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# Senolytics: targeting senescent cells for age-associated diseases

**Iman M. A. Al-Naggar, PhD**[1,*], **George A. Kuchel, MD**[1], **Ming Xu, PhD**[1,2]

[1]UConn Center on Aging, UConn Health, Farmington, Connecticut, USA

[2]Department of Genetics and Genome Sciences, UConn Health, Farmington, Connecticut, USA

### Keywords

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## Introduction

Growing evidence indicates that most, if not all, chronic diseases and geriatric syndromes share common pathways with aging and that these mechanisms may be modified. This Geroscience Hypothesis arose from the observation that aging is the greatest risk factor for most chronic diseases, such as cardiovascular disease, type II diabetes, cancer and many degenerative and neurodegenerative disorders[1]. The main advantage of looking at aging through the lens of the Geroscience Hypothesis is that, if true, then by targeting these shared aging mechanisms, it may be possible to prevent, delay and perhaps even reverse multiple aging-related conditions and phenotypes simultaneously [2–4] Such interventions would increase the human healthspan (quality, healthy, independent, productive years of life), while decreasing the burden of long disease-ridden years later in life on both caretakers and economy[5, 6]. Several molecular pathways contributing to aging have been identified collectively called “the Hallmarks of Aging” [7].More recently, these have been grouped into 4 main categories, or fundamental aging mechanisms: 1) chronic, low-grade sterile inflammation and fibrosis, 2) Macromolecular/organelle dysfunction, 3) Stem and progenitor cell dysfunction, and 4) cellular senescence[8, 9]. Several studies show that these mechanisms are somewhat intertwined: initiating one can activate others and targeting one usually affects others. Genetically and pharmacologically targeting cellular senescence, in

*Corresponding author: 263 Farmington Avenue, EM013, Farmington, CT 06030-1835, alnaggar@uchc.edu.

Competing Financial Interests

Dr. Xu has financial interest related to senolytics. Patents on senolytic drugs (including PCT/US2016/041646, filed at the US Patent Office) are held by Mayo Clinic.

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Human and Animal Rights

All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

particular, has been the focus of much recent research with very promising pre-clinical results [10–24]. The goal of this review is to describe advances in discovering senolytics, drugs that eliminate senescent cells (**SnCs**) to delay or alleviate geriatric diseases and syndromes, introduce techniques and models used for their discovery, and where we are in terms of clinical translation.

## Cellular Senescence

Like apoptosis and differentiation, cellular senescence is a cellular fate[25]. SnCs are dysfunctional cells that have permanently exited the cell cycle in response to stress or molecular damage. They are resistant to apoptosis, accumulating in many organs 1) with aging, 2) as a result of therapies that cause DNA damage such as chemotherapy or radiation in cancer patients, and 3) at sites of pathology of chronic diseases such as atherosclerosis, obesity, Alzheimer's and idiopathic pulmonary fibrosis (IPF). Their most detrimental aspect is believed to be their secretome, the senescence-associated secretory phenotype, SASP[25, 26]. The SASP is highly inflammatory, and is composed of many pro-inflammatory cytokines, chemokines and proteases that can be damaging to organs, ultimately resulting in dysfunction. The SASP is so noxious that a small percentage of SnCs might cause substantial tissue damage. This is supported by our studies showing that transplanting just 1 million SnCs (<0.03% of cells throughout the body) intraperitoneally into young, healthy mice, can cause the onset of aging-like symptoms, increase senescence elsewhere and decrease lifespan[27, 10]. Additionally, studies done in transgenic animals, where SnCs (those highly expressing the cyclin-dependent kinase inhibitor p16) were ablated using genetic approaches showed marked improvements in several aging phenotypes[18, 19, 22, 28]. Additionally, senescence in stem cell niches reduces the regenerative capacity of tissues, leading to deterioration and pathology[29, 13].

Cumulatively, pre-clinical data suggest that reducing SnC burden or preventing their accumulation may alleviate or delay the onset of aging-related conditions. Several methods for accomplishing this are being developed and include senolytics (drugs that specifically target and induce the death of SnCs), senomorphics (drugs that dampen or inhibit the SASP), promoting reactivation of proliferation of SnCs (but this may accidentally induce tumorigenesis) and immunotherapy (boosting the immune system to re-instate its ability to combat SnCs as in young organisms)[30].

## Senolytics

Ideal senolytics for translation to human use would be drugs that can specifically target and kill harmful SnCs without affecting other cells or harming the individual.[31, 32] The discovery of such senolytics, however, has been made difficult by several factors: 1) Most of our knowledge about SnCs comes from *in vitro* studies using SnCs induced by irradiation or treatment with DNA-damaging drugs, and although we showed that these *in vitro*-generated SnCs replicate some of the key altered pathways in naturally occurring SnCs, they may not reflect precisely what occurs *in vivo*[33]; 2) The percentage of SnCs in various tissues *in vivo* is relatively small (0.5–20%); 3) There are no sensitive, specific or standard biomarkers for identifying SnCs, and the most routinely used markers such as senescence-associated

beta-galactosidase, p16 and p21 are not found in all SnCs and can also be found in non-SnCs (p16 is expressed in many terminally differentiated cell types); 4) SnCs might be as heterogeneous as the cell types they arise from, and may therefore require targeting in terms of matching specific drug combinations with cell type.

Administration of senolytics is envisioned to be intermittent. This proposed “hit-and-run” nature of senolytic treatment reduces the possibility of undesirable off-target effects involved in traditional continuous administration of a drug[34]. Damaged cells take 2–6 weeks to senesce, and a certain threshold of SnCs has to be reached in the body before they can cause damage. Thus, senolytics, designed to kill a large portion of SnCs in a few doses, only needs to be repeated when enough SnCs have returned. The rate of re-accumulation of SnCs is expected to vary among different people, and thus sensitive and reliable biomarkers for SnCs are desired to measure the SnC load in individuals, allowing for more tailored senolytic therapy (precision medicine)[35]. With all of these considerations in mind, we will review most major classes of senolytic drugs developed to date.

## Dasatinib and Quercetin

Zhu et al. used a hypothesis-driven approach combined with bioinformatics to identify Dasatinib and Quercetin as the first senolytic agents[12]. They reasoned that SnCs, reported to be resistant to apoptosis, have upregulated pro-survival pathways and down-regulated pro-apoptotic pathways[36]. Comparing proteomic and transcriptomic profiles of senescent and non-SnCs allowed them to identify several Senescent Cell Anti-Apoptotic Pathways (SCAPs). Using RNA interference studies to target these pathways, they identified ones that specifically induced death of SnCs rather than proliferating cells, and realized that different SCAPs need to be targeted to induce apoptosis in different cell types. Dasatinib, a tyrosine kinase inhibitor used in cancer therapy, interferes with members of the ephrin survival-regulating dependence receptors (EFNB) appears to exhibit specificity in terms of killing senescent preadipocytes. In contrast, quercetin, a flavonoid that inhibits kinases (including PI3K), serpines, and certain BCL-2 family members, demonstrates greater specificity for killing senescent endothelial cells. Furthermore, combining the two (D+Q) kills more senescent cell types than either alone by simultaneously targeting multiple SCAPs. The *in vivo* effects of D+Q senolytic therapy have been tested under dozens of conditions in mouse models including physical dysfunction, osteoporosis, insulin resistance, Alzheimer’s, kidney dysfunction, vasomotor dysfunction, liver steatosis, pulmonary fibrosis, anxiety and lifespan reduction and *ex vivo* on human tissue with promising results [14, 37–41, 11].

D+Q has moved to human trials of incurable diseases where SnCs have been found to play a causal role such as idiopathic pulmonary fibrosis (IPF), diabetic kidney disease (DKD) and early stage symptomatic tau$^{+}$ Alzherimer’s disease to determine safety, tolerability and effectiveness (Figure 1) [42, 43, 34, 44, 37, 45, 46]. Early proof of concept human studies reported no serious drug effects, reduced SnC burden, reduction in SASP factors and improved physical function[42, 43, 34]. Follow-up double blinded placebo-controlled trials will be needed before it becomes possible to study the ability of D+Q to prevent or reverse age-related conditions and improve healthspan in older adults.

## Fisetin

Fisetin, a flavonoid very similar in structure to Quercetin, was identified in a screen of flavonoids for more potent senolytics[17, 47]. Fisetin was found to clear SnCs of different lineages and reduce SASP *in vivo* in several mouse aging models, reduce senescence in human *ex vivo* tissue explants, and increase lifespan and healthspan. It alleviated frailty in mice even when administered late in life (starting at 20 months, equivalent to approximately 60 human years). Fisetin is attractive for therapy because it is widely available as a supplement with few apparent side effects and better bioavailability, making it eminently translatable. Additionally, it has other desirable effects such as being anti-inflammatory, anti-oxidant, anti-carcinogenic and its targets include PI3 kinase δ, some BCL-2 family members, nuclear factor-kappa B (NF-κB), and mTOR [48–51]. Furthermore, Fisetin was described to be a caloric restriction mimetic, an intervention shown to increase lifespan in mammals[52]. Its well-established safety profile and many benefits have made fisetin one of the quickest senolytics to enter into human clinical trials, with studies currently underway examining its potential benefits in frailty, osteoarthritis and chronic kidney diseases (Figure1).

## Targeting the BCL-2 family (Navitoclax (ABT-263), UBX1967/1325, ABT737, EF24)

In a bioinformatics screen for the identification of pathways that may be conferring the resistance of SnCs to apoptosis, Zhu et al. identified upregulation of the BCL-2 pro-survival pathway as one of the SCAPs[12]. This family includes the anti-apoptotic proteins BCL-2, BCL-W, BCL-XL, MCL-1 and A1. Soon thereafter, two reports identified Navitoclax (or ABT-263) as a senolytic[13, 53]. Navitoclax can target several BCL-2 family members (BCL-2, BCL-W and BCL-$X_L$), which are upregulated in senescent HUVECs and IMR-90 cells, but not in primary human preadipocytes. Consequently, Navitoclax was found to be senolytic in HUVECs and IMR-90s but not preadipocytes, with promising *in vivo* results for rejuvenating stem cells of the hematopoietic and skeletal muscle systems[13]. However, Navitoclax has undesirable effects, mainly toxicity to platelets and neutrophils, making it complicated for translation. Because this undesired off-target toxicity may be caused by specific inhibition of BCL-2, drugs that target other BCL-2 family members, were tested with the hope of less toxicity. A1331852 and A1155463, which specifically target BCL-$X_L$ (but not BCL-2) were later found to be senolytic [13, 47, 54, 53]. It is currently unknown whether these two drugs might have less deleterious side effects.

Several *in vivo* mouse studies have since shown the ability of ABT-263 to kill SnCs and attenuate several diseases or conditions in which SnCs have been shown to accumulate. ABT263 eliminated senescent astrocytes and improved cognitive function in an accelerated brain aging model induced by whole brain irradiation, ameliorated hyperglycemia and improved β-cells in an aging model, high fat diet model and insulin resistance models of Type II Diabetes, cleared senescent cardiomyocytes in hearts of aged mice, alleviated myocardial remodeling, attenuated expression of profibrotic mediators and improved the maintenance of cardiac function following MI, resulting in decreased post-MI mortality, attenuated tau phosphorylation and aggregation in a mutant tau protein model of

neurodegeneration, protected lung tissues from chemically induced pulmonary emphysema and improved radiation induced pulmonary fibrosis in mice[55, 21, 23, 56, 57].

ABT-737 is another BCL-2 family inhibitor reported to be senolytic (targets BCL-2, BCL-W and BCL-$X_L$) and to functionally rejuvenate hematopoietic stem cells in naturally aged mice and mice exposed to sublethal radiation[54].

EF24, a curcumin analog screened for its senolytic actions based on curcumin's reported anti-aging effects in *C. elegans* and *D. melanogaster*, was shown to be senolytic in many cell types induced to senesce by different methods[58]. EF24 induced apoptosis of SnCs, and caused a reduction in Bcl-xl and Mcl-1 proteins but not their mRNA, pointing to a possible post-transcriptional mechanism. Proteasome degradation of the BCL-2 family appeared to be the mechanism by which EF24 was senolytic. EF24 combined with ABT-263 had synergistic effects, possibly allowing the lowering of the dose of ABT-263 to decrease its side effects (thrombocytopenia), making it useful for clinical translation.

Some BCL-2 family inhibitors (UBX1967 and UBX1325, Unity Biotechnology) are being prepared for testing in human trials for targeting diseases of the aging eye, such as age-related macular degeneration and glaucoma (Figure1). However, the evidence of efficacy of these drugs in animal models is very limited.

## Piperlongumine

Piperlongumine is a natural product made by species from the genus Piper (pepper plants or pepper vines). It was identified as a senolytic in the same screen of a small library of structurally diverse, rationally selected small molecules that target pathways predicted to be important for survival of SnCs that identified ABT-263 by Chang et al[13]. Piperlongumine has low toxicity in mice, kills SnCs by apoptosis, and has significant synergistic senolytic effect when combined with ABT-263, suggesting a different mechanism of action of Piperlongumine from ABT-263[59]. However, the exact mechanism is still unknown for piperlongumine to induce apoptosis in SnCs, and it appears to target several signaling and survival pathways in SnCs.

### Targeting p53 (UBX0101, FOXO4 peptide and USP7 inhibitors)

P53 is a transcriptional factor whose levels must be tightly controlled in cells because it regulates various cellular processes including apoptosis, senescence, and proliferation[60]. It can induce apoptosis in a transcription-dependent way (by inducing the expression of pro-apoptotic genes) or transcription-independent mechanism (translocates to the mitochondria and interferes in the interaction between anti-apoptotic BCL-family proteins and pro-apoptotic proteins)[61, 62]. It is also downregulated in many tissues with aging, which may drive higher cancer incidence and SnC accumulation. P53 is upregulated upon activation of the DNA damage response in many cell types, but sometimes goes down in cells that upregulate p16 to maintain their proliferation arrest and senescence, whereas its activity may stay up in cells that do not upregulate p16. Evidence also exists for a role of p53 in SASP suppression by a mechanism involving p38MAPK and NF-κB, and for inducing apoptosis of SnCs, making the activation of p53 an attractive goal for senotherapy[63–65].

Baar et al. also hypothesized that anti-apoptotic pathways must be up-regulated in SnCs, making them resistant to apoptosis and performed RNA sequencing on proliferating and senescent IMR90 human cells[66, 36]. Their transcriptomics results didn't fully support that hypothesis (eg, senescent IMR-90s had higher PUMA and BIM (pro-apoptotic) and lower BCL-2 (anti-apoptotic) than non-senescent IMR-90s), and so they reasoned that transcription factors may be interfering with the execution of the apoptotic program and focused on FOXO4, a transcription factor linked to apoptosis and target of Insulin/IGF signaling. They found FOXO4 expression to be increased in SnCs and that its inhibition could lead to apoptosis of SnCs. They showed that FOXO4 inhibits apoptosis by binding p53 thus inhibiting p53-mediated apoptosis, causing cells to senesce instead of die in response to DNA damage. They designed a cell permeable peptide to interfere with the FOXO4-p53 interaction and showed that it selectively induced apoptosis in SnCs IMR-90 fibroblasts, by excluding p53 from the nucleus and sending it to the mitochondria, resulting in transcription-independent apoptosis[67, 68]. Using three *in vivo* senescence models, they show the FOXO4 peptide (FOXO4-DRI) could be clinically useful for use against conditions associated with SnCs (Table 1).

Another means of post-transcriptionally activating p53 is by preventing its interaction with the murine double minute 2 (MDM2), a ubiquitin ligase[69]. MDM2 and p53 are connected by a negative-feedback loop, where elevated p53 increases MDM2 expression, which in turn promotes the ubiquitination and proteasome degradation of p53, reducing its activity[70]. He et al. show that small molecule inhibitors of the ubiquitin-proteasome system 7 (UPS7), which deubiquitinates MDM2 preventing its degradation by UPS, are senolytic[71]. USP7 inhibitors activate p53 and cause apoptosis in cancer cells by promoting MDM2 auto-ubiquitination and degradation[72]. This indirect inhibition of MDM2 is well tolerated in mouse studies, compared to direct inhibition of MDM2, making it a more attractive target. In their studies, they find that USP7 inhibitors upregulated p53 and led to apoptosis only partly by MDM2 degradation, indicating other p53 transcription-dependent and independent mechanisms might also contribute. USP inhibitors successfully killed SnCs in a doxorubicin-treated mouse model of senescence and decreased SASP expression in several organs.

UBX0101, a senolytic that completed a Phase 1 human trial for safety and tolerability (Figure 1) to target moderate to severe painful osteoarthritis (OA) of the knee, is stated on Unity Biotechnology's website (not by peer-reviewed literature) to be a p53/MDM2 interaction inhibitor. Jeon et al showed a causative role of SnCs in trauma-and aging-induced OA, and that eliminating SnCs genetically and pharmacologically with UBX0101 can attenuate OA progression and symptoms[20]. UBX0101 alleviated pain, decreased articular erosion and increased cartilage regeneration in knees of mice that developed post-traumatic OA following anterior cruciate ligament transection (ACLT), while decreasing many senescence markers and SASP molecules in both articular cartilage and synovioum of the knee joint. It is important to note that MDM2 inhibitors are toxic, and may only be useful therapeutically in local (versus systemic) administration as is being tested for OA in humans.

## HSP-90 inhibitors

Fuhrmann-Stroissnigg et al. took advantage of the accelerated senescence of DNA damage repair deficient Mouse Embryonic Fibroblasts (MEFs) from Ercc$^{-/-}$ mice (progeroid mouse model) to establish a screen for senotherapeutics[73, 74]. They observed that, when grown at atmospheric oxygen (20%), approximately 50% of these cells became senescent as measured by several senescence markers. They also showed that an SA β-galactosidase (SABG)-based assay using the substrate $C_{12}$FDG, which fluoresces upon cleavage by β-galactosidase, could accurately determine the proportion of senescent to non-senescent Ercc$^{-/-}$ MEFs. They used both flow cytometry and automated confocal microscopy to quantify senescent and non-SnCs, and performed screens using the microscopy detection method. Because their cultures of Ercc$^{-/-}$ MEFs contained both senescent and non-SnCs, they could determine the effect of different drugs on both cells in the same well, and showed the assay could differentiate between senomorphics (Rapamycin and NDGA, which reduced the number of SnCs without affecting the total number of cells) and senolytics (D+Q and Navitoclax, reduced both the number of SnCs and total cell number, suggesting the SnCs were being killed). They screened a library of 97 autophagy regulators, and identified 13 compounds as senotherapeutics, which can modulate cellular senescence. After excluding compounds that were highly toxic, known to affect lysosomal pH (would yield a false positive in their SABG-based screen), or were senomorphic but not senolytic, they identified two promising candidates, tanespimycin (17AAG) and geldanamycin, both of which are heat shock protein (HSP90) inhibitors. Testing 7 HSP90 inhibitors from different classes showed that all were senolytic, and that they could kill SnCs from different human and mouse cell types induced to senesce with different methods. Specifically, they appeared to induce apoptosis of SnCs by disrupting the stabilization of active, phosphorylated AKT (anti-apoptotic factor upregulated in SnCs with important role in regulating the PI3K/AKT anti-apoptotic pathway) by HSP90. Finally, to establish the *in vivo* senolytic activity of HSP90 inhibitors, they tested the effect of 17-DMAG (improved, more water soluble, clinically tested geldanamycin derivative) on age-related phenotypes in the Ercc1$^{-/\Delta}$ progeroid mouse model, and found a significant decrease in a composite score of aging symptoms as well as a reduction in p16 expression in kidneys (but not liver) of drug-treated mice, signifying a positive effect on healthspan, as previously observed with D+Q (Table1).

## Fibrates

Nogueira-Recalde et al used high throughput screening to identify drugs that can simultaneously modulate senescence and autophagy, both shown to play a role in aging-associated articular cartilage degeneration and OA[75]. They used a human chondrocyte-based cell culture imaging assay, where senescence was induced by IL-6 treatment, causing an increase in SABG. After screening 1120 compounds, they found that 279 had senotherapeutic activity. They then tested the effect of these identified senescence modulators on autophagy, and found that 14/279 also increased autophagic flux. They focused on fenofibrate (FN), a peroxisome proliferator-activated receptor alpha (PPARα) agonist and therapeutic target for lipid metabolism dysfunction, because it was previously shown to be important for chondrocyte homeostasis. In addition to being senolytic in human chondrocytes (by inducing apoptosis), they found FN to be senolytic in human IMR90 lung

fibroblasts and Ercc1$^{-/-}$ MEFs. Furthermore, they showed that genetic ablation of PPARα induces senescence and SASP, and reduces autophagic flux, confirming its role in senescence and autophagy. They also showed a decrease in PPARα-positive chondrocytes in knee cartilage from surgical OA mice, naturally aged mice, and in samples from OA-patients. Finally, they compared genetically matched subjects from the Osteoarthritis Initiative (OAI) cohort taking fibrates to ones not taking fibrates and found a significant difference in self-reported function, fewer knee replacements and a trend towards less pain in the fibrate-treatment group. Their results suggest fibrates show promise in alleviating OA symptoms, perhaps through their simultaneous effect on senescence and autophagy in chondrocytes.

Interestingly, their primary screen also identified Digitoxigenin, a cardiac glycoside (CG), as a senotherapeutic. Three months later, CGs were reported to be senolytic simultaneously by 2 independent studies[76, 77].

## Cardiac glycosides

Guerrero et al. screened the LOPAC 1,280 library of pharmacologically active compounds, in various senescent cells [76]. They found that Ouabain and other CGs could specifically induce apoptosis of SnCs in all conditions tested for inducing senescence in IMR-90 fibroblasts. They showed that Ouabain could be used *in vivo* as a senolytic using a model of tumor initiation in the liver and a pituitary tumor model, in which SnCs have been shown to accumulate. They also tested the suitability of CGs in the elimination of SnCs produced as a result of treatment with chemotherapy, radiation therapy and targeted anti-cancer drugs and found that, indeed, they sensitized these therapy-induced SnCs to undergo apoptosis, both in *in vivo* models (looking at SnCs accumulating in the lungs of irradiated mice or mice treated with doxorubicin, Table1) and in several cancer cell culture models. Finally, they saw improvements in metabolic blood markers, markers of physical function, decreased p16 expression in liver, heart and kidneys and decreased immune infiltration in the liver of old Ouabain treated mice relative to controls (Table1).

Triana-Martinez et al screened the Prestwick chemical library in bleomycin-induced senescent cells, and identified the CG Proscillaridin A as a senolytic[77]. As Proscillaridin is not currently being used clinically, they chose to test the CGs Digoxin, which is commonly used for treatment of heart conditions, and Ouabain. They confirmed the senolytic activity of these CGs in multiple senescent cell types (tumor and primary cells) via different induction methods. Screening several additional libraries, they found many more CGs to be senolytic. They also showed Digoxin acted via apoptosis induction (not ferroptosis or necroptosis) for inducing SnC death. To test whether Digoxin could be used for killing cancer-therapy-induced SnCs *in vivo*, they used mouse models to show that combining CGs with senescence-inducing cancer drugs significantly decreased tumor size. Finally, they found that Digoxin could kill SnCs in a model of lung fibrosis, and alleviate fibrosis in the lungs (Table 1). This finding further supports the use of senolytics in IPF patients (Figure 1)[78–81].

Data from both studies suggest that the senolytic activity of CGs might come from inhibiting the $Na^+/K^+$ ATPase. SnCs were more sensitive to changes in membrane potential caused by Digoxin, which causes loss in membrane potential and cellular acidification, because their membranes are slightly depolarized and they are more acidic than proliferating cells at baseline. CGs appear to tip SnCs past a threshold, inducing apoptosis.

## Other Methods for killing SnCs *in vivo*

In addition to senolytic drugs described above, several other methods are currently being developed to kill SnCs *in vivo*. Muñoz-Espín et al described the use of nanoparticles coated with galacto-oligosaccharides and containing cytotoxic agents to specifically deliver poisons to senescent cells, many of which have a high lysosomal content and lysosomal β-galactosidase, allowing the digestion of the coat and release of the encapsulated drug[82].

Guerrero et al also took advantage of the higher β-galactosidase activity of SnCs to test whether galactose-modified duocarmycin cytotoxic prodrugs would be selectively toxic to SnCs[83]. They showed successful senolysis *in vitro* and in *in vivo* mouse models (irradiation model and cancer model).

Another delivery method (Lipid Nanoparticles) taking advantage of a different aspect of the biology of many SnCs (high expression of p16) is being developed by Oisín Biotechnologies. Their SENSOlytics® will deliver an apoptotic gene under control of a p16 promoter. This gene will only be expressed in cells with high p16 expression, and they will undergo cell death.

Although both methodologies have resulted in a marked *in vivo* decrease in SnC burden in animal models, they both suffer from the shortcoming that the aspect of biology they target (high lysosomal content or high p16 activity) might not be specific to SnCs, and may cause side effects by targeting non-senescent, terminally differentiated cells that express p16 or cells with high lysosomal content as part of their biology such as macrophages. On the other hand, these strategies might have the unique advantage of reducing the potential off-target toxicity once their precise targets have been optimized. Furthermore, these methods could be combined with senolytics to achieve additive benefits.

## Conclusions

Although senolytics are being tested for treatment of varied chronic diseases of aging and geriatric syndromes, it may be a while before these compounds can be safely used in healthy individuals for the purpose of prevention, delaying or reversing normal aging phenotypes. Nevertheless, the field of senolytic discovery is moving rapidly through more efficient and specific screens, followed by testing and validation of promising candidates in various preclinical models, and ultimately rigorous testing in clinical trials. Our understanding of the basic biology of SnCs *in vivo* is still limited, but new techniques such as single cell sequencing are offering new capabilities for better understanding these small populations and then facilitating next-generation senolytic drugs development. Using senolytics for treating diseases where SnCs play a well-established role may soon become a reality, with several clinical trials under way for determining safety and efficacy (Figure 1). In the

meantime, these senolytics will certainly improve prognosis for cancer patients who suffer from premature aging likely caused by therapy-induced accumulation of SnCs, providing more evidence for their power against a fundamental aging mechanism.

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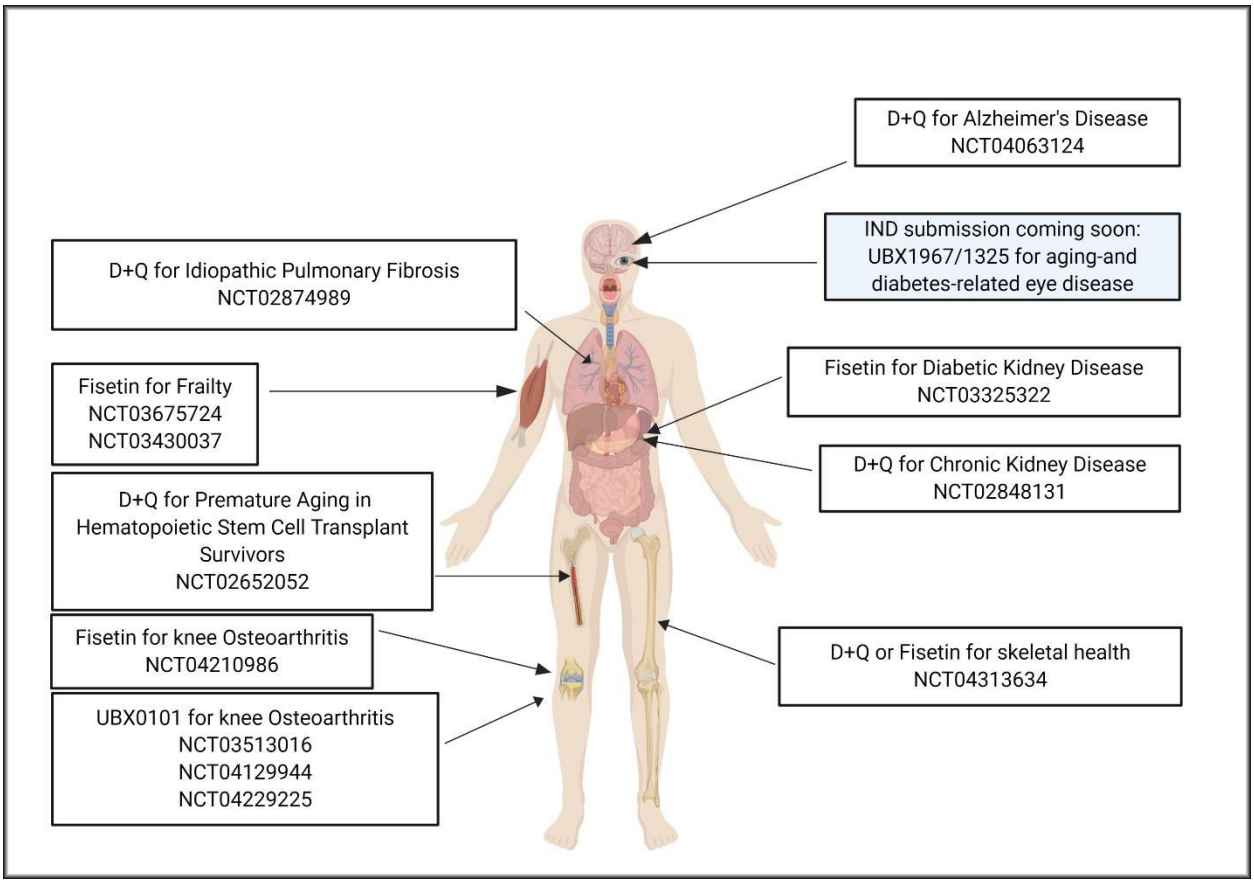


**Figure 1. Senolytics Clinical Trials in Humans.**

Shown are some completed, recruiting, ongoing and planned trials. Before being tested in healthy adults for delaying or alleviating aging-related diseases and syndromes, senolytics are being tested for safety and efficacy of removing senescent cells from patients with serious conditions involving senescent cell accumulation for which there are currently no other cures. These include idiopathic pulmonary fibrosis (IPF), diabetic/chronic kidney disease, tau$^{+}$ Alzheimer's disease, accelerated aging resulting from hematopoietic stem cell transplantation, frailty and osteoarthritis. Some completed Phase I trials have reported no serious adverse effects (eg, D+Q, UBX0101) and are moving on to Phase II trials. (Image created using Biorender.)

**Table 1.**

Models Used for Successfully Testing Senolytics in the Different Senolytics Discovery Studies Presented.

| | Senolytic | TIS Mouse Model (Radio- or Chemotherapy) | Progeroid Mouse Models | Other Mouse Models | Natural Aging in Mice | Human Samples (ex vivo) | Ref. |
|---|---|---|---|---|---|---|---|
| Kinase inhibitors | Dasatinib and Quercetin | Irradiated single mouse leg | DNA damage repair deficient Ercc1$^{-/\Delta}$ progeroid mouse model (models human XFE progeria) | | Improved cardiovascular function, Improved blood albumin, phosphate and amylase levels Improved rotarod activity | | 12 |
| | Fisetin | | DNA damage repair deficient Ercc1$^{-/\Delta}$ progeroid mouse model (models human XFE progeria) with a p16 reporter | | Decreased senescent cells of different lineages and in many tissues Increased healthspan and lifespan in mice, even when started very late in life | Adipose tissue explants | 17 |
| Fibrates | Fenofibrate | | | | | Aged and osteoarthritic chondrocytes | 75 |
| BCL-2 family inhibition | ABT-737 | Irradiated mice | | p53 activation induced senescence in skin | | | 54 |
| | Navitoclax (ABT-263) | Irradiated mice (models premature aging of hematopoietic system) | | | Cleared senescent cells in bone marrow, lungs and muscle, reducing SASP Improved defects in HSC clonogenicity, long-term repopulating ability and imbalances in multinlineage differentiation (myeloid skewing). Improved muscle stem cell clonogenicity | | 13 |
| HSP90 inhibition | 17-DMAG | | DNA damage repair deficient Ercc1$^{-/\Delta}$ progeroid mouse model (models human XFE progeria) with a p16 reporter | | | | 73 |
| Cardiac Glycosides | Ouabain | 1) Irradiated mice 2) Doxorubicin-treated mice | | | Decreased senescent cells in several tissues Improved blood albumin, phosphate and amylase levels Improved rotarod activity Decreased immune infiltration in liver | | 76 |
| | Digoxin | Injected human cancer cells into immunosuppressed mice, administered senescence-inducing cancer drugs combined with Digoxin | | Intratracheally administered senescent or proliferating IMR90 fibroblasts into lungs of immunosuppressed mice to model lung fibrosis. | | SABG positive osteoarthritic chondrocytes | 77 |

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| | Senolytic | TIS Mouse Model (Radio- or Chemotherapy) | Progeroid Mouse Models | Other Mouse Models | Natural Aging in Mice | Human Samples (ex vivo) | Ref. |
|---|---|---|---|---|---|---|---|
| p53 activation | USP7 inhibitors (P5091) | Doxorubicin-treated p16-3MR mice | | | | | 71 |
| | UBX0101 | | | Post-traumatic osteoarthritis induced by ACLT surgery in mice (intra-articular injections) | Naturally aged mice with injury-induced OA- reduced SnCs in articular cartilage and synovium, reduced pain, reduced p16, p21 and SASP factors | SABG positive osteoarthritic chondrocytes | 20 |
| | FOXO4-DRI | Doxorubicin-treated mice | Human progeroid syndrome model (Trichothiodystrophy) | | IP injected FOXO4-DRI into naturally aged mice Reduced p16+ve senescent cell burden<br>Restored renal filtering capacity<br>Improved fur density and responsiveness | | 66 |

Only *in vivo* mouse models and human samples are shown in this table, which excludes *in vitro* cell studies for lack of space. As seen in this table, therapy-induced senescence (TIS) which includes mouse irradiation (usually total body irradiation) with a sublethal radiation dose (6–8 Gray) or treatment with a chemotherapeutic drug (such as doxorubicin), results in DNA damage that causes upregulation of the senescent cell load in several tissues and organs, including the hematopoietic system, and leads to premature aging phenotypes. Accelerated aging models of human diseases (progeroid) where senescent cell accumulation has been established are also used for testing senolytics. Naturally aged mice (19–24 months used in studies described in this review) are the gold standard for testing senolytics in mice, with some of the effects of senolytics described in the studies presented shown. Some groups are coming up with different ways to model human aging phenotypes correlated with senescent cell accumulation to test whether senolysis would attenuate disease (eg, transplanting senescent cells, but not proliferating ones, in mouse lungs intratracheally caused fibrosis, which was reversed by treatment with the senolytic Digoxin, a cardiac glycoside).