A Senescence-Centric View of Aging

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Review

A Senescence-Centric View of Aging: Implications for Longevity and Disease

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Cellular senescence is a state of stable cell cycle arrest associated with macromolecular alterations and secretion of proinflammatory cytokines and molecules. From their initial discovery in the 1960s, senescent cells have been hypothesized as potential contributors to the age-associated loss of regenerative potential. Here, we discuss recent evidence that implicates cellular senescence as a central regulatory mechanism of the aging process. We provide a comprehensive overview of age-associated pathologies in which cellular senescence has been implicated. We describe mechanisms by which senescent cells drive aging and diseases, and we discuss updates on exploiting these mechanisms as therapeutic targets. Finally, we critically analyze the use of senotherapeutics and their translation to the clinic, highlighting limitations and suggesting ideas for future applications and developments.

Highlights

There is increasing evidence of the detrimental role of senescent cells in aging.

Clearance of senescent cells has been shown to improve age-associated pathologies in animal models, leading to promising new clinical trials.

Different mechanisms of senescent cells can be exploited pharmacologically to develop new therapeutic targets.

Cellular Senescence: From Physiology to Pathology

Cellular senescence (see Glossary) is a state of stable and generally irreversible growth arrest that acts as a potent tumor-suppressive mechanism. A major regulator of the senescence-associated cell cycle arrest is a chronic DNA damage response (DDR), which derives from unresolved DNA lesions and triggers activation of cell cycle inhibitors. Other common features observed in senescent cells are profound changes in (epi)genetic landscape and gene expression, persistent macromolecular damage, and aberrant metabolism and activation of a hypersecretory phenotype [1,2].

The hypersecretory phenotype is defined as the **senescence-associated secretory phenotype (SASP)**, a collection of chemokines, cytokines, matrix remodeling proteases, and extracellular vesicles (EVs) [2]. The SASP has been hypothesized to link cellular senescence and **inflammaging** [3], and to participate in tissue dysfunction. The SASP is a highly heterogeneous program whose composition depends on various intrinsic and extrinsic factors [4,5].

Restricted and localized SASP contributes to various beneficial functions. It favors correct organ patterning during embryogenesis, halts malignant transformation by reinforcing cell cycle arrest and activating tumor immunosurveillance, and promotes tissue repair [6]. By contrast, persistence of senescent cells and SASP has been associated with chronic inflammation, age-related pathologies, and a cancer-permissive microenvironment [7]. In this regard, the evolutionary theory of antagonistic pleiotropy is used to explain the senescence phenotype [8]. According to this theory, a biological trait can be beneficial for survival and reproduction in early life, at the cost of reduced **healthspan** at later stages. In recent years, the generation of transgenic mouse models reporting and inducibly eliminating senescent cells allowed for a direct demonstration of the pleiotropic biological functions of senescence (Box 1). Consistently, in a young healthy individual senescent cells can be induced successfully upon damage, to ensure correct tissue function and repair, and counteract incipient oncogenic stimuli, whereas their improper activation and disposal followed by progressive accumulation with age leads to disease (Figure 1). For this

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Box 1. Senescent Cell Clearance in Genetic Mouse Models

p16-3MR mice [40]: $p16^{INK4A}$ promoter reporter with functional domains of a synthetic *Renilla* luciferase (detection by luminescence), monomeric (m)RFP and truncated herpes simplex virus 1 thymidine kinase (HSV-TK). This construct allows the identification and isolation of $p16^{INK4a}$ positive cells by *in vivo* luminescence and mRFP fluorescence. In addition, HSV-TK allows the specific elimination of $p16^{INK4a+}$ cells by treatment with ganciclovir (GCV).

INK-ATTAC mice [69]: $p16^{INK4a}$ promoter reporter followed by GFP to allow $p16^{INK4a+}$ cells, and with the fusion protein construct consisting of a FK506-binding protein (FKBP) and caspase 8 (Casp8). In this mouse model, administration of the synthetic molecule AP20187 induces the dimerization of FKBP-Casp8 and $p16^{INK4A+}$ cells are selectively killed by caspase-dependent apoptosis.

INK-NTR mice [99]: a modification of the INK-ATTAC mouse, where FKBP-Casp8 is replaced with the *NTR* gene. Metrodinazole (Mtz), a nontoxic prodrug, is administered to these mice and then converted into a cytotoxic metabolite by NTR, hence eliminating $p16^{INK4a}$+ cells.

ARF-DTR mice [123]: promoter of p19-Arf, together with luciferase gene and diphtheria toxin (DT) receptors, allowing the detection by luminescence *in vivo* of Arf^{+} cells as well as their elimination by DT administration.

reason, interventions selectively targeting cellular senescence hold the potential to delay **aging** and alleviate multiple age-related dysfunctions.

Senescence as a Basic Aging Mechanism

Senescent cells exhibit and integrate several hallmarks of aging (Figure 2) [9]. Progressive telomere erosion leads to the induction of senescence via p53-dependent DDR, coordinated by the activity of ataxia telangiectasia mutated (ATM) and ATM and Rd3-related (ATR) [10]. Stable p53 leads to cell cycle exit in part by inducing the expression of the cyclin-dependent kinase (CDK)2 inhibitor $p21^{WAF1/Cip1}$, which suppresses the phosphorylation of pRB. Increased protein expression of the CDK4/6 inhibitor $p16^{INK4a}$ also blocks pRB phosphorylation, which induces cell proliferation arrest by inhibiting the activity of E2F protein members. Telomere attrition induces alteration of DNA structure, referred to as telomere uncapping, causing additional DNA breaks and genome instability [11]. DNA lesions, such as those induced by oxidative stress, radiation (e.g., UV light and ionizing radiation) and genotoxic agents (e.g., chemotherapeutic) can be converted to DNA double-strand breaks (DSBs) [12], to which cells rapidly respond and attempt repair by activating DDR. Persistence of DDR initiates the secretion of SASP factors [13]. Like telomeres, dysfunctional mitochondria lead to senescence [14]. This is partially due to increased mitochondrial mass, changes in fusion and fission rates, and altered membrane potential [14]. **Mitochondrial dysfunction-associated senescence (MiDAS)** leads to hyperproduction of reactive oxygen species (ROS) which in turn drive DDR and SASP factors secretion [15,16]. MiDAS and increased mammalian target of rapamycin (mTOR) activity can also derive from deregulation of nutrient sensing molecules. In particular, low NAD^{+}/NADH ratio and depletion of mitochondrial malic enzymes can activate AMP-activated protein kinase (AMPK), which can in turn trigger cell cycle arrest via p53 [17]. Increases in AMP:ATP and ADP:ATP ratios also contribute to functional decline of sirtuin proteins and poly-ADP ribose polymerase (PARP), which are important modulators of SASP and NF-κB [15,16]. Despite much evidence on the activity of mTOR in SASP regulation, its role in autophagy in senescent cells is still not clear. In accordance with the conventional role of mTOR being an inhibitor of autophagy, studies have shown that suppression of autophagy can induce senescence [18]. Paradoxically, increased autophagic flux is necessary to counteract the endoplasmic reticulum stress and unfolded protein response (UPR), in part deriving from the high load of SASP secretion and thus contributing to survival of senescent cells [19]. This suggests the possibility of general and selective autophagy having opposing roles during senescence activation [20]. Altered protein function results from the generation of ROS and enhanced DDR. The majority of protein containing serine/threonine or cysteine residues

Glossary

Age-associated diseases: diseases whose incidence increases with aging, most of them sharing an inflammatory pathogenesis and correlating with increased levels of cell senescence.
Aging: molecular and cellular damage accumulation over time leading to a progressive decline in physical and mental capacity, and to an increased risk for disease and death.
Apoptosis: programmed and controlled cell death that regulates growth, development, tissue homeostasis, and tumor suppression.
Cellular senescence: heterogeneous cell state in response to different stress stimuli, characterized by stable cell cycle arrest, as well as morphological, structural, and functional changes, including enhanced expression and secretion of proinflammatory mediators.
Frailty: clinical syndrome observed in older adults that predispose to poor health, onset and progression of diseases and decreased capacity to cope with cellular and tissue stress.
Geroconversion: conversion from reversible cell cycle arrest (quiescence) to irreversible cell cycle arrest (senescence).
Healthspan: period of life where an individual has good health, free of disabilities and diseases.
Immunosenescence: gradual age-associated functional decline of the immune system, especially of the adaptative immune system, that contributes to increased risk of morbidity and mortality.
Inflammaging: low-grade chronic inflammation, not induced by pathogens, causing higher risk of morbidity and mortality in elderly.
Lifespan: measure of populations average survival time between birth and death.
Mitochondrial dysfunction-associated senescence (MiDAS): cellular senescence originated from dysfunctional mitochondria, likely as a result of the accumulation of ROS.
Mitophagy: regulated degradation of dysfunctional mitochondria by autophagy.
Paracrine senescence: cellular senescence originated in a non-cell autonomous manner via SASP factors secreted by neighboring senescent cells.
Progeroid: syndrome or phenotype mimicking premature aging.

can be susceptible to oxidative damage and activate senescence via the extracellular signal-regulated kinases (ERK) signaling cascade [21]. Increased ROS levels can also affect correct protein folding and aggregation which are characteristic of most neurodegenerative disorders. Of note, enhanced ROS correlate with altered and reduced enzymatic activity of different chaperones (such as heat shock proteins, HSPs) implicated in the UPR [22], which is coupled to aberrant autophagic-mediated protein degradation during senescence. A common characteristic of aging is chronic low-grade inflammation, also known as sterile inflammation (activation of immune response in absence of pathogens). Increased senescence burden influences macrophages, T cells, and natural killer function favoring a switch toward a more immunosuppressive function between young and old tissue microenvironments [23,24]. As the major T lymphoid organ, the thymus regulates T cell repertoire and immune tolerance. Thymic involution with age, is associated with contraction of naïve T cells and decreased capacity of immune response to infection [25]. Senescence occurs during thymic involution, contributing to tissue atrophy, inflammation, and thymopoietic decay [26]. Importantly, senescent T cells bearing dysfunctional mitochondria, have been shown to trigger a type I cytokine storm in peripheral organs, causing an accelerated aging phenotype in mice [27]. Furthermore, increased mTOR activity and mitochondrial oxidative stress have been linked to senescence of hematopoietic progenitor cells in elderly people [28].

Thus, **immunosenescence** contributes to altered inflammatory response and impaired stem cell function [3,9], which might be explained by incipient senescence activation, sustained secretion of SASP factors and senescence-induced inflammasome activation [29]. Accumulation of senescence and secretion of SASP factors during aging fosters inflammatory responses, alters cell-to-cell communication, and limits regeneration thus contributing to tissue dysfunction, **frailty**, and disability [30–32].

Senescence-associated secretory phenotype (SASP): robust and heterogeneous secretion of soluble modulators by senescent cells, including cytokines, chemokines, growth factors, proteases, and EVs.
Senotherapeutics: molecules and strategies that target cellular senescence, which can be classified as **senolytics** (selective elimination of senescent cells via programmed cell death) and **senomorphics/ senostatics** (modulation of senescence-associated phenotypes without senolysis).

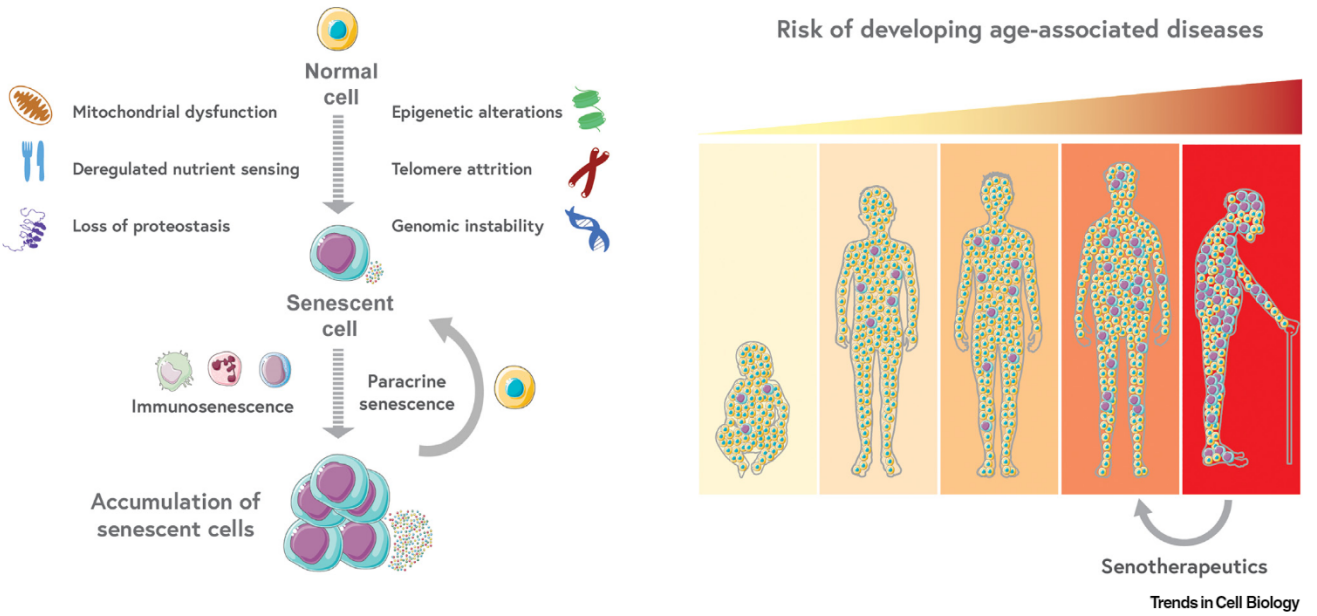

Figure 1. Senescence-Centric View of Aging. Some of the hallmarks of aging (mitochondrial dysfunction, deregulated nutrient-sensing, loss of proteostasis, epigenetic alterations, telomere attrition, and genomic instability) induce normal cells to become senescent, which in turn can induce **paracrine senescence** in nearby normal cells through senescence-associated secretory phenotype (SASP). Senescence-promotion through SASP together with a decline in the immune system activity, converge to induce organismal accumulation of senescent cells. In aged individuals, chronic accumulation of senescent cells contributes to tissue dysfunction and increased risk of age-associated diseases development. Nevertheless, senescent cells elimination with different senotherapeutic approaches can improve healthspan in aged individuals.

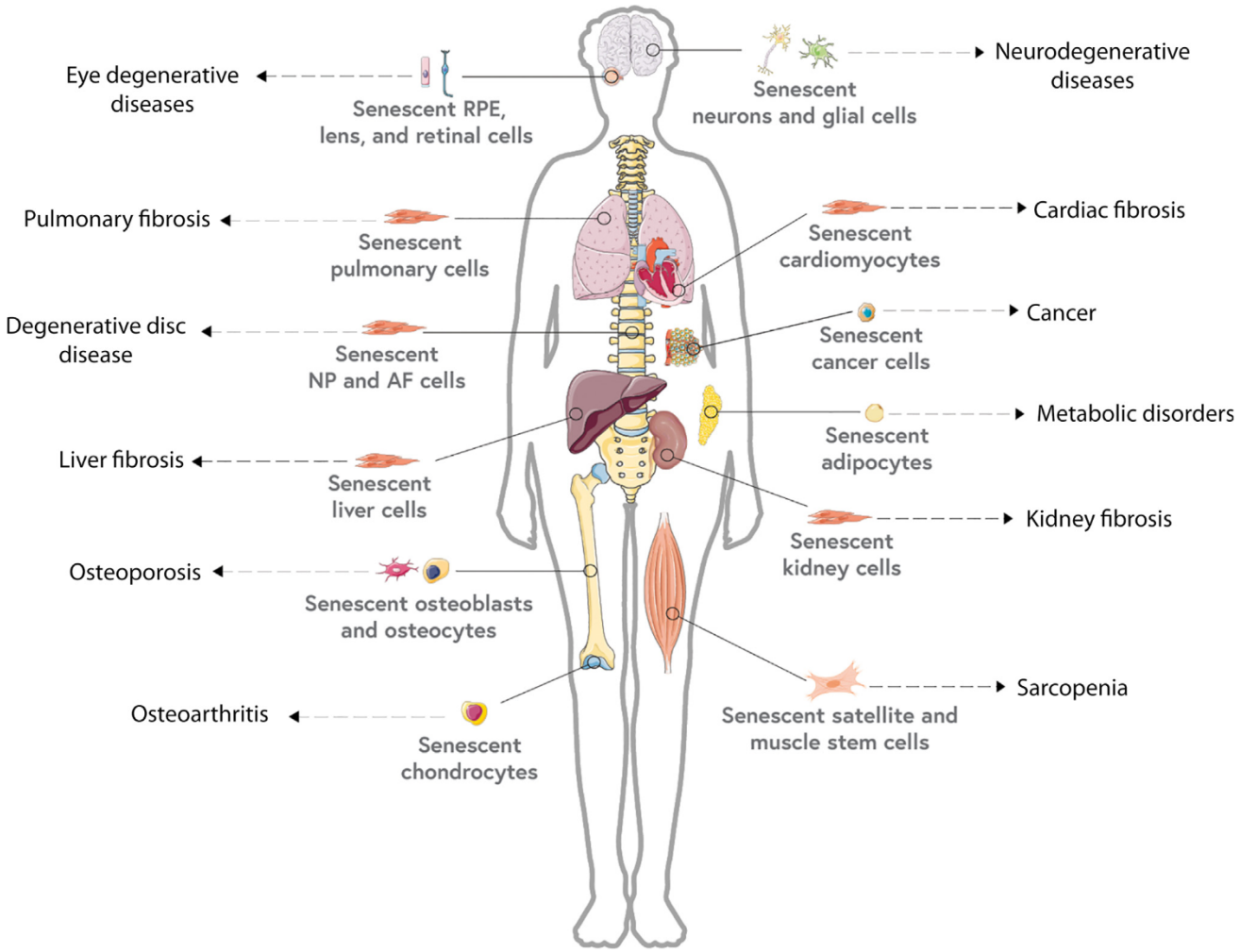

Figure 2. Senescent Cells Play a Role in Age-Associated Diseases. Elimination of senescent cells had led to a beneficial impact on the indicated age-related diseases. Some of the senescent cells described in the literature as implicated in the disease development have also been depicted. Abbreviations: AF, annulus fibrosus cells; NP, nucleus pulposus cells; RPE, retinal pigment epithelium.

Targeting Senescence in Age-Related Diseases

Preclinical studies have highlighted that specific clearance of senescent cells in genetic animal models (Box 1) or with **senotherapeutics** with different molecular targets (Table 1) alleviates **age-associated diseases** and frailty (Figure 2). In the following sections we discuss the most relevant findings in these areas.

Musculoskeletal Dysfunctions

Osteoarthritis

Osteoarthritis (OA), a disorder that involves the movable joints, is the leading cause of chronic pain and disability in elderly people [33]. The accumulation of senescent cells in the aged cartilage and their involvement in OA has been reported in many studies [34–39]. In fact, the causal role of senescence in OA was demonstrated by the injection of senescent cells into the knee of mice, which led to a state resembling OA [39]. Senescent cells arise in the cartilage during aging, but also as a consequence of traumatic injuries in an attempt to promote tissue

Table 1. Senotherapeutics and Their Molecular Targets[a]

Drug	Function	Molecular targets	Involvement	Refs
Dasatinib + quercetin	Senolytic	Ephrin receptors + PI3K/AKT/ROS/P53/p21/serpine/ HIF-1a	Osteoporosis, disc degeneration; obesity; glaucoma; frailty, AD; lung fibrosis	[46–48,77,84,85,94,96,124]
Navitoclax (ABT-263)	Senolytic	Bcl-2/Bcl-x family	AD, cancer; stem cell rejuvenation, lung fibrosis, atherosclerosis	[77,99,114,125,126]
ABT-737	Senolytic	Bcl-2/Bcl-x family	Lung function, accelerated aging, liver regeneration	[75,127,128]
ABT-199	Senolytic	Bcl-2	Diabetes type 1	[97]
PZ15227	Senolytic	Bcl-xl	Bone loss, myeloid skewing	[115]
A1331852	Senolytic	Bcl-2/Bcl-x family	Huvec, IMR90	[129]
A1155463	Senolytic	Bcl-2/Bcl-x family	Huvec, IMR90	[129]
UBX0101	Senolytic	P53/mdm2	Osteoarthritis	[36]
Fenofibrates	Senolytic	PPARα agonist	Osteoarthritis	[41]
AT-406	Senolytic	IAP1/2/XIAP inhibitors	Osteoarthritis	[42]
FOXO4-DRI	Senolytic	p53/foxo4 interaction/Bcl-x family	Frailty, nephropathy, natural aging	[130]
Cardiac glycosides	Senolytic	Na^+/K^+ ATPase pump	Cancer; lung fibrosis	[131,132]
Fisetin	Senolytic	Bcl-2/Bcl-x family/ PI3/AKT	Osteoarthritis, aging	[133,134]
Piperlongumine	Senolytic	Bcl-2/Bcl-x family/ PI3/AKT	Senescent lung fibroblasts	[135]
Curcumin	Senolytic	Broad spectrum (e.g., BCL-2, NF-KB)	Disc degeneration	[66]
17-DMAG	Senolytic	HSP-90 inhibitor	Frailty/healthspan	[136]
ARV825	Senolytic	BET family inhibitor - NHEJ	Hepatocarcinoma	[137]
KU-60019	Senolytic	ATM	Wound healing	[138]
SSK1-Gemcitabine	Senolytic	Lysosomal β-galactosidase	Lung/liver fibrosis, frailty	[70]
2-DG	Senolytic	Lysosomal V-ATPases	Cancer	[19]
Rapamycin	**Senomorphic**	mTOR	Osteoarthritis, sarcopenia	[60,139]
Metformin	Senomorphic	AMPK activator	Osteoarthritis, disc degeneration	[43,140]
Ruxolitinib	Senomorphic	JAK1/2 inhibitor	Osteoporosis, frailty	[47,53]
NBD peptide/mimetics	Senomorphic	NF-κB inhibitor	Aging, osteoporosis	[141]

[a]Abbreviations: 17-DMAG: 17-dimethylaminoethylamino-17-demethoxygeldanamycin; 2-DG: 2-deoxy-D-glucose; NHEJ, non-homologous end joining.

regeneration [36,40]. In a post-traumatic OA mouse model selective clearance of senescent cells, achieved by a genetic (p16-3MR; Box 1) or pharmacological (UBX0101; Table 1) strategy, halted OA progression, with increased synthesis of extracellular matrix (ECM) components, reduced expression of matrix metalloproteinase (MMP)-13 and interleukin (IL)-1β, and reduced pain [36]. In addition, elimination of naturally age-accumulated $p16^{INK4a}$ cells resulted in reduced cartilage degeneration. In OA human chondrocytes, UBX0101 treatment improved the synthesis of ECM proteins, promoting a proregenerative environment [36], while another **senolytic** compound, Fenofibrate (a PPARα agonist), reduced proteoglycan loss in IL-1β-treated human cartilage explants [41]. Moreover, local elimination of senescent cells by inhibition of the antiapoptotic proteins cellular inhibitor of **apoptosis** protein (c-IAP)1/2 and X-linked inhibitor of apoptosis protein (XIAP) with the molecule AT-406 was shown to reduce SASP secretion and promote regeneration in a post-traumatic OA rat model [42]. Finally, increasing evidence suggests that molecules targeting signaling networks involved in SASP (e.g., rapamycin and metformin) promote a regenerative environment in cartilage and exert beneficial effects in preclinical models [43,44].

Osteoporosis

Osteoporosis is characterized by the decrease in the total bone mass accompanied by increasing risk of bone fractures. During aging, bone loss derives from reduced bone formation and increased formation of bone marrow fat. Higher levels of $p16^{INK4a}$ expression are observed in osteoblast progenitors, osteoblasts, and osteocytes in old mice, and in human biopsies [45]. Treatment of the **progeroid** mouse model Ercc1(-/Δ), which accumulates DNA damage due to impaired nuclear genome repair capacity, with the senolytic cocktail dasatinib (D; anticancer drug with several functions; Table 1) plus quercetin (Q; polyphenol with several functions; Table 1) improved bone mineral content and density [46]. Moreover, genetic (INK-ATTAC mice; Box 1) or pharmacological (D + Q) approaches improved both the antiresorptive and anabolic pathways leading to increased bone mass and strength in old mice [47], and reduced radiotherapy-induced bone loss [48,49]. Several preclinical studies have shown that molecules that inhibit oxidative stress can result in reduced osteocyte senescence and SASP, and improved bone structure [50–52]. For example, the FDA-approved Janus kinase (JAK 1/2) and signal transducer and activator of transcription protein 3 (STAT3) inhibitor, ruxolitinib, shown to be a SASP inhibitor [53], reduce bone resorption while promoting new bone formation [47].

Sarcopenia

Aging is associated with pronounced loss of skeletal muscle mass and function, a process defined as sarcopenia, which significantly contributes to frailty and increased mortality in the geriatric population [54]. A role for senescence in promoting muscle weakness and frailty was demonstrated by a study that showed improved physical function (walking speed, endurance, and grip strength) of aged mice treated with the **senolytics** D+Q [55]. Mechanistically, recent studies have suggested that satellite cell senescence is key in the development of sarcopenia. Satellite cells are resident muscle stem cells that are required for skeletal muscle regeneration and growth after injury and exercise, after which these cells break quiescence, proliferate, and contribute to muscle fiber repair and growth [56,57]. In skeletal muscles of geriatric (28–32 months old) and progeroid mice satellite cell activation is impaired by **geroconversion** [58] (i.e., transition from quiescence to senescence), as demonstrated by increased senescence associated (SA)-β galactosidase staining and $p16^{INK4a}$ and Igfbp5 expression [59]. Senescence in geriatric satellite cells was found to be caused by decreased **mitophagy** and increased ROS production [60], and $p16^{INK4a}$ or ROS inhibition or autophagy activation restored satellite cell function and muscle regeneration [59,60]. Additionally, transforming growth factor (TGF)-β signaling is also implicated in satellite cell senescence by promoting a Smad3-mediated increase of CDKs (most notably $p15^{INK4B}$ and $p21^{WAF1/Cip1}$), and inhibition of TGF-β signaling improves muscle regeneration in aged mice [61]. Senescent fibroadipogenic progenitor cells [62] and postmitotic muscle fibers [63] have been also detected in skeletal muscles of exercised and aged mice, but the contribution of these senescent subsets to sarcopenia remains to be determined. In addition, senescence in other cell types in the body may contribute to sarcopenia, as demonstrated by transplantation of senescent preadipocytes in young mice, which resulted in marked physical dysfunction and weaker muscles [55]. Finally, satellite cell senescence has been also reported in mouse models of degenerative muscle wasting diseases such as muscular dystrophies [64,65], suggesting that cellular senescence also plays a role in the pathology of other muscle wasting conditions.

Intervertebral Disc Degeneration

Intervertebral disc degeneration (IVDD) is considered as a natural progression in the aging process and is often associated with chronic back pain. Clearance of senescent cells in progeroid mice with D+Q increases the levels of proteoglycans in the nucleus pulposus (NP) of the IVD, suggesting an improvement in the ECM of the tissue [46]. Clearance of senescent human NP cells with curcumin or o-vanillin increases the number of Ki-67-positive cells and reduces SASP

expression, as well as promoting the synthesis of ECM components [66]. Specific elimination of $p16^{INK4a}$-positive cells in old p16-3MR mice ameliorates the aggrecan fragmentation and histological score of IVD when compared with young mice, with increased expression of aggrecan and inhibition of MMP-13 expression [67].

Frailty

Frailty represents an age-associated syndrome involving vulnerability and a progressive decline in multiple physiologic systems. Senescent cells accumulation seems to be an important risk factor in frailty. Transplantation of a small number of senescent cells in both young and aged mice is enough to induced a frailty state (physical dysfunction) and reduced **lifespan** [55]. Clearance of senescent cells in INK-ATTAC mice [68,69] or with D+Q [46,55], as well as targeting SASP with JAK inhibitors [53], alleviates frailty symptoms in old mice, and correlates with delayed aging-related pathologies and increased healthspan. In fact, in a more recent study, clearance of naturally occurring senescent cells in old mice with the SSK1 prodrug, decreased the overall chronic and systemic inflammation and led to increased physical performance and reduced expression of age-associated gene signatures [70].

Fibrotic Diseases

Primary Sclerosing and Biliary Cholangitis

Liver cholangitis is a condition of progressive tissue dysfunction manifested with portal inflammation and varying degree of fibrosis and necrosis. The increased burden of both parenchymal and biliary senescent cells is considered a driver of chronic liver diseases in humans [71]. Resolution of liver fibrotic scars is mediated by crosstalk with resident immune cells. Indeed, blockade of immune-ligand specific clearance of senescent hepatic stellate cells enhanced their accumulation exacerbating fibrosis [72]. In a p21- bile duct-inducible mouse model, senescent cholangiocytes aggravate biliary damage through SASP secretion, exacerbating collagen deposition and reducing parenchymal regenerative function [73]. Blockade of TGF-β receptor signaling or hepatocyte-dependent SASP secretion with senolytics upon acute injury shows a reduction in jaundice, resolution of necrosis, and restored regeneration [74,75].

Idiopathic Pulmonary Fibrosis

Characterized by alveolar wall disruption and progressive inflammation, idiopathic pulmonary disease (IPF) severity is associated with incipient senescent mesenchymal and epithelial cells [76]. In IPF models, clearance of senescent epithelial cells in INK-ATTAC mice or by the senolytics D+Q, improves overall pulmonary function and elastance [77] as seen also by reductions in ECM deposition and the associated profibrotic SASP [78].

Chronic Kidney Disease

The gradual loss of glomerular filtration capacity is a characteristic of chronic kidney disease (CKD) that is often associated with diabetes and hypertension in humans. Increased senescence of tubular kidney cells has been associated with CKD as well as chronic allograft nephropathy in both mouse models and humans [79]. The unilateral ureteral obstruction injury (UUO) is an acute model in which a transient activation of senescence has been shown to be beneficial, by directly limiting hypertrophy and inflammation [80]. Additionally, in an ischemia–reperfusion mouse model, removal of cells expressing $p16^{INK4a}$ ameliorates interstitial fibrosis and tubular atrophy [81].

Neurodegeneration

Alzheimer's Disease

Alzheimer's disease (AD), whose main risk factor is advanced age, is a common and multifactorial neurodegenerative disease causing progressive dementia [82]. $p16^{INK4a}$ upregulation has been

described in astrocytes of AD patients [83], and staining for senescence markers in human AD tissues [84] and transcriptional upregulation of senescence-associated genes in laser capture microdissected cortical neurons containing neurofibrillary tangles from AD brains [85] provide further evidence of the involvement of senescence in AD. Increased $p16^{INK4a}$ levels are found in hippocampal neurons [86] and in oligodendrocyte progenitor cells [84] in amyloid mouse models. In addition, elimination of senescent oligodendrocyte progenitor cells improves cognitive function and reduces amyloid load [84]. In AD models of dysfunctional tau protein, genetic or pharmacological elimination of senescent cells have also been shown to improve disease progression [85,87].

Parkinson's Disease

The prevalence of Parkinson's disease (PD), the second most common neurodegenerative disorder, increases with advanced age [88]. A recent study with an incident PD cohort identified that both inflammatory and senescence markers ($p16^{INK4a}$) derived from blood are valuable predictors of clinical progression in PD patients [89]. In mouse models, elimination of senescent cells improves neurological functions. Neurotoxin-induced PD has been shown to be accompanied by accumulation of senescent cells, while the elimination of senescent astrocytes by the use of a transgene protects against neuropathology [90]. Furthermore, inhibition of astrocyte senescence with the antioxidant astragaloside IV confirms the beneficial effect of removing senescent astrocytes in PD [91]. Senescent dopaminergic neurons have been detected in a model of familial PD [92].

Diabetes

Type 2 Diabetes

Characterized by insulin resistance in peripheral organs, type 2 diabetes (T2D) is highly associated with age. Individuals predisposed to T2D diabetes and obesity often develop comorbidities such as hypertension, osteoporosis, anxiety, and skeletal fragility. Senescent cells can negatively impact the proper function of adipocyte progenitor cells and osteocytes via secretion of MMPs, activin A, tumor necrosis factor (TNF), and macrophage-stimulating chemokines [93]. Clearance of senescent adipocytes in mice fed a high-fat diet or knockout for the leptin receptor improves insulin sensitivity and reduces fat hypertrophy, possibly as the consequence of reduced inflammatory SASP factors interferon (IFN)-γ, IL-1β, and macrophage colony-stimulating factor (M-CSF) [94]. Senolysis of $p16^{INK4a}$-expressing cells also restored β-cell function, and improved glucose tolerance and insulin activity in liver, fat, and muscle tissues [95]. In addition to ameliorating several metabolic parameters, treatment with D+Q in obese mice also reduced diabetic nephropathy, leading to an improvement in kidney proteinuria and glomerulopathy [96].

Type 1 Diabetes

Cellular senescence is also implicated in the pathogenesis of type 1 diabetes (T1D), a disease characterized by insulin deficiency due to the progressive immune-mediated elimination of pancreatic β cells (PBCs). A recent study demonstrated that targeted elimination of a subset of senescent PBCs is sufficient to protect from T1D development [97].

Cardiovascular Disease

Atherosclerosis

Atherosclerosis is characterized by the formation of growth plaques in the arterial lumen, leading to blood flow reduction and increasing the risk of cardiovascular diseases and stroke [98]. Direct involvement of cellular senescence in atherosclerosis was suggested by the detection of senescence markers in endothelial-like cells, vascular-smooth-muscle-like cells, and

Table 2. List of Completed, Ongoing, and Planned Clinical Trials with Senotherapeutic Compounds

Study identifier[a]	Type	Condition	Participants	Senolytic	Status (Refs)
NCT02848131 (Mayo Clinic)	Phase II R[c], OL	Diabetic kidney disease	*n* = 9 (female *n* = 2, male *n* = 7, mean age: 68.7 yr)	D+Q (3 consecutive days) D: 100 mg/day (d) Q: 1000 mg/d	Completed [113]
NCT02874989 (Wake Forest University)	Phase I R, OL, P	Idiopathic pulmonary fibrosis	*n* = 14 (≥50 yr)	D+Q (3 consecutive days for 3 wk) D: 100 mg/d Q: 1250 mg/d	Completed [112]
NCT02652052 (Mayo Clinic)	Pilot R, OL	Stem cell transplant	*n* = 10 (HSCT survivors ≥18 yr)	D+Q (3 consecutive days) D: 100 mg/d Q: 1000 mg/d	Recruiting
NCT04063124: SToMP-AD (Texas Health Science Center)	Phase I/II OL	AD	*n* = 5 (>65y)	D+Q (intermittent: 2 d on, 14 d off for 12 wk)	Not yet recruiting
NCT04313634 (Mayo Clinic)	Phase II R, OL	Healthy (aging)	*n* = 120 (female ≥70 yr)	D+Q or Fisetin (5 dosing periods repeated every 28 d over 20 wk) D: 100 mg/d (2 d) Q: 1000 mg/d (3 d) Fisetin: 20 mg/kg/d (3 d)	Not yet recruiting
NCT03325322 (Mayo Clinic)	Phase II R, DB, P	CKD	*n* = 30 (40–80 yr)	Fisetin: 20 mg/kg/d for 2 consecutive days	Recruiting
NCT03430037: AFFIRM (Mayo Clinic)	Phase II R, DB, P	Frail elderly syndrome	*n* = 40 (female ≥70 yr)	Fisetin: 20 mg/kg/d for 2 consecutive days/wk (2 mo)	Recruiting
NCT03675724: AFFIRM-LITE (Mayo Clinic)	Phase II R, DB, P	Frail elderly syndrome	*n* = 40 (adult ≥70 yr)	Fisetin: 20 mg/kg/d for 2 consecutive days (single dose)	Recruiting
NCT04210986 (Steadman Philippon Research Institute)	Phase I/II R, DB, P	OA (knee)	*n* = 72 (adult 40–80 yr)	Fisetin: 20 mg/kg/d for 2 consecutive days/wk	Recruiting
NCT03513016 (Unity Biotechnology)	Phase I R, DB, P	OA (knee)	*n* = 78 (adult 40–85 yr)	UBX0101: dose-finding study (single dose)	Completed
NCT04129944 (Unity Biotechnology)	Phase II R, DB, P	OA (knee)	*n* = 180 (adult 40–85 yr)	UBX0101: 0.5, 2.0, or 4.0 mg single dose	Active
NCT04349956 (Unity Biotechnology)	Phase II R, DB, P	OA (knee)	*n* = 180 (adult 40–85 yr)	No intervention: Long-term follow-up study patients NCT04129944	Enrolling by invitation
NCT04229225 (Unity Biotechnology)	Phase I R, DB, P	OA (knee)	*n* = 36 (adult 40–85 yr)	UBX0101: 8.0 mg single dose or 2 × 4.0 mg repeat dose	Recruiting
Study identifier[a]	**Type**	**Condition**	**Participants**	**Senostatic**	**Status (Refs)**
NCT01649960: CARE (Mayo Clinic)	Phase I OL	Coronary artery disease	*n* = 13 (adult ≥60 yr)	Rapamycin: 0.5, 1, or 2 mg daily for 12 wk	Completed [142]
NCT02874924 (The University of Texas Health Science Center)	Phase II R, DB, P	Aging	*n* = 34 (adult 70–95 yr)	Rapamycin (Rapamune/sirolimus): 1 mg daily for 8 wk	Completed
NCT03103893 (Drexel University)	Phase I/II OL, P	Dermal atrophy	*n* = 36 (adult 40–100 yr)	Rapamycin (topical on skin) 0.5 ml daily (10 μM cream)	Completed [143]
NCT01462006 (NHLBI, University of Virginia)	Pilot R, DB, P	Idiopathic pulmonary fibrosis	*n* = 32 (adult 25–85 yr)	Rapamycin (sirolimus) concentration unknown	Active
NCT04200911: CARPE DIEM (The University of Texas Health Science Center)	Phase I	AD	*n* = 10 (adult 55–85 yr)	Rapamycin (Rapamune/sirolimus): 1 mg orally daily for 8 wks	Not yet recruiting [144]
NCT02432287: MILES (Albert Einstein College of Medicine)	Phase IV R, DB, P	Aging (impaired glucose tolerance)	*n* = 16 (adult ≥60 yr)	Metformin: 1700 mg daily	Completed

(continued on next page)

Table 2. (continued)

Study identifier[a]	Type	Condition	Participants	Senostatic	Status (Refs)
NCT02570672	Phase II R, DB, P	Frailty	*n* = 120 (adult 65–90 yr)	Metformin: 1000 mg twice daily	Recruiting [145]
NCT03451006 (Mayo Clinic)	Phase II R, DB, P	Frailty	*n* = 12 (adult ≥60 yr)	Metformin: up to 2 g daily for 1 yr	Recruiting
TAME[b]	R, DB, P	Aging and age-associated disease	*n* = 3000 (adult 65–80 yr)	Metformin: 850 mg twice daily	Not yet recruiting [122]
NCT03309007 (University of New Mexico)	Phase III R, DB, P	Prediabetes	*n* = 25 (adult 30–70 yr)	Metformin: 1500 mg daily for 1 mo	Active

[a]Clinical trials. gov identifier.
[b]No identifier (not yet recruiting).
[c]Abbreviations: DB, double-blind; HSCT, hematopoietic stem cell transplantation; OL, open label; P, placebo-controlled; R, randomized.

foamy macrophages in plaques from mouse models of this disease [99–101]. Importantly, depletion of senescent cells using transgenic mouse models (Box 1) or senolytics (navitoclax; Table 1) results in decreased plaque formation and plaque size and reduced expression of proatherogenic SASP factors [99]. Moreover, ablation of senescent cells in established plaques increases plaque stability, suggesting that removal of senescent cells is beneficial at all stages of atherosclerosis [99].

Cardiac Dysfunction

Cardiac aging, even in the absence of other systemic risk factors, leads to structural and functional aberrations that can lead to heart failure. Recent studies showed that senescent cells also contribute to age- and metabolic-syndrome-associated cardiac dysfunction. Senescent cardiac progenitor cells are known to accumulate upon aging [102]. In addition, a recent study suggested that with age postmitotic cardiomyocytes enter a senescence-like state, induced by telomeric damage independent from telomere length, and contribute to cardiac fibrosis and hypertrophy [63]. Importantly, ablation of senescent cells in aged mice using the INK-ATTAC mouse model or senolytics (D+Q or navitoclax) resulted in a decrease of fibrosis and hypertrophy and increase in smaller proliferating cardiomyocytes, suggesting that removal of senescent cardiomyocytes and/or cardiac progenitor cells could rescue age-associated cardiac remodeling [63,68,102]. In addition, clearance of senescent cells using the p16-3MR mouse model improved cardiac function in obese mice and in mice treated with the chemotherapeutic doxorubicin, which induces senescence [94,103].

Cancer

Cancer is characterized by an uncontrollable proliferative potential and by the capacity to evade from the tissue of origin and migrate to distal tissues. Similar to other diseases, aging and senescence play key roles in cancer development [104]. By contrast to the obvious tumor-suppressive function of the senescence-associated growth arrest, it has been shown that senescent cells have a paradoxical protumorigenic potential mediated by both cell and non-cell autonomous mechanisms. Senescence-associated stemness is a cell-autonomous feature that exerts highly aggressive growth potential upon escape from cell-cycle blockade, and has been shown to be enriched in relapsed tumors [105]. Genomically unstable cancer cells can evade the toxicity of anticancer treatments by acquiring a senescent-like-phenotype (dormant state), and then bypass growth arrest, recovering the aggressive and uncontrolled proliferation [106]. Hence, strategies that combine senescence-inducing cancer therapies with senolytics might prevent the regrowth of senescent cancer cells [107]. SASP factors secreted by senescent cells can promote several

processes involved in tumorigenesis, including proliferation migration/invasiveness, promotion of epithelial-to-mesenchymal transition, vascularization, protection from immunosurveillance and cancer relapse [108]. Systemic accumulation of senescent cells due to anticancer therapies can not only promote tumorigeneses, but also promote the development of other age-associated diseases such as cardiovascular or neurodegenerative diseases [109]. Taking into consideration the detrimental effect of senescent cell accumulation in cancer, senotherapeutics seem a promising strategy to reduce cancer growth and relapse [103,107,110,111], as well as the adverse effects of cancer interventions [49,103].

From Bench to Bedside

Preclinical findings that clearance of senescent cells or inhibiting the SASP using senotherapeutics can improve healthspan in mice and other animal models led to the first clinical trials in humans (Table 2). In a single-arm, open-label pilot study without placebo (NCT02874989), repeated D+Q treatment of IPF patients was safe, with only mild to moderate reversible adverse events, and resulted in significant improvement of functional measures, such as 6-min walking distance, 4-m gait speed, chair-stands, and Short Physical Performance Battery scores [112]. In a different Phase I study (NCT02848131), a single 3-day course of D+Q in diabetic patients with CKD was also found to be safe and resulted in significant decreases in senescent cells in skin and adipose tissue, and a decrease of circulating SASP factors (IL-1α, IL-2, IL-6, IL-9, MMP-2, MMP-9, and MMP-12) [113]. The senolytic compound UBX0101 is being tested in clinical trials for OA treatment. In the first completed Phase I clinical trial performed (NCT03513016), a one-time intra-articular administration of UBX0101 was found to have no serious adverse effects but the effectiveness was not statistically significant, mainly due to the low number of patients involved (https://acrabstracts.org/abstract/). A Phase II clinical trial (NCT04129944) to explore the effect of a single dose of UBX0101 in a bigger cohort of approximately 180 patients is expected to be completed soon. In parallel, a second Phase I clinical trial (NCT04229225) that includes two cohorts of OA patients – one treated with a single dose, one with repeated doses – is currently ongoing. Several other clinical trials are currently planned, ongoing, or recruiting patients for OA, CKD, and frail elderly syndrome (Table 2). In addition, two other senolytic compounds that target BCL (UBX1967/UBX1325) are currently in an investigational new drug (IND) enabling phase for treatment against age-related macular degeneration, diabetic macular edema, and diabetic retinopathy, even if the evidence for a direct role of cellular senescence in these diseases remains scant.

Concluding Remarks

The development of more sophisticated tools and models, also favored by increased financial investments in the field, have improved our understanding of the mechanisms regulating the proaging and prodisease function of senescent cells. Targeting senescence holds the potential to significantly improve healthspan and alleviate age-associated dysfunctions, frailty, and tissue fibrosis, therefore its impact in the population can be tremendous. However, the development and application of senotherapies is far from completed, and important questions remain (see Outstanding Questions).

First, current senotherapies mainly involve repurposed drugs with on- and off-target effects. For example, targeting BCL2 family members has on-target toxicity on certain immune cells and platelets, and can result in thrombocytopenia and lymphopenia. Improving selectivity of these compounds by targeting more senescence-specific mechanisms might alleviate toxicities [114,115].

Second, senotherapies are not taking heterogeneity into account and, consequently, indiscriminately target both beneficial and detrimental senescent cells.

Outstanding Questions

Is it possible to target mechanisms that are unique to senescent cells to avoid side effects?

How heterogeneous is the phenotype of age-related senescence? Can we specifically interfere with detrimental senescence?

What are the biomarkers that can be exploited for detection of senescent cells *in vivo* and to monitor the efficacy of senotherapies?

Will the selective elimination of senescent cells have a systemic health improvement?

How do we evaluate the effectiveness of senescent cells removal during aging?

How frequent and from which age should senotherapies be provided to achieve maximum healthspan improvement?

What are the effects of combining senotherapies with other rejuvenation strategies?

We still lack a comprehensive phenotypical characterization of age-associated senescent cells *in vivo*. *Ex vivo* experiments have suggested that senescence can be heterogeneous, and that several senescence-associated features, including the SASP, are dependent on intrinsic and extrinsic factors including cell type, senescence inducer, tissue of origin, and environmental conditions [4,5,116]. The phenotypical heterogeneity suggests the possibility that different subsets of senescent cells could coexist, and that not all senescent cells might be detrimental. Identification of mechanisms associated to specific subtypes of senescent cells might help to develop more specific and better tolerated therapies.

Third, established senescence markers such as $p16^{INK4a}$, $p21^{WAF1/Cip1}$, and SA-β galactosidase can be used to identify senescent cells, but these markers are neither specific nor universal [75,117]. Recent studies of gene signatures in different cell types and *in vitro* senescence models have suggested that general senescence-associated gene signatures might exist, but these signatures remain to be validated at the protein level in humans [5,116]. Novel and more reliable biomarkers are essential to evaluate therapy efficacy, and might help to monitor the elimination of the most detrimental subsets of senescent cells. There is increasing evidence that a small fraction of senescent cells in a population is the main source of SASP factors [118] and inflammatory response. If specific subtypes of senescent cells can be distinguished this will be also relevant for biomarker development. A limiting factor on the study of subtypes of senescence derives from technical hurdles, but the continuous advancements in single cell technologies might minimize this issue. Moreover, past studies have largely focused on the transcriptome/proteome of senescent cells. The investigation of other levels of complexity in circulating factors such as EVs, long noncoding RNAs, and metabolites will probably add to the source of biomarkers. It is conceivable that senescence-derived circulating factors might function as biomarkers to recognize and selectively target senescent cells exhibiting the hypersecretory phenotype while maintaining other cell types that are beneficial.

Fourth, elimination of senescent cells has shown the potential to be a general anti-aging strategy achieving generalized improvement of healthspan and reduction of multiple age-related dysfunctions at the same time. However, translation of these findings remains difficult, since aging as such is not considered to be a disease or syndrome by regulatory agencies. This is in contrast with obesity, the other major risk factor for chronic diseases, which is officially recognized as a disease. Therefore, surrogate readouts need to be used to evaluate the efficacy of antiaging strategies. One approach is to use frailty as an aging indicator [119], and frailty indices to evaluate the effect of therapeutic interventions [120] (Table 2). A major limitation remains the definition of strict parameters to measure frailty in humans, but attempts to overcome this issue are ongoing [121]. An additional approach to evaluate antiaging therapies is to monitor their effect on multiple diseases in the same study. This approach is being pioneered by the Targeting Aging with Metformin (TAME) trial, which aims to determine if metformin treatment in elderly people can delay accumulation of age-associated diseases rather than targeting individual diseases [122] (Table 2).

Fifth, if senotherapies can be tested as antiaging interventions in humans, then more studies on the treatment regimen and frequency need to be performed. The few available experiments in mouse models suggest that intermittent dosing of senolytics starting at middle age could have the largest benefit. On the one hand, such a strategy would avoid a significant burst of senescence throughout the lifetime, thus limiting any temporary detriment of senescent cells and their SASP. On the other hand, it would limit the chance to incur serious adverse effects due to elimination of beneficial senescence. However, the time needed for senescent cells to reaccumulate after a cycle of clearance is not known, and this might well be dependent on the age of the individual. Moreover, it remains to be established if subsets of senescent cells might

develop resistance to senotherapies and become even more detrimental, a phenomenon commonly observed in cancer cells that acquire resistance to cancer therapies.

Last, it remains debatable whether senotherapies represent the 'fountain of youth'. Mouse experiments suggest that the sole removal of senescent cells helps to delay age-related phenotypes and significantly improve health- and lifespan, but with only a partial rejuvenation effect. The achievement of full anti-aging potential should take into account the combination of senotherapy with additional strategies such as stem cell transplantation or tissue reprogramming. Thus, in parallel to evaluate the tolerability and efficacy of senotherapeutic approaches, more research should be dedicated to combining multiple approaches, at least in preclinical settings.

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