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Targeting normal and cancer senescent cells as a strategy of senotherapy

Ewa Sikora*,[1], Anna Bielak-Zmijewska[1], Grazyna Mosieniak[1]

Laboratory of Molecular Bases of Ageing, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

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ABSTRACT

Senotherapy is an antiageing strategy. It refers to selective killing of senescent cells by senolytic agents, strengthening the activity of immune cells that eliminate senescent cells or alleviating the secretory phenotype (SASP) of senescent cells. As senescent cells accumulate with age and are considered to be at the root of age-related disorders, senotherapy seems to be very promising in improving healthspan. Genetic approaches, which allowed to selectively induce death of senescent cells in transgenic mice, provided proof-of-concept evidence that elimination of senescent cells can be a therapeutic approach for treating many age-related diseases. Translating these results into humans is based on searching for synthetic and natural compounds, which are able to exert such beneficial effects. The major challenge in the field is to show efficacy, safety and tolerability of senotherapy in humans. The question is how these therapeutics can influence senescence of non-dividing post-mitotic cells. Another issue concerns senescence of cancer cells induced during therapy as there is a risk of resumption of senescent cell division that could terminate in cancer renewal. Thus, development of an effective senotherapeutic strategy is also an urgent issue in cancer treatment. Different aspects, both beneficial and potentially detrimental, will be discussed in this review.

1. Introduction

In the modern world the population of very old people is increasing and we have realized that successful intervention in the lifespan is possible. A desirable outcome of such intervention would be ageing free of age-related diseases, such as metabolic and cardiovascular disease, neurodegeneration, cancer and many others. Geroscience aims to understand the relationship between ageing, chronic age-related diseases (ARDs) and geriatric syndromes (GSs) and assumes that ageing and ARDs/GSs share a common set of basic biological mechanisms (Franceschi et al., 2018). It has been suggested that these mechanisms include cell senescence. Indeed, recently it has been proposed that elimination of senescent cells in genetically modified mice can rejuvenate the organism (Childs et al., 2015). The just coined term "senotherapy" refers to the process of body rejuvenation by pharmacological treatment with senolytic drugs selectively eradicating senescent cells (Sturmlechner et al., 2017; Zhu et al., 2015).

2. Cellular senescence

The term "cellular senescence", introduced almost 60 years ago, referred to the limited capacity of cell population to divide in culture (Hayflick and Moorhead, 1961), which later on has been attributed to telomere shortening (Harley et al., 1990). Since then, it has been observed that not only telomere shortening, but also oncogenes and some sorts of stress were able to stop cell divisions and induce cell senescence. These phenomena were termed OIS (oncogene-induced senescence) (Kuilman et al., 2008) and SIPS (Toussaint et al., 2000) (stress-induced premature senescence), respectively.

Many molecular mechanisms and signalling pathways, involved in cell senescence induction and acquisition of the senescence phenotype, have been recognized (de Magalhaes and Passos, 2018). However, irreversible loss of the capability of proliferation-competent cells to divide, is the most important feature, that define senescent cells and discriminates them from quiescent (temporally arrested in the G0 phase) and terminally differentiated (so called post-mitotic) cells. Thus, cell cycle inhibitors, such as p21 and p16 proteins, along with diminished DNA replication capability, are the main hallmarks of cell senescence (Campisi, 2007). One of the earliest described and the most commonly measured (although not fully specific) marker of senescent cells is the increased activity of a lysosomal enzyme- the Senescence-Associated β-Galactosidase (SA-β-gal) (Dimri et al., 1995). Other important features of senescent cells include increased number of DNA double strand breaks (DSBs), increased level or activity of key proteins

* Corresponding author.
E-mail address: e.sikora@nencki.gov.pl (E. Sikora).
[1] All authors equally participated in the preparing the manuscript.

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involved in DNA damage response, such as ATM and p53, chromatin remodeling, dysfunctional mitochondria and lysosomes, and increased capacity to secret many factors, including pro-inflammatory cytokines (Bielak-Zmijewska et al., 2018). The latter feature is commonly called senescence associated secretory phenotype, SASP (Rodier et al., 2009). SASP is the most undesirable feature of cell senescence as it is able to induce bystander senescence and create low grade inflammatory state, which is at the root of many age-related diseases (Franceschi and Campisi, 2014). However some beneficial aspects of SASP, such as improvement of wound healing (Rodier and Campisi, 2011) and protection from fibrosis (Krizhanovsky et al., 2008) cannot be neglected.

Replicative senescence was for the first time described in human fibroblasts and these cells are still the favorites of many researchers. Moreover, in this model of senescence, the resistance of senescent cells to apoptosis was documented for the first time (Wang, 1995) and later on proved for other cell types (reviewed in Yosef et al., 2016). Beside fibroblasts, also other cell types, like keratinocytes and melanocytes (Bandyopadhyay et al., 2001), lymphocytes (Brzezinska et al., 2003; Effros et al., 2003), epithelial cells (Romanov et al., 2001), endothelial cells (Erusalimsky, 2009), adipocytes (Tchkonia et al., 2010), vascular smooth muscle cells (Bielak-Zmijewska et al., 2014; Gorenne et al., 2006), mesothelial cells (Ksiazek et al., 2006) and mesenchymal stem cells (Shibata et al., 2007) undergo replicative and stress-induced senescence.

Using different sets of markers of senescence, senescent cells were found *in vivo* in human, baboon and mouse skin, human and rodent vascular endothelial cells, smooth muscle cells, skeletal muscle, fat tissue and liver (Jeyapalan and Sedivy, 2008), in skeletal muscle of rodents and primates (Kreiling et al., 2011), in human immune system (Brzezinska et al., 2004) and in hematopoietic stem cells (Akunuru and Geiger, 2016). Cellular senescence is also associated with age-related diseases (Childs et al., 2015; Sikora et al., 2011). There is experimental evidence of accumulation of senescent cell at sites of such pathologies as type 2 diabetes, atherosclerosis, hypertension, chronic pulmonary disease, cataracts, glaucoma (Naylor et al., 2013), kidney (Valentijn et al., 2018) and liver (Sheedfar et al., 2013) diseases. Moreover, accumulation of senescent cells in tissues with age promotes tumor formation and growth *via* SASP and age-dependent impairment of immune function, which in turn escalates senescent cell accumulation and neoplastic surveillance, establishing a pro-tumorigenic environment (Sieben et al., 2018).

3. Transgenic mouse models used in senotherapy

In recent years, several transgenic mouse models have been developed that make possible the visualization, assessment and eradication of senescent cells *in vivo*. In these models the promoter of p16INK4A gene was used to drive the expression of genes encoding proteins, which induce death of senescent cells upon administration of small molecules (transgene activator). Beside death-inducing product of the transgene also expression of fluorescent proteins can be regulated by p16INK4A promoter in some transgenic mouse models. Thus, it is possible to visualize senescent cells in living animals. In INK-ATTAC animals, the p16INK4A promoter drives the expression of an inducible caspase-like transgene, which encodes an apoptotic protein that is activated by a small ligand, AP20187 (Baker et al., 2011). Subsequently the INK-ATTAC model was used in other studies including those in which INK-ATTAC mice were made double transgenic: INK-ATTC; db/db (obesity model) (Ogrodnik, 2019; Palmer et al., 2019) and tau MAPT P301S PS19 :ATTAC (taopathy model) (Bussian et al., 2018). Two additional models, both under control of the p16INK4A promoter, carrying suicide transgenes for a truncated herpes simplex virus thymidine kinase (p16-3MR mouse) and for nitroreductase (INK-NTR), have been developed. In these cases, selective killing of senescent cells was accomplished through treatment with ganciclovir (GCV) in the p16-3MR mouse (Demaria et al., 2014) and metronidazole in the INK-NTR mouse (Childs et al., 2016). The p16-3MR mouse model was also used as a double transgene, *e.g.Xpd*$^{TTD/TTD}$:p16-3MR and Ldlr−/− p16-3MR mice (Baar et al., 2017; Childs et al., 2015). The only published study to use a p19ARF-directed CDKN2A promoter sequence employed transgenic expression of the diphtheria toxin receptor (ARF-DTR) (Hashimoto et al., 2016).

Thus, these animal models confirm that senescent cells accumulate during ageing and in response to environmental stresses as well as at sites of age-related pathologies and in response to tissue damage or injury. Importantly, using the transgenic models that permit selective elimination of senescent cells, it has been shown that age-related pathologic symptoms could be alleviated and, consequently, the healthspan and even the lifespan could be improved (reviewed in Campisi, 2016; Schmitt, 2017). On the other hand, transplantation of even a small number of senescent cells (preadipocytes) into young mice led to spreading of cellular senescence to neighboring tissues and caused persistent physical dysfunctions. This effect could be reduced by oral administration of senolytics (Xu et al., 2018). Senolytic originates from the words “senescence” and “lytic”.

Studies using genetically altered mice established the crucial role of senescence in age-related disorders. Also the senolytic strategy, without genetic manipulations, has already been applied in many mice models. Table 1 present the compilation of so far published studies including evidences for reduced the burden of senescent cells and physiological effects in both genetic and pharmacological approaches. There is a rapidly growing body of evidence showing that the variety of disorders can be at least alleviated in these models.

4. Senolytics *in vitro* and *in vivo*

Senescent cells are resistant to apoptosis. On the other hand some SASP factors are pro-apoptotic. Moreover, pro-apoptotic pathways were shown to be up-regulated in senescent cells (Zhu et al., 2015), To solve this paradox the hypothesis was tested that senescent cells depend on pro-survival pathways, which inhibit apoptosis, in order to defend themselves against their own pro-apoptotic SASP. Using bioinformatics approaches based on the RNA and protein expression profiles of senescent cells, five Senescent-Cell Anti-Apoptotic Pathways (SCAPs) were identified: BCL-2/BCL-$X_{L,}$ PI3K/AKT/ceramide metabolic network, MDM2/p53/p21/serpine elements, Ephirins/dependence receptors/tyrosine kinases and hypoxia inducible factor (HIF-1α) pathway. The requirement of SCAPs for senescent cell viability was verified by RNA interference studies, in which key proteins in these pathways were reduced. Knocking-down expression of these proteins (“Achilles’ heels”) caused death of senescent but not of non-senescent cells. Since the discovery of the first five SCAPs, another one was identified, namely HSP-90-dependent pathway (Fuhrmann-Stroissnigg et al., 2017). This approach was subsequently used to identify putative senolytic targets (reviewed by Kirkland et al., 2017). Many of these senolytics are effective in *vitro* and some were already tested in *vivo* (Table 1).

To this group belongs a polyphenol, quercetin (a flavonoid with antioxidant properties), and dasatinib (chemotherapeutic, a pan-tyrosine kinase inhibitor). Both of them are most effective if they are applied in a cocktail, and are able to reduce expression of markers of senescence (SA-β-Gal and p16) in fat and liver tissue from old mice (Ogrodnik et al., 2017; Xu et al., 2018; Zhu et al., 2015). Moreover, Hsp90 inhibitors (Alvespimycin-17-DMAG, Geldanamycin) (Fuhrmann-Stroissnigg et al., 2017; van Willigenburg et al., 2018), piperlongumine, a natural product isolated from a variety of species of Piper (preferentially killed senescent human WI-38 fibroblasts *in vitro* only) (Wang et al., 2016), Navitoclax (also known as ABT-263) and UBX0101 (chondrocytes in *vivo* and in *vitro*) (Jeon et al., 2017) are able to specifically induce apoptosis of senescent cells.

It seems that, so far, the most promising natural compound with senolytic properties is a flavone, fisetin (Yousefzadeh et al., 2018; Zhu

Table 1
Physiological outcome of senescent cells elimination analyzed in different mouse models *in vivo*.

Mouse	Examined condition	Elimination compound		Outcome		Reference
		Transgene activator	senolitics	Evidences for eliminating senescent cells	Physiological effect/changes in phenotype	
BubR1H/H;INK-ATTAC mice	Progeria	AP20187 **(D)**		Reduction in INK-ATTAC and GFP transcript levels, reduced SA-β-gal in inguinal adipose tissue (IAT), skeletal muscle, eye (but not in tissues in which endogenous p16Ink4a is not induced e.g. liver and heart)	Delayed onset of accelerated aging: reduced lordokyphosis, premature muscle weakness, cataract, lipodystrophy, improved exercise capacity	(Baker et al. 2011)
3-4-mo-old **p16-3MR** irradiated mice	Accelerated ageing	GCV **(A)**		Reduction of luminescence, reduced SA-β-gal and mRNAs for *p16Ink4a, mRFP, Il-6, Mmp-3* in fat tissue, kidney, lung	Reduced number of senescent cells	(Demaria et al. 2014)
18-24-mo-old **p16-3MR** mice,	Natural ageing	GCV		Reduced luminescence, SA-β-gal staining, and *p16Ink4a* mRNA levels in visceral fat		
wounded, 3-4-mo-old **p16-3MR** mice		GCV **(A)**		Elimination of senescent cells (reduced luminescence) and reduced mRNA levels encoding *p16Ink4a, mRFP,* and *p21* at the injury site, less *Mmp-2* but significantly more *Mmp-10* and *Mmp-13*	Delayed skin wound healing, more fibrotic tissue	
>24-mo-old WT mice	Natural ageing		D+Q (**A**)	Reduction of SA-β-gal, *p16Ink4a* mRNA in fat tissue from old mice and from liver	Improved cardiovascular functions	(Zhu et al. 2015)
4- old mice, leg irradiation	muscle injury		D+Q (**A**)	Decreased *p16Ink4a* expression in quadriceps muscles and cellular SA-β-gal in inguinal fat	Improved exercise capacity	
6-wk-old **Ercc1**−/Δ mice	Progeria		D+Q (**C**)	Reduced expression of *p16Ink4a* and SA-β-gal activity in liver, kidney and disc nucleus pulposa	Improved physical function, significant reduction in the composite score of age-related symptoms such as improvement of cardiovascular function and exercise endurance, reduced osteoporosis and frailty, extended healthspan	
18-mo-old **INK-ATTAC** mice	Natural ageing	AP20187 **(B)**		Decreased expression of *IL6, p16Ink4a* and *Cdkn1a* gene and decreased number of SA-β-gal-positive cells in fat tissue	Reduced lipodystrophy, improved fat tissue function (increased expression of C/EBPa and PPARg, markers of adipogenesis), glucose homeostasis and insulin sensitivity, reduction of circulating avidin A (increased with age, secreted by progenitor fat cells)	(Xu et al. 2015)
22-24-mo-old WT mice	Natural ageing		Ruxolitinib (INCB) (**C**)		Enhanced adipogenesis (increased markers of adipogenesis, PPARg, C/EBPa, FABP4, adipo-Q and GPAT4), reduced loss of fat tissue, reduced circulating activin A, reduced lipotoxicity, and enhanced insulin sensitivity, increased activity of old mice	

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6-8 wk-old mice irradiated **K5-rtTA/tet-p14** transgenic mice (human p14ARF gene is indelibly expressed in the basal layer of the skin epidermis)			ABT-737 ABT-737	Decreased expression of senescence markers (SA- β-gal, γH2AX, p53, p21) and increased caspase-3 cleavage Reduced expression of SA-β-gal, decreased number of p14-expressing cells, increased caspase-3 cleavage and number of Ki-67 positive cells in hair follicle bulges, increase in the numbers of proliferating hair-follicle stem cells in the bulge		(Yosef et al. 2016)
12-mo-old **ARF-DTR** mice 20-22-mo-old **ARF-DTR** mice	 Natural ageing	Diphtheria toxin **(B)**		Reduction of luminescence in lungs, reduced *ARF*, *INK4a*, and *Cdkn1a* mRNA levels in the lung tissue Lack of luciferase signal from lung tissue	Restoration of lung function (recovered lung elasticity) and morphology, decreased expression of MMP-10 and -12, rejuvenated gene expression profile in lung tissue	(Hashimoto et al. 2016)
24-mo-old mice 24-mo-old **INK-ATTAC** mice 24-mo-old **ApoE -/-** mice	Natural ageing Natural ageing Atherosclerosis	 AP20187 **(C)**	D+Q **(C)** D+Q **(B)**	Reduction of TAF (telomere associated foci) and γH2AX foci per cell in aorta	Improved vasomotor function Decreased intimal plaque calcification and improved vasomotor function	(Roos et al. 2016)
Sublethally irradiated 2-3-mo-old **p16-3MR** mice 21-22-mo-old WT mice	Premature ageing Natural ageing	GCV **(B)**	ABT-263 **(B)** ABT-263 **(B)**	Reduction of luminescence and *Cdkn2a* mRNA level and SA–β-gal activity, γH2AX and p-p38 level in HSCs and MuSCs	Improved clonogenicity of HSCs and MuSCs, diminished number of senescent cells in lung tissue, reduced SASP	(Chang et al. 2016)
10-wk-old Ldlr−/− **p16-3MR** HFD mice 70-day-old Ldlr-/- **INK-ATTAC** HFD mice 70-day-old Ldlr-/- **INK-NTR** HFD mice	Atherosclerosis	GCV **(A, B, D)** AP20187 **(D)** Metronidazole **(D)**	ABT-263 **(D)**	Elimination of senescent cells in atherosclerotic plaques measured by SA- β-gal activity, *p16Ink4a, p19Arf, Cdkn1a, IL1α, Mmp3, Mmp13* mRNA level	Decreased atherosclerotic lesion formation (reduction in plaque number and size, smaller fatty streaks and protection from plaques rupture)	(Childs et al. 2016)
2.5-8-mo-old **INK-ATTAC** bleomycin-treated mice	Fibrotic pulmonary disease	AP20187 (B)	D+Q (B)	Reduced expression of *p16Ink4a*, SASP factors *Mcp1, Il6, Mmp12* and reduced expression of profibrotic factors *Col1a1* and *Tgfb* in lung, lowered number of macrophages, lymphocytes and neutrophils present in bronchoalveolar lavage (BAL) fluid, dimished level of IL6 and MCP1 in BAL	Functional improvements of lung function as measured by whole-body plethysmography, increased body weight and improved exercise capacity	(Schafer et al., 2017)

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WT mice (thoracic irradiation)	pulmonary fibrosis (PF) an outcome of radiotherapy		ABT263 **(B)**	Reduced expression of *Cdkn2a, Bcl2, Il1a*, and *Il1b* mRNA, reduced number of SA- β-gal-positive AECIIs cells	Reversed persistent pulmonary fibrosis	(Pan et al. 2017)
24-mo-old **INK-ATTAC**	Natural ageing	AP20187 **(C)**	D+Q **(C)**	Reduced karyomegalic and TAF-positive hepatocytes, a significant reduction in hepatic fat deposition	Reduced hepatic steatosis	(Ogrodnik et al. 2017)
6-mo-old **INK-ATTAC** HFD	Obesity	AP20187 **(C)**		Decreased the frequency of senescent markers in hepatocytes, including TAF, karyomegaly, mRNA expression of *p16Ink4a* and SA- β-gal activity		
4-mo-old **db/db** mice	type 2 diabetes		D+Q **(C)**	Reduced TAF-positive hepatocytes		
20-22-mo-old **INK-ATTAC** mice	Natural ageing	AP20187 **(D)**		Reduced *p16Ink4a* mRNA and fluorescence in the bone, reduced senescence-associated distention of satellites –SADS-positive osteocytes, reduced *p16Ink4a* mRNA level and number of SA-β-gal-positive cells in adipose tissue reduction of SASP	Higher bone mass and strength, better bone microarchitecture due to lower bone resorption with either maintained (trabecular) or higher (cortical) bone formation, reduction of osteoporosis and frailty	(Farr et al. 2017)
20-mo-old male WT mice			D+Q **(D)**			
22-mo-old WT mice			Ruxolitinib (INCB) **(C)**			
10-wk- or 19-mo-old **p16-3MR** mice	Induced post-traumatic osteoarthri-tis (OA)	GCV **(B)**	UBX0101 **(B)**	Reduced luminescence and *p16Ink4a* mRNA and protein expression, reduced expression of *Cdkn2a*, *Cdkn1a*, *Il6* and *Mmp13*	Inhibition of articular cartilage erosion Blocking of SASP	(Jeon et al. 2017)
10-wk-old WT mice with induced post-traumatic OA			UBX0101 **(B)**	A higher number of cells expressing Ki-67 and proliferating cell nuclear antigen (PCNA), fewer p16Ink4a - and MMP13-positive chondrocytes, reduced levels of *Cdkn2a, Cdkn1a, Il6 and Mmp13* mRNAs	Reduced development of post-traumatic osteoarthritis (OA) and related pain, and establishment of a prochondrogenic environment	
12-mo-old **INK-ATTAC** mice	Natural ageing	AP20187 **(D)**			Reduced age-related cartilage degeneration	
10–16-wk-old doxorubicin treated **p16-3MR** mice	Chemotherapy- induced cytotoxicity	GCV **(A)**		Reduced bioluminescence and the expression of *p16INK4a* , reduced number of cells with DNA damage foci and decreased expression of selected SASP factor genes associated	Reduced burden of circulating inflammatory factors, functional recovery of HPCs and prevention of cardiac dysfunction, limitation of drug toxicity, prevention or delay of cancer relapse and metastasis; improvement of physical activity	(Demaria et al. 2017)
10–16-wk-old doxorubicin treated **p16-3MR** mice with implanted cancer	Cancer relapse	GCV **(A)**	ABT263 **(A)**			

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Table 1 (*continued*)

10–40 wk- old doxorubicin-treated **p16-3MR** mice	Chemotoxicity	GCV **(A)**	FOXO4-DRI **(A)**	Reduced luminescence in liver	Reduced liver toxicity	(Baar et al. 2017)
26–60 wk-old ***Xpd*** $^{TD/TTD}$**:p16-3MR mice**	Accelerated ageing	GCV **(A)**	FOXO4-DRI **(A)**	Reduced luminescence in mice, reduced SA-β-gal activity and IL-6 expression and increased apoptosis (TUNEL ex vivo) in senescent cells in kidneys.	Restored fitness (reduced features of frailty), hair density and renal function	
115–130 wk-old **p16-3MR** mice	Natural ageing	GCV **(A)**	FOXO4-DRI **(A)**	Increased number of LMNB1 positive cells, reduced *Il-6* expression	Restored kidney function	
5- mo-old **ARF-DTR** elastase-treated mice	emphysema	Diphtheria Toxin **(B)**		Reduced level of *ARF* and *DT-INK4a* mRNA in lung tissues in treated ARF- DTR mice but not in wild -type mice	Attenuation of chemically induced emphysema	(Mikawa et al. 2018)
6- mo-old **ARF-DTR** elastase-treated mice			ABT-263 **(C)**			
13–14-mo-old **ARF-DTR** mice		Diphtheria Toxin **(B)**			Decreased lung compliance	
12-wk-old multidrug resistance 2 gene knockout (Mdr-/-) mice	biliary liver fibrosis		A-1331852 **(B)**	Decreased number of p16-positive cholangiocytes and decreased SASP level in serum	Reduction of fibrosis-inducing growth factors and cytokines, decrease of α-smooth muscle actin–positive ASFs, and significant reduction of liver fibrosis	(Moncsek et al. 2018)
17-mo-old WT mice with transfected senescent preadipocytes LUC+			D+Q **(A)**	Reduced luminescence	Alleviated physical dysfunction	(Xu et al. 2018)
20-mo-old mice	Natural ageing		D+Q **(D)**	Lower expression of several key SASP components in the visceral adipose tissue	Alleviated physical dysfunction	
24-27-mo-old mice			D+Q **(D)**		36% higher median post-treatment lifespan and lower mortality hazard	
26-28-mo-old **INK-ATTAC** mice		AP20187 **(D)**		Reduction of the burden of p16-positive cells	Alleviated physical dysfunction	
3-wk-old **MAPT P301S PS19 :ATTAC** mice	tau-dependent neurodegenerative disease	AP20187 **(D)**	ABT-263 **(D)**	Reduced expression *of p16Ink4a, p19Arf, Cdkn1a* and the pro-inflammatory genes *Pai1* (Serpine1), *Il-6, and Il-1β,* in hippocampus, reduced SA-β-gal activity and *p16Ink4a* expression in astrocytes and microglia,	Prevention of gliosis, hyper-phosphorylation of both soluble and insoluble tau leading to neurofibrillary tangle (NFT) deposition, and degeneration of cortical and hippocampal neurons, preserved cognitive function	(Bussian et al. 2018)
6-mo-old **MAPT P301S PS19 :ATTAC** mice		AP20187 **(A)**	ABT-263 **(D)**	prevention of the upregulation of senescence-associated genes; attenuated tau phosphorylation		

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10-wk-old **Ercc1−/Δ; p16Ink4a-** luciferase mice	Progeria		Fisetin **(C, D)**	Reduced luminescence and expression of *p16Ink4a*, *Cdkn1a* and *Il1β, Il6, Il10, Tnfα, Cxcl2, Mcp1, and Pai1* in white fat tissue		(Yousefzadeh et al. 2018)
22-24-mo-old WT mice	Natural ageing		**(A)**	Reduced number of SA-β-gal positive cells in white fat tissue. (*p16Ink4a* and *Cdkn1a*) and lowered senescence-associated secretory phenotype (SASP) (*Il1β, Il6, Il10, Tnfα, Cxcl2, Mcp1, and Pai1*) in many tissues		
22–24-mo-old **INK-ATTAC** mice	Natural ageing		**(A)**	Reduced *p16Ink4a* expression in mesenchymal stem/ progenitor, immune, and endothelial cells	Extended lifespan and reduced age-related pathology of many tissues	
85-wk-old (>20 mth) WT mice	Natural ageing		**(D)**	Reduced expression of senescence and SASP markers in multiple tissues; reduction of senescence and SASP factors expression in peripheral CD3+ T cells		
8-mo-old **p16-3MR** paraquat (PQ)-treated mice	neuropathological features of Parkinson's disease	GCV **(A)**		Abrogated markers of senescence in SN: *p16INK4a* and *Il-6* mRNA, Lamin B1 (astrocytes), HMGB1	Abrogated PQ-mediated neuropathology and motor deficits.	(Chinta et al. 2018)
3-mo-old Rapidly Aging SAMP8 mouse	sporadic AD and dementia		Fisetin **(D)**		Reduced cognitive deficits in old SAMP8 mice, restored level of multiple markers associated with impaired synaptic function, stress, and inflammation.	(Currais et al. 2018)
20-mo-old **Tau$_{NFT}$-**	Taupathy model		D+Q **(D)**	Reduction in neurofibrillary tangle (NFT) burden and SASP expression	Improvements to brain structure and cerebral blood flow and neuroprotection (NeuN, Synaptosin, PSD95)	(Musi et al. 2018)
8-mo-old **INK-ATTAC** mice (2 mo HFD)	Diet-induced obesity	AP20187 **(C)**		Decrease of senescence markers SA-β-gal, *p16INK4a* expression, and TAF in perigonadal adipose tissue; decreased level of SASP factors in plasma, reduction of the number of periventricular glia cells accumulating lipid droplets and positive for TAF, IL-6 and CXCL1, clearance of p16-positive and TAF-positive cells in hypothalamus and amygdala	Alleviated anxiety-related behavior	(Ogrodnik 2019)
db/db mice	Obesity and type 2 diabetes		D+Q **(C)**		Restored neurogenesis and alleviated anxiety-related behavior	
INK-ATTAC;db/db mice	Obesity type 2 diabetes	AP20187 **(C)**		Decrease of senescence markers SA-β-gal, *p16INK4a*, and TAF in perigonadal adipose tissue; decreased number of lipid droplets in ependymal cells and lower level of the senescence marker TAF in glia in close proximity to the LV	Alleviated anxiety-related behavior	
23-34-mo-old **INK-ATTAC** mice	Natural ageing	AP20187 **(B)**	D+Q **(B)**	Decreased expression of *p16INK4a* mRNA in heart	Activation of cardiac precursor cells	(Lewis-McDougall et al. 2019)

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p16-3MR DIO mice	Diet-induced obesity (DOI)	GCV **(C)**		Decreased senescence in adipose tissue measured, depending on the model, by luminescence, expression of eGFP component, mRFP and *p16INK4a*, SA-β-gal activity, percent of stromal vascular fraction (SVF) cells highly expressing FLAG (a component of the *p16Ink4a* promoter-driven ATTAC fusion protein), CENP-B, and p21	Improved glucose tolerance and reduced hemoglobin A1c (HbA1c), a marker of long-term glucose control, improved peripheral insulin sensitivity, lower level of insulin in response to a glucose challenge, alleviated metabolic and adipose tissue dysfunction;	(Palmer et al. 2019)
INK-ATTAC DIO mice	obesity	AP20187 **(A, B)**	D+Q **(A, C)**		Decreased macrophage homing to adipose tissue	
INK-ATTAC; *db*/*db* mice	obesity	AP20187				
p16-3MR db/db mice	DIO	GCV				
WT DIO mice	obesity		D+Q			
db/db mice			D+Q			
5-mo-old **APP/PS1** AD mice	familial AD		D+Q **(B)**	Reduced SA-β-gal activity associated with Aβ, as well as the levels of Olig2 and p21, decrease in the levels of Aβ-plaque-associated *p16INK4a* mRNA , active caspase 3 in SA- β-gal-positive cells	Reduced neuroinflammation, lessened Aβ load and ameliorated cognitive deficits	(Zhang et al. 2019)
3.5-mo-old **APP/PS1** AD mice			**(C)**			
27-mo-old **INK-ATTAC** mice	Natural ageing	AP20187 **(B)**		Reduced TAF, decreased *p16INK4a* expression	Reduced cardiac hypertrophy and fibrosis	(Anderson et al. 2019)
2-mo-old **INK-ATTAC** mice (thoracic irradiation)	a model of cardiac hypertrophy induced by thoracic irradiation.	AP20187 **(D)**		Reduced TAF induced by thoracic irradiation,	Rescued hypertrophy	
23-mo-old WT mice	Natural ageing		ABT263 **(B)**	reduced TAF in cardiomyocytes, increased cell proliferation	Reduced hypertrophy and fibrosis, increased regenerative properties	
23-mo-old WT mice	Natural ageing, myocardial infarction		Navitoclax **(B)**	Decreased expression of *p16INK4a* and *Cdkn1a*, reduction of SASP (TGFbeta2)	Elimination of senescent cardiomyocytes and attenuation of profibrotic protein expression in aged mice, improved myocardial remodeling, diastolic function and overall survival following acute myocardial infarction	(Walaszczyk et al. 2019)
12-wk-old non-obese diabetic (NOD) mice	Spontaneous autoimmune diabetes		ABT-737 **(B)**	Decrease in the percentage of *Cdkn2a* expressing beta cells, elimination of SASP	Inhibition of the progression of immune-mediated beta cell destruction and prevention of T1D by preserving beta cell mass.	(Thompson et al. 2019)
			ABT-199 (venetoclax) **(B)**	reduction of SASP (Igfbp3, IL-6, and Serpine1), reduction of SA-β-gal- positive cells, lowered expression of *Cdkn1a* and *Cdkn2a*		

Table presents summarized data published up to May 2019. Letters in the brackets in columns presenting gene activator or senolitics used in experiments, indicate different timing of compound application: (A) acute or up to 1 week treatment, (B) – up to one month, (C) – 1–3 months, (D) – more than 3 months. Rows marked in grey present data, in which physiological effect of elimination of senescent cells in transgenic animals was phenocopied by senolitic treatment.

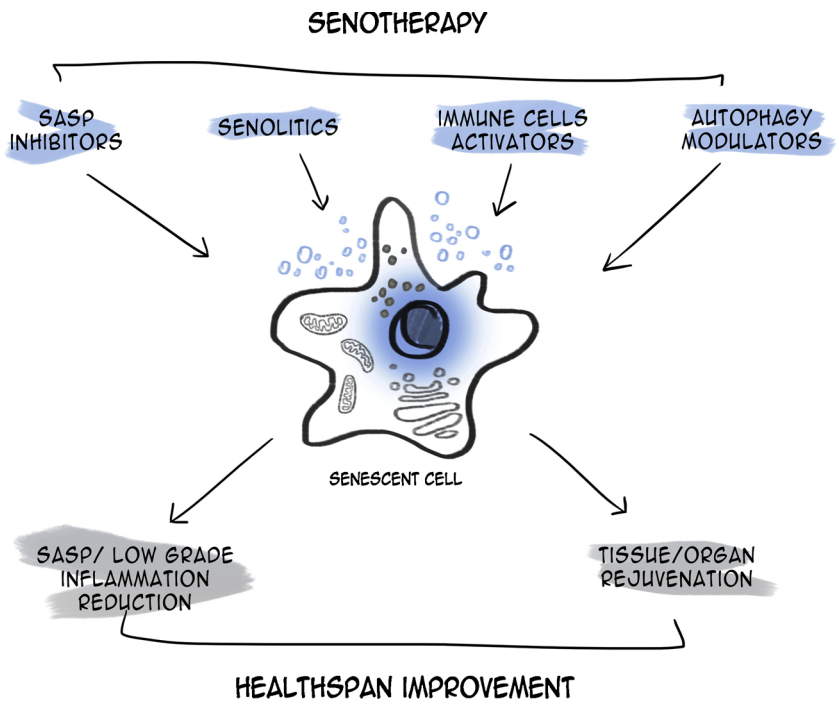

Fig. 1. Senotherapy as antiageing strategy targeting senescent cells and leading to the improvement of healthspan. Upon senolytic treatment senescent cells can be removed. Alternatively, the harmful influence of senescent cells on the tissues can be alleviated by modification of their SASP, activation of immune cells or by modification of autophagy.

et al., 2017). It has been shown that fisetin is able to elongate the lifespan and healthspan in both naturally aged and progeric mice. Administration of this compound reduced the burden of senescent cells in multiple tissues (the most specific is the adipose tissue) (Yousefzadeh et al., 2018). A weak activity was demonstrated in murine fibroblasts *in vitro*, for two other polyphenols, namely, for the flavonoid luteolin and the non-flavonoid curcumin (Yousefzadeh et al., 2018). Our results show no senolytic activity of curcumin *in vitro* (unpublished) however, a plethora of data obtained both in *vitro* and in animal models denotes curcumin as an effective pro-health agent (Salvioli et al., 2007). This discrepancy could be due to the fact that curcumin is metabolised and, in fact, the positive effect on the organism is exerted by its metabolites.

Another promising senolytics seems to be Foxo4 D-Retro-Inverso (also known as Proxofim), a peptide causing disruption of p53 and FOXO4 interaction (FOXO transcription factor is essential for survival of senescent cells), what allows p53 to induce apoptosis (Baar et al., 2017). Proxofim efficiently killed senescent cells without affecting non-senescent ones.

The main message from *in vitro* studies is that the effectiveness of senolytic drugs is very often cell type specific. None of the individual agents discovered so far proved to be potent in all senescent cell types. For example, Navitoclax is senolytic for replicatively senescent human MSCs (mesenchymal stromal cells), HUVECs (Human Umbilical Vein Endothelial Cell) and for IMR-90 human fetal lung fibroblasts but not primary adult lung human fibroblasts or human preadipocytes (Grezella et al., 2018; Schafer et al., 2017; Zhu et al., 2015). However, another group claims that Navitoclax can act in a cell type– and species-independent manner, as it has been shown for senescent IMR-90 cells, human renal epithelial cells (RECs) and mouse embryonic fibroblasts (MEFs), which were more sensitive than their non-senescent counterparts (Chang et al., 2016). Moreover, Navitoclax was shown to be selective for senescent hematopoietic stem cells - HSCs (isolated from whole-body irradiated mice) but had no effect on HSCs from non-irradiated control mice (Chang et al., 2016). In turn, fisetin selectively induces apoptosis in senescent HUVECs, but is not effective in senescent IMR90 cells, or primary human preadipocytes (Zhu et al., 2017). Others have shown that it reduced the burden of senescent cells in many different tissues (Yousefzadeh et al., 2018). A1331852 and A1155463 act as senolytics for HUVECs and IMR90 cells, but not for preadipocytes (Zhu et al., 2017). More comprehensive description of known senolytics is presented by Kirkland et al. (2017).

5. Senotherapy in alleviation of age-related diseases

The results obtained in mouse transgenic models and upon treatment with senolytics give hope that many age-related diseases in humans can be cured. The potential outcome of senotherapy is outlined on Fig. 1 and detailed description of the results obtained in animal experiments, both genetic and pharmacological, are presented in Table 1. Their number is growing quite rapidly. Some of these results are discussed below.

5.1. Renal diseases

Recent studies demonstrate that senescent cell depletion through INK-ATTAC transgene- or cell-penetrating FOXO4-DRI peptide induced forced apoptosis, reduced age-associated damage and dysfunction in multiple organs, in particular, in kidney (reviewed by Valentijn et al., 2018).

Moreover, senescent cells seem to be a good target to improve renal transplantation outcome (reviewed in Sturmlechner et al., 2017; van Willigenburg et al., 2018). Kidney transplants derived from aged donors show an increased number of senescent cells, which secrete a range of pro-inflammatory proteins. The high number of senescent cells negatively influences the local environment and reduces the regenerative capacity of kidney, which can lead to impaired functioning and lower survival. Moreover, several studies show that transplantation by itself induces senescence. Treatment with senolytics might reduce the chronic senescent cell burden and lower the risk of transplant rejection. However, newly generated senescent cells promote cutaneous wound healing during the healing period after implantation. Thus, taking this into account, anti-senescence strategies should be avoided in the initial phase after kidney transplantation.

5.2. Liver diseases

Non-alcoholic fatty liver disease (NAFLD) is more frequent in the elderly and can be the consequence of steatosis and fibrosis that lead to

hepatocellular carcinoma. A close correlation between hepatocyte senescence and fat accumulation was observed. Induction of senescence in hepatocytes leads to liver steatosis and senescent fibroblasts and hepatocytes have decreased ability to metabolize fat. Elimination of senescent cells, both in the genetically modified INK-ATTAC mouse or by using senolytic drugs, a cocktail of dasatinib with quercetin, reduced hepatic steatosis in ageing, obese and diabetic mice (Ogrodnik et al., 2017). Depletion of senescent cells may be a novel strategic approach to reduce steatosis.

Senescent cells have been considered as a cause of primary sclerosing cholangitis (PSC), a rare progressive liver disease characterized by multi-focal bile duct strictures. A large number of cholangiocytes in liver of patients suffering from PSC exhibit a senescent phenotype and SASP. SASP of cholangiocytes led to activation of stromal fibroblasts and induction of senescence of remaining cholangiocytes and in ASF (activated stromal fibroblasts), which resulted in liver fibrosis. A-1331852 was able to deplete both senescent ASF and cholangiocytes from fibrotic tissue to reduce biliary fibrosis. It is suggested that targeting of both ASFs and senescent cholangiocytes with Bcl-2 inhibitors can be a novel therapeutic strategy (Moncsek et al., 2018).

5.3. Lung diseases

Senescent fibroblasts and epithelial cells have been observed in human and mouse lung tissue affected by idiopatic pulmonary fibrosis (IPF), which leads to a dramatic shortening of lifespan (Hashimoto et al., 2016). IPF can be also induced by radiotherapy, an essential treatment for lung cancers. Using bleomycin-induced lung injury as a model of IPF in INK-ATTAC mouse, it has been shown that depletion of senescent cells reduced the symptoms of disease (Schafer et al., 2017). Elimination of senescent cells improved pulmonary function and physical health. Senolytic cocktail composed of dasatinib and quercetin (D + Q) replicates the benefits of clearance of senescent cells in a transgenic mouse model. Secretory phenotype of senescent fibroblasts increased fibrogenesis while selective removal of such fibroblasts by senolytics alleviated the harmful effect of SASP. Lung senescent cells were efficiently eliminated by induction of apoptosis as a result of BCL-W and BCL-XL inhibition by the small molecules, ABT-737 and ABT-263 (Mikawa et al., 2018; Pan et al., 2017; Yosef et al., 2016). Also, treatment with D + Q eliminated senescent alveolar epithelial cells in lung tissue in an experimental model of murine lung fibrosis and in primary alveolar epithelial type (AT) II cells derived from human IPF (Lehmann et al., 2017). Depletion of senescent epithelial cells in three-dimensional lung tissue cultures decreased fibrotic markers and proved that senescence of alveolar epithelial cells may contribute to pathogenesis of the disease. These results have clearly proved that IPF can be reversed by a senolytic drug even after it becomes progressive and that senotherapy can be a good candidate for a new treatment for IPF. Indeed, the results of the first human pilot study have already been published (Justice et al., 2019).

5.4. Musculoskeletal impairment

In both humans and rodents the reduced number of post-mitotic osteoblasts, which are short-living cells that need to be constantly replaced with new ones, are a cause of osteoporosis. Osteoblasts arise from mesenchymal osteoblast progenitors and are responsible for the synthesis and mineralization of the bone matrix. The age-related intrinsic defects in osteoblast progenitors cause a decrease in bone mass. Application of ABT-262 in transgenic (Osx1-Cre;TdRFP) mice attenuated age-related changes in the degree of SASP and eliminated senescent osteoprogenitor cells (Kim et al., 2017). Similarly, elimination of senescent cells, either in transgenic INK-ATTAC mice or using pharmacological approach (D + Q), prevented age-related bone loss (Farr et al., 2017). Senolytics inhibited bone resorption without any reduction in bone formation. In animal models higher bone mass and strength as well as a better bone microarchitecture were observed. This can be beneficial for protection against osteoporosis but also for reduction of age-related frailty. Decline in senescent cell population also reduced the number, perimeter, and volume of bone marrow adipocytes.

Sarcopenia is an age-related dramatic decline in body mass, loss of skeletal muscle mass and strength, which affects regenerative capacity (result of satellite cells activation) and correlates with physical disability and poor quality of life. Both motor neurons and mature myofibers are impaired and muscle fibrosis is increased. In aged muscles the number and function of satellite cells (MuSCs) decline (Sousa-Victor et al., 2015). It has been shown that eradication of senescent MuSCs led to rejuvenation of the functioning of the remaining cells (Chang et al., 2016).

5.5. Osteoarthritis

Ageing is a risk factor for osteoarthritis, a chronic degenerative joint disease, characterized by a gradual loss of articular cartilage, distorted bone growth and joint inflammation. All these changes lead to pain, physical disability and immobility. Senescent chondrocytes were found in cartilage tissue isolated from patients during replacement surgery. It was found that senescent cells accumulated in the articular cartilage and synovium after cruciate ligament injury, and that selective elimination of these cells attenuated the development of post-traumatic osteoarthritis, reduced pain and increased cartilage development. Intra-articular injection of a senolytic molecule (UBX0101) that selectively killed senescent cells validated these results in transgenic, non-transgenic and aged mice (Jeon et al., 2017). On the other hand, transplantation of senescent fibroblasts into the knee joint area of wild-type young mice resulted in cell accumulation around joints and caused osteoarthritis (Xu et al., 2017).

5.6. Cardiovascular diseases

Atherosclerosis, the leading cause of vascular disease, is characterized by gradual accumulation of lipid- and protein-filled plaques in the inner walls of arteries. Advanced atherosclerotic lesions contain senescent cells, which promote plaque instability due to elastic fibre degradation and fibrous cap thinning. Unstable plaque is more susceptible to rupture, which, in consequence, can lead to thrombosis and vessel occlusion, heart attack or stroke. Elimination of senescent cells from plaques in atherosclerosis-prone, low-density lipoprotein receptor–deficient (Ldlr−/−) transgenic p16-3MR mice, has shown that these cells are detrimentally associated with the pathogenesis of the disease (Childs et al., 2016). By using this approach all types of senescent cells: endothelial, vascular smooth muscle and foam cells, derived from activated macrophages, were efficiently removed. This reduced both plaque formation and promoted some features of stable plaques (Childs et al., 2015). Prolonged oral administration of dasatinib plus quercetin reduced senescent cell burden in aorta and improves vasomotor function in naturally ageing mice and mice with chronic hypercholesterolemia (Roos et al., 2016).

Cardiomyocytes are post-mitotic non-dividing cells, however, some marks of senescence were documented in these cells. Senescence has been shown to occur in the heart during ageing and it contributes to the pathophysiology of a number of CVDs. Very recently it has been documented that genetic and pharmacological elimination of senescent cardiomyocytes improved myocardial remodeling, diastolic function and overall survival in ageing mice following acute myocardial infarction (Anderson et al., 2019; Walaszczyk et al., 2019) and activation of cardiac precursor cells (Lewis-McDougall et al., 2019).

5.7. Neurological disorders

Ageing is the main risk factor for Alzheimer (AD) and Parkinson

disease (PD). At present it seems unquestionable that cell senescence can play a crucial role in ageing and age-related neurodegeneration. The hallmarks of cell senescence were found in glial cells, and so far collected data suggest that senescence of astrocytes and microglia can be involved in normal brain ageing as well as in AD and PD (Bhat et al., 2012; Luo et al., 2010; Tan et al., 2014). PD symptoms in a mouse model (p16-3MR) could be induced by paraquat (it induces astrocytic senescence and SASP *in vitro* and *in vivo*). Injection of ganciclovir, which allowed selective depletion of senescent cells in such modified mice model, improved motor function and restored adult neurogenesis (Chinta et al., 2018). It has been found that the $MAPT^{P301S}$PS19 mouse (model of tau-dependent neurodegenerative disease) accumulates p16-positive senescent astrocytes and microglia. Clearance of these cells as they arise in INK-ATTAC transgenic mice prevents gliosis and hyperphosphorylation of both soluble and insoluble tau, thus preserving cognitive function. Pharmacological intervention with the ABT-263 senolytics resulted in similar effects as genetic intervention in PS19 mice (Bussian et al., 2018). Another study performed by Currais et al. (Currais et al., 2018) showed that fisetin can improve cognitive ability in rapidly ageing SAMP8 mice, which are the model of sporadic AD and dementia. Subsequently Zhang et al. (Zhang et al., 2019) proved that D + Q was able to reduce neuroinflammation, lessen Aβ load and ameliorate cognitive deficits in the familial mice model of AD, that is, APP/PS1 AD mice. Interestingly this was possible by selective removal of senescent oligodendrocytes from the plaque environment.

Senescent glial cells can also affect other, namely, neuropsychiatric functions of the brain. To investigate the role of senescence in obesity-related neuropsychiatric dysfunction, Ogrodnik et al. (Ogrodnik, 2019) used the INK-ATTAC mouse model, from which p16Ink4a-expressing senescent cells can be eliminated. They found that obesity in mice led to accumulation of senescent glial cells in the proximity of the lateral ventricle, a region in which adult neurogenesis occurs. Clearing senescent cells from high fat-fed or leptin receptor deficient obese mice by treatment with D + Q restored neurogenesis and alleviated anxiety-related behavior. This study proved that senescent cells are major contributors to obesity-induced anxiety and that senolytics represent a potential new therapeutic avenue for treating neuropsychiatric disorders (Ogrodnik, 2019).

So far only one published study documented that clearance of senescent neurons (D + Q) containing neurofibryllary tangles in mouse model of tauopathy was neuroprotective and improved brain structure and cerebral blood flow (Musi et al., 2018).

5.8. Impairment of adipose tissue

By late middle age the capacity of adipogenesis, adipose tissue mass and metabolic functions begin to decline, as the result of senescent cell accumulation and stem cell dysfunction. Firstly, it has been shown that elimination of senescent cells can actually reverse age-related fat loss in progeroid BubR1H/H animals (Baker et al., 2011). Further studies showed that primary human senescent fat cell progenitors inhibited adipogenesis in non-senescent progenitor cells by secreting activin A. The production of activin A in fat tissue increases with age of the animal. Elimination of senescent cells from naturally-aged INK-ATTAC mice reduced the level of circulating activin A, which in consequence prevented lipodystrophy, reduced fat loss, enhanced adipogenesis and metabolic function, reduced lipotoxicity and increased insulin sensitivity in old age (Xu et al., 2015).

6. Senotherapeutic approach to treat cancer and to alleviate side-effects of anticancer therapy

Increasing age is one of the strongest risk factors for cancer development. On the other hand, cell senescence is an important barrier to cancer development as it protects against spreading cells with harmful mutations.

At the end of the previous century Serrano and colleagues showed that *in vitro*overexpression of the Ras oncogene in normal cells leads to senescence (OIS, *oncogene induced senescence*), while transfection of immortalized cells with the same oncogene facilitates cell transformation (Serrano et al., 1997). Later on, using different mouse models of cancer initiation and development, it was demonstrated that cell senescence is induced at the early stages of carcinogenesis *in vivo* (Chen et al., 2005; Collado et al., 2005; Michaloglou et al., 2005). Since cellular senescence can significantly halt the process of carcinogenesis, cells must bypass OIS to progress to full-blown cancer. Respectively, genes encoding p53, p16 and pRb proteins that are involved in cell senescence, are frequently mutated in many types of cancer (Hanahan and Weinberg, 2000).

Interestingly, despite this, many cancer cells remain sensitive to induction of senescence upon anticancer treatment (Roninson, 2003). Senescent cancer cells as non-proliferating ones, seemed to be completely safe for the body, thus therapy-induced senescence (TIS) became a desirable outcome for cancer treatment (Ewald et al., 2010; Nardella et al., 2011). However, this assumption became recently questionable. It seems that the old maxim: “the only good cancer cell is a dead cancer cell” takes on a new meaning, as anticancer treatment leading to cancer cell senescence, can be considered as a form of resistance to treatment.

The first reason of that is the ability of senescent cells to secrete many factors that modify microenvironment for that favouring cancer development. Namely, several activities have been pointed as responsible for the pro-tumorigenic effect of SASP. One is degradation of extracellular matrix facilitating invasion of cancer cells (Coppe et al., 2008), driving epithelial-to-mesenchymal transition, reinforcing cancer invasion (Laberge et al., 2012) and promoting angiogenesis through proangiogenic factors and indirectly through chemokines, that recruit macrophages, which adopt a proangiogenic phenotype (Davalos et al., 2010; Kelly et al., 2007). Moreover, macrophages can support immunosuppressive conditions due to attracting myeloid-derived suppressor cells that inhibit CD8+ cytotoxic T lymphocytes (Toso et al., 2014) and creating a state of low grade inflammation that reinforces tumor growth (Kuilman et al., 2008).

However, secretion of many biologically active proteins that promote carcinogenesis is not the only danger that was linked with senescent cancer cells. Recently, a serious doubt has arisen whether senescence is really the terminal state of growth arrest. The question is – can senescent cells resume proliferation? and the answer to this question is absolutely fundamental in the context of both cancer development and therapy-induced senescence. Indeed, escape from senescence was observed in many models of OIS, in which acquisition of certain mutations promoted cancer progression into malignancy (Damsky et al., 2015; Patel et al., 2016; Vredeveld et al., 2012). Reversibility can be also observed in chemotherapeutic drug-induced senescence. According to others (Chitikova et al., 2014; Erenpreisa and Cragg, 2013; Mansilla et al., 2009; Puig et al., 2008; Sabisz and Skladanowski, 2009; Wang et al., 2013; Weihua et al., 2011) and our (Mosieniak et al., 2015; Sliwinska et al., 2009) observations, senescence of cancer cells can be associated with polyploidization (Jackson et al., 2013; Milanovic et al., 2018; Was et al., 2018). Subsequently, after the period of growth arrest, polyploid senescent cells regain the ability to proliferate, which is associated with depolyploidization. It was also revealed that cancer cells undergoing senescence upregulate stem cell genes. Therefore, it was postulated that senescence-associated stemness is a cell-autonomous feature that exerts its detrimental, highly aggressive growth potential upon escape from cell cycle blockade (Jackson et al., 2013; Milanovic et al., 2018; Was et al., 2018). Thus, it was hypothesized that TIS could represent a mechanism of adaptation (resistance) to evade the toxicity of chemotherapy and radiation, enabling cancer recurrence (Chakradeo et al., 2015). For these reasons interventions that aim at efficient elimination of cancer senescent cells are definitely required.

Although, there are still not many proof-of-concept experiments, senolytics, especially in combination with pro-senescence drugs, are

considered as an attractive and very promising anticancer strategy. Panobinostat, FDA-approved histone deacetylase inhibitor, was shown to have senolytic activity in Non-Small Cell Lung Cancer (NSCLC) and Head and Neck Squamous Cell Carcinoma (HNSCC) (Samaraweera et al., 2017). Similarly, it has been shown that ABT-263, selectively kills senescent melanoma and lung cancer cells (Wang et al., 2017). Notably, many senolytis, such as dasatinib, quercetin, Navitoclax, fisetin, A1331852, and A1155463, among others, induce apoptosis of cancer cells, thus they could exert an anticancer effect by itself.

Interestingly, there are also new strategies aim to kill selectively senescent cancer cells. Dörr et al. (2013) revealed that upon induction of senescence by chemotherapeutic treatment, cancer cells undergo metabolic reprogramming. Increased glucose utilization and much higher ATP production were observed in senescent cancer cells. These changes were linked to massive proteotoxic stress, which is a consequence of SASP. Toxic proteins were targeted for autophagy in an acutely energy consuming fashion. In consequence, senescent cancer cells were highly sensitive to blockage of glucose utilization or autophagy and underwent apoptosis. Thus, altering the metabolism of senescent cancer cells can be considered as an important target for senotherapy.

Another way to eliminate senescent cancer cells is reprogramming SASP in order to strengthen/induce the natural mechanisms of killing senescent cells by immune cells. Indeed, it was documented that pharmacological inhibition of the Jak2/Stat3 pathway reprogrammed SASP in Pten-loss-induced senescence by reducing the overall level of immunosuppressive cytokines while retaining unchanged, or even increased, the levels of immunostimulatory cytokines (Toso et al., 2014).

An alternative strategy could be to target normal senescent cells present in the tumor stroma. Laberge et al. (Laberge et al., 2015) revealed that inhibition of mTOR by rapamycin influenced SASP of senescent fibroblasts. Importantly, rapamycin suppressed the ability of senescent fibroblasts to stimulate prostate tumor growth in mice. Similar positive tumor-inhibitory effect was observed when senescent fibroblasts were treated with simvastatin - an HMG-CoA reductase inhibitor. Simvastatin decreased SASP of senescent fibroblasts by suppressing the activity of Rho family GTPases - Rac1 and Cdc42. Moreover, simvastatin mitigated the effects of senescent cells conditioned media on breast cancer cell proliferation and endocrine resistance (Liu et al., 2015).

Importantly, another aspect of anticancer therapy is a side effect of normal cell senescence. Indeed, there is a very well described phenomenon of accelerated ageing in people, particularly children who were exposed to anticancer therapies (Ness et al., 2018). Survivors have an earlier onset and higher incidence of chronic comorbidities, including endocrinopathies, cardiac dysfunction, osteoporosis, pulmonary fibrosis, secondary cancers and frailty than the general population (Cupit-Link et al., 2017). Accordingly, it has been demonstrated that chemotherapeutic drugs induced persistent presence of senescent cells in normal noncancerous tissues. Elimination of senescent cells in either genetically modified p16-3MR mice or using a senolytic drug, ABT-263, attenuated side effects of chemotherapy, such as cardiac dysfunction, bone marrow suppression and chemotherapy-induced fatigue, and prevented or delayed cancer relapse and metastasis to distal tissues (Demaria et al., 2017). Generally, senolytics might prevent and potentially even revert premature frailty in cancer survivors by blocking accelerated senescence following therapy (Short et al., 2019).

7. Autophagy in senotherapy

Autophagy is a catabolic process, in which proteins, other macromolecules, and organelles are degraded and recycled, thus providing metabolites to maintain energy supply in the cell. It also prevents “waste” accumulation in a tissue. Three main types of autophagic processes have been described to date: macroautophagy, microautophagy, and chaperone-mediated autophagy (Gomez-Sintes et al., 2016). As macroautophagy has been the best recognized type of autophagy, the term autophagy usually refers to macroautophagy. A characteristic morphological and functional feature of autophagy is the formation of autophagosomes, which fuse with lysosomes wherein their cargo is degraded and recycled. Ageing, as a process characterized by accumulation of molecular damage and disturbed proteostasis, is considered to be connected with autophagy dysfunctions (Ogrodnik et al., 2018). The connection between active autophagy and longevity has emerged at the beginning of this century and stems from studies on C. elegans (Melendez et al., 2003). Till this time it has been documented in many studies that, indeed, age-related disorders such as cardiovascular and neurodegenerative diseases, are characterized by defective autophagy and activation of autophagy in model organisms can lead to their rejuvenation (Nakamura and Yoshimori, 2018). It has been demonstrated, for example, that spermidine, which activates autophagy, extends lifespan across species, promotes cardio- and neuroprotection, stimulates antineoplastic immune response and allows cells to avoid immunosenescence by stimulating memory T-cell formation (Madeo et al., 2019). Rapamycin, resveratrol and metformin are other modulators of autophagy, which possess anti-ageing propensity (Gurau et al., 2018).

However, the connection between autophagy and senescence on the cellular level is still an unsolved problem (Kang and Elledge, 2016). Nonetheless, lipofuscin accumulation and impaired mitophagy (selective autophagy of mitochondria) (Hyttinen et al., 2018) clearly denote autophagy dysfunction in senescent cells. It has been shown that defective autophagy of vascular smooth muscle cells (VSMC) accelerates not only the development of stress-induced premature senescence but also atherogenesis, albeit without worsening plaque stability (Grootaert et al., 2018). Failure of autophagy in aged stem cells promoted senescence, loss of proteostasis, increased mitochondrial dysfunction and oxidative stress. Re-establishment of lysosome function/autophagy cleared protein aggregates and reversed senescence (Garcia-Prat et al., 2016; Leeman et al., 2018). Recently, it has been documented that defective association of autophagosomes and lysosomes with minus-end-directed motor proteins, might be a driver of the decline in autophagy in aged hepatocytes (Bejarano et al., 2018). Also recently it has been demonstrated that autophagy impairment is crucial for oxidative stress-induced senescence of mouse fibroblasts; thus, restoring autophagy activity could be a promising way to retard senescence (Tai et al., 2017). However, some studies also indicated that autophagy promotes cellular senescence by facilitating SASP (Young et al., 2009). These seemingly opposite roles of autophagy may reflect a complex picture of autophagic regulation of cellular senescence, which includes different types of autophagy and its unique spatiotemporal activation. Thus, a better understanding of the autophagy process will allow us not only to elucidate the conundrum of the dual role of autophagy in the regulation of cellular senescence but also help to develop new therapeutic strategies for many human diseases associated with cell senescence (Kwon et al., 2017).

Thus, since the majority of studies point to the active autophagy as the mechanism protecting cells against senescence, senotherapy, besides inducing apoptosis, activating immune system and alleviating SASP, may also act *via* activation of autophagy in senescent cells.

Modulation of autophagy is a very important part of cancer therapy (Sieben et al., 2018), but the connection between autophagy and senescence in cancer cells is far more complicated than in normal cells. First, the basal levels of autophagy can both promote and suppress development of cancer depending on the genetic background and cancer microenvironment. This complicates therapeutic intervention and raises the question whether to avoid potential aggravation of the disease we should interfere in the process of autophagy. Second, there are many controversies in the literature concerning the role of autophagy in cancer cell senescence and, as pointed out (Gewirtz, 2013), it is difficult to judge whether these two processes are dependent or occur collaterally but independently. Thus, the fundamental question is: how

to modulate autophagy in cancer cells undergoing senescence? As we agreed, autophagy in normal cells seems to be cytoprotective, including protection against senescence, thus it restores cell proliferation. However, in the case of cancer cells, the more desirable outcome would be autophagic cell death. We have shown that curcumin-induced senescence of cancer cells was accompanied by autophagy, and that autophagy inhibition using si*ATG5* decreased the number of senescent cells, but did not lead to increased cell death (Mosieniak et al., 2006). However, others (Jakhar et al., 2018) have documented that inhibition of autophagy by quinazoline in cancer cells, which underwent senescence following nocodazole or paclitaxel treatment, facilitates escape from senescence and induces cell death. These studies showed that the outcome of autophagy inhibition depends on the cell type and experimental approaches. Nonetheless autophagy was inhibited in both studies. It cannot be excluded that activation of autophagy in senescent cancer cells could lead to cell death, although, again, it may be context dependent. Thus, autophagic cell death must be studied more thoroughly to become a target of cancer cell senotheraphy needs more studies.

8. Questions and remarks

Pharmacological and nutraceutical elimination of senescent cells from the body seems to be very promising in postponing ageing and age-related diseases. However, results obtained so far originate mainly from animal and tissue culture studies, even though the first human pilot study has been successfully completed (Justice et al., 2019). Namely, fourteen patients with stable mild severe idiopathic pulmonary fibrosis (IPF) were treated with 9 oral doses of D + Q over 3 weeks. Physical function and clinical parameters were measured before and 5 days after the last D + Q dose, well beyond these drugs' elimination half-lives. Statistically significant (within-subject) and clinically meaningful improvement in physical function following treatment was observed. IPF is a fatal disease, the incidence of which rises with advancing age with median survival of less than four years in patients diagnosed over 65 years of age (Raghu et al., 2014). From this clinical study it is impossible to predict long lasting potential effects, either beneficial or adverse. Nonetheless such study give hope for rapid progress in this field and probably many other clinical trials are ongoing. Thus, it is quite urgent to answer some scientific questions, which are still open. The many fundamental questions and indications for future studies have already been thoroughly discussed in excellent reviews becoming from the laboratories directly involved in studies on genetic and pharmacological eradication of senescent cells (Kirkland and Tchkonia, 2017; Short et al., 2019; Tchkonia and Kirkland, 2018; van Deursen, 2019). Here, we focus only on some aspects.

First, none of the individual senolytic induced apoptosis in all types of senescent cells. Second, even if senolytics cause very similar effects to genetic approach (Table 1), this phenocoping doesn't necessarily means that they have exactly the same targets. We can expect that there are off-target effects, which should be seriously taken into consideration.

The goal of senotherapy is to eliminate detrimental effects of senescent cells. The goal of senolytic therapy is to eliminate senescent cells, which are at the heart of ageing and age-related disease. From Table 1 it emerges that in mice both genetic and pharmacological approaches reduced the burden of senescent cells identified on the basis of commonly accepted characteristics of these cells. Visualization of p16-positive cells in transgenic animals and their disappearance is really very spectacular. Generally, the main hallmarks of senescence used in genetic and pharmacological experiments showing clearance of senescent cells are SA-β-gal activity, p16-positivity and SASP. All these measurement together with others (*e.g.*TAF, telomere-associated foci) were used in so far published results. In a few studies the active caspase 3, which is the main executor of apoptosis was evidenced in SA-β-gal-positive cells proving that senescent cells were killed (Yosef et al., 2016; Zhang et al., 2019). Disrupting an interaction between FOXO4 and p53 with a FOXO4 D- retro reverse peptide in p16-3MR mice killed senescent cells as it was shown *ex vivo* by TUNEL assay (Baar et al., 2017).

Certainly, it should be admitted that with the development of our knowledge, the doubts concerning the nature of senescent cells are increasing instead of decreasing. The most commonly used senescence marker is SA-β-gal activity measured at pH 6.0. However, even under normal physiological conditions, SA-β-gal activity is enriched in particular cell types, such as mature tissue macrophages and osteoclasts, and it is detected in cells undergoing increased lysosomal activity during autophagy. Contact-inhibited quiescent cells, maintained for prolonged periods in culture, can upregulate SA-β-gal activity (reviewed in Sharpless and Sherr, 2015). We have shown strong activation of SA-β-gal in rat neurons during long term culture and in mice hippocampus. However, for the lack of other unambiguous marks of cell senescence (instead of REST which appeared independently from SA-β-gal activity), we doubt whether these SA-β-gal positive neurons can be considered as senescent ones (Piechota et al., 2016). Recently, it has been documented that the SA-β-gal activity is not observed in the brain of MAPT P301S PS19:ATTAC mice (Bussian et al., 2018). In turn, in a seminal paper by Jurk et al. (2012) SA-β-gal-positive neuronal cells were documented upon DNA damage.

The rational of using genetic models is based on the targeting p16-positive cells. Indeed, the main effect observed after mice treatment with molecules activating caspase-8 is reduction of luminescence and *Cdkn2a* mRNA level (see Table 1). The cyclin-dependent kinases inhibitor, p16, similarly to SA-β-gal, is considered to be one of the best hallmark of senescence. However, recently, it has been argued that macrophages can acquire characteristic of cell senescence (SA-β-gal and p16 positivity) due to polarization or the bystander effect, which could be induced also by senescent cells in young animals (Hall et al., 2016, 2017). These studies were extended by Sharpless's group (Liu et al., 2019), which generated a fluorescence-based reporter allele with tandem-dimer Tomato (tdTom) knocked into the endogenous p16INK4a locus. This allele enables the identification and isolation p16INK4a-(tdTom +) of activated cells at a single-cell level in cultured cells and *in vivo*. They showed that cells harboring activation of the p16INK4a promoter (including macrophages) accumulate with ageing and during inflammation *in vivo*, and display characteristic features of senescence, such as increased activity of SA-β-gal, elevated level of p16, SASP and decreased replication. Moreover, using these genetically modified mice the existence of senescence-associated macrophages (SAMs) has been proved. Altogether, these experiments indicated that SAMs could be a real target for senolysis. Interestingly, this is quite a new argument for the inflammageing theory, which originally referred to macrophages as a real culprit of ageing and age-related diseases (Franceschi et al., 2000). It was also suggested in experiments performed by Childs et al (Childs et al., 2016) that macrophages can substantially contribute to SA-β-gal staining, and might be killed *via* suicide genes driven by $p16^{ink4a}$ promoters, thus leading to decreased atherosclerotic lesion formation (Bennett and Clarke, 2016). The suggestion that the main target of senolytics are macrophages, does not exclude other targets and does not undermine the positive effects of genetic approaches on mice health improvement, but certainly this opens new directions in the field of senotherapy. However, two papers reported no luciferase activity in bronchoalveolar lavage fluid (BALF) (which contain macrophages and other immune cells) of ARF-DTR mice (Hashimoto et al., 2016; Mikawa et al., 2018).

Notably, recently a paper has been published showing for the first time that elimination of senescent cells can prevent type 1 diabetes, which is not an age-related disease (Thompson et al., 2019). Senescent β-cells from pancreas of non-obese diabetic (NOD) mice where successfully cleared by senolytics (ABT-199 and ABT-739), which inhibit anti-apoptotic proteins from the Bcl-2- family. Moreover, it has been documented that ABT-739 did not influence the number of macrophages in pancreatic islets of treated mice, suggesting that macrophages, even if SA-β-gal- and p16-positive (although not analyzed in

this study), are not prone to anti-apoptotic treatment by senolytics (at least in young animals). Taken together it seems that macrophages can be targeted by a senolytic therapy only in some circumstances.

The major challenge for senotherapy is to target the right cells at the right time and to avoid side effects in other organs. In some animal experiments (Table 1) senolytics were applied for a short time, but this was enough to substantially reduce the burden of senescent cells. Even if we are aware of some side effects caused by them (*e.g.*in case of Navitoclax) there should be enough time after treatment to eliminate them. In other experiments the beneficial effects were achieved by intermitted and/or rather long lasting treatment. To our knowledge there are no follow up studies, but frequently the subjects of experiments were old animals. The clinical potential of senolytic drugs has been thoroughly discussed by Kirklad and co-workers and we refer the reader to this work (Kirkland et al., 2017).

A purely hypothetical question is: what would happen if we were able to completely prevent/eliminate cell senescence? Will we not get old at all? As cell senescence exerts some positive effects, such as body shaping during embryogenesis, improving wound healing and preventing fibrosis and, last but not least, creating a barrier against cancer, it cannot be excluded that we would not be able to survive without senescent cells. Interestingly, in the longest-lived rodent, naked mole rat (NMR), that is resistant to a variety of age-related diseases and does not show ageing phenotype until very late stages of life, developmentally programmed cellular senescence has been observed in multiple tissues. NMR cells also underwent cellular senescence when transfected with oncogenic Ras. In addition, cellular senescence was detected in NMR embryonic and skin fibroblasts subjected to γ-irradiation (IR). However, NMR cells required a higher dose of IR for induction of cellular senescence (Zhao et al., 2018). Thus, the question is: would it be possible to prolong the NMR life even further by eradicating senescent cells?

The assumption that cells are the basic building blocks of the body and thus senescent cells might be the culprit of organismal ageing, at least in case of mice and humans, seems to be correct but, concomitantly, a reductionist one. One can argue that multicellular organisms, such as the human body, are not a simple medley of cells, but a very complex and complicated “living machine”, which represents the added value of the sum of cells. Moreover, organismal functioning is regulated on many levels of organization including the microbiome. Nonetheless, it seems that, indeed, cell senescence can be a major process in the development and progression of various diseases. This may include metabolic diseases, such as obesity and type-2 diabetes, which are a major risk factor in the development of additional pathological conditions, such as cardiovascular disease, kidney disease and cancer. Senescent adipocytes can drive disease development, and obesity and diabetes can potentially create an environment that accelerates cell senescence within other tissues. This can consequently manifest as age-related biological impairments and secondary diseases (Burton and Faragher, 2018). Thus, senescent cells might be part of a pathogenic loop in diabetes, as both the cause and the consequence of metabolic changes and tissue damage. The question is: are senlotytics able to reverse all dysfunctions and deterioration of many tissues caused by such severe disorder? So far, experiments performed on mouse models showed that, indeed, eradication of senescent cells can significantly improve healthspan. It cannot be excluded that in a milieu deprived of senescent cells and SASP, the repair processes begin, including the filing of the empty space by proliferating stem cells. However, stem cells, also undergo senescence (Goodell and Rando, 2015) and their pool is better preserved in young organism, thus it may limit the rebuilding capacity in older organisms. Ageing of the hematopoietic system is a consequence of reduced number of hematopoietic stem cells (HSCs) (reviewed in Akunuru and Geiger, 2016), however, there are also data showing that, in mice, ageing is associated with a qualitative, but not a quantitative, reduction in tissue stem cells (including HSCs and MuSCs) (Chang et al., 2016). Clearance of senescent cells by oral administration of ABT-263, to either sublethally irradiated mice or normally aged mice, effectively depleted senescent bone marrow hematopoietic stem cells, but it was suggested that such treatment led to rejuvenation of aged tissue stem cells (Chang et al., 2016). Interestingly, ABT-737 was also able to reduce the number of senescent cell formed in the epidermis. It led to an increase in hair-follicle stem cell proliferation (Yosef et al., 2016).

It is obvious, however, that for our comfort and for purely economic reasons it would be much better to prevent a disease than to cure it, but what is the best time for prevention? Even if we agree that ageing is not a programmed but simply a stochastic process starting after the developmental program ends (Hayflick, 2007), it is not so straightforward to predict when precisely it starts. We are still far away from being able in a simple way to define biological age, which is different from the chronological age for each of us (Burkle et al., 2015). Moreover, senescent cells can play a different role in a young and in an old organism. For instance, it is commonly accepted that senescent cells sustain a barrier to cancer but when they accumulate in the elderly they can create pro-cancerogenic milieu (Campisi, 2003). This is also the case for other age-related disorders. Senescent cells accumulate with age due to permanent stress increasing over time. Such cells should be eliminated by the immune system, but immune cells also senesce and become less efficient over time (Ovadya and Krizhanovsky, 2018). Thus, again, the fundamental question is: in which moment of our life rejuvenating senotherapy should start? Are we able to protect ourselves against age-related disorders in advance? Studies performed on transgenic mice, both progeroid and wild type, showed that dasatinib plus quercetin, or Navitoclax, or other apoptosis inducers, such as A1331852, can reduce many age-related disorders and improve mice healthspan even if applied at late age (Yousefzadeh et al., 2018 and literature there). How to translate these results to humans in terms of treatment course and dosage, and to avoid adverse effects? For example, Navitoclax (which is an anticancer agent) treatment in patients has some common toxic, dose-dependent side effects such as transient thrombocytopenia and neutropenia (Ng and Davids, 2014). One solution could be to directly target senescent cells and avoid off-target effects. Recently, a drug delivery system based on the encapsulation of drugs with galacto-oligosaccharides, which can be substrates for lysosomal SA-β-gal, has been described. It has been shown that gal-encapsulated fluorophores were preferentially released within senescent cells in mice. In a model of chemotherapy-induced senescence, gal-encapsulated cytotoxic drugs targeted senescent tumor cells and improved tumor xenograft regression in combination with palbociclib. Moreover, in a model of pulmonary fibrosis in mice, gal-encapsulated cytotoxic drugs targeted senescent cells, reduced collagen deposition and restored pulmonary function (Munoz-Espin et al., 2018).

Interestingly, recent studies have shown that fisetin, which is a natural polyphenol with a structure very similar to quercetin, is the most potent senolytic, among many other analyzed natural compounds, which seems not to exert side effects (Yousefzadeh et al., 2018). However, we should take into account that murine lifespan is only 3 years, thus, the treatment is relatively short in comparison to what must be applied in humans. In other studies, showing that fisetin can improve cognitive ability of SAMP8 mice, a model of sporadic Alzheimer disease and dementia with accelerating ageing, the supplementation lasted 7 months, which sustained nearly half of the lifespan of these mice (Currais et al., 2018). Moreover, fisetin, like other natural polyphenols, has been shown to interfere with many signaling pathways and possess antioxidant, anti-inflammatory and anti-proliferative activities, which places it among potent anticancer and anti-neurodegenerative agents (Kashyap et al., 2018; Maher, 2015, 2017). The very interesting scientific question is: why is fisetin a better senolytic than other natural polyphenols? From Yousefzadeh et al. (Yousefzadeh et al., 2018) study it emerges that also another polyphenol, curcumin, can be a promising

senolytic with slightly lower activity *in vitro* than fisetin. However, our own experiments, also performed *in vitro,* showed that curcumin is not able to postpone cell senescence or induced cell death in senescent cells (manuscript in preparation), but interestingly it can elevate Sirt1 (Grabowska et al., 2016), considered as a member of the "youthful" protein family (Grabowska et al., 2017). Translation of the results obtained on animal models is still a real puzzle as, generally, polyphenols have different bioavailability, are differentially metabolized by animal and human tissues and, moreover, by their microbiomes. Moreover, any compounds, including natural ones (e.g curcumin) (Demirovic and Rattan, 2011), can exert hormetic effects, which means that their dosage is crucial. It was documented for example that arsenite, which is a strong toxin, in a low dose prolonged the lifespan of C. elegans (Schmeisser et al., 2013). Thus, only clinical trials can be decisive and give real grounds for supplementation of potential senolytics.

Another question is: how do senolytics affect neuronal functions if neurons are not proliferation-competent cells and do not undergo canonical cell senescence? The term "cell senescence" originally referred to cell division arrest of previously proliferation-competent cells. Senescence of post-mitotic cells, such as neurons and skeletal muscle cells, goes beyond this definition. Moreover, age-related disorders include neurodegeneration and sarcopenia, manifested by loss of neurons and muscle cells. These diseases aggravate the age-dependent decline of these cells in the organism.

Recently Burton and Stolzing have proposed to discriminate "cellular senescence", which is a programmed change in cell state associated with permanent growth inhibition, from "cellular ageing" which is a stochastic process of gradual accumulation of damage over time (Burton and Stolzing, 2018). However, ageing of non-dividing post-mitotic cells, which fit to the term "cellular ageing" could also share some common characteristics with "cell senescence". Indeed, others (Geng et al., 2010; Jurk et al., 2012) and we (Piechota et al., 2016) have found SA-β-gal-positive cells in ageing mice brain. Contrary to studies by Jurk et al. (Jurk et al., 2012), which showed that neuronal senescence is strictly connected with cdks inhibitor, p21, we were not able prove it in neuronal *in vitro* culture (not shown). Thus, the question is: whether senolytics will be able to recognize "ageing cells" as a target? Surprisingly the answer is: yes, but another question arises: how to protect post-mitotic cells against age-related degeneration? It cannot be excluded that clearance of senescent non-neural cells from brain can be protective enough against neurodegenerative diseases (Chinta et al., 2018; Zhang et al., 2019). Moreover, induction of cytoprotective autophagy in post-mitotic cells would be a desired target of senotherapy.

Interestingly, a recent paper by Musi et al. (Musi et al., 2018) seems to dispel our fears concerning senotherapy of post-mitotic neurons. The authors showed that Tau-containing neurofibrillary tangle (NFT) accumulating in the brain of AD patient and AD transgenic mice, are associated with features of cell senescence, such as apoptosis resistance, upregulation of *Cdkn2a* encoding p16, upregulation of *Glb1* encoding SA-β-gal (but not SA-β-gal activity) and increased SASP. In dasatinib plus quercetin treated mice, MRI brain imaging and histopathological analyses indicated a reduction in total NFT density, neuron loss, ventricular enlargement and SASP alleviation. However, this study did not give us a clue whether this treatment could prevent dementia development in people suffering from AD. Also, very recently, it has been documented that senescent mouse post-mitotic cardiomyocytes, are involved in age-associated cardiac hypertrophy and fibrosis and that their clearance may induce a compensatory cardiomyocyte regeneration (Anderson et al., 2019; Walaszczyk et al., 2019).

Overall our enthusiasm for beneficial effects of senotherapy, and particularly senolytics, should be balanced by justified skepticism. Moreover, as discussed by (Kirkland and Tchkonia, 2017), clinical trials should follow the map of procedures for translating the intervention from bench to the bedside. It includes many analyses, which should be performed to prove that senescent cells are cleared and that the use of senolytics in humans is safe.

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Declaration of Competing Interest

The authors confirm no conflict of interest

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