals, infections, mechanical or shear stresses that deform cells, resistance exercise and factors secreted by other senescent cells[18,38–47]. Many such stressors activate DNA damage response signaling and activation of the p53/p21$^{CIP1/WAF1}$, p16^{INK4a}/retinoblastoma protein or other pathways, resulting in cell cycle arrest and the development of a senescence-associated secretory phenotype (SASP)[24,40,48–53] (Fig. 1). Through upregulation of pro-survival and antiapoptotic pathways such as SRC kinases, the PI3K–AKT signaling pathway, heat shock protein (HSP) pathways, serpines, mitochondrial pathways or apoptosis regulator BCL-2-related proteins, those senescent cells with a proapoptotic SASP can survive, despite the cytotoxic microenvironment they create[6,7,14,34,35,54–58].

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Table 1 | Senolytic clinical trials: completed, current or planned

Study title	Senolytic	Study design	Identifier	Status
Targeting pro-inflammatory cells in idiopathic pulmonary fibrosis: a human trial	D + Q	Phase 1, randomized, open-label	NCT02874989	Completed[63]
Senescence in chronic kidney disease	D + Q	Phase 2, randomized, open-label	NCT02848131	Current, preliminary report published[48]
Hematopoietic stem cell transplant survivors study (HTSS)	D + Q	Randomized, open-label	NCT02652052	Current
ALSENLITE: senolytics for Alzheimer's disease	D + Q	Phase 1/2, open-label	NCT04785300	Current
Senolytic therapy to modulate the progression of Alzheimer's disease (SToMP-AD) study	D + Q	Phase 1/2, open-label, pilot study Phase 2, randomized, double-blind, placebo-controlled	NCT04063124 and NCT04685590	Current
Senolytics to improve cognition and mobility in older adults at risk of Alzheimer's disease	D + Q	Single-arm, open-label, pre–post pilot study	Pending	Pending
An open-label intervention trial to reduce senescence and improve frailty in adult survivors of childhood cancer	D + Q; F	Phase 2, randomized, open-label	NCT04733534	Current
Targeting cellular senescence with senolytics to improve skeletal health in older humans	D + Q; F	Phase 2, randomized, open-label	NCT04313634	Current
Quercetin in coronary artery by-pass surgery (Q-CABG)	Q	Phase 2, randomized double-blind, placebo-controlled	NCT04907253	Current
Use of senolytic and anti-fibrotic agents to improve the beneficial effect of bone marrow stem cells for osteoarthritis	F	Phase 1/2, randomized, double-blind, active-control	NCT04815902	Current
Senolytic drugs attenuate osteoarthritis-related articular cartilage degeneration: a clinical trial	F	Phase 1/2, randomized, double-blind, placebo-controlled	NCT04210986	Current
COVID-FISETIN: pilot in SARS-CoV-2 of fisetin to alleviate dysfunction and inflammation	F	Phase 2, randomized, double-blind, placebo-controlled	NCT04476953	Current
Alleviation by fisetin of frailty, inflammation and related measures in older women (AFFIRM)	F	Phase 2, randomized, double-blind, placebo-controlled	NCT03430037 and NCT03675724	Current
Inflammation and stem cells in diabetic and chronic kidney disease	F	Phase 2, randomized, double-blind, placebo-controlled	NCT03325322	Current
COVID-19 pilot study of fisetin to alleviate dysfunction and decrease complications (COVFIS-HOME)	F	Phase 2, randomized, double-blind, placebo-controlled	NCT04771611	Current
Pilot in COVID-19 (SARS-CoV-2) of fisetin in older adults in nursing homes (COVID-FIS)	F	Phase 2, randomized, double-blind, placebo-controlled	NCT04537299	Current
Targeting senescence to reduce osteoarthritis pain and cartilage breakdown (ROPE)	F	Phase 1/2, randomized, double-blind, placebo-controlled	NCT04770064	Current
Senolytic agent improve the benefit of platelet-rich plasma and losartan	F	Phase 1/2, randomized, double-blind, placebo-controlled	NCT05025956	Current
Safety and tolerability and long-term follow-up studies of patients with osteoarthritis of the knee treated with UBX0101 or placebo	UBX0101 (nutlin-3a or related)	Phase 2, randomized, double-blind, placebo-controlled	NCT03513016 and NCT04349956	Completed; failed to achieve primary endpoint
A study to assess the safety and efficacy of a single or repeat doses of UBX0101 in patients with osteoarthritis of the knee	UBX0101 (nutlin-3a or related)	Phase 1, randomized, double-blind, placebo-controlled Phase 2, randomized, double-blind, placebo-controlled	NCT04229225 and NCT04129944	Current
Safety and tolerability study of UBX1325 in patients with diabetic macular edema or neovascular age-related macular degeneration	UBX1325 (N or related)	Phase 1, open-label Phase 2, randomized, double-blind, sham-controlled	NCT04537884 and NCT04857996	Current

D + Q, dasatinib and quercetin; F, fisetin; N, navitoclax; Q, quercetin.

The senescence-associated secretory phenotype. Most cells undergoing senescence develop a SASP (Fig. 1). In 30–70% of senescent cells, this SASP entails pro-inflammatory, proapoptotic and pro-fibrotic factors[7,31,40,59–64], some of which can cause previously non-senescent cells to become senescent both locally and at a distance in an endocrine manner[24,39,47]. This proapoptotic SASP can have detrimental effects both locally and systemically due to senescent cell accumulation and persistence[24]. In the other 30–70% of senescent cells, the SASP appears to comprise growth and other regenerative factors, potentially causing less apoptosis, tissue destruction and fibrosis than proapoptotic, pro-inflammatory senescent cells and can even contribute to tissue repair (for example, through the growth factors VEGF-A and PDGF-AA)[61,65].

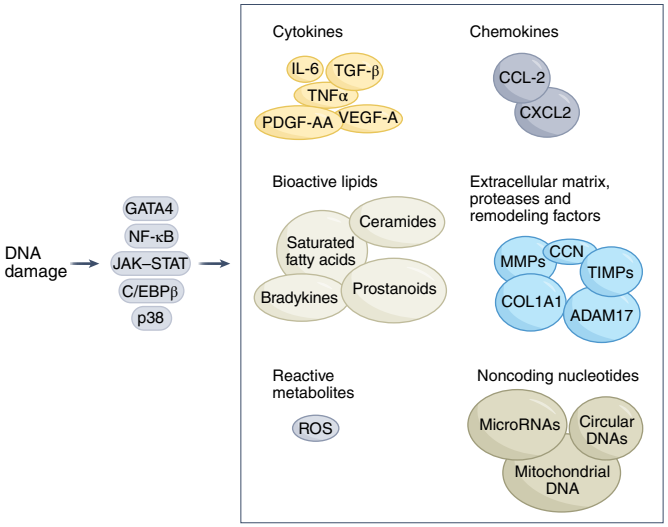

Fig. 1 | Senescence-associated secretory phenotype. The SASP is a key feature of cellular senescence. Cellular stressors induce DNA damage response signaling, which activates key transcription factors and pathways including NF-κB, CCAAT/enhancer binding protein-β (C/EBPβ), GATA binding protein 4 (GATA4), p38 and JAK–STAT, which can drive and modulate the SASP[31,48,60,63,64,103,157,214–218]. The various forms of the SASP can comprise chemokines, extracellular matrix proteases, remodeling factors, bioactive lipids, noncoding nucleotides and reactive metabolites[7,31,59–64]. IL-6, interleukin-6; ROS, reactive oxygen species; TGF-β, transforming growth factor beta; TIMPs, tissue inhibitors of metalloproteinases; TNF, tumor necrosis factor.

If senescent cells are present transiently, beneficial functions of both the proapoptotic and pro-growth types of SASP can orchestrate tissue remodeling (during fetal development, growth of younger individuals, and after cell or tissue damage), induce immune responses during infections or tissue injury, promote parturition by SASP factors released by placental senescent cells, and induce clearance of those senescent cells with a pro-inflammatory SASP because they attract, anchor and activate immune cells[10,11,14,61,62,65].

Adverse impacts of persistent, proapoptotic SASP-expressing senescent cells. Normally, senescent cells appear to be cleared within days to weeks after they develop by natural killer cells and other immune cell types[62,66–69]. However, if a threshold burden of senescent cells is exceeded, senescent cells can accumulate, perhaps because proapoptotic SASP-expressing senescent cells induce paracrine and endocrine spread of senescence at a rate exceeding immune clearance of preexisting and newly formed senescent cells[24,62] (Fig. 2). Once senescent cell burden surpasses this threshold, continuing increases in proapoptotic/pro-inflammatory senescent cell burden may contribute to tissue destruction and hence development or progression of multiple diseases and age-related disorders (Table 2) as well as immune dysregulation, further amplifying senescent cell accumulation in a feed-forward loop[36,38,62]. Although not every senescent cell develops a proapoptotic, inflammatory SASP, accumulation and persistence of such senescent cells can induce a chronic low-grade, pro-fibrotic inflammatory state (usually associated with aging and chronic diseases), known as 'inflammaging'[70]. This sterile inflammatory state can provoke dysfunction of neighboring and distant non-senescent cells, such as progenitor cells, contributing to impaired tissue function and reduced regenerative capacity[18,21,24,26,34]. Consistent with this, persistence of senescent cells has been implicated in causing disorders related to tissue inflammation, fibrosis and extracellular matrix degradation, adipose tissue insulin resistance, reduced muscle hypertrophy after resistance exercise and impaired fracture repair in older individuals, as well as promoting malignant transformation[6,18,22,23,48,71–77].

The SASP of senescent cells is not static; it can change over time[78–80] and varies depending on the type of cells that became senescent and how senescence was induced[16,44,59,60,81]. The intracellular and extracellular environment can modulate which SASP factors are produced and their abundance. Persistent senescent cells appear to be highly responsive to extracellular cues, such as damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), which can exacerbate the proapoptotic, pro-inflammatory qualities of the SASP[16]. For example, in the case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, some of these extracellular cues are mediated through Toll-like receptor 3 and angiotensin converting enzyme-2, contributing to coronavirus disease 2019 (COVID-19) morbidity[44,82]. Intracellular cues can exacerbate damaging, pro-inflammatory properties of the SASP several weeks or months after senescence has been induced. These include retrotransposable elements (for example, LINE-1), cytosolic mitochondrial DNA, or circular DNA—all of which can activate the cytosolic DNA-sensing (cGAS)–STING pathway, triggering the expression of pro-inflammatory genes[79–81].

Discovery and development of senolytic drugs

The first senolytic drugs were identified using a hypothesis-driven, mechanism-based drug discovery approach (Fig. 3). Because those 30–70% of senescent cells that have a proapoptotic, tissue-destructive SASP are themselves resistant to apoptosis, it was hypothesized that such senescent cells depend on antiapoptotic, pro-survival pathways to avoid self-destruction[14,83]. Analysis of proteomic and transcriptomic datasets revealed there is indeed upregulation of one or more senescent cell antiapoptotic pathways (SCAPs) in senescent cells. SCAP pathways are similar to those that protect certain types of cancer cells, such as B cell lymphoma or chronic lymphocytic leukemia cells, which also release tissue-destructive proapoptotic factors but evade undergoing apoptosis themselves[84,85]. Transiently disabling SCAP pathways results in apoptosis of the senescent cells with a tissue-destructive SASP, while non-senescent cells or those senescent cells with a pro-growth, non-apoptotic SASP remain viable (U. Tripathi, S.C., L.G.P.L. Prata, T.T. and J.L.K., unpublished data).

Bioinformatic analyses identified 46 compounds that target SCAP pathways as being potentially senolytic[14]. The first senolytic agents intentionally selected for further investigation were ones that: (1) target several SCAPs, rather than adhering to the traditional drug development approach of one drug/one molecular target/one disease, (2) can be administered orally, and (3) are natural products with known safety profiles or are already approved by the US Food and Drug Administration (FDA) for other indications, to facilitate translation from bench to bedside. These included the SRC/tyrosine kinase inhibitor dasatinib (D), which has been approved and extensively used since 2006 and has a quite good safety profile, and the natural flavonoids quercetin (Q) and fisetin (F), which are present in fruits and other foods[14,86].

In some types of senescent cells, SCAPs can be redundant, so that targeting a single SCAP may not eliminate such cells—but combination treatment targeting multiple SCAPS may be effective. As an example, senescent mesenchymal embryonic fibroblasts from *Ercc1*$^{-/-}$ mice and bone marrow mesenchymal progenitors from old mice are not eliminated by either D or Q alone, but are eliminated by the combination of these agents[14]. Consistent with the heterogeneity of SCAPs across different senescent cell types, senescent human fat cell progenitors (preadipocytes or mesenchymal stromal cells) are sensitive to D but not Q or F, while senescent human umbilical vein endothelial cells are sensitive to Q or F but not D[14]. Since the first SCAPs were discovered, others have been identified and, based on

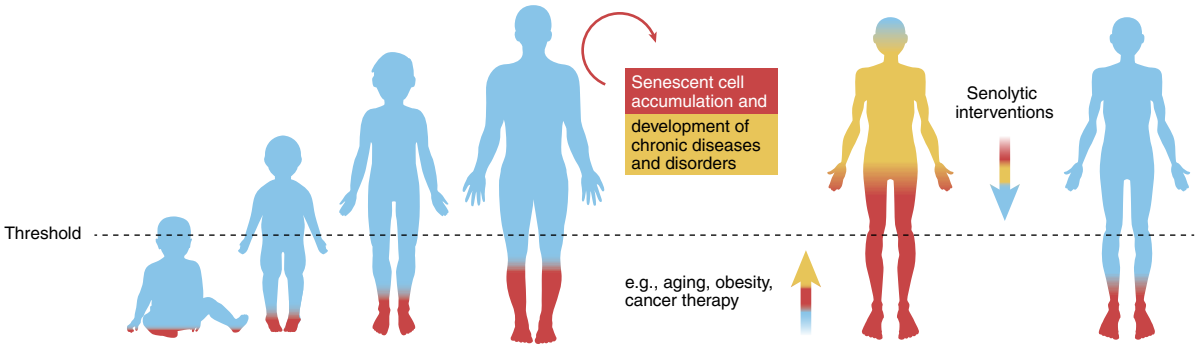

Fig. 2 | The threshold theory of senescent cell accumulation. This theory postulates that once senescent cell burden exceeds a threshold, self-amplifying paracrine and endocrine spread of senescence through the SASP outpaces clearance of senescent cells by the immune system[34,219]. Additionally, increased abundance of SASP factors may impede immune system function[62,78], further amplifying accumulation of senescent cells. Senescent cell accumulation may also accelerate other fundamental aging mechanisms. In studies of effects of transplanting senescent versus non-senescent cells into middle-aged mice, a minimum number of transplanted senescent cells was necessary to cause accelerated aging-like phenotypes[24]. In conditions in which senescent cell burden is already high, such as obesity, fewer senescent cells need to be transplanted to induce the same effect as in lean mice of the same age[23,24,151]. Consistent with this, in human childhood cancer survivors who have had DNA-damaging anticancer therapy, a subsequent accelerated aging-like phenotype can occur at a considerably earlier age than in older individuals who do not have a history of childhood cancer treatment[169]. Hence, senescent cells with a proapoptotic, inflammatory SASP may need to exceed a threshold to exert detrimental effects. Systemic clearance of senescent cells by genetic or pharmacologic means tends to attenuate the other pillars of aging and can delay, prevent or alleviate multiple age-related disorders and diseases[23,24,30,49].

these, more senolytic strategies have been developed. For example, forkhead box O4 (FOXO4) retains p53 in the nucleus, so peptides interfering with this interaction can lead to p53-mediated apoptosis in some types of senescent cells[87]. HSP90 prevents proteasomal degradation of AKT, hence inhibiting HSP90 disables pro-survival signaling through this SCAP node and results in elimination of some senescent cell types[54]. Consistent with this, certain HSP90 inhibiting drugs, such as geldanamycin, are senolytic against particular senescent cell types.

In some cases, senolytic compounds that target a single SCAP node, such as the BCL-2 pathway inhibitors, N (ABT-263), A1331852 or A1155463, tend to induce apoptosis in a restricted range of senescent cell types[57,86]. However, it is worth noting that N can cause thrombocytopenia and neutropenia, even after brief exposures[57,88–91]; this raises the question of whether agents that target a single molecular pathway have a greater risk of toxicity due to off-target effects associated with the high dosing required to fully suppress a single SCAP node. Perhaps by using agents or combinations that 'lightly' impact a number of nodes, it may be feasible to target a broader range of senescent cell types while using lower doses of each agent, thereby potentially improving the side-effect profiles of these agents. The latter approach has been used to improve tolerability of antibiotic treatments, for example.

Second-generation senolytics are now being identified using high-throughput library screens and other approaches[54]. One approach stems from the increase in lysosomal mass and senescence-associated β-galactosidase activity in many senescent cells, whereby galacto-oligosaccharide-coated nanoparticles and β-galactosidase-activated prodrugs appear to eliminate at least some of these cells[92,93]. Another approach is based on the high lysosomal activity of some senescent cells that renders them sensitive to lysosomal ATPase inhibitors[94]. Due to ruptured lysosomal membranes, at least some senescent cells depend on glutamine metabolism as a pH-buffering system, inhibition of which renders them vulnerable to apoptosis[95].

Other strategies for decreasing age-related senescent cell burden and pathologic conditions involve modulating immune clearance of senescent cells[62]. Certain cell surface proteins tend to be more highly expressed by senescent cells than most other cell types, which prompted development of engineered chimeric antigen receptor (CAR) T cells, vaccines and antibody–drug conjugates targeting these cell surface markers. Each of these approaches eliminates senescent cells, although in some cases, activated macrophages and other non-senescent cell types are also affected[96–99]. It is not yet clear if these approaches eliminate primarily those senescent cells with a proapoptotic, inflammatory, tissue-destructive SASP, those with a mainly growth-promoting SASP, or both forms of senescent cells. A possible advantage of small-molecule senolytics over vaccines or transplanted CAR T cells is that if a need for senescent cells occurs, for example, during wound healing, tissue remodeling or pregnancy, then treatment can be discontinued—whereas the continued elimination of senescent cells induced by vaccines or CAR T strategies may not readily be switched off. Furthermore, CAR T cell therapy is expensive, generally has to be specifically formulated for each individual being treated, and can lead to graft versus host disease, necessitating prolonged immunosuppressive therapy with all its attendant risks.

It should be noted that in commonly used preclinical models, senescence is abolished by means of $p16^{INK4a}$-based or $p21^{CIP1/WAF1}$-based genetic clearance and this senescence-targeting approach acts through mechanisms that are distinct from first-generation, SCAP-targeting small-molecule senolytics; as a result, there appear to be differences in the types of senescent cells targeted. For example, cell types such as activated macrophages, which may not be classically senescent, can have high $p16^{INK4a}$ expression[100], but are not targeted by D + Q[24]. Unpublished work from our own laboratory suggests that there may be differences in the phenotypic effects (such as those relating to wound healing and healthspan) of targeting senescent cells in transgenic mice compared to the effects of senolytic agents in wild-type mice. This is in agreement with a recent report showing that the removal of senescent cells expressing high levels of $p16^{INK4a}$ can lead to fibrosis in mice[101], whereas small-molecule senolytics appear to reduce fibrosis in several mouse tissues[22,71,73,75,102]. Indeed, work to develop senolytics began before genetic models of senescent cell clearance were published and did not depend on those models[34,56]. These genetic models have been useful in pinpointing those cells expressing particular senescence-linked markers (for example, $p16^{INK4a}$ or $p21^{CIP1/WAF1}$) and as complementary tools in senolytic proof-of-concept studies (Table 2). However, given their inability to eliminate the naturally

Table 2 | Disorders and diseases linked to senescent cell accumulation and alleviated by senolytics in preclinical models

Disorders and diseases	Genetic model	Pharmacologic agent	Phenotype of intervention
Diabetes/obesity/age-related lipodystrophy[23,25,42,48,144–152]	*PLD*[145] *INK-ATTAC*[23,25,150] *p16-3MR*[25]	D + Q[25] N[150]	Improved metabolic and adipose tissue function[23,25,145,150], reduced inflammation[25], improved adipogenesis[25], improved beta cell function[150]
Cardiac dysfunction[14,26,153–159]	*INK-ATTAC*[26,157–159] *p16-3MR*[159]	Q[153] N[155–157,159] D + Q[14,26,158]	Activation of resident cardiac progenitor cells and cardiomyocyte formation[26], alleviated myocardial hypertrophy and fibrosis[155,157], improved left ventricular ejection fraction[14,155], increase in myocardial vascularization[155], increased survival after myocardial infarction[156]
Vascular hyporeactivity/calcification/ arteriovenous fistulae[158,160]	*INK-ATTAC*[158]	D + Q[158,160]	Improved vasomotor function, reduced aortic calcification[158]
Frailty/sarcopenia/muscular dystrophy/ fibrodysplasia[14,24,49,71,124,161–164]	*PLD*[49] *INK-ATTAC*[24,71]	N[163] F[124] D + Q[14,24,71]	Improved and delayed age-associated physical dysfunction[14,24,49,71,163], extended median and maximum lifespan[124]
Age-related impairment of muscle hypertrophy after resistance exercise[18]	NA	D + Q[18]	Improved muscle growth[18]
Response to and sequelae of chemotherapy/ radiation[9,14,24,71,87,88,165–169], cancers[24,168,170–172], bone marrow transplantation[169]	*p21-ATTAC*[165] *INK-ATTAC*[24,71] *p16-3MR*[88,168]	D + Q[14,24,71] N[88,167,168] FOXO4-DRI[87]	Prevented radiation-induced osteoporosis[165], alleviated physical dysfunction[24,87,167], reduced adverse effects of chemotherapy or radiation[71,88,167,168], rejuvenation of aged tissue progenitor cells following radiation[88]
Sequelae of organ transplantation[31,74,145,173–176]	NA	D + Q[31,145]	Prolonged survival of old cardiac allografts[31], mitigated insulin resistance following xenotransplantation[145]
Age-related cognitive impairment/ Alzheimer's/ Parkinson's/ALS/ anxiety[27,30,33,177–183]	*p16-3MR*[177,182] *INK-ATTAC*[30,33,181]	N[177,181] D + Q[27,30,33,178,180]	Activation of neural progenitor cell proliferation and neurogenesis[33,177], enhanced spatial memory[177], reduced microglial activation[30], improved cognitive function[180,181], alleviated anxiety-related behavior[33], reduced tau aggregation[27,181], improved learning and memory[178], improved cognitive function[30,178], reduced neuroinflammation[180], reduced Aβ load[180]
Renal dysfunction[87,102,184–186]	*INK-ATTAC*[185]	N[186] D + Q[185] Q[102] FOXO4-DRI[87]	Improved renal function[87,185,186], reduced damage[102] and fibrosis[102,185,186]
Osteoporosis/osteoarthritis/rheumatoid arthritis/intervertebral disc disease/fracture healing[21,176,187–192]	*p16-3MR*[190,191] *INK-ATTAC*[21]	D + Q[21,188] UBX0101 (nutlin-3a or related)[191]	Attenuated the development of osteoarthritis[191], reduced pain and increased cartilage development[191], higher bone mass and strength and better bone microarchitecture[21], attenuated age-dependent intervertebral disc degeneration[190]
COPD/IPF/tobacco/hyperoxic lung damage/ pulmonary arterial hypertension[63,71,193–199]	*INK-ATTAC*[71]	N[193] Digoxin[194] D + Q[71]	Improved pulmonary function[71] and reduced fibrosis[194]
Hepatic steatosis/cirrhosis/primary biliary cirrhosis[22,75,200]	*INK-ATTAC*[75]	A1331852[22] D + Q[75]	Reduced hepatic steatosis[75] and liver fibrosis[22]
Progerias[87,124]	*INK-ATTAC*[201]	F[124] FOXO4-DRI[87]	Delayed onset and progression of age-related pathologies[87,124,201]
Intestinal inflammation/microbiome[32]	NA	D + Q[32]	Reduced inflammation and microbial dysbiosis[32]
Preeclampsia/uterine fibrosis/ovarian involution/vaginal dysplasia[73,202,203]	NA	D + Q[73]	Reduced fibrosis[73]
Cataracts/macular degeneration/glaucoma/ ocular hypertension/diabetic retinopathy[204–211]	*INK-ATTAC*[204] *p16-3MR*[206]	D[206] UBX1967 (N or related)[204]	Prevented loss of retinal function and cellular structure[206]
Progenitor growth, activation or differentiation[21,23,25,26,30,33,154,177,203]	*p16-3MR*[25,177] *INK-ATTAC*[21,23,25,26,30,33]	N[177] D + Q[21,25,26,30,33]	Increased neural precursor cell[30,33,177]/cardiac progenitor cell proliferation[26], improved proliferative and differentiation potential of adipocyte progenitor cells[23,25], enhanced osteoblastic progenitor function[21]
Lifespan/healthspan[24,55,124,201]	*INK-ATTAC*[24,201]	Procyanidin C1 (ref. [55]) F[124] D + Q[24]	Delayed onset and progression of age-related pathologies, reduced frailty[24,55,124,201], increased maximum lifespan[124]
COVID-19 (refs. [16,44,45,47])	*INK-ATTAC*[16]	N[45,47] D + Q[16,45] F[16]	Reduced mortality[16], reduced inflammation[16,45,47], increased antiviral antibodies[16]
Down syndrome[13,212]	NA	D + Q[13]	Alleviated transcriptional, molecular and cellular dysfunction[13]
Skin disorders/chronic wound healing[213]	NA	D + Q, F (publications in preparation)	Studies underway

ALS, amyotrophic lateral sclerosis; COPD, chronic obstructive pulmonary disease; FOXO4-DRI, forkhead box O transcription factor 4-D-retro-inverso; *INK-ATTAC*, *p16*^{Ink4a} apoptosis through targeted activation of caspase; NA, not assessed; *p16-3MR*, *p16*^{Ink4a}-trimodality reporter; *p21-ATTAC*, *p21*$^{Cip1/Waf1}$ apoptosis through targeted activation of caspase; *PLD*, *p21*-Cre/+;LUC (floxed loxP-flanked STOP cassette between a *Gt(ROSA)26Sor* promoter and firefly luciferase)/DTA (floxed-STOP cassette followed by diphtheria toxin A driven by ROSA promoter) mice. Adapted from ref. [34]. Selected references, but not all publications, are cited.

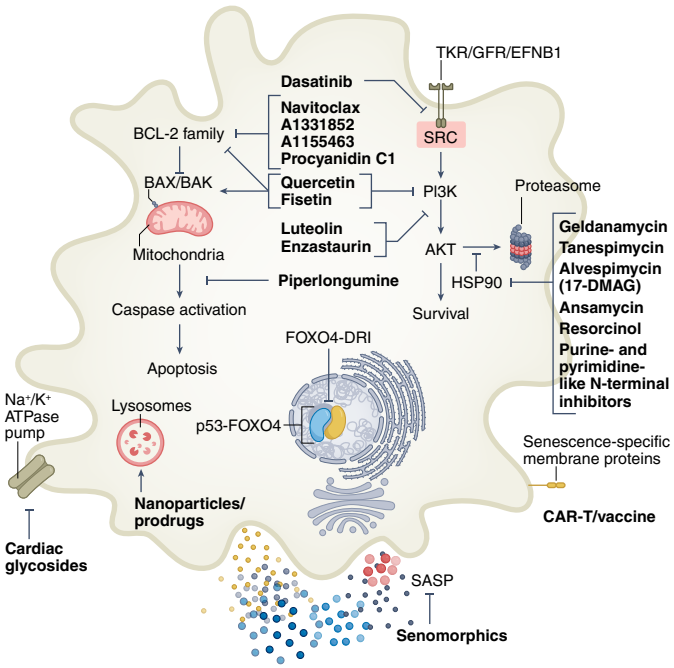

Fig. 3 | First- and second-generation senolytic strategies. First-generation senolytics target different SCAPs, including tyrosine kinase receptors (TKRs), growth factor receptors (GFRs), ephrin receptor B1 (EFNB1), SRC kinases, PI3K–AKT, HSP90, BCL-2 family members, caspase inhibition and p53 modulation[14,54,57,86–88]. High-throughput library screens and other approaches have informed second-generation senolytic strategies, including lysosomal and SA-β-gal-activated prodrugs and nanoparticles[54,92,93,220], sodium–potassium pump (Na^+/K^+-ATPase)-dependent apoptosis[194,221], SASP inhibition[103–106] and immune-mediated clearance by CAR T cells, antibody–drug conjugates or vaccines[62,96–99].

occurring heterogeneous senescent cell pool (that is, elimination of both $p16^{INK4a}$-expressing or $p21^{CIP1/WAF1}$-expressing cells or senescent cells that express neither) and the fact that also non-senescent cells such as activated macrophages are targeted[100], these genetic models are of limited use to assess the translational potential of senolytic agents—but are useful for mechanistic insights nonetheless.

SASP inhibitors

Suppressing the SASP without eliminating senescent cells is an alternative therapeutic approach for alleviating cellular senescence-related phenotypes or diseases. SASP inhibitors (senomorphics) can directly or indirectly attenuate the SASP of senescent cells by inhibiting transcription factor nuclear factor (NF)-κB, the JAK–STAT signal transduction pathway, the serine/threonine protein kinase mTOR, mitochondrial complex-1-related or 4-related targets, or other pathways involved in the induction and maintenance of the SASP[103–106]. In vitro and in vivo, inhibitors of NF-κB (mediating the cell response to inflammation), can reduce pro-inflammatory SASP cytokines and chemokines[104]. In addition, an RNA-mediated interference screen revealed that targeting alternative splicing in senescent cells may be a viable approach for inhibiting the SASP[107]. Rapamycin and its analogs (so-called 'rapalogs') suppress the SASP by inhibiting mTOR and appear to extend healthspan and lifespan in mice[105,108,109]. The antidiabetic drug metformin, which, among other activities, inhibits the SASP, alleviates several age-related conditions and chronic diseases[110–114]. A clinical trial (TAME, Targeting Aging with Metformin) is planned to test if metformin delays the time for a second age-related disease to occur in patients who already have a single age-related condition[115].

Advantages and disadvantages of senolytics versus SASP inhibitors

There are important differences between SASP inhibitors and senolytics. Whereas SASP inhibitors potentially suppress both the growth-promoting as well as the proapoptotic, inflammatory, tissue-destructive sets of SASP factors, the first-generation senolytics target the underlying cause of detrimental SASP factor production by eliminating those senescent cells that release pro-apoptotic factors. In the case of SASP inhibitors, continuous treatment is needed to maintain suppression of the SASP, although some agents, such as rapamycin, can have prolonged effects after a brief course of administration[116,117]. This may be a result of inhibiting SASP-mediated spread of senescence, thereby allowing the innate and adaptive immune system to further reduce senescent cell burden, consistent with the Unitary Theory (Fig. 2). With senolytics, intermittent administration appears to be as effective as continuous treatment for attenuating senescent cell burden[21]. This intermittent 'hit-and-run' strategy of senolytic administration could serve to reduce side effects of agents such as D, which generally appear after weeks to months of continuous administration[118,119]. In this regard, the advantages of D, Q or F over some other senolytics are their brief half-lives (4, 11 or 3–4 h, respectively, in humans) and rapid elimination[120–122]. The greater need for continuous administration of SASP inhibitors could lead to more side effects than seen with intermittently dosed senolytics and could also lead to off-target effects due to suppression of cytokine secretion—even when such cytokines are needed—by non-senescent cells such as innate or adaptive immune cells. Furthermore, some SASP inhibitors can have agent-specific off-target effects, for example, rapamycin, which can cause nephrotoxicity, metabolic impairment and susceptibility to infections, at least at higher doses in mice[109].

Senolytics cause SASP-expressing senescent cells to undergo apoptosis, and such senescent cells are present at sites of dysfunction. Interestingly, a study involving transplanted mesenchymal stromal cells, which have therapeutic effects in a wide range of disease models, may hint at a possible mechanism for the beneficial effects of senolytic-induced apoptosis. The study suggested that apoptosis of mesenchymal stromal cells is required for their therapeutic effects, possibly by means of downstream immunosuppressive effects of apoptotic processes[123]. This raises the intriguing hypothesis that senolytic-induced apoptosis of destructive SASP-expressing senescent cells, which are concentrated at sites of pathology, might contribute to the beneficial effects of senolytics, which has not been directly tested in preclinical models in currently available reports.

Consideration of the different cell populations affected by senolytic and SASP inhibitor interventions, whether expressing a detrimental tissue-destructive or beneficial pro-growth factor secretory phenotype, is crucial to the successful development of senotherapeutic interventions[61]. Elimination of all senescent cells or general inhibition of the SASP might be detrimental in some instances in which senescent cells are beneficial. However, interventions that predominantly target the persisting, tissue-destructive SASP-expressing senescent cells might have superior therapeutic potential and fewer off-target effects.

Senolytics in preclinical models

First-generation senolytics, such as D + Q, have been tested in several preclinical models of aging and diseases, including type 2 diabetes, and bone, heart, kidney, liver, lung, muscle and neurological disorders (Table 2). Whereas transplanting senescent cells decreases healthspan and lifespan in preclinical models, eliminating transplanted or endogenous senescent cells increases healthspan, thereby fulfilling Koch's postulates for causality[24,124].

In mouse models of diet-induced obesity, reducing the burden of senescent cells in adipose tissue by administering D + Q

attenuates adipose tissue inflammation, alleviates metabolic dysfunction, and restores the capacity of preadipocytes to differentiate into functional, mature, insulin-responsive fat cells[25]. High-fat diets (HFDs) and obesity result in senescent cell accumulation and lead to impaired function of multiple organs. For example, HFD-induced senescent mouse kidney cells are linked to renal fibrosis and functional impairment; Q treatment reduces senescent cell burden and SASP marker expression, alleviates renal fibrosis and improves kidney function[102]. In livers of HFD-fed mice, oral D + Q reduces the burden of senescent hepatocytes and hepatic steatosis[75]. Obesity also leads to accumulation of senescent cells near the third ventricles of the murine brain, together with development of neuropsychiatric dysfunction, particularly anxiety, which results from altered function of the limbic system situated near the third ventricles. Oral D + Q reduces this neuroinflammation, increases markers of neurogenesis, reduces gliosis and alleviates anxiety in obese mice[33]. Fibrosis can be a senescence-driven progressive process that contributes to reduced organ function; in a mouse model of idiopathic pulmonary fibrosis (IPF), D + Q treatment improved lung compliance and reduced frailty[71]. In age-related osteoporosis linked to senescent-like osteocytes and bone marrow cells in mice, D + Q reduced development of bone-resorbing osteoclasts, while increasing differentiated bone-forming osteoblasts, with restoration of bone mass[21]. After resistance exercise, senescent cells develop in muscle of old mice in tandem with impaired exercise-induced muscle hypertrophy compared to young mice[18]. D + Q alleviates this negative impact of aging on muscle growth. Recently, senescent cells have been implicated in accentuating the severity of viral infections due to amplification of SASP factor secretion, predisposing to cytokine storm and multi-organ failure. In mice infected with a murine coronavirus, the mortality rate was reduced following administration of D + Q or F[16].

Transplanted senescent cells or organs from old mice have been shown to spread the senescent phenotype to distant sites in recipient mice, predisposing to detrimental outcomes after transplantation[24,31]. D + Q treatment of either old donor mice, the organs harvested from the old donors before transplantation, or the recipients, led to decreased senescent cell burden and increased survival of recipient mice – so that outcomes resembled those observed in mice receiving a transplant from a young donor[31]. Hence, a potential new application of senolytics may entail ex vivo perfusion of organs from older donors that are currently being discarded because of increased risk of organ failure or allograft rejection, potentially offsetting shortages of organs for transplantation[125].

A recent study implicated cellular senescence and the beneficial effects of senolytics for Down syndrome (trisomy 21)[13]. A third copy of chromosome 21 in neural progenitor cells leads to transcriptional and nuclear organizational changes similar to those in senescent cells. Interestingly, D + Q alleviated transcriptional changes and cellular dysfunction in an in vitro human neural cell Down syndrome model. An accelerated aging-like phenotype has been observed in astronauts who are exposed to radiation, high G-forces, zero gravity and cabin air quality[126]. Thus, senolytics might support long-distance space exploration by eliminating the senescent cells caused by cosmic and solar radiation, especially outside the van Allen radiation belts. In April 2022, effects of space travel on senescence biomarkers in astronauts and cultured human fat cell progenitors were tested during the Axiom-1 mission to the International Space Station; data are pending[127].

Systemic versus local administration

According to the threshold theory of cellular senescence, senescent cells can be present without clinical manifestations but, if their abundance exceeds a threshold, senescent cell burden can increase further and contribute to development of local and systemic dysfunction and multiple diseases (Fig. 2). Senescent cells can spread even to distant sites because of their SASP; therefore, systemic, intermittent administration of senolytics for senescence-associated diseases is potentially more promising than local administration, with the exception of perhaps eye, skin or dental topical or other local applications[34]. Perhaps there may not need to be strict cell type specificity of senolytics administered in vivo. Arguably, once systemic senolytic treatment has reduced overall senescent cell burden to below a threshold, the immune system might clear remaining senescent cells, including those resistant to that particular senolytic. This could be especially important for older individuals or those with chronic diseases, who already have a high systemic senescent cell burden[24,128]. Strategies for systemic senolytic administration include oral or intravenous routes, with orally active senolytics generally being more accepted by patients and less expensive to administer. The timing, the particular senolytic agent used, and the age, sex, and other characteristics of the individual may impact effectiveness of senolytics[24]. For example, F may be beneficial but D detrimental in healthy young female mice that have not yet accumulated many senescent cells[129].

Clinical trials and future directions

Based on promising results in preclinical models, over 20 clinical trials of senolytic therapies are completed, ongoing or planned[34] (Table 1). Because side effects of senolytics in humans are not yet fully known, and to maximize benefit–risk ratios, the first clinical trials are underway in patients with serious health conditions, such as diabetic kidney disease, Alzheimer's disease, frailty and IPF[34]. The first in-human trial of senolytics (D + Q), the Hematopoietic Stem Cell Transplant Survivors Study, is still underway (NCT02652052; first patient dosed on 1 April 2016). The first senolytic clinical trial published was an open-label pilot study in which 14 patients with IPF were treated with intermittent D + Q on 3 d per week for 3 weeks[63]. Results suggested that senolytics improved physical function in these frail patients. Furthermore, post hoc analysis of a study involving 20 patients with IPF showed that urine levels of the 'geroprotective' factor α-Klotho were higher after oral D + Q than before treatment[19]. In an open-label phase 1 pilot study in 9 patients with diabetic kidney disease, a 3-d course of oral D + Q was sufficient to decrease adipose tissue senescent cell burden, inflammation, fibrosis and circulating SASP factors for at least 11 d after the last dose of senolytics, indicating target engagement and suggesting that an intermittent dosing regimen may be effective in humans[48]. These early data warrant evaluation in larger randomized, double-blind, placebo-controlled trials for senescence-associated disorders and diseases, some of which are underway[34] (Table 1).

It should be noted that a phase 2, randomized, double-blind, placebo-controlled clinical trial (NCT04349956)—in which the senolytic agent was a p53-destabilizing protein MDM2 inhibitor, UBX0101 (also known as nutlin-3a)—did not achieve its primary endpoint of improving pain in patients with osteoarthritis of the knee in a 12-week follow-up. In our hands and others, nutlin-3a does not show or shows only weak senolytic activity and, in some cases, can even induce cellular senescence[95,130]. Furthermore, in a mouse model of osteoarthritis, a combination of UBX0101 with N was necessary to restore aged joint structure[131]. Thus, the failure of this clinical trial seems related to the particular agent administered, which may not have been a fully effective senolytic.

Biomarkers of senescent cell burden and senolysis: gerodiagnostics. Identification and monitoring of senescent cell burden in situ, especially during clinical trials to assess safety and efficacy, can be challenging if solely based on analysis of tissue biopsies given the contextual and complex regulation of the senescent cell fate. However, SASP factors, senescence markers and markers of other fundamental aging processes (for example, α-Klotho) can be assayed in body fluids such as urine, saliva, blood or cerebrospinal

fluid[7,19,48,59,61,64,132,133]. Additionally, ongoing efforts are underway to identify and define biomarkers of senescent cell accumulation (senescence biomarkers) and destruction of senescent cells during senolytic treatment (senolysis biomarkers) that meet requirements of regulatory authorities. Panels of SASP factors and senolysis markers for clinical trials and, ultimately, clinical practice will be important as diagnostics/predictors for multiple disorders and diseases, monitoring target engagement and individualizing senolytic regimens for patients. Indeed, to facilitate developing interventions targeting fundamental aging processes and for eventual use in clinical practice, such indicators will need to be more than mere 'biological clocks'. Indicators are needed that are reproducible, reliable, feasible and inexpensive to measure, and reflect not only biological age, but also correlate with clinical function and/or disabilities. These should also change in response to interventions, predict or reflect changes in clinical function caused by these interventions, and indicate which senolytic or SASP inhibitor may be best for an individual. Such biomarkers or composite scores of biomarkers are known as 'gerodiagnostics'. Indeed, the first gerodiagnostic score sensitive to senolytic administration in humans was a blood composite score published in 2019 (ref. [48]). Additionally, we propose the concept of 'gerodiagnostic ratios', whereby gerodiagnostic indicators that increase with aging or disorders linked to acceleration of fundamental aging mechanisms, could be summed into a composite in the numerator (for example, markers of senescent cells, SASP factors, CD38 and mTOR activity indicators), while beneficial geroprotective factors (for example, α-Klotho or perhaps nicotinamide adenine dinucleotide (NAD^+) or Sirt-6) could be in the denominator. This approach could both enhance sensitivity and reduce 'denominator effects'; for example, creatinine is often used as a dominator for urine analytes, introducing the confounding variable of muscle mass, upon which creatinine levels depend.

Clinical trajectory for the next decade. Large, randomized controlled trials to assess and ensure safety, benefit and target engagement of senolytics are needed to validate preliminary results from early-phase clinical trials (Table 1). If safety and effectiveness of senolytics are first demonstrated in patients with serious diseases for which current treatments are inadequate, it could become acceptable to test them for less severe senescence-linked disorders. If safe and effective for such conditions, senolytics might then be tested for prevention of age-related dysfunction and diseases in older individuals, using a strategy like that which is planned for metformin in the TAME study[115]. TAME will test if metformin delays the appearance of a second age-related disorder in patients who already have one such disorder; it will not be a trial including completely healthy older adults[115]. If attempts to extend human healthspan are effective, future studies might conceivably aim to evaluate the role of senolytic therapies in extending human lifespan.

Combination therapeutic strategies. Clinical studies of geroscience interventions targeting the other pillars of aging are currently underway or being planned. Dietary interventions, such as caloric restriction and intermittent fasting, might be protective against development of age-related dysfunction and diseases based on studies in animal models[134,135]. Exercise may delay senescent cell formation and reduce inflammation, frailty and chronic disease onset in mice[136,137]. Metformin, resveratrol and rapalogs (agents related to rapamycin) are SASP inhibitors and appear to impact some of the same basic processes as caloric restriction or exercise[110,138]. Sirtuins facilitate DNA damage repair, partially relying on oxidized NAD^+ to do so. Senescent cells can decrease NAD^+ through SASP activation of the NAD-degrading enzyme CD38 on the surface of macrophages and, remarkably, senolytics partially restore tissue NAD^+ levels[139,140]. NAD^+ or NAD precursors can increase healthspan in experimental animals[141]. The non-feminizing estrogen, 17α-estradiol, declines with aging in females and males and 17α-estradiol treatment extends healthspan and alleviates age-related metabolic dysfunction and inflammation in mice[142]. As considered above, senolytics can increase α-Klotho, which is geroprotective, neuroprotective and linked to healthspan in mice[19]. Interestingly, α-Klotho overexpression in mice increases healthspan and lifespan by up to 30% (ref. [143]). Hence, consistent with the Unitary Theory, interventions targeting the different fundamental aging mechanisms appear to alleviate aging phenotypes, delay, prevent or treat multiple diseases, and extend healthspan in preclinical models. Testing combinations of these lifestyle, nutritional, natural product and pharmacologic interventions may be informative. However, if the Unitary hypothesis is correct, they may contribute less-than-additive effects because fundamental aging mechanisms are tightly interconnected. A more promising strategy could be to combine geroscience with disease-specific interventions.

Box 1 | Scientific, logistical and regulatory obstacles

- Methods to stop effects of interventions such as vaccines or CAR T cells need to be devised, in case the fundamental aging mechanism that they target becomes necessary in a patient, for example, in the context of tissue repair, wound healing or pregnancy.
- Interventions that appear effective in preclinical models, for example, in mice, often fail in clinical trials[222]. There will be inevitable clinical trial failures, which could become a psychological barrier and tarnish the field. A strategy to mitigate this could involve the conduct of multiple, parallel smaller trials of different geroscience interventions (for example, senolytics versus SASP inhibitors versus NAD^+ precursors versus sirtuin agonists versus placebo) for different indications, each linked to fundamental aging mechanisms. These could be carried out across multiple institutions, but with shared outcome variables (for example, blood, saliva or urine gerodiagnostics, imaging studies, assessments of physical findings, activity, or other indicators and/or questionnaires).
- There are currently no FDA-recognized gerodiagnostics to serve as primary outcomes of clinical trials, nor any consensus-based recommendations to inform study design.
- There is a lack of ICD codes for relevant clinical states such as frailty, multimorbidity or sarcopenia.
- Given the importance and potential of the geroscience field, there are insufficient funding opportunities and incentives to spur progress.
- There are few academic geriatricians with expertise in basic or preclinical geroscience research or interventional clinical trials, and there are no resources to train sufficient numbers of such individuals[223].
- Occasional exaggerated 'antiaging' claims and profiteering have caused skepticism.
- Many companies and entrepreneurs are not interested in lifestyle, natural product or repurposed off-patent agents that may be effective geroscience interventions, but for which intellectual property protection is unattainable.
- Drug regulatory agencies often lack sufficient expertise and familiarity with geroscience interventions, which can drastically slow bench-to-bedside translation.
- Academic or financial ambitions, incentives and rivalries among investigators, disciplines and institutions can hinder discovery and testing of geroscience interventions.

Conclusions

The elimination of senescent cells has emerged as a plausible therapeutic strategy for preventing, delaying or alleviating multiple

diseases and age-related dysfunction. Promising results of senolytics in preclinical models suggest therapeutic and preventive opportunities for delaying multimorbidity and increasing healthspan. While randomized controlled trials will define the safety and potential benefits of senolytic strategies, scientific and regulatory challenges must be addressed in the near term if senolytics are to be used in the clinic (Box 1).

A key priority should be the identification of reliable, sensitive and specific gerodiagnostics—biomarkers to quantify senescent cell abundance, the SASP and senolysis as well as other pillars of aging. Interventions modulating the SASP (including those that specifically upregulate or downregulate tissue-destructive factors versus growth factors) or topical senolytic agents, especially those that eliminate senescent cells with a proapoptotic SASP, could accelerate wound healing, a possibility that needs to be experimentally tested and correlated with predictive gerodiagnostics.

The lack of WHO International Classification of Disease (ICD) codes for multimorbidity, sarcopenia, healthspan or the geriatric syndromes represents a barrier to clinical development. Such ICD codes would facilitate regulatory approvals, recording of conditions linked to fundamental aging processes in hospital and insurance records, epidemiological studies, physician and hospital reimbursement and engagement of the pharmaceutical industry. The possible interdependencies among fundamental aging mechanisms need to be investigated to deepen our knowledge about basic aging mechanisms and disease etiologies and to develop treatment strategies to reduce multimorbidity and enhance healthspan.

Finally, a note of caution is important. Even though preclinical data are promising, unless and until carefully monitored, rigorous clinical trials demonstrate safety and effectiveness of senolytics or SASP inhibitors, they should not be endorsed for the prevention or treatment of diseases over-the-counter or in clinical practice. In the meantime, the results of ongoing and planned clinical trials will yield informative data and insights into the role of cellular senescence as a therapeutic target for age-related disorders, potentially enabling translation of small-molecule senolytics into the clinic in the near future.

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Author contributions

J.L.K. conceptualized this Review. All authors contributed to the writing and approved the submitted version of the manuscript. S.C. created the figures using BioRender.com.

Competing interests

T.T. and J.L.K. have a financial interest related to this research, including patents and pending patents covering senolytic drugs and their uses that are held by Mayo Clinic. S.C. has received royalties from Rejuveron Senescence Therapeutics. This research has

been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with the Mayo Clinic's conflict of interest policies.

Additional information

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