The costs and benefits of senotherapeutics for human health

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Cellular senescence is a major contributor to age-related diseases in humans; however, it also has a beneficial role in physiological and pathological processes, including wound healing, host immunity, and tumour suppression. Reducing the burden of cell senescence in animal models of cardiometabolic disorders, inflammatory conditions, neurodegenerative diseases, and cancer using pharmaceutical approaches that selectively target senescent cells (ie, senolytics) or that suppress senescence-associated secretory phenotype (ie, senomorphics) holds great promise for the management of chronic age-associated conditions. Although studies have provided evidence that senolytics or senomorphics are effective at decreasing the number of senescent cells in humans, the short-term and long-term side-effects of these therapies are largely unknown. In this Review, we systematically discuss the senolytics and senomorphics that have been investigated in clinical trials or have been used off-label, presenting their various adverse effects. Despite the potential of senotherapeutics to transform anti-ageing medicine, a cautionary approach regarding unwanted dose-dependent side-effects should be adopted.

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Introduction

Cellular senescence is a process that occurs in response to different triggers, including DNA damage, oncogene activation, and telomere dysfunction. This process has been linked to fundamental mechanisms, such as embryogenesis, regeneration, tissue repair, tumour suppression, and physiological ageing of organisms. In 1961, it was first shown that human fibroblasts divide a finite number of times before irreversibly arresting their growth.[1] Although senescent cells exist in a state of permanent growth arrest, they remain metabolically active and undergo physiological transformations, including alterations of paracrine signalling. In this respect, the senescence-associated secretory phenotype (SASP), a hallmark of senescent cells that mediates their pathophysiological effects, is characterised by the increased secretion of some bioactive molecules, including cytokines, chemokines, proteases, and growth factors.[2] Over the past decade, it has become clear that tissue ageing is caused by the accumulation of senescent cells, which alters the physiological responses in the surrounding microenvironment in an autocrine and paracrine fashion through SASP.[2,3] Tools have since been developed, such as the colorimetric assay for senescence-associated β-galactosidase activity, proving that accumulation of senescent cells promotes organ and organismal ageing. In addition to marking ageing, cellular senescence can suppress tumorigenesis by limiting the malignant transformation of preneoplastic cells and by hampering the proliferation of tumour cells. Nevertheless, the incidence of cancer increases when people get older because of the increased burden of cell senescence in organs, partly due to an impaired immune system, which results in a reduced clearance of senescent cells.[4] Furthermore, SASP contributes to persistent chronic inflammation (known as inflammaging).[5] The body of evidence showing that elimination of senescent cells seemed to be largely beneficial[6,7] led to huge research efforts to identify novel agents that eliminate senescent cells in humans.[8–10] These senotherapeutic strategies can be broadly categorised into two categories: pharmacological agents termed senolytics, which eliminate senescent cells, and those termed senomorphics, which prevent the detrimental cell-extrinsic effects of senescent cells by selectively targeting and suppressing the development of SASP, which is associated with increased age and medical risk in humans.[11] Senolytics decrease the number of naturally occurring senescent human cells in vitro, and improve physical function and increase the lifespan of aged mice.[12,13] Over the past 5 years, senotherapeutic research has progressed exponentially with the demonstration that these drugs can be used as a potential approach to improve transplantation outcomes and transplant availability in both animal models and in humans,[14] or to reduce mortality from a SARS-CoV-2-related murine-β-coronavirus in an aged mouse model.[15]

However, the repurposing of existing drugs and the use of new senotherapeutics are associated with various side-effects; incomplete functional characterisation of peripheral tissues at systemic administration; an absence of standardised guidelines for timing, dose, and route of administration; and a paucity of efficacy and safety data from clinical trials. Therefore, the full potential of senotherapeutics has been hampered in clinical applications. The scope of this Review is to summarise the state-of-art literature on the benefits and risks of senotherapeutics (particularly senolytics) and on their use in patients with ageing-related disease. We will not address the use of these agents in chemotherapy-induced senescence, given that this has already been reviewed by Prasanna and colleagues.[16] We will break this evidence down to single physiological functions and organ systems, both in patients and in murine models (figure).

Effects of senotherapeutics on nutrient metabolism and inflammation

Obesity-related insulin resistance and diabetes are associated with inflammation and dysfunction of adipose tissue. Given that senescent cells accumulate in the adipose tissue of both humans and rodents with obesity, it has been suggested that these cells are also involved

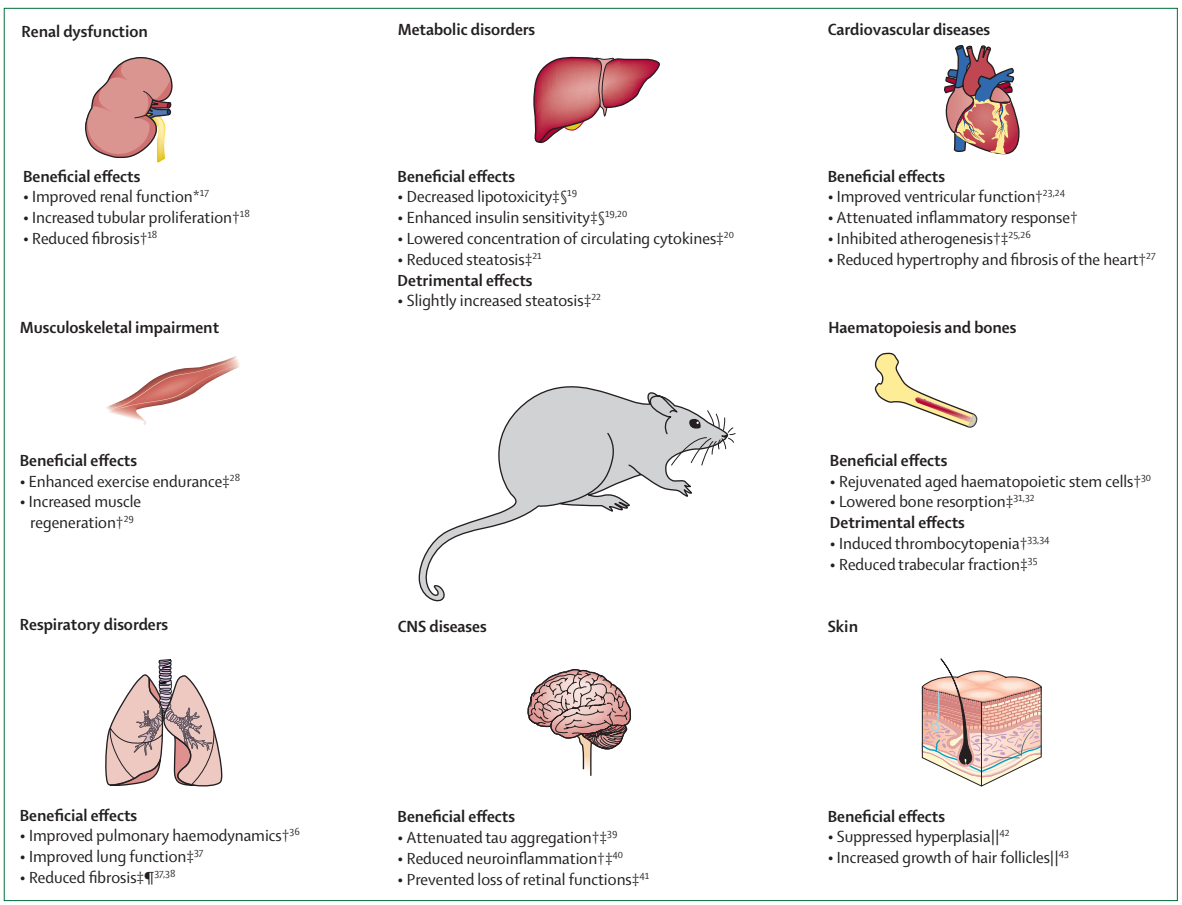

Figure: **Effects of senotherapeutics in murine models**
*FOXO4-DRI. †ABT263. ‡Dasatinib and quercetin. §Ruxolitinib. ¶Cardiac glycosides. ||ABT737.

the development of diabetes. In 2015, researchers from the Mayo Clinic showed that senescent adipocyte progenitors secrete activin A, a member of the transforming growth factor superfamily, and directly inhibit adipogenesis in non-senescent progenitors.[44] The researchers also observed that the concentration of activin A increased with age in the adipose tissue of mice. Therefore, a group of mice aged 22 months was administered with the senotherapic ruxolitinib (a Janus kinase 1 or 2 inhibitor), at a concentration of 60 mg/kg daily for 8 consecutive weeks. The researchers found that this treatment lowered the number of senescent cells and circulating concentration of activin A, preserved fat mass, decreased lipotoxicity, and enhanced insulin sensitivity.[19] In 2019, the same research group used a cocktail of two senolytic compounds, dasatinib and quercetin, which were already known for their synergic killing effects on different types of senescent cells. Following one daily dose of dasatinib (5 mg/kg) and quercetin (50 mg/kg) for 5 consecutive days in obese mice, the researchers observed a reduced burden of senescent cells in adipose tissue, resulting in improved glucose tolerance, enhanced insulin sensitivity, decreased circulating concentration of inflammatory mediators, and a recovery of the adipose tissue functions that promote adipogenesis.[20] Additionally, clearance of senescent cells reduced the number of macrophages in visceral adipose tissue and prevented the migration of monocytes. Concurrently, renal and cardiac functions were improved after administration of senotherapeutics.[20] Given that use of dasatinib and quercetin has not always been efficacious in every mouse model of metabolic disease, its efficacy seems to be controversial. Although this senolytic cocktail was shown to decrease the burden of senescent cells and reduce hepatic steatosis in one study,[21] it failed to promote clearance of senescent cells and prevent progression of non-alcoholic fatty liver disease in lean mice and in mice with obesity induced by a high-fat diet.[22]

These experimental findings in mice indicate that cellular senescence could be a potential causal factor in obesity-related inflammation and in metabolic imbalance, and that senolytic agents could be a promising treatment for obesity-related metabolic dysfunction and its complications. Nevertheless, the discordant findings and the paucity of studies on the potential long-term toxic

effects of senolytic agents have cast some doubts over their usefulness as a reliable treatment. Conveniently, because there is evidence that it can take up to 6 weeks for a cell to become fully senescent,[10] therapies with senolytics can be planned with a so-called hit-and-run approach, which involves intermittent administration of the drug. Consequently, drug safety is improved and the risk of side-effects is reduced.

Furthermore, an altered secretory profile of adipose tissue contributes to obesity and diabetes in humans. The link between the senescent status of adipose tissue and metabolic complications in obesity was investigated in a prospective cohort of 227 individuals with severe obesity. Senescence-associated β-galactosidase activity was seven times higher in subcutaneous tissue than in omental adipose tissue and was correlated with altered glycaemic status; however, this activity was not associated with body-mass index or chronological age.A subset of human adipose tissue biopsies was then treated with a cocktail of dasatinib (1 μmol/L) and quercetin (20 μmol/L), which decreased senescence-associated β-galactosidase activity in subcutaneous tissue and omental adipose tissue.[45] In a small, open-label, phase 1 pilot study of seven patients with diabetic kidney disease, administration of once daily oral dasatinib (100 mg) and quercetin (1000 mg) for 3 days, which have elimination half-lives of up to 11 h, significantly reduced the burden of senescent cells in adipose tissue within 11 days.[46]

Obesity and diabetes are risk factors of degenerative ocular diseases, such as diabetic macular oedema and age-related macular degeneration, which are the most common causes of vision loss in the global population. UBX1325 (Unity Biotechnology, San Francisco, CA, USA), a small molecule inhibitor of Bcl-xL, was assessed in a phase 1 clinical study to establish its safety profile (table). This open-label, single-ascending dose study enrolled 12 patients with advanced diabetic macular oedema or age-related macular degeneration. UBX1325 showed a favourable acute safety profile supporting further clinical development; there were no dose-limiting toxicities and two non-serious adverse events unrelated to the drug. Additionally, six patients who received high doses had a gain in best corrected visual acuity at 2 weeks. Six of 12 patients had a decrease in central subfield thickness, and three of four patients with wet age-related macular degeneration saw an improvement in disease-relevant pathology and a reduction in subretinal or intraretinal fluid.[47] A phase 2 study on the tolerability of UBX1325 activity in patients with diabetic macular oedema is ongoing (NCT04857996; table). These pilot senolytic approaches were mostly developed on a hit-and-run basis and took place in a narrow time window. Long-term monitoring of efficacy and safety, as well as randomised controlled trials (even with administration of a single dose of senolytic), are necessary.

Effects of senotherapeutics on the cardiovascular system

Cardiovascular disease has a prevalence of 70–75% in the older population (aged 60–69 years).[48] It has been shown that senescent cells accumulate in the heart with age and contribute to related pathologies in animal models.[27] Clearance of senescent cells in aged mice and in mice with atherosclerosis using genetic and pharmacological approaches improves vascular and myocardial function and attenuates age-dependent remodelling.[25,27] In a 2019 study, mice aged 22 months were treated with the BCL2 and Bcl-xL inhibitor, ABT263 (Navitoclax, Selleckchem, Houston, TX, USA), or vehicle alone by oral gavage at 50 mg/kg per day for 7 days per cycle, for two cycles with a 1-week interval.[27] Treatment with ABT263 showed a significant reduction in heart hypertrophy and fibrosis, together with a compensatory regeneration in cardiomyocytes. However, ABT263 had no significant effect on cardiac function, left ventricle mass, or ventricle wall rigidity. Subsequently, it was shown that ABT263 was able to rescue the functional decrease in ejection fraction occurring after myocardial infarction in aged mice, to improve left ventricular function, to increase myocardial vascularisation, to decrease scar size, and to attenuate the inflammatory response in a young mouse model of ischaemia–reperfusion.[23,24]

The incidence of atherosclerotic coronary artery disease increases with age and is present in over 50% of people older than 60 years.[49] The two most frequently used genetic mouse models of atherosclerosis are the *Apoe*-knockout model and the *Ldlr*-knockout model, which differ in their dietary conditions for developing atherosclerosis.[50] A study in mice aged 10 weeks with *Ldlr* deficiency, fed a high-fat diet (a reliable model of atherosclerosis in mice), and treated once daily with 100 mg/kg ABT263 for 5 days followed by 14 days off treatment on a repeating cycle for 88 days, showed that elimination of senescent cells led to inhibited atherogenesis and reduced the number and average size of plaques.[26] Similar results were obtained after administering the dasatinib (5 mg/kg) and quercetin (10 mg/kg) cocktail as senolytic treatment once monthly for 3 months by oral gavage in the *Apoe*-knockout mouse model of atherogenesis. Specifically, mice with *Apoe* deficiency on a high-fat diet developed atherosclerotic plaques containing an increased number of senescent cells. Treatment with dasatinib and quercetin decreased the burden of senescence and plaque calcification, even though no change in plaque size was observed.[25]

Effects of senotherapeutics on the musculoskeletal system

Skeletal muscle is among the most age-sensitive tissues in mammals. Considerable changes occur in resident stem cells, myofibers, and the extracellular matrix, leading to a decrease in tissue homoeostasis, function, and regenerative capacity. Regeneration of skeletal

	Effects	Stage	Recruiting period	Trial registration number
UBX1325 (Bcl-xL inhibitor)				
Patients with diabetic macular oedema and age-related macular degeneration	Improved visual acuity and reduction in central subfield thickness	Phase 1	From August, 2020, to October, 2021	NCT04537884
Patients with diabetic macular oedema	NA	Phase 2	From May, 2021, to June, 2022	NCT04857996
Dasatinib (tyrosine kinase inhibitor)				
Patients with scleroderma	Decrease in skin expression of SASP	Phase 1 and 2	From January, 2009, to April, 2011	NCT00764309
Healthy participants	NA	Phase 2	From June, 2020, to March, 2023	NCT04313634
Quercetin* (PI3K/AKT pathway inhibitor)				
Patients with idiopathic pulmonary fibrosis	Improved physical function, respiratory symptoms, and skin irritation	Phase 1	From December, 2016, to June, 2019	NCT02874989
Patients with Alzheimer's disease	NA	Phase 1 and 2	From August, 2019, to August, 2023	NCT04063124
Patients with Alzheimer's disease	NA	Phase 2	From December, 2021, to December, 2031	NCT04685590
Stem-cell transplant recipients	NA	NA	From March, 2016, to December, 2021	NCT02652052
Patients with diabetic chronic kidney disease	Reduced skin and circulating SASP factors	Phase 2	From July, 2016, to June, 2022	NCT02848131
Patients with frailty	NA	Phase 2	From October, 2021, to July, 2024	NCT04733534
Healthy participants	NA	Phase 2	From June, 2020, to March, 2023	NCT04313634
Patients with coronary artery disease	NA	Phase 2	From June, 2021, to June, 2022	NCT04907253
UBX0101 (p53/MDM2 interaction inhibitor)				
Patients with osteoarthritis of the knee	Not effective	Phase 2	From January, 2020, to September, 2020	NCT04229225
Patients with osteoarthritis of the knee	Not effective	Phase 2	From October, 2019, to August, 2020	NCT04129944
Patients with osteoarthritis of the knee	NA	NA	From April, 2020, to November, 2020	NCT04349956
Patients with osteoarthritis of the knee	NA	Phase 1	From May, 2018, to April, 2019	NCT03513016
Fisetin (PI3K/AKT pathway inhibitor)				
Patients with osteoarthritis of the knee	NA	Phase 1 and 2	From January, 2020, to December, 2022	NCT04210986
Patients with osteoarthritis of the knee	NA	Phase 1 and 2	From June, 2022, to May, 2024	NCT04770064
Patients with osteoarthritis of the knee	NA	Phase 1 and 2	From March, 2021, to May, 2025	NCT04815902
Patients with frailty	NA	Phase 2	From January, 2018, to November, 2021	NCT03675724
Patients with frailty	NA	Phase 2	From February, 2018, to June, 2022	NCT03430037
Patients with frailty	NA	Phase 2	From October, 2021, to July, 2024	NCT04733534
Patients with femoroacetabular impingement	NA	Phase 2	From September, 2021, to August, 2024	NCT05025956
Healthy participants	NA	Phase 2	From June, 2020, to March, 2023	NCT04313634
Healthy participants	NA	Phase 2	From June, 2020, to March, 2023	NCT04313634
Patients with COVID-19	NA	Phase 2	From August, 2020, to July, 2022	NCT04476953
Patients with COVID-19	NA	Phase 2	From October, 2021, to December, 2023	NCT04537299
Patients with COVID-19	NA	Phase 2	From July, 2021, to June, 2023	NCT04771611

NA=not available. SASP=senescence-associated secretory phenotype. *Used in combination with dasatinib.

Table: **Clinical trials on senotherapeutics**

muscle is carried out by the satellite stem-cell population that remains in a quiescent state. In aged mice, resting satellite cells lose the ability to repress *P16ink4a* (*Cdkn2a*), switching from the reversible quiescent state to an irreversible pre-senescence state. When injured, these cells fail to activate and expand, entering full senescence.[51]

A study showed that administration of dasatinib and quercetin in a murine model of irradiation-induced senescence ameliorated muscle function.[28] After 12 weeks of leg irradiation (10 Gy), mice aged 4 months showed impaired capacity in a treadmill exercise and an increased expression of senescent markers in the leg muscles. 5 days after a single dose of dasatinib (5 mg/kg) and quercetin (50 mg/kg) by oral gavage, expression of senescent markers was reduced and exercise endurance was better than in vehicle-treated controls.[28] These differences were maintained for 7 months following treatment. Senotherapy has also proven to be effective in a rat model of Duchenne muscular dystrophy,[29] a progressive disease characterised by chronic muscle degeneration and inflammation. The administration of senolytic ABT263 (18·75 mg/kg per day for 7 days

per cycle, for two cycles with a 2-week interval) in rats reduced the expression of senescence markers, prevented the loss of bodyweight and muscle strength, and increased muscle regeneration, even at the late stage of Duchenne muscular dystrophy.[29]

In humans, skeletal muscles are among the largest organs in the human body, generating movement and maintaining metabolic homoeostasis. Muscle regeneration and maintenance are facilitated by resident mesenchymal progenitors and muscle stem cells. Skeletal muscle mass and function decline with ageing, culminating in sarcopenia, and are linked to an increased burden of senescent cells with involvement of the immune system.[52,53] The effects of senolytic therapy in the context of age-associated functional decline of skeletal muscle and sarcopenia in humans have not yet been investigated. However, a wealth of literature shows that exercise or physical activity might be the most cost-effective senolytic therapy, although there is large heterogeneity among published studies.[54]

Effects of senotherapeutics on the skin

Skin ageing is associated with the onset of several pathological changes, including loss of insulation with decreased protection from pathogens, increased irritation, impaired wound healing, and increased susceptibility to cancer.[55] Senescent cells accumulate with age in the dermis and epidermis, which can be accelerated by various harmful factors, such as DNA-damaging agents (eg, x-rays, ultraviolet radiation, and cigarette smoke) and mitochondrial dysfunction.[56] In mice, cellular senescence in the epidermis due to persistent mitochondrial dysfunction has also been associated with epidermal thinning with age. However, the role of senescent cells in skin function is complex because they seem to have both beneficial and detrimental effects, depending on the context.[57] In mice, senescent cells have shown the capacity to limit fibrosis and to promote proper wound healing through their secretion of platelet-derived growth factor AA in SASP,[58] and chronic induction of cellular senescence might contribute to stem-cell loss with age.[59] Only a few studies have investigated the senotherapeutic approach for treatment and prevention of skin ageing. In 2020, Azazmeh and colleagues[42] showed that prolonged expression of transgenic p16INK4a in the epidermis of mice induced hyperplasia and dysplasia through a paracrine-mediated activation of the Wnt pathway, contributing to the development of premalignant skin lesions. Intraperitoneal administration of the ABT263 analogue, ABT737 (75 mg/kg six times over 9 days after 6 months of *Cdk2a* induction), eliminated p16-positive cells, inactivated the Wnt pathway, and subsequently suppressed hyperplasia.[42] Another study, investigating the age-related increase of hair loss, used a double-transgenic mouse model, in which human p14ARF gene expression was induced, leading to an increase of senescent cells in the epidermis and the growth arrest of hair follicles in the telogen stage.[43] Only a small number of stem cells in bulge areas maintained their proliferative capacity in this model. Intraperitoneal administration of 75 mg/kg ABT737 on 2 or 4 consecutive days resulted in clearance of senescent cells in the epidermis and hair follicles, increasing non-senescent bulge capacity for proliferation and repopulation.[43]

Nevertheless, it is still unclear whether senotherapy could be helpful in reducing the deleterious effects of skin ageing, and further testing of dosage and timing needs to be investigated.

Lämmermann and colleagues[60] identified an extract from the plant *Solidago virgaurea* alpestris, which exhibited weak senolytic activity in fibroblasts and keratinocytes isolated from skin biopsies of healthy adult donors. This plant extract reduced the expression of various SASP components, ameliorating the negative paracrine crosstalk between these cells.[60]

A single-arm, open-label clinical trial in 12 patients with interstitial lung disease associated with systemic sclerosis administered dasatinib treatment for approximately 9 months, performed in parallel gene expression profiling in the skin.[61,62] Only three (25%) patients showed some clinical improvement, which correlated with a decrease in skin expression of SASP and other senescence-related gene sets.[61] In an open-label phase 1 pilot study,[46] administration of once daily oral dasatinib (100 mg) and quercetin (1000 mg) for 3 days was shown to reduce p16INK4a-positive cells by 38% and p21CIP1-positive cells by 30% in the epidermal layer of the skin and adipose tissues. Beyond medical applications and systemic effects, cosmetic use of therapeutic senolytics are likely to find applications as topically administered skin anti-ageing products.[63–65] In both medical and cosmetic applications, clinicians should consider the important physiological role of senescence in wound healing when offering senolytic therapy, especially in patients who plan to undergo or are recovering from surgery to avoid disrupting the normal wound healing process.

Effects of senotherapeutics on the nervous system

The brain ageing process leads to progressive impairment in memory, orientation, attention, and cognition.[66] In addition, ageing is a major risk factor for the onset of many neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease.[67]

Age-associated cognitive decline has been associated with multiple molecular processes, such as chronic inflammation, altered autophagy, oxidative stress damage, and mitochondrial dysfunction.[68,69] Data have shown that various parts of the brain in patients with neurodegenerative diseases are characterised by increased expression of senescence markers.[70] Clearance of senescent cells has been shown to enhance brain function in healthy aged mice[71] and in various murine models of

neurodegenerative diseases, such as Parkinson's disease,[72] neurodegeneration related to amyloid β,[40] tau-dependent neurodegenerative iseases,[39] and neuropsychiatric disorders.[73] In most of these studies, ABT263 (50 mg/kg) or the dasatinib (5–12 mg/kg) and quercetin (50 mg/kg) cocktail were used as the senotherapeutic, with different cycles of treatment and washout over a period of 11 weeks to 6 months. Senotherapy has been shown to ameliorate phenotypes in all of these mouse models, attenuating tau phosphorylation and aggregation,[39] reducing neuroinflammation and amyloid β plague load, and improving cognitive deficits.[40] Collectively, these findings show that senescence has a key role in brain ageing and in related diseases in mice, and that senolytics can improve brain performance.

One of the most common chronic neurodegenerative diseases in humans is glaucoma, which is a leading cause of irreversible blindness worldwide. Although a study in a mouse model showed that dasatinib prevented glaucoma-dependent loss of retinal functions and cellular structure,[41] a retrospective study assessing the effects of senolytic drugs on vision in 28 patients with glaucoma showed that senolytic exposure did not affect visual acuity or intraocular pressure, and did not have toxic effects.[74] This pilot study implies that senolytic drugs might not have clinically significant toxicity and, therefore, could be safe for use in humans.

It is currently unknown whether senolytics have beneficial effects on age-associated neurological and cognitive impairments, such as those observed in Parkinson's disease or Alzheimer's disease. Nevertheless, following convincing preclinical studies, phase 1 and 2 clinical trials exploring first-generation senolytics (eg, dasatinib and quercetin) are ongoing in ophthalmology and neurology (table).[75,76]

Effects of senotherapeutics on immune system and pulmonary function

Lung ageing is associated with structural remodelling, decline of respiratory function, and increased susceptibility to acute and chronic lung diseases, including asthma, obstructive pulmonary disease, and idiopathic pulmonary fibrosis.[77] Several external factors, such as cumulative exposures to environmental pollutants, allergens, smoke, and respiratory infections, accelerate lung impairment.[78] Furthermore, advanced age is associated with many age-related changes in innate and adaptive immunity,[79] including phagocytotic function altered by macrophages and neutrophils, reduced activity of natural killer cells, increased serum concentrations of proinflammatory cytokines, an increased number of airway neutrophils, and reduced T-cell activation. These changes support the development of chronic pulmonary diseases in older people, which is associated with a poor prognosis in cases of comorbidities, such as infection and inflammatory disease.[80] The increased burden of senescent cells, together with the release of inflammatory cytokines associated with SASP, contribute to chronic inflammation in the lung.[81] In particular, interleukin (IL)-6 and IL-8, the most common cytokines associated with SASP, are elevated in the bronchoalveolar lavage of patients aged 65 years and older.[82] IL-6 is a chemotactic and prosurvival factor for monocytes and neutrophils.[83] In macrophages, p16 expression is associated with proliferation of and differentiation into the proinflammatory M1 macrophage,[84] leading to increased IL-6 secretion. Senolytic drugs can eliminate p16-positive macrophages[85] and senescent vascular cells, exerting a dual approach to reverse vascular remodelling. Van der Feen and colleagues[36] showed that intraperitoneal administration of ABT263 (10 mg/kg daily for 7 days) reversed vascular remodelling and improved pulmonary haemodynamics in the end stage of shunt-induced pulmonary arterial hypertension in a rat model.[36] A study linked the senescent fibroblast secretome to fibrogenic activity in a mouse model of idiopathic pulmonary fibrosis, a fatal disease characterised by interstitial remodelling and compromised lung function, induced by administration of bleomycin.[37] The use of dasatinib (5 mg/kg) and quercetin (50 mg/kg) by oral gavage once a week for 3 weeks improved pulmonary function and physical health, although lung fibrosis was visibly unaltered.[37] Interestingly, data show that other senotherapeutics, belonging to the cardiac glycoside family, resulted in effective elimination of senescence-induced lung fibrosis in female immunodeficient NMRI nude mice (a mouse model named after the Naval Medical Research Institute) aged 8 weeks.[38]

A two-centre, open-label study in 14 patients with idiopathic pulmonary fibrosis showed that intermittent dasatinib (100 mg/day) and quercetin (1250 mg/day) treatment for 3 days per week over 3 weeks had a tolerable safety profile and caused significant ameliorations in physical function, assessed with a 6-min walk distance and 4 m gait speed. By contrast, pulmonary function, circulating concentrations of biochemical markers of senescence and profibrotic factors, frailty index scores, and reported health were unchanged.[86]

Patients with idiopathic pulmonary fibrosis are extremely susceptible to developing COVID-19 after infection with SARS-CoV-2.[87,88] Similarly, COVID-19 survivors are susceptible to developing pulmonary fibrosis-like symptoms.[87,88] A shared genetic aetiology between idiopathic pulmonary fibrosis and severe COVID-19 has been proposed.[89] A *Science* report showed that aged mice acutely infected with pathogens, including murine-β-coronavirus related to SARS-CoV-2, had increased senescence and 100% mortality. Targeting senescent cells with either fisetin (20 mg/kg per day) or dasatinib (5 mg/kg) plus quercetin (50 mg/kg) on days 3, 4, 11, and 12 after pathogen exposure significantly reduced mortality (by 50%), cellular senescence, and inflammation, and increased antiviral responses.[15] This mouse study did not analyse lung function. A clinical trial funded by the National Institutes of Health is in

progress to investigate whether fisetin is able to decrease pulmonary pathological progression and morbidity related to SARS-CoV-2 in hospitalised older patients with COVID-19 (NCT04537299).[90] Supporting these data with larger randomised controlled trials in patients with pulmonary fibrosis would constitute a major breakthrough for public health.

Effects of senotherapeutics on the haematopoietic system and bone health

Bone ageing is major risk factor for primary osteoporosis, an age-related disease characterised by altered bone metabolism that suppresses bone formation and promotes bone resorption,[91] impairing bone homoeostasis.[92] Bone homoeostasis relies on a dynamic balance between osteogenesis, carried out by osteoblasts, and osteoclastogenesis, carried out by osteoclasts.[93] It is well established that haematopoiesis, the generation of new blood cells that takes place in the bone marrow, involves both haematopoietic and non-haematopoietic cells.[94] Non-haematopoietic stromal cells, including osteoblasts and osteoprogenitor cells, promote the maintenance of haematopoietic stem cells.[95] Ageing and genotoxic stress induce cellular senescence of these stem cells[96,97] and osteoprogenitor cells,[98,99] with subsequent decline in the functions of these cells in both mice and humans.[100]

In the past few years, various studies have assessed the possible beneficial effects of senotherapeutic approaches on bone ageing and haematopoiesis ageing, yielding different findings. In 2016, Chang and colleagues[30] showed that oral administration of ABT263 in sublethally irradiated mice and naturally aged mice resulted in a mitigation of premature ageing of the haematopoietic system induced by total body irradiation and a rejuvenation of aged haematopoietic stem cells.[30] However, several other studies indicated that ABT263 and other Bcl-xL-inhibitory BH3 mimetics induced thrombocytopenia and a transient thrombocytopathy, which could impair the haemostatic function of platelets.[33,34] Studies on the effects of senolytics on bone remodelling have also showed divergent results. Some studies have shown that targeting senescent cells in aged mice (aged 20–24 months) with established bone loss by use of dasatinib (5 mg/kg) plus quercetin (50 mg/kg) by oral gavage once monthly for 4 months,[31] or by ABT263 (41 µmol/kg) daily for 5 days,[105] lowered bone resorption, with maintained trabecular bone formation and enhanced cortical bone formation.[31,32] However, another study showed that, despite decreasing the burden of senescent cells, ABT263 treatment in aged female and male mice reduced both trabecular bone volume fraction (by 60·1% in females and by 45·6% in males) and the ability of osteoblasts derived from bone marrow stem cells to produce mineralised matrix (by 88% in females and by 83% in males).[35] Therefore, it is not clear from these murine studies whether senotherapy could be a suitable approach to counteract age-related bone loss and impairments in haematopoietic renewing, and further studies are required to assess the safety and efficacy of these drugs.

Nevertheless, several pilot studies in humans testing the potential beneficial effects of senolytics in recipients of haematopoietic stem-cell transplantation, patients with age-related osteoporosis, and patients with osteoarthritis are currently recruiting or ongoing (table). However, the leading compound, UBX0101 (Unity Biotechnology, San Francisco, CA, USA), failed a phase 2 study in patients with osteoarthritis in the knee, a major setback for this early stage research into senotherapy. It is not known whether UBX0101 was able to remove senescent cells; however, the drug did not improve clinical symptoms. Therefore, further studies are needed to evaluate the usefulness of senolytics in patients with haematopoietic disorders or bone disorders.

Effects of senotherapeutics on renal function

Kidneys of older people are characterised by decreased function, altered homoeostasis, maladaptive repair, and increased susceptibility to both acute and chronic kidney injury. Over the past decade, the pivotal role of cellular senescence in driving ageing and age-related diseases in multiple organs, including the kidney, has emerged. Experimental data support the notion that tubular epithelial cells are frequently implicated in renal senescence. In 2016, Baker and colleagues[6] studied the effect of genetic ablation of senescent cells using the INK-ATTAC transgenic mouse model. INK-ATTAC (INK-linked apoptosis through targeted activation of caspase) is a mouse model developed by Baker's research team, in which senescent cells expressing p16INK4a can be selectively eliminated in an inducible fashion. The researchers showed that senescence occurred in proximal tubular epithelial cells with increasing age. In another murine model, after ischaemia–reperfusion injury, nuclear $p21^{cip1}$ (CDK-interacting protein 1) was localised in proximal and distal nephrons, but not in the glomerular area.[101] However, other kidney cell types, such as parietal epithelium, podocytes, and vascular smooth muscle cells, showed positive straining for senescence markers.[102] Clearance of chronically senescent cells from aged or irradiated animal models benefits multiple organs, including the kidney.[6,30] However, kidney senescence is not always detrimental. For example, acute senescence has been observed to have beneficial effects and to contribute to antifibrotic mechanisms in murine models of unilateral ureteral obstruction, which leads to tubular injury and subsequent renal fibrosis. Studies in INK4-knockout mice subjected to unilateral ureteral obstruction showed that p16INK4A has an important role in reducing inflammation and cell proliferation.[103]

The results of experiments based exclusively on transgenic mice might not fully characterise the spectra of senescent cells seen in vivo, suggesting that the use of senotherapeutics could be a valid alternative to explore

Search strategy and selection criteria

We searched PubMed from June 23, 2021, to Aug 31, 2021, using both MeSH terms (controlled language) and free-text terms, including "senolytics" OR "cellular senescence". We then searched for more specific combinations, such as "senolytics" AND "neurodegenerative" OR "cancer" OR "toxicity". We did not restrict the search by language, date, or publication status. To collect information of current clinical trials, we searched www.ClinicalTrials.gov from Aug 25, 2021, to Nov 8, 2021 using the search query that included the name of the specific senolytic AND "senescence" OR "senolytic" OR "aging".

the effect of eliminating senescent cells in the kidney. However, to date, only a few studies have investigated use of these drugs. The most important contribution came from Baar and colleagues,[17] who designed the interfering peptide, FOXO4-DRI (5 mg/kg injected every 2 days for 6 days), which was shown to trigger selective apoptosis in senescent cells induced by ageing and by chemotherapy, resulting in an improvement in mouse health, including protection of renal structure and function. Additionally, Mylonas and colleagues[18] showed that treatment of aged and irradiated mice with ABT263 reduced the number of senescent cells and restored regeneration of the kidneys, with increased tubular proliferation, improved function, and reduced fibrosis after ischaemia–reperfusion injury.

In an open-label, phase 1 pilot study, 3 days of oral dasatinib (100 mg) and quercetin (1000 mg) were administered orally to nine patients with obesity and diabetic kidney disease.[104] Excisional biopsies from abdominal subcutaneous adipose tissue were acquired 11 days after completing treatment. The main findings were a significant decrease in senescent cell markers and macrophage content, a significant increase in adipogenic progenitors, a significant decrease in SASP, and no serious adverse events. This study did not report effects on metabolic and renal function, and can be considered preliminary.

Future directions and conclusions

The safety profile and efficacy of senotherapeutics in patients are yet to be fully investigated in clinical trials, and it is likely that the best senotherapeutic against age-associated diseases and malignancies is yet to be discovered. Dasatinib, quercetin, and other senolytics were discovered using a mechanism-based approach.[7,26,105] High throughput screening technology, which allows for automated testing of thousands of molecules present in chemical compound libraries[106] in in-vitro senescence models, could assist with the discovery of new effective senolytics. To date, high throughput screening of commercial chemical compound libraries has led to the discovery of new families of senolytics: HSP90 inhibitors,[107] the BET family protein degraders,[108] and cardiac glycosides.[37] Furthermore, the safety and potency of existing senolytics can be improved by molecular engineering and drug delivery approaches. For example, the use of ABT263 is limited due to dose-limiting platelet toxicity. He and colleagues[34] devised a proteolysis-targeting chimera technology to reduce the platelet toxicity of ABT263 by converting it into PZ15227. Compared with ABT263, PZ15227 was shown to be less toxic to platelets, but was a more potent senolytic in vitro and in vivo.[34] Similar strategies might be useful to improve the efficacy and the safety profile of other toxic or repurposed senolytic agents. Eradicating senescent cells in an adult organism is not always beneficial. For instance, senescence can be induced in macrophages as part of a polarisation in response to reversible immunomodulatory stimuli,[109] and a senescence-like phenotype is present in various post-mitotic cells of mice entering middle age in the absence of disease or advanced ageing.[110] Grosse and colleagues[111] reported that genetic removal of liver sinusoidal endothelial cells with high expression of p16 hampered the healthspan of mice because the procedure induced fibrosis in the liver and systemic perivasculature. Interestingly, given the high expression of p16, dasatinib and quercetin treatment removed senescent macrophages, but was ineffective against senescent liver sinusoidal endothelial cells in mice.[111]

Future therapeutic approaches based on novel nanotechnology-based strategies for cargo delivery specific to cell type, biomarker, and phenotype are also being developed to increase the specificity and reduce the side-effects of senolytics.[9,112]

In conclusion, senolytic drugs have shown promising results in the elimination of senescent cells and in alleviating various diseases in animal models (figure). However, in patients, there is a paucity in data on the efficacy and safety of senotherapeutics from clinical trials, including systemic effects and side-effects. In this regard, as highlighted in a workshop delivered by the National Institutes of Health on the consideration of senolytics for clinical trials, it is important to assess the specificity of senolytics in killing targeted senescent cells and their cytotoxic effects, to identify reliable markers for intervention responses, to elucidate interactions with comorbidities and other drugs, and to standardise administration protocols.[75]

Contributors

MR contributed to writing of the original draft, methodology, and visualisation. MV contributed to writing of the original draft, reviewed and edited the manuscript, and supervised the project.

Declaration of interests

We declare no competing interests.

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