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# Strategies for targeting senescent cells in human disease

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**Cellular senescence represents a distinct cell fate characterized by replicative arrest in response to a host of extrinsic and intrinsic stresses. Senescence facilitates programming during development and wound healing, while limiting tumorigenesis. However, pathologic accumulation of senescent cells is implicated in a range of diseases and age-associated morbidities across organ systems. Senescent cells produce distinct paracrine and endocrine signals, causing local tissue dysfunction and exerting deleterious systemic effects. Senescent cell removal by apoptosis-inducing senolytic agents or therapies that inhibit the senescence-associated secretory phenotype have demonstrated benefit in both preclinical and clinical models of geriatric decline and chronic diseases, suggesting that senescent cells represent a pharmacologic target for alleviating effects of fundamental aging processes. However, senescent cell populations are heterogeneous in form, function and tissue distribution, and even differ among species, possibly explaining issues of bench-to-bedside translation in current clinical trials. Here we review features of senescent cells and strategies for targeting them, including immunologic approaches, as well as key intracellular signaling pathways. Additionally, we survey current senolytic therapies in human trials. Collectively, there is demand for research to develop targeted senotherapeutics that address the needs of the aging and chronically ill.**

Chronological age is the preeminent risk factor for the leading causes of morbidity and mortality worldwide[1,2]. Chronological and biological aging are often correlated[3]; however, the latter can be accelerated by the coexistence of multiple chronic diseases and geriatric syndromes, which in turn account for interindividual heterogeneity in terms of risk for development of new diseases, disability or death[4]. With these considerations in mind, there is now a growing effort to understand and modulate the root causes of aging to alleviate multimorbidity at a global scale. Cellular senescence has garnered increasing attention as a fundamental driver of chronic conditions and functional decline in late life. Despite its historic discovery in the context of in vitro cell growth arrest following serial passage[5], the ramifications of cellular senescence have only recently been appreciated following its implication in a host of age-related and chronic diseases spanning most organ systems. In tandem with our expanding appreciation of senescence in pathology, there is an equally evolving understanding of the role of cellular senescence in basic physiology. This inherent duality in the senescence program alongside our limited understanding has increased research fervor and incited controversies ranging from appropriate laboratory methods for studying senescent cells to their potential role as therapeutic targets.

Here we survey the current field of cellular senescence research with regards to its inherent heterogeneity to address the diverse roles of this cell fate with respect to both physiology and pathology. We first explore the molecular underpinnings of senescence to emphasize how senescent cells are currently defined in light of their underlying diversity. We then explore how this heterogeneity is further compounded by the senescent cell secretome. Finally, we consider the therapeutic implications of and approaches for targeting various types of senescent cells as well as the current clinical trials of senolytic drugs.

## Cellular senescence

Despite the controversies surrounding the role of cellular senescence in both health and disease, there is a degree of consensus involving its basic definitions and principles. At its core, cellular senescence represents a cell fate that is characterized by stable proliferative arrest in response to various stressors and, frequently, production of an accompanying secretome termed the senescence-associated secretory phenotype (SASP). Inducing stressors vary and include replicative stress with associated telomere shortening, activation of DNA damage pathways, epigenetic changes, oxidative stress, mitochondrial dysfunction, radiation, oncogene induction, and mechanical and shear stress, among others that have been thoroughly reviewed[6]. Following stress exposure, cell cycle checkpoint blockading factors are upregulated that halt replicative progression. Although these cells accumulate damage products, including DNA breaks, and upregulate DNA damage response (DDR) pathways[7,8] in part due to mitochondrial dysfunction and increased reactive oxygen species (ROS) production[9], they do not undergo apoptosis. Instead, they have upregulated pro-survival pathways called senescent cell anti-apoptotic pathways (SCAPs) while key apoptotic mediators are downregulated[10–13]. In addition to their internal homeostatic perturbations, senescent cells can induce an inflammatory state that provokes both local and systemic inflammation and tissue damage through their SASP[14–16].

This cascade of stress, senescence, and subsequent SASP signaling and immune activation is critical for normal development and health. From early development, cells with senescent features are important for body axis patterning, with the associated SASP directing growth of structures such as the limb bud and orchestrating regression of transient features through macrophage-mediated clearance[17–19]. Furthermore, as senescent cells produce factors that promote tissue remodeling and recruit associated immune components, spatiotemporally regulated senescence is important for

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mediating the balance between healing and fibrosis in regenerative processes including wound and organ repair[20–25]. Interestingly, senescent cells can also promote dedifferentiation and plasticity of neighboring cells via paracrine mechanisms[26,27]. Additionally, owing to its induction in response to both DNA damage and oncogenic stress, the senescence program may also serve as a tumor suppression mechanism, although its inflammatory properties and DNA damage induction are also associated with increased tumor burden when dysregulated[28–30].

While there is substantial interest in the beneficial roles of senescent cells, much of cellular senescence research has been aimed at their roles in disease and emphasized their contributions to geriatric decline. This modern wave of attention was promoted by the discoveries that markers of cellular senescence accumulate with aging and accumulation is delayed by interventions that increase healthspan and lifespan[31], removal of senescent cells increases healthspan in progeroid mouse models[32], and transplantation of a relatively small number of senescent cells into previously healthy animals provokes multisystem dysfunction similar to what is seen in aged animals[33,34]. Furthermore, it was found that the correlation of senescent cell accumulation with disease extends to humans[35–37], and that senescent cell burden can be safely reduced in a clinical context[38]. On the basis of these points, it appears crucial to understand the roles of senescence in morbidity and mortality. To date, deleterious effects of senescent cells have been implicated in a causative or proximal capacity for diseases encompassing most organ systems, including the leading causes of morbidity, death and healthcare burden, such as cardiovascular, pulmonary, neurologic, renal, hepatic, infectious, musculoskeletal and endocrine diseases[29,39–51].

**Table 1 | Select features of senescent cells**

| | |
|---|---|
| Morphologic features | Increased size[56] |
| | Increased granularity[56,57] |
| Cell cycle blockade | p21–p53 (ref. [52]) |
| | p16–RB (ref. [52]) |
| Mitochondrial changes | Increased size/number[9] |
| | Increased ROS production[62] |
| | Decreased membrane integrity[62] |
| Lysosomal changes | Increased size/number[58] |
| | Increased SA-β-gal activity[59] |
| | Lipofuscin accumulation[61] |
| Nuclear changes | Telomere shortening[6,52,63] |
| | DDR (telomere-associated foci, γ-H2AX)[8,63] |
| | Lamin B1 loss[65] |
| | Senescence-associated heterochromatin foci[66] |
| | Decreased DNA replication[6] |
| Additional selected features | Cytosolic DNA/cGAS–STING activation[79–81] |
| | LINE-1 retrotransposon de-repression[67] |
| | Remodeling of SASP-associated super-enhancers[84] |
| | Other senescence transcriptional changes[55,69,70] |

## Molecular basis and markers of cellular senescence

Senescence is a cell fate that is widely regarded as a stress response. Senescence is distinct from other non-proliferative cell fates including quiescence, the latter being reversible with appropriate mitogens, or terminal differentiation, in which cells develop from defined lineages to fulfill specific roles and arrest growth through diverse pathways. In response to internal or external factors such as telomere shortening or ionizing radiation, senescent cell cycle arrest signals primarily converge on the p53–Cdkn1a (p21) and/or retinoblastoma (RB)–Cdkn2a (p16) pathways[52]. Increased levels of the cyclin-dependent kinase inhibitors (CDKIs) p21 or p16 can contribute to G1/S cell cycle blockade, mediated by inhibition of the formation of the cyclin–cyclin-dependent kinase complex and failure to inactivate RB with associated transcriptional repression[53]. Despite the convergence of senescent growth arrest pathways, there is growing appreciation that different inducing stressors can result in unique senescent subpopulations[54]. This complexity is further compounded by the fact that currently no single known marker defines senescence with high sensitivity and specificity, although this lack of a gold standard is not unique to the field. Current practices rely on a combination of phenotypes and sets of markers to suggest cellular senescence. For example, although accumulation of p16 and p21 has been biologically implicated in senescence and they are extensively used as molecular markers, various in vitro stressors induce differential expression of these CDKIs, even within the same cell line[55], indicating that neither is fully sufficient or reliable on its own. To complicate this further, the same study also revealed that different cell types can differentially alter their transcriptome in response to the same inducing stressor. Unlike the well-controlled constraints of cell culture models, different stressors can act simultaneously in vivo with both aging and disease. Thus, it is hypothesized that senescent populations are especially diverse in vivo.

Besides p16 and p21, a number of other markers can be used to identify senescent cells, although sensitivity and specificity varies for each (Table 1). Several morphologic features are suggestive of senescence in vitro and can be assessed using approaches such as brightfield microscopy. Compared to their counterparts, senescent cells have increased size and granularity[56,57], likely reflecting their altered metabolism and organelle homeostasis. In particular, both mitochondria and lysosomes accumulate in cells exposed to senescence-inducing stressors[9,58]. Whether causative of, or occurring in response to the stress response, or both, these changes provide an added means for detecting senescent cells. Lysosomal abundance in senescent cells is one of the most commonly used features, as lysosomal senescence-associated beta-galactosidase (SA-β-gal) activity assays are relatively quick and straightforward to conduct[59]. However, there are limitations to the specificity of this marker[60] and it should be paired with others considered here. Lipofuscins, which are aggregates of lysosomal byproducts, also accumulate within senescent cells and can conveniently be assayed, although such assays have similar limitations to specificity to those of SA-β-gal assays[61]. Mitochondrial abundance increases in senescent cells together with altered membrane potential and increased ROS production, oxidative phosphorylation and oxygen consumption[62]. Hence, assays of redox state, mitochondrial function and mitochondrial biogenesis can be used to enhance senescent cell phenotyping.

Nuclear alterations are prominent features of senescent cells and largely result from activation of the DDR pathways that lead to *p16* and *p21* activation. These pathways also lead to increased phosphorylation and activation of the DDR orchestrator histone, H2AX, which coordinates DNA repair following genotoxic stress and double-strand breaks[63]. This has led to the use of immunohistochemical techniques to label phosphorylated (γ) H2AX in senescence assays. As this pathway can repair DNA double-strand breaks across most of the chromosome, it potentially lacks sensitivity owing to its transient nature at most loci. However, double-strand breaks are persistent at telomeric loci owing to localized inhibition of non-homologous end-joining pathways developed to prevent chromosomal end-to-end fusions[64]. As a result, assays for

telomere-associated foci of DNA damage, assessed by assaying for colocalization of telomeric probes and γ-H2AX antibodies, which accumulate in cells following senescence-inducing stress, are relatively sensitive and specific for detecting senescent cells[8]. Other nuclear alterations include loss of the nuclear intermediate filament protein and the epigenetic modulator, lamin B1 (ref. [65]), specific chromatin remodeling patterns termed senescence-associated heterochromatin foci[66] and decreased DNA replication, each of which can be assessed using immunofluorescence techniques. Additional genetic and epigenetic markers include de-repression of LINE-1 retrotransposons[67] and upregulation of the histone acetyltransferase KAT7 (ref. [68]).

The cellular senescence research field is currently being propelled by omic and bioinformatic approaches that may elicit a better understanding of the senescence landscape and provide new molecular insights with a particular emphasis on heterogeneity. Transcriptomic approaches yield unique perspectives into the dynamics of senescent cells while suggesting novel markers. It was recently revealed that, regardless of the inducing stress and cell type, there appear to be shared profiles of differential gene expression in senescent cells[55]. This includes a shared downregulation of histone-associated transcripts and upregulation of the long noncoding RNA PURPL that is involved in p53 signal modulation. However, at single-cell resolution, transcriptomic analysis reveals that even a single cell faced with identical stressors yields unique senescent subtypes with distinct profiles of differential gene and SASP expression[69], calling into question the possibility of ever finding a single gold standard senescence marker. Regardless, single-cell transcriptomics allow naturally occurring senescent cell populations to be examined by leveraging a multitude of marker transcripts. For example, this technique has been used to show that adipose-derived mesenchymal stem cells accumulate senescence markers with natural aging and altered senescence-associated pathways[70]. Coupled with epigenomic insight[71], and proteomic[72] and metabolomic[73] data, our understanding of the senescent cell fate is growing exponentially, and we are on the path to a comprehensive profiling database.

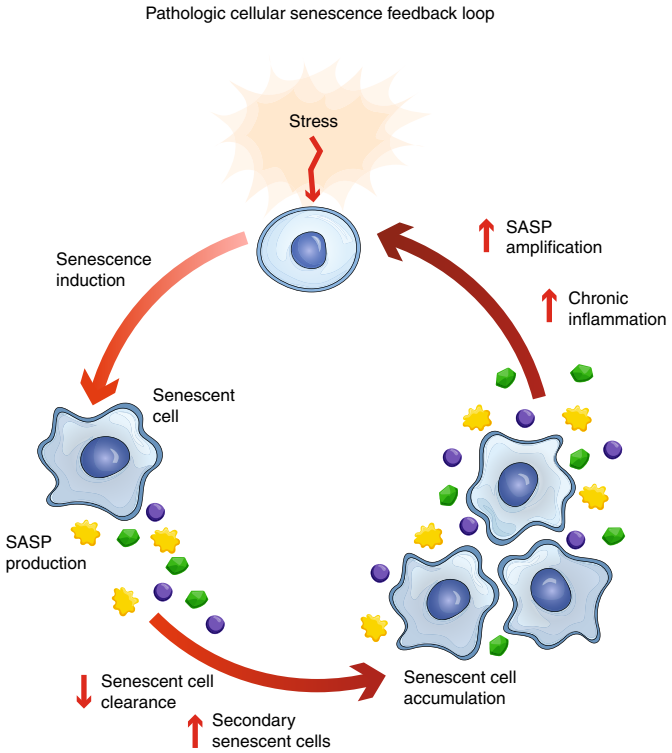


**Fig. 1 | Senescent cell accumulation positive feedback loop.** Cellular senescence is induced following exposure to an initial stress. The resulting senescent cells produce a SASP that can potentiate further senescent cell accumulation and impair clearance. In turn, the SASP can be amplified, eliciting an environment of chronic inflammation and additional senescence-inducing stressors that drive a feed-forward cycle of senescent cell accumulation.

## The SASP

In addition to the effects mediated by their proliferative arrest, the SASP is a key contributor to the combined physiologic and pathologic roles of senescent cells. The SASP represents a myriad of cytokines, matrix metalloproteinases, microRNAs, chemokines, growth factors and small-molecule metabolites that are directly secreted or exosomally packaged[6,15,74] and are largely under the control of the p53, NF-κB, CEBPB, JAK–STAT and GATA4 transcription factors[75–78]. Beyond the transcriptional level, there is an evolving understanding that SASP regulation is multilayered, including contributions from pre-transcriptional signaling cascades such as the cGAS–STING pathway[79–81] as well as epigenetic regulation[82,83] with particular remodeling noted in the super-enhancer landscape[84]. Efforts are being made, such as by the SASP Atlas, to map and record the resulting senescence secretome, and such analyses already indicate that the SASP varies with different stressors[72]. For example, pathogen-related factors, such as lipopolysaccharide or severe acute respiratory syndrome coronavirus 2 S1 antigen, can substantially amplify the SASP of existing senescent cells, potentially contributing to the increased risks for cytokine storm and mortality in the elderly and those with chronic diseases that are associated with a high burden of senescent cells due to infections[50].

SASP components fulfill many functions, but are classically associated with the chronic inflammatory state of aging[85,86]. Several SASP components are immunomodulatory[87]. SASP chemokines such as interleukin 1 (IL-1), IL-8 and tumor necrosis factor (TNF) recruit immune cells including macrophages, neutrophils and T cells[88–90]. This allows for senescent and neighboring cells that have accumulated ROS, DNA damage or oncogenic stress to be destroyed, consistent with the protective role of senescence in tumor prevention. Conversely, these same SASP components (alongside others) when chronically or uncontrollably expressed may perpetuate tumor seeding and invasion through matrix and vascular remodeling. This could explain the apparently contradictory findings implicating senescence in both cancer prevention and progression[91–94]. Furthermore, the SASP is not only a product of senescent cells, it also induces and reinforces senescence itself through paracrine and autocrine signaling mechanisms[34,95,96]. In light of this, it is thought that age-associated losses in immune competency in a chronically inflamed milieu may allow senescent cells to evade removal[97–99], although this is not fully understood. Additionally, SASP signals confer senescent cells with resistance to immune-mediated clearance (for example, through upregulation of ligands such as HLA-E, which can inhibit $CD8^+$ T cell and natural killer cell function[100]). Regardless of the exact underlying mechanism of immune interplay, senescent cell accumulation can perpetuate a feed-forward cycle of failed clearance coupled with increased SASP signals and resulting secondary senescent cell induction (Fig. 1). Of note, though, there is debate over whether cells that are secondarily induced into senescence are similar to those becoming senescent owing to a primary stressor, with evidence indicating differences in SASP dynamics between primarily and secondarily induced senescent cells[101]. Additionally, senescence and damaging SASP signals are further augmented by aged immune system components. For example, T cells with mitochondrial dysfunction, like those present with aging, cause global increases in senescent cell burden that are associated with increases in frailty and multimorbidity and that appear to be partially mediated by TNF signaling[102]. Furthermore, introducing a construct that interferes with DNA repair into hematopoietic

progenitors in transgenic mice, so that immune cells prematurely become senescent, causes early development of multiple age-related phenotypes and increases senescent cell burden in multiple tissues in transgenic mice[103].

SASP heterogeneity, reflecting the complexity of the underlying senescence program, is further recapitulated in how the SASP impacts the regenerative potential of non-senescent cells. When expressed transiently, NF-κB pathway SASP factors can promote stemness and enhance regenerative potential in keratinocyte regeneration models in vivo[27]. However, interruption of this signal or prolonged exposure to it reduces stemness markers and impairs regenerative capacity[27]. This highlights the need to consider SASP kinetics and illustrates how chronic senescent cell accumulation and signaling can impair ability to respond to physiologic insults. Opposing roles of the senescent secretome are also evident in the liver. Senescence and associated transforming growth factor-β signaling have been linked with impaired regeneration and biliary fibrosis[104], while genetic ablation of senescent cells and ensuing SASP reduction can induce fibrosis in vascular structures[105]. Although these findings may be artifacts of the underlying model systems used, including continuous elimination of senescent cells, limited specificity of p16 as a senescence marker, and potentially vascular cell populations with associated vascular leakage in the latter study[105], there appears to be contextual variability in features of senescence.

## Targeting senescent cells

A 2004 report[31] of an inverse relation between senescent cell burden and healthspan in mice prompted the initiation of efforts to develop drugs that selectively eliminate senescent cells. The subsequent development of the transgenic *INK-ATTAC* mouse model[32], in which age-related phenotypes were alleviated through selectively removing cells expressing p16 at high levels from progeroid mice, further supported these efforts. As senescent cells are highly heterogeneous in both their molecular biology and their physiological function, targeted strategies are needed that ideally preserve senescent cells in beneficial contexts while eliminating effects that are detrimental. Broadly, these therapies can be broken down into the major categories of senomorphic and senolytic drugs, although this classification might be arbitrary as agents with senomorphic effects in one cell type or context may be senolytic in another and vice versa. Senomorphic compounds target pathologic SASP signaling, while senolytics eliminate the underlying senescent cells that release damaging SASP factors. Senomorphics are discussed elsewhere, but in brief senomorphics prevent the production of, antagonize or neutralize SASP components[106,107], and usually require continuous administration. We will instead focus on emerging senolytic strategies that address a root cause of senescence pathology, senescent cells, yielding pleiotropic benefits with intermittent administration. The current senolytic class originates from work demonstrating senescent cell clearance with the combination of the Src kinase inhibitor dasatinib and the flavonoid quercetin (D + Q)[10] and, subsequently, BCL-2 family inhibitors and others[108–112]. While the first senolytics were developed using a bioinformatically informed approach aimed at disrupting SCAPs and other pro-survival networks[10], the class has expanded to take advantage of additional senescence features and enhance immune-mediated clearance (Fig. 2). Broadly, first-generation agents act by transiently disabling SCAPs, causing those senescent cells with a tissue-damaging SASP to kill themselves. Importantly, while all senolytic strategies may elicit off-target effects or interfere with beneficial populations, these can often be limited as most therapeutics are amenable to intermittent 'hit-and-run' dosing strategies that do not require daily, or even weekly, administration. This interval dosing strategy is hypothesized to be efficacious given that senescent cells take 7 days or more (at least in vitro) to accumulate and develop a SASP even when faced with persistent and harsh senescence-inducing stress in vitro[67,113], and could potentially take as long to start re-accumulating in vivo.

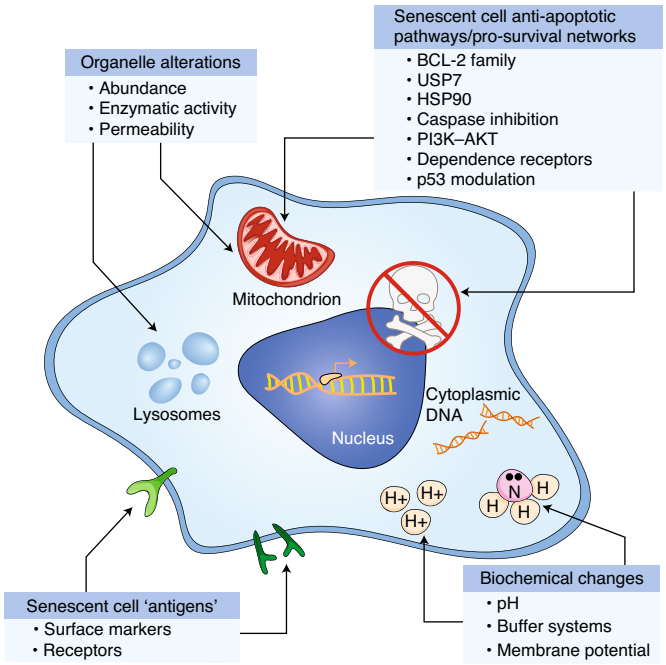


**Fig. 2 | Senescent cell features targeted by senolytics.** Select features of senescent cells have been leveraged to specifically reduce their abundance. Broadly, these targets include unique surface markers, SCAP and other survival networks, biochemical adaptations and changes in organelle characteristics.

**Extracellular targets and immune-mediated clearance.** Characterization of senescent cells has revealed unique markers that serve as senescence-associated self-antigens. These can be co-opted for immune system–mediated senolytic activity and clearance. A recent study[114] took advantage of this using chimeric antigen receptor (CAR) T cells targeted against the urokinase-type plasminogen activator receptor (uPAR) in a mouse model. uPAR is associated with extracellular matrix remodeling that is upregulated at the cell surface of senescent cells during replicative, oncogene-induced and toxicity-induced senescence. Cytotoxic CAR T cells were able to selectively clear uPAR-expressing senescent cells in vitro and in vivo. CAR T cell–mediated clearance of senescent cells led to survival and histopathologic benefit in murine models of both carbon tetrachloride– and diet-induced liver fibrosis, suggesting the feasibility and potential of this clearance strategy. However, supratherapeutic CAR T cell dosing is associated with a proinflammatory cytokine profile, weight loss and hypothermia, suggesting that careful attention would need to be paid to initial dosing strategies, particularly in geriatric populations where a compromised immune system together with ongoing chronic inflammation may restrict the therapeutic window. Additionally, CAR T cell approaches in humans involve initial immune system suppression with considerable attendant morbidity and are expensive. Although senolytic CAR T cells appear to be self-limiting[114], their expansion and contraction occurs over a period of days, which is a risk in the case of an acute to subacute adverse reaction. Overall, this strategy is highly promising given its preclinical efficacy and senescent cell specificity, but future optimization and testing is necessary before clinical administration.

Co-opted senescence antigens provide an additional strategy for cell clearance through vaccination. Senescent T cell populations accumulate in obese adipose tissue, contributing to inflammation

with both local and systemic effects[115]. These T cell populations bear known senescence-associated markers and are characterized by $CD4^+$, $CD44^{high}$, $CD62L^{low}$, $PD1^+$ and $CD153^+$ expression. Immunization against these cells with a CD153 peptide conjugate promotes senescent T cell clearance from adipose tissue with associated improvements in metabolic function. As with other immune-mediated clearance strategies, vaccination against senescence epitopes will require careful consideration for clinical translation especially in the context of their lasting effects that might be difficult to reverse. As CD153 is implicated in mycobacterial clearance[116], neutralizing antibodies or elimination of positive cells may be contraindicated in those with a history of or enhanced susceptibility to mycobacterial infection. Additionally, immunosenescence may limit vaccine response and memory in the geriatric population[117].

**Modulating SCAPs.** Prototypic senolytic drugs were developed to target SCAP networks[10]. In contrast to a one-drug, one-target approach, SCAP inhibition may interface with several pro-survival signals at once. As a result, these early senolytics typically possess several pharmacologic mechanisms of action that interact synergistically. Prime examples of this are the flavonoid fisetin[118] as well as D + Q, which have been utilized and reviewed thoroughly[112]. In brief, although the precise mechanism of action is unknown (as is the case for most agents), the D + Q combination exerts broad spectrum senolytic activity through interference with several pro-survival networks, including ephrin dependence receptor signaling, PI3K–AKT and BCL-2 members. The BCL-2 family members (BCL-2, BCL-XL, BCL-W and so on), which prevent activation of pro-apoptotic mitochondrial signaling cascades, cytochrome *c* release and downstream caspase activation, are targeted by other senolytics. These compounds, such as navitoclax (ABT-263), A1331852 and A1155463, display in vitro activity against senescent human lung fibroblasts and umbilical vein endothelial cells[10,119] amongst other cell types[120]. However, in contrast to D + Q, these drugs do not eliminate all senescent populations in vitro, with particular resilience demonstrated by senescent pre-adipocytes that are capable of causing widespread metabolic dysfunction and harm in vivo[34]. Additionally, the clinical utility of BCL-2 inhibitors as senolytics is limited by off-target effects on platelets[121], neutrophils[122] and potentially T cells[123], which can be idiosyncratic (that is, can occur after a single dose) and might impair hemostasis and further compromise immune function in at-risk populations, although intermittent dosing may help. Additional SCAPs that can be drug targeted include modulation of p53-associated pathways and interruption of the anti-apoptotic transcription factor FOXO4 (ref. [12]), inhibition of the peptidase USP7 (ref. [124]), inhibition of the HSP90 chaperones[125], increased apoptotic drive by caspase activation[126] and others that are being studied.

**Other approaches.** Additional aspects of senescent cells are advantageous for directed senolysis. One such feature is their increased lysosomal enzyme activity. This can be leveraged with the use of prodrugs that are cleaved and activated by the lysosomal enzyme SA-β-gal or by loading cytotoxic chemicals into galacto-polymer-coated nanoparticles that can be preferentially released into senescent cells[127,128]. This strategy could suffer from off-target effects in non-senescent cells with high SA-β-gal activity, such as activated macrophages[129]. Lysosomal abundance in senescent cells also offers the opportunity for senolysis through increased autophagy. Autophagy is normally involved with the turnover of organelles and other cellular components that are directed to the lysosome for molecular repurposing. Outside its role as a cellular recycling system, autophagy can lead to activation of cell death pathways when highly activated under persistent stress[130]. Autophagy is inhibited within senescent cells[131], but senescent cells are primed for cell death following an autophagic push. This is demonstrated by autophagy induction and subsequent senolysis with the use of fibrate drugs[132], metformin[133], mTOR complex 1 (mTORC1) inhibitors[134], bromodomain and extraterminal domain (BET) inhibition[135] and lysosomal acidification with ataxia telangiectasia mutated (ATM) inhibitors[136]. Beyond organelle targeting, even minor chemical changes can be exploited for preferential senolysis. This is demonstrated by the senolytic activity of cardiac glycosides, which take advantage of changes in membrane potentials and proton concentrations within senescent cells[137,138]. Furthermore, compensatory responses to these chemical changes in senescent cells may be targeted. For example, senescent cells resist lowered pH produced by lysosomal alterations and other factors by upregulating buffering systems including the glutaminase product ammonia[139]. Interruption of these buffering systems can induce preferential senescent cell death, and the enzymes that maintain buffer concentrations could be targeted by small-molecule drugs.

**Table 2 | New and ongoing clinical trials for senolytic therapies**

| Senolytic therapy | Indication | Trial |
|---|---|---|
| D + Q | Diabetic kidney disease | NCT02848131 |
| | Alzheimer's disease | ALSENLITE, NCT04785300 |
| | | SToMP-AD, NCT04685590 |
| | Accelerated age-like state post bone marrow transplantation | HTSS, NCT02652052 |
| | Accelerated age-like state in childhood cancer survivors | SENSURV, NCT04733534 |
| | Age-related osteoporosis | NCT04313634 |
| Fisetin | Frailty in older women | AFFIRM, NCT03430037 |
| | Diabetic and chronic kidney disease | NCT03325322 |
| | Accelerated age-like state in childhood cancer survivors | SENSURV, NCT04733534 |
| | Age-related osteoporosis | NCT04313634 |
| | Osteoarthritis | NCT04210986 |
| | Coronavirus disease 2019 (COVID-19) in nursing home patients | COVID-FIS, NCT04537299 |
| | COVID-19 in hospitalized patients | COVID-FISETIN, NCT04476953 |
| | COVID-19 in outpatients | COVFIS-HOME, NCT04771611 |
| UBX1325 | Diabetic macular edema | NCT04537884 |

## Clinical trials of senolytics

The first clinical study of senolytics published was a pilot, open-label study of 14 patients with idiopathic pulmonary fibrosis[140] (ClinicalTrials.gov identifier NCT02874989). They were administered 9 doses of oral D + Q over 3 weeks. Five days after the final dose, the participants had improved 6-minute walk distance, walking speed, chair rise ability and short physical performance battery. This may have been related to the study drug, learning effects or other factors inherent in open-label study designs. On the basis of these initial data, a larger, placebo-controlled clinical trial of D + Q for idiopathic pulmonary fibrosis is planned. Interim results were recently reported from a phase 1, open-label, clinical trial of D + Q in individuals with diabetic kidney disease (NCT02848131). By 11 days after the last day of a 3-day oral course of D + Q, 9 individuals

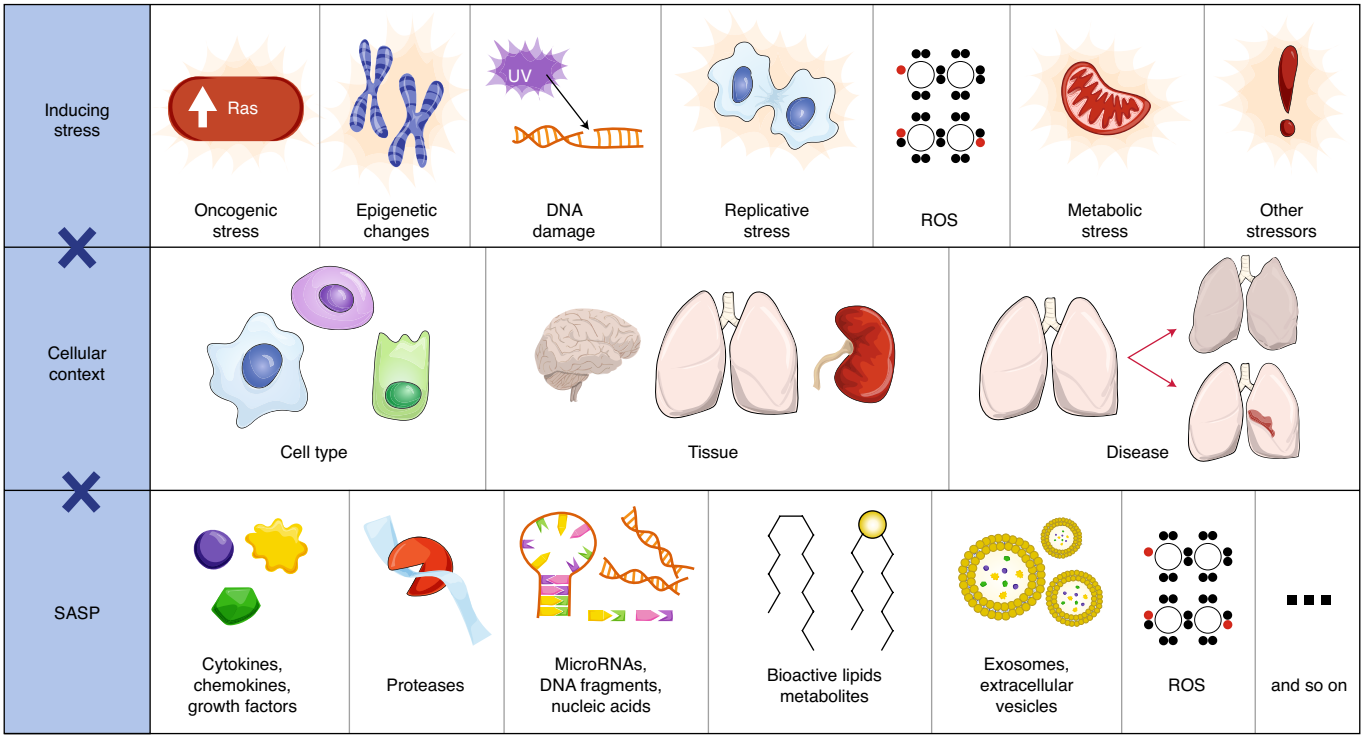


**Fig. 3 | Layers of heterogeneity in cellular senescence.** Senescent cells exhibit context-dependent phenotypic diversity. Numerous stimuli can induce a stress response sufficient to induce cellular senescence. Furthermore, susceptibility to these stressors and senescent cell characteristics can depend on cell type, tissue, disease state and other context. The resulting senescent cells display a spectrum of convergent and divergent phenotypes in their secretome and consequent functionality.

with diabetic kidney disease had decreased numbers of adipose tissue $p16^{INK4A}$-positive and SA-β-gal$^+$ cells compared to those in biopsies before D + Q was given[38,141]. Additionally, 11 days after completing the 3-day senolytic intervention, a composite score of 10 circulating SASP factors was significantly decreased as was activated CD68$^+$ macrophage adipose tissue infiltration and adipose tissue crown-like structures, which are due to fibrosis. In this continuing trial (goal = 30 individuals), no serious or severe side-effects have emerged so far. Multiple other clinical trials are underway or about to begin (Table 2). These include, among others, trials of D + Q for Alzheimer's disease (ALSENLITE, NCT04785300; SToMP-AD, NCT04685590), the accelerated aging-like state in bone marrow transplant survivors (HTSS, NCT02652052), the accelerated aging-like state in survivors of childhood cancer (SENSURV, NCT04733534) and age-related osteoporosis (NCT04313634), and trials of fisetin for frailty in older women (AFFIRM, NCT03430037), diabetic and chronic kidney disease (NCT03325322), childhood cancer survivors (compared to D + Q in SENSURV), age-related osteoporosis (compared to D + Q in NCT04313634), osteoarthritis (NCT04210986) and coronavirus in individuals in nursing homes, hospitalized individuals and outpatients (COVID-FIS, NCT04537299; COVID-FISETIN, NCT04476953; and COVFIS-HOME, NCT04771611, respectively). In addition to D + Q and fisetin, a trial of the Bcl-xL inhibitor UBX1325 for the treatment of diabetic macular edema is currently recruiting (NCT04537884). A clinical trial of the proposed senolytic compound UBX0101, an inhibitor of the murine double minute 2 (MDM2) negative regulator of p53, for treatment of osteoarthritis (NCT03513016) was recently halted in phase 2 following failure to outperform the placebo in interim analysis. This is speculated to be due to several factors including that MDM2 inhibitors have senomorphic activity, which could represent their primary mechanism of action and would require continuous administration[142]. Additionally, UBX0101 required a treatment course of multiple injections in preclinical models[143] in contrast to the single injection in the clinical trial. Potential benefits of the UBX0101 arm of the trial may also have been masked due to the clinical benefits of intra-articular saline injection on patient-reported outcomes in osteoarthritis[144]. Until results and data about benefits as well as adverse or off-target events are available from these and other studies, senolytic agents should not be administered outside carefully monitored clinical trials.

## Conclusions and future directions

In the last decade there has been exponential growth in cellular senescence research due to its pervasive and widespread implications. Additional field-defining discoveries are certain to follow over the coming years. However, substantial gaps and key questions still remain. Senescent cells exhibit hysteresis, in that their phenotypes are dependent on the context of prior stress. Furthermore, senescent cells are heterogeneous in both form and function. Senescent phenotypes vary by cell type, tissue of origin, tissue of residence, functional impact and how senescence was induced (Fig. 3). This is further complicated by differences between in vitro models and translation from tissue cultures studies to in vivo animal models and ultimately to humans. However, few models exist to study these differences (Table 3). Current animal models such as *INK-ATTAC* (ref. [32]) or *p16*-3MR mice[25] and other *p16*-dependent systems[105,145–147] are limited because $p16^{Ink4a}$ is not expressed by every senescent cell and non-senescent, frequently abundant cell types, such as activated macrophages, can express $p16^{Ink4a}$ at high levels[129]. Murine systems using *p21*-based constructs have recently been developed to address these issues and explore uncharacterized senescent cell populations[148]. However, it is ultimately unknown whether senescent cells in mice are representative of those found in humans. We have begun to address this through transplantation studies[34], in which human senescent cells are introduced into murine hosts, but this question

**Table 3 | CDKI construct–based models to study senescent cells**

| Model | Construct | Utility | Ref. |
|---|---|---|---|
| *p16 INK-ATTAC* | Transgenic *p16* promoter driving drug (AP20187)-inducible caspase 8 activation/ cell death and GFP | GFP fluorescent tracking and controlled elimination of p16-positive cells via AP20187 administration | Ref. [32] |
| *p16*-3MR | Transgenic *p16* promoter driving trimodality reporter of luciferase, monomeric RFP and herpes simplex virus 1 thymidine kinase | Bioluminescence assays, RFP fluorescent cell tracking and controlled elimination of p16-positive cells via ganciclovir administration | Ref. [25] |
| *p16*-Cre | Knock-in of constitutively active Cre, thymidine kinase and tdTomato to end of endogenous *p16* third exon | Cre-mediated conditional gene expression (that is, fluorescent reporter, suicide gene encoding diptheria toxin fragment A (DTA)) in cells expressing p16; of note, this line is crossed with reporter and DTA mouse lines owing to reported low p16 expression in vivo and resulting lack of tdTomato expression or ganciclovir sensitivity | Ref. [105] |
| *p16*-Cre | Inducible Cre knock-in at first exon of endogenous *p16* locus | Cre-mediated conditional gene expression (that is, fluorescent reporter) in cells expressing p16 | Ref. [145] |
| *p16*-tdTom$^+$ | Knock-in of tdTomato to first exon of endogenous *p16* | Tracking of p16-expressing cells via fluorescent reporter | Ref. [146] |
| *p16*-luciferase | Knock-in of firefly luciferase to translational start site of endogenous *p16* locus | Bioluminescence assays, and luciferase expression tracking to assess for p16 expression | Ref. [147] |
| *p21*-Cre | Transgenic *p21* promoter driving inducible Cre and GFP | Cre-mediated conditional gene expression (that is, fluorescent signal tracking via cross with floxed tdTomato reporter line, targeted ablation via DTA) in cells expressing high level of p21, following tamoxifen administration; tamoxifen-independent tracking of p21 expression via GFP | Ref. [148] |

remains open. The field is in clear need of novel animal models, senescent cell culture systems and additional human translational experiments to push forward. Amid this evolving appreciation of senescent cell heterogeneity, it is likely that there are yet undefined populations and subpopulations of senescent cells that may have distinct phenotypes, possess unique spatiotemporal dynamics and fulfill distinct physiologic and pathogenic roles in a context-dependent manner. In particular, few studies have been conducted to assess the characteristics of naturally occurring senescent cells[70], which are likely to be the most relevant to aging phenotypes. While there is an abundance of preclinical evidence indicating that senolytics may treat various disease processes or even supplement regenerative therapies[149], it is unknown how modulating different senescent cell populations with senotherapeutic strategies will translate into clinical outcomes or whether senolytics differ in efficacy when used preventatively or after a disease process has already begun. Ideally, as our understanding of senescence heterogeneity expands, it will be increasingly possible to use rational design strategies to target and eliminate only the most detrimental senescent cell subpopulations (for example, by leveraging single-cell differential gene expression profiles across senescent cells). However, these translational questions are challenging to address considering the inherent variations between animal models and humans, which are convoluted by differences in immune function and kinetics of senescent cell accumulation. Collectively, these gaps need to be addressed by the burgeoning cellular senescence field and are likely to evoke interest for the foreseeable future.

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## Competing interests

Patents on senolytic drugs and their uses are held by Mayo Clinic. This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic Conflict of Interest policies.

## Additional information

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