# Chapter 4
# Senolytics Target Senescent Cells and Improve Aging and Age-Related Diseases


**Tony Buffard and Gerardo Ferbeyre**

**Abstract** Accumulation of senescent cells has emerged as a major pathogenic factor in aging and multiple age-related diseases. Studies on the biology of senescent cells have identified vulnerabilities to eliminate them using a novel class of drugs called senolytics. These drugs kill senescent cells by blocking their resistance to apoptosis, by reactivating latent p53 or by increasing oxidative stress. Other compounds inhibit the senescence associated secretory phenotype or SASP. Senolytics and SASP modulators have been effective to improve natural aging and age-related diseases in mice models leading to ongoing clinical trials in humans.

**Keywords** Senolytics · Senescence associated secretory phenotype (SASP) · BCL2 family · Apoptosis · P53 · Metformin

## 4.1 Introduction

Cellular senescence is a programmed response triggered by both physiological or pathological factors that results in a phenotype characterized by an inability to respond to proliferative signals, resistance to apoptosis and the secretion of a variety of proteins and lipids with potent proinflammatory activity (Ferbeyre 2018; Lopes-Paciencia et al. 2019). In vivo, senescent cells can be divided into three distinct categories: embryonic, acute and chronic. Embryonic senescent cells help to shape developing tissues in mammals, fish and amphibia (Davaapil et al. 2017; Yun et al. 2015; Storer et al. 2013; Munoz-Espin et al. 2013; Villiard et al. 2017) whereas acute senescent cells are a protective response to abrupt stress such as a wound or an oncogenic signal. Both acute and embryonic senescent cells are beneficial and are eliminated through the immune system. Chronic senescence, on the other hand, may result from slowly accumulating damage at the macromolecular level and is associated to

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aging, cancer and age-related diseases (Childs et al. 2015). Accumulation of DNA damage, including damage in telomeric regions, have been linked to aging (Sedelnikova et al. 2004; Liu et al. 2005; Lombard et al. 2005; Herbig et al. 2006; Sahin and Depinho 2010; Hewitt et al. 2012). DNA damage activates the DNA damage response, which is measurable using antibodies that recognize proteins phosphorylated by DNA damage-activated kinases and includes the histone variant γ-H2AX (Mallette et al. 2007; Mallette et al. 2007; Halazonetis et al. 2008; Di Micco et al. 2006). However, the precise triggers of DNA damage and senescence during aging are still unknown.

## 4.2 Evidence of Senescent Cell Accumulation in Vivo

A lot of research confirms that accumulation of senescent cells is a hallmark of aging (López-Otín et al. 2013; He and Sharpless 2017). This might be the consequence of an increased generation of senescent cells with aging and/or a decrease in senescent cell clearance as the immune system gets older (Childs et al. 2015). In fact, senescent cells have a longer half-life in old animals (Karin et al. 2019), suggesting that their clearance becomes less effective with aging. Accumulation of senescent cells has been demonstrated using several senescence biomarkers in old zebrafish (Kishi et al. 2008) and many mammals (Jeyapalan and Sedivy 2008).

A biomarker commonly used to detect senescent cells is the staining for the Senescence-Associated β-Galactosidase (SA-β-Gal), a lysosomal enzyme upregulated in senescent cells (Kurz et al. 2000; Bandyopadhyay et al. 2005). The other standard biomarker is the induction of the cyclin-dependent kinase inhibitor (CKI) $p16^{Ink4a}$ mRNA levels. Expression of this tumor suppressor is undetectable in young rodents, but it increases with age in older tissues (Krishnamurthy et al. 2006; Berkenkamp et al. 2014; Burd et al. 2013) including stem cells (Janzen et al. 2006; Molofsky et al. 2006). $P16^{Ink4a}$ is mechanistically connected to senescence since inhibition of its expression in stem cells reduces their aging phenotype and allows faster tissue repair (Janzen et al. 2006; Molofsky et al. 2006). Telomere shortening, another well-known cause of senescence, can be measured using in situ hybridization with telomeric probes and can be used as a biomarker in some tissues. Telomere length decreases with age in the gut and liver in mice (Hewitt et al. 2012) and primates (Jeyapalan et al. 2007). Given the lack of a universal marker for senescence, quantification of senescent cells should use methods combining several biomarkers. For example, by combining the SA-β-Gal assay, DNA damage response markers and the depletion of HMGB1 from the cell nucleus, Biran et al. found senescent cells to be 10–20 times more abundant in old than in young mice (Biran et al. 2017). Using a similar strategy based on several biomarkers, Herbig et al. showed that the percentage of senescent cells in baboons' skin increased exponentially from 2% in young individuals to more than 15% in aged ones (Herbig et al. 2006). This was confirmed by another study and it was suggested that it would be the case for any mitotic tissue (Jeyapalan et al. 2007).

In humans, the levels of $p16^{Ink4a}$ and p27 (another CKI) are accurate biomarkers of aging in kidneys (Chkhotua et al. 2003; Melk et al. 2004), while the abundance of SA-β-Gal positive cells correlated with age in skin samples (Dimri et al. 1995). This has been confirmed using other senescence-associated markers in the skin (Wang and Dreesen 2018), bones, mesenchymal stem cells (Zhou et al. 2008; Farr and Khosla 2019) and human peripheral blood T lymphocytes (Liu et al. 2009). All of this research convincingly shows that senescent cells do accumulate with age in mammals. Importantly, senescent cells are also more readily detected in many ailing tissues and age-related conditions (Table 4.1) (Jaul and Barron 2017; Franceschi et al. 2018). This suggests that drugs acting on senescent cells will have a major impact in gerontology and healthy aging.

## 4.3 Elimination of Senescent Cells: Senolytics

Elimination of senescent cells using suicide genes in genetically modified mice or drugs that kill senescent cells, called senolytics, improve many age-related diseases (Table 4.2). Senolytics are thus posed to have broad medical applications. Here, we will discuss them according to their mechanism of action, illustrated in Fig. 4.1.

### *4.3.1 Senolytics that Inhibit the Bcl2 Family*

In multicellular organisms, cells can be eliminated by a process of programmed cell death called apoptosis. Apoptosis can be triggered in two ways. The extrinsic pathway involves a death receptor situated on the cytoplasmic membrane that can be activated by several death effector cytokines. The intrinsic pathway is triggered by endogenous damage that engages mitochondria to release pro-apoptotic factors such as cytochrome c. In both cases, Bcl-2 family proteins (Bcl-2, Bcl-xl, Bcl-w, Bfl-1 or Mcl-1) antagonize this process (Azmi et al. 2011).

Senescent cells are particularly resistant to apoptosis. For example, senescent human fibroblasts express high levels of Bcl-2 family members and can last as long as four weeks in media lacking serum without signs of apoptosis (Wang 1995). Senescent cells also secrete many cytokines and lipids, collectively known as the Senescence-Associated Secretory Phenotype (SASP), that may have anti-apoptotic functions. Together these Senescent Cell Anti-apoptotic Pathways protect senescent cells from cell death (Kirkland and Tchkonia 2017) and targeting them could be a promising way to selectively kill senescent cells.

Recent publications have shown several compounds that could act as effective senolytics via inhibition of antiapoptotic pathways. ABT-263, also known as Navitoclax, preferentially induces apoptosis in senescent fibroblasts and vein epithelial cells by inhibiting Bcl-2, Bcl-xl and Bcl-w (Zhu et al. 2016). These results were also observed in vivo in different mice models (Chang et al. 2016, 2016; Pan et al. 2017).

**Table 4.1** Senescent cells in human diseases. ABT-263: inhibitor of the Bcl2 family, D + Q: Dasatinib + Quercetin, Ink-ATTAC: mouse model for clearance of p16Ink4a positive cells, HSV-TK: human herpes simplex virus thymidine kinase, JAKi: JAK kinase inhibitor, Rapamycin: mTOR inhibitor to suppress IL6 and the senescence-associate secretory phenotype

| Disease | System affected | References |
|---|---|---|
| Presbycusis | Hearing | Watson et al. (2017) |
| Sarcopenia | Muscle | Snijders and Parise (2017), Sousa-Victor et al. (2014), Sousa-Victor et al. (2014) |
| Immunosenescence and AIDS | Immune | Fülöp et al. (2017), Palacio et al. (2019), Lanna et al. (2014), Bestilny et al. (2000) |
| Heart diseases | Cardiovascular | Rotter Sopasakis et al. (2019), Shimizu et al. (2019), Balint et al. (2019) |
| Hypertension | Cardiovascular | Guzik and Touyz (2017) |
| Atherosclerosis | Cardiovascular | Minamino et al. (2002), Sasaki et al. (2019), Thorin and Thorin-Trescases (2009), Voghel et al. (2010), Childs et al. (2016), Garrido and Bennett (2016), Roos et al. (2016) |
| Benign Neoplasia | Multiple | Choi et al. (2000), Castro et al. (2003), Nakamura and Nishioka (2003), Castro et al. (2004), Maldonado et al. (2004), Michaloglou et al. (2005), Vernier et al. (2011), Deschenes-Simard et al. (2013, 2019), Burd et al. (2013) |
| Osteoarthritis | Skeletal | Franceschi et al. (2018), Jeon et al. (2017), Xu et al. (2016) |
| Osteoporosis | Skeletal | Saeed et al. (2011), Farr et al. (2017) |
| Rheumatoid arthritis | Skeletal | Chalan et al. (2015), Fessler et al. (2018), Petersen et al. (2019) |
| Intervertebral disc degeneration | Skeletal | Patil et al. (2019) |
| Alzheimer's disease | Nervous | Franceschi et al. (2018), Bhat et al. (2012), Garwood et al. (2014), Zhang et al. (2019) |
| Parkinson's | Nervous | Franceschi et al. (2018), Chinta et al. (2013) |
| Diabetes | Metabolic | Palmer et al. (2015), Aguayo-Mazzucato et al. (2019) |

(continued)

**Table 4.1** (continued)

| Disease | System affected | References |
|---|---|---|
| Obesity | Adipose and Metabolic | Palmer et al. (2019), Tchkonia et al. (2010), Yoshimoto et al. (2013), Loo et al. (2017) |
| Chronic lung disease | Respiratory | Franceschi et al. (2018), Barnes et al. (2019), Noureddine et al. (2011) |
| Hepatitis and fatty liver | Liver | Papatheodoridi et al. (2019), Paradis et al. (2001) |
| Cirrhosis | Liver | Wiemann et al. (2002), Gutierrez-Reyes et al. (2010) |

TW-37, an inhibitor of Bcl-2, Bcl-xl and Mcl-1, was less senolytic than ABT-263 (Zhu et al. 2016), suggesting that Bcl-w plays an important role in protecting senescent cells from apoptosis. ABT-263 was ineffective against human senescent primary preadipocytes (Zhu et al. 2016), demonstrating that senolytics act in a tissue specific manner, a factor that should be taken into account for their use. ABT-737, an inhibitor of Bcl-xl and Bcl-w, preferentially kills senescent cells induced by DNA damage in the lung and senescent cells induced by $p14^{ARF}$ expression in the epidermis (Yosef et al. 2016). Interestingly, the elimination of senescent cells in the epidermis led to an increase in hair follicle stem cell proliferation (Yosef et al. 2016). Of note, the anti-Bcl2 family of drugs cause neutropenia and thrombocytopenia (Roberts et al. 2012), side effects that could limit their application in healthy old individuals.

Other inhibitors of the Bcl2 family with senolytic activity include fisetin, a flavone molecule that induces apoptosis in senescent fibroblasts and endothelial cells but not in senescent preadipocytes (Zhu et al. 2017). In progeroid $Ercc1^{-/\Delta}$ mice, fisetin killed senescent cells and reduced senescence biomarkers. In old naturally aged C57BL/6 mice, a 5-day diet of fisetin was able to significantly reduce the proportion of senescent cells in different tissues and extend median and maximal lifespan even when the treatment was initiated in old animals (Yousefzadeh et al. 2018). In cancer cells, fisetin can cause apoptosis by activating both the intrinsic and the extrinsic pathways and had beneficial effects to treat inflammation and metastasis (Kashyap et al. 2018). Fisetin is present in many fruits and vegetables, suggesting that it can be safely used as a senolytic and anti-aging agent in humans (Kashyap et al. 2018). Epigallocatechin gallate, a phytochemical found in green tea, inhibits both the anti-apoptotic Bcl-2 family and mTOR. The latter controls the SASP by regulating the translation of mRNAs coding for inflammatory cytokines (Herranz et al. 2015; Laberge et al. 2015). Epigallocatechin can thus act both as a SASP modulator by inhibiting mTOR and as a senolytic (Kumar et al. 2019).

Panobinostat is a deacetylase inhibitor used to treat multiple myeloma. Panobinostat is particularly potent against all deacetylases of class I, II and IV (Laubach

**Table 4.2** Diseases improved by senolytics

| Disease | Models | | Senolytic used | References |
|---|---|---|---|---|
| | In vitro | In vivo | | |
| Diabetes | Mice β cells Human β cells | Ink-ATTAC Mice | Ink-ATTAC and ABT-263 | Aguayo-Mazzucato et al. (2019) |
| Age-related bone loss | | Mice | Ink-ATTAC and D + Q | Farr and Khosla (2019) |
| Cancer | | Mice | siRNA against HSP47, Ink-ATTAC, Drug delivery system | Muñoz-Espín et al. (2018), Yoshimoto et al. (2013), Baker et al. (2016) |
| | Human cervical cancer | | Rapamycin (to suppress IL-6 from SASP) | Laberge et al. (2015) |
| Pulmonary fibrosis | Human and murine lung cells | Ink-ATTAC mice | ABT-263, D + Q | Pan et al. (2017, Muñoz-Espín et al. (2018), Schafer et al. (2017) |
| | | Human | D + Q | Justice et al. (2019) |
| Hepatic steatosis | | Mice | Ink-ATTAC and D + Q | Cellular senescence drives age-dependent hepatic steatosis. Nat Commun.(2017) |
| Atherosclerosis | | Mice | Ink-ATTAC and ABT-263 | Childs et al. (2016) |
| Osteoarthritis | | Mice | Ganciclovir with HSV-TK | Jeon et al. (2017) |
| | | Human | Fenofibrate | Nogueira-Recalde et al.(2019) |
| Alzheimer's disease | | Mice | D + Q | Zhang et al. (2019) |
| Tau-mediated neurodegeneration | | Mice | Ink-ATTAC, ABT-263, D + Q | Mendelsohn and Larrick (2018, Bussian et al. (2018) |
| Osteoporosis | | Ink-ATTAC Mice | Ink-ATTAC, JAKi | Farr et al. (2017) |
| Dysglycemia | | Mice | JAKi | Xu et al. (2015) |
| Cardiovascular diseases | | Mice | Ink-ATTAC, D + Q, ABT-263 | Roos et al. (2016, Walaszczyk et al. 2019) |

(continued)

**Table 4.2** (continued)

| Disease | Models | | Senolytic used | References |
|---|---|---|---|---|
| | In vitro | In vivo | | |
| Glomerulosclerosis | | Ink-ATTAC Mice | Ink-ATTAC | Baker et al. (2016) |
| Fatty liver disease | | Mice | JAKi, D + Q | Papatheodoridi et al. (2019) |
| Renal diseases | | Mice | Ink-ATTAC | Valentijn et al. (2018) |

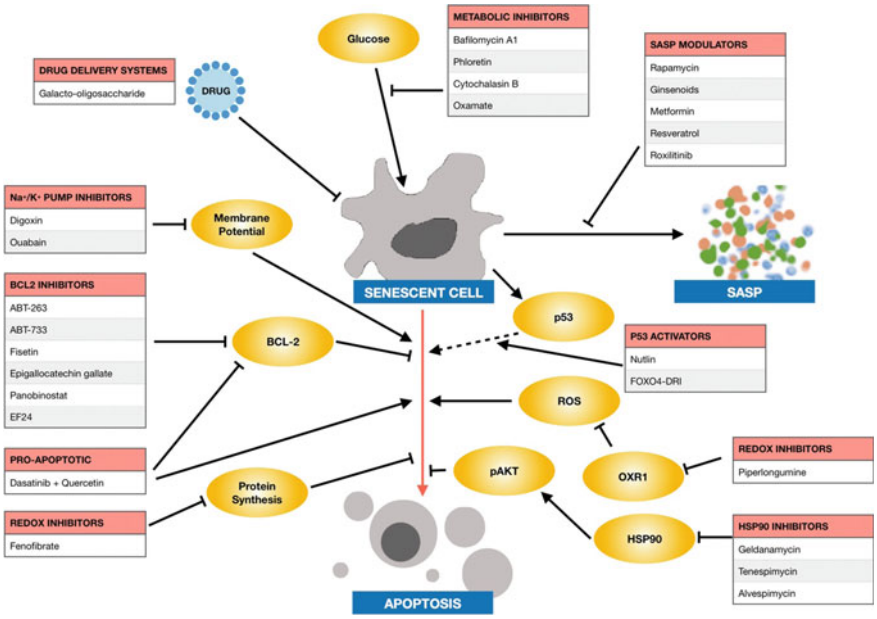


**Fig. 4.1** Schematic overview of the different strategies pursued to eliminate senescent cells or alleviate the detrimental effects of the SASP

et al. 2015). In non-small cell lung cancer and head and neck squamous cell carcinoma, senescent cells have altered H3 acetylation and Bcl-xl expression. Panobinostat inhibits Bcl-xl and kill senescent cells induced by chemotherapy (Samaraweera et al. 2017). However, this drug may cause a few adverse effects, including diarrhea, asthenia and a lower count of immune blood cells (Van Veggel et al. 2018; Hennika et al. 2017).

Finally, EF24, a natural compound found in turmeric (*Curcuma longa*) that is similar to curcumin, can kill senescent cells by downregulating Bcl-xl (Li et al. 2019). EF24 could be used in synergy with ABT-263 to kill senescent cells more effectively, and at the same time, prevent ABT-263's cytotoxic effects (Li et al. 2019).

### 4.3.2 Proapoptotic Cocktail Dasatinib + Quercetin

Based on the fact that senescent cells are resistant to apoptosis induced by serum deprivation and other stresses, Zhu et al. hypothesized that it could be possible to kill them by inhibiting their antiapoptotic pathways. A screening using molecules that block these pathways showed that the drugs dasatinib and quercetin were particularly efficient to kill senescent cells and improves health span in progeroid mice (Zhu et al. 2015). The synergistic combination proved to be efficient to counteract the effect of irradiation in vivo, and to extend lifespan in naturally-aged mice (Xu et al. 2018). Dasatinib is a competitive inhibitor of tyrosine kinases and is currently used to treat leukemia (Zarbock 2012). The drug strongly suppresses the SASP and genes related to senescence in human subjects with systemic sclerosis (Martyanov et al. 2019). Quercetin is a flavonol found in some fruits and vegetables that has anti-inflammatory properties (Li et al. 2016). The cocktail with both drugs targets different anti-apoptotic pathways in senescent cells (Kirkland and Tchkonia 2017). It significantly lowered the number of senescent cells in human adipose tissues without killing macrophages. This subsequently had a positive impact on inflammation and frailty (Xu et al. 2018). However, senescent hepatocellular carcinoma cells resist the effect of the combination dasatinib/quercetin (Kovacovicova et al. 2018) indicating again the tissue specific mode of action of senolytics.

### 4.3.3 Senolytics that Activate P53: Nutlin and FOXO4DRI

Most types of stress that trigger senescence also activate the tumor suppressor p53 (Qian and Chen 2010). P53 acts as a transcription factor inducing the expression of genes such as promyelocytic leukemia (PML) (de Stanchina et al. 2004; Ablain et al. 2014), p21 (Fang et al. 1999), PAI1 (Kortlever et al. 2006) and E2F7 (Aksoy et al. 2012) that mediate the growth arrest phenotype of senescent cells. P53 can also trigger cell death but the key molecular switches that control cell fate downstream of p53 activation are not well understood (Macip et al. 2003). Reactive oxygen species (ROS) can convert a p53-dependent senescence response into apoptosis but the mechanism has not been totally elucidated (Macip et al. 2003). It has been proposed that p53 must overcome a concentration threshold to trigger apoptosis (Kracikova et al. 2013). Another pertinent characteristic of p53 in this regard is its ability to translocate to mitochondria (Mihara et al. 2003) and inhibit Bcl-2 and Bcl-xl, preventing their anti-apoptotic activities (Hagn et al. 2010). P53 translocation to mitochondria is inhibited by the nucleolar protein nucleophosmin (Dhar and St Clair 2009) and the E3 ubiquitin ligase TRAF6 (Zhang et al. 2016).

Nutlin (Nutley inhibitor) was designed by Vassilev and colleagues to prevent the binding of Mdm2 to p53. The nutlin binding site is situated in a deep hydrophobic pocket in the Mdm2 protein (Vassilev et al. 2004). Mdm2, an E3 ubiquitin ligase, inhibits p53 through three mechanisms: (1) degradation of p53 by poly-ubiquitylation

and targeting it to the proteasome; (2) export of p53 out of the nucleus by mono-ubiquitylation and (3) direct binding to p53 preventing its activity as a transcription factor (Wu and Prives 2018). Since p53 is needed to express Mdm2, levels of p53 are autoregulated in a feedback loop (Lessel et al. 2017).

Nutlin is not genotoxic and does not promote p53 modifications associated to DNA damage, it only stabilizes p53 by protecting it from Mdm2. Although senescent cells activate p53, this activation is limited (Huang and Vassilev 2009) suggesting that p53 cannot attain the levels needed to trigger apoptosis. It is then quite possible that nutlin could stabilize latent p53 in senescent cells and trigger apoptosis. This has been shown in cultures of senescent chondrocytes obtained from patients with osteoarthrosis or in vivo in a mouse model of osteoarthrosis triggered by transection of the anterior cruciate ligament (Jeon et al. 2017). However, p53 reactivation could lead to toxic effects in normal cells, including death by apoptosis (Burgess et al. 2016), or could generate a selective pressure for mutations of p53 (Aziz et al. 2011). Also, nutlin could potentially bind to other protein pockets with similar shapes and physicochemical properties, leading to potential toxic effects (Nguyen et al. 2019).

Proteolysis Targeting Chimera (PROTAC) consist of two protein binding fragments, one capable of binding to a target protein and another that binds to an E3 ubiquitin ligase (Bondeson et al. 2018). PROTACs having an Mdm2-binding fragment such as nutlin could have a double effect. First, they can activate p53 by preventing its inhibition by Mdm2. Second, they could target another protein for degradation by the proteasome by promoting its interaction with Mdm2. Such a PROTAC targeting BRD4 was shown to be very effective against cancer cells with wild type p53 (Hines et al. 2019) but it could also be modified by coupling nutlin to anti-Bcl2 family compounds such as ABT-273 to better kill senescent cells.

FOXO4 is a member of the Forkhead box O (FOXO) family of transcription factors which are negatively regulated by insulin or IGF-1 via AKT-dependent phosphorylation and cytoplasmic retention (Martins et al. 2016). In the nucleus, FOXO factors can localize to PML nuclear bodies (Trotman et al. 2006) which are particularly induced in senescent cells (Bourdeau et al. 2009). FOXO factors are involved in resistance to stress, metabolism, cell cycle arrest and apoptosis (Martins et al. 2016).

In response to acute damage, FOXO4 was shown to favor senescence instead of apoptosis. During senescence, p53 is phosphorylated by ATM (Ataxia-Telangiectasia Mutated), which would prevent its inhibition by Mdm2 (Mallette et al. 2007). In this situation, p53 localizes to chromatin having persistent DNA damage (DNA-SCARS), next to PML bodies containing FOXO4. FOXO4 would then limit p53's ability to promote apoptosis by sequestering p53 in PML bodies (Baar et al. 2017). Based on this, Baar and colleagues synthesized a FOXO4-derived peptide that would prevent the binding of FOXO4 to p53 (Baar et al. 2017). This peptide was designed as a D-retro inverse isoform (DRI) so that it would have a better potency than its natural L-isoform counterpart. The FOXO4-DRI peptide was effective in relocating p53 to mitochondria, promoting apoptosis selectively in senescent cells both in vitro and in vivo. This peptide had potent anti-aging effects both in progeroid and wild-type mice illustrating once again the causal relationship between senescent cells and aging (Baar et al. 2017). FOXO4-DRI showed a tenfold selectivity to senescent cells

compared to normal cells. Although the peptide was safe in rodents upon repeated administration, long living humans may require even more injections. It would be preferable to optimize this peptide to achieve a higher degree of selectivity to avoid potential toxicities in humans (Baar et al. 2018).

### *4.3.4 Metabolic Inhibitors: Targeting Glycolysis and REDOX Metabolism*

Senescent cells have a dramatic upregulation of glucose utilization in association to their mitochondrial dysfunction (Moiseeva et al. 2009). For instance, therapy-induced senescence (TIS) in lymphoma cells is accompanied by an increase in glucose utilization and autophagy that together support the ATP and metabolic demands of senescent cells. A combination of deoxyglucose with the autophagy inhibitor bafilomycin A1 selectively killed these senescent cells (Dörr et al. 2013). The glucose transport inhibitors phloretin and cytochalasin B or the lactate dehydrogenase inhibitor oxamate were also selectively toxic for TIS cells (Dörr et al. 2013). This was actually the first demonstration of a pharmacological approach to kill senescent cells.

The screening of a library of small molecules supposed to target important pathways for senescent cells showed that piperlongumine was a promising candidate as a senolytic (Wang et al. 2016). The drug induced apoptosis selectively in senescent fibroblasts compared to control cells. A significant synergy between piperlongumine and ABT-263 could allow for a lower dose of ABT-263. The latter causes thrombocytopenia and neutropenia because of inhibition of Bcl-xl in platelets (Wang et al. 2016). Although piperlongumine's mode of action is not fully understood, the drug was shown to bind the protein Oxidation Resistance 1 (OXR1) leading to its degradation by the ubiquitin-proteasome system (Liu et al. 2018). Targeting OXR1 kills senescent cells by promoting oxidative stress (Yang et al. 2014; Zhang et al. 2018).

Senescent chondrocytes have been linked to osteoarthritis, a disease for which there is no cure. Nogueira-Recalde and colleagues found fenofibrate, an agonist of PPARα (peroxisome proliferator-activated receptor alpha) as a senolytic after interrogating the Prestwick chemical library for molecules that kill senescent cells. They showed that fenofibrate effectively and selectively killed senescent chondrocytes by promoting apoptosis in vitro. Since this drug was found by screening chemical compounds it is not yet clear how it selectively kills senescent cells. The authors correlated the effects of fenofibrate with inhibition of the mTOR effector S6 kinase (Nogueira-Recalde et al. 2019). Since mTOR is required for protein synthesis, this strategy may work by inhibiting the expression of anti-apoptotic proteins. In a retrospective study, human osteoarthritis patients taking fenofibrate reported a significant decrease in disability and pain leading to fewer joint surgeries (Nogueira-Recalde et al. 2019). The use of fenofibrate as a senolytic could be however limited by its hepatotoxicity (Hedrington and Davis 2018).

### 4.3.5 HSP90 Inhibitors

Robbins and colleagues screened a library of autophagic regulators for compounds that killed senescent *ercc1* null murine embryonic fibroblasts. They identified the HSP90 inhibitors geldanamycin, 17-AAG (tenespimycin) and 17-DMAG (alvespimycin) as potent senolytics that triggered apoptosis in senescent cells (Fuhrmann-Stroissnigg et al. 2017). The mechanism of senolytic activity of HSP90 inhibitors included the destabilization of phospho-AKT (Fuhrmann-Stroissnigg et al. 2017). Of note, treating progeroid *ercc1*$^{-/\Delta}$ mice with 17-DMAG reduced the expression of senescence biomarkers in the kidneys and delayed the onset of age-related phenotypes (Fuhrmann-Stroissnigg et al. 2017).

### 4.3.6 Sodium/Potassium ATPase Inhibitors

Produced naturally by many plants, cardiac glycosides have been recently found to be senolytic agents. They prevent cytoplasmic transmembrane $Na^+/K^+$ pumps to maintain the resting potential across the membrane by binding to their alpha 1 subunit (Langford and Boor 1996). Using a high throughput screening method on compounds found in the Prestwick library, Triana-Martinez et al. identified 9 cardiac glycosides as senolytics. Among them, digoxin had the highest senolytic index (Triana-Martinez et al. 2019). Cardiac glycosides selectively trigger apoptosis of senescent cells by increasing intracellular concentration of $Na^+$ ions. This in turn would inhibit $Na^+/Ca^{2+}$ and $Na^+/H^+$ exchangers, leading to increasing concentrations of $Ca^{2+}$ and $H^+$. Since senescent cells already have a lower cytosolic pH than normal cells, digoxin could activate both the intrinsic and the extrinsic apoptosis pathways in these cells only (Majdi et al. 2016). In a similar fashion, ouabain, another cardiac glycoside, was also found to have senolytic properties (Guerrero et al. 2019). Interestingly, in this study ouabain was shown to induce the expression of the proapoptotic protein Noxa, suggesting that changes in gene expression underpin the senolytic activity of cardiac glycosides.

### 4.3.7 Immunotherapy

Human senescent fibroblasts express higher levels of the cell surface marker dipeptidyl peptidase 4 (DPP4) (Kim et al. 2017). An antibody-dependent cell-mediated cytotoxicity (ADCC) assay showed that NK cells can recognize and selectively kill these DPP4 positive senescent fibroblasts (Kim et al. 2017). As senescence can also occur in immune cells, the immune system can become impaired, leading to accumulation of senescent cells. Thus, targeting specifically immune senescent cells could have an indirect senolytic effect on senescent cells from other tissues. In mice,

removal of senescent hematopoietic stem cells had a rejuvenating effect on aged tissues (Chang et al. 2016). Also, clearance of senescent cells in irradiated mouse spleen restored the functions of T cells and macrophages (Palacio et al. 2019). It seems also possible to engineer immune cells against senescent cells expressing IL-6, and then promote their death via cell fusion (Qudrat et al. 2017).

### *4.3.8 Drug Delivery System Targeting Senescent Cells*

Another interesting way of killing senescent cells is to encapsulate a cytotoxic compound in a shell that would preferentially target these cells. Since they have a high level of lysosomal β-galactosidase compared to normal cells, Muñoz-Espín et al. used nanoparticles covered with galacto-oligosaccharides on a silica scaffold. These beads are integrated by most cells via endocytosis and then quickly released via exocytosis. However, in the case of senescent cells, β-galactosidase will digest the polysaccharide allowing the release of drugs inside the nanoparticles before exocytosis. Fluorophores were used to show that these nanoparticles identified senescent cells in vivo and when loaded with doxorubicin they selectively killed senescent cells (Muñoz-Espín et al. 2018).

### *4.3.9 SASP Modulation*

Another way to fight the detrimental effects of senescent cells is to attenuate their inflammatory secretions. The hundreds of cytokines, chemokines, growth factors and metalloproteases come mainly from two distinct pathways: NF-κB and C/EBPβ (Paez-Ribes et al. 2019). Targeting these pathways or their upstream regulators could help reduce inflammation linked to aging. Rapamycin decreases secretion of interleukin-6 (IL-6) and other inflammatory cytokines by inhibiting their translation (Herranz et al. 2015; Laberge et al. 2015). MAPK pathway inhibitors, such as ginsenosides, were able to suppress the SASP in senescent astrocytes (Hou et al. 2018) or hematopoietic stem cells (Tang et al. 2015). The NF-κB pathway could also be inhibited by the antidiabetic drug metformin, preventing the expression of several SASP cytokines (Moiseeva et al. 2013; Oubaha et al. 2016). Resveratrol, a polyphenol, was able to inhibit the SASP through SIRT1/NF-κB pathway on melanoma cells (Menicacci et al. 2017) or in the gut of the fish *N. guentheri* (Liu et al. 2018). Administration of a JAK inhibitor, ruxolitinib, reduced inflammation in aged mice by down-regulating the C/EBPβ pathway (Xu et al. 2015). Glucocorticoids such as corticosterone or cortisol were shown to suppress IL-6 as well as several other SASP components. They inhibit IL-1α signaling upstream of NF-κB, which in turn, stimulates the expression of IL-1α, in a positive feedback loop (Laberge et al. 2012).

## 4.4 Clinical Trials and Future Directions

Since aging is still not recognized as a disease, very few of the aforementioned senolytics have made their ways to clinical studies. Unity biotechnology is currently testing the effect of a compound called UBX0101 on osteoarthritis. After a successful phase I (NCT03513016) they are recruiting patients for a phase II trial (NCT04129944). UBX0101 is supposed to eliminate senescent cells that accumulate in joints, which should decrease local inflammation and alleviate the pain. The Mayo clinic is testing the cocktail D + Q in 2 different clinical trials. One is aimed at chronic kidney diseases (NCT02848131) and the other one is targeted against Alzheimer's disease (NCT04063124). Mayo clinic is also investigating the senolytic effect of fisetin on frail elderly syndrome (NCT03430037). They are currently recruiting for phase II on these 3 studies. A preliminary report of the phase I trial with D + Q in 9 patients with diabetic kidney disease claims a reduction in adipose tissue senescent cells burden 11 days after completion of a 3-days senolytic treatment (Hickson et al. 2019). If confirmed in a large number of patients, this study suggests that all the beneficial effects observed in mice treated with senolytics will be also attained in humans.

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