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Is it the time of seno-therapeutics application in cardiovascular pathological conditions related to ageing?

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ABSTRACT

It rates that in 2030, the cardiovascular diseases (CVD) will result in 40% of all deaths and rank as the leading cause. Thus, the research of appropriate therapies able to delay or retard their onset and progression is growing. Of particular interest is a new branch of the medical science, called *anti-ageing medicine* since CVD are the result of cardiovascular ageing. Senescent cells (SC) accumulate in cardiovascular system contributing to the onset of typical age-related cardiovascular conditions (i.e., atherosclerosis, medial aorta degeneration, vascular remodeling, stiffness). Such conditions progress in cardiovascular pathologies (i.e., heart failure, coronary artery disease, myocardial infarction, and aneurysms) by evocating the production of a proinflammatory and profibrotic senescence-associated secretory phenotype (SASP). Consequently, therapies able to specifically eliminate SC are in developing. The *senotherapeutics* represents an emerging anti-SC treatment, and comprises three therapeutic approaches: (a) molecules to selectively kill SC, defined *senolytics*; (b) compounds able in reducing evocated SC SASP, acting hence as SASP suppressors, or capable to change the senescent phenotype, called *senomorphics*; (c) inhibition of increase of the number of SC in the tissues. Here, it describes them and the emerging data about current investigations on their potential clinical application in CVD, stressing benefits and limitations, and suggesting potential solutions for applying them in near future as effective anti-CVD treatments.

1. Introduction

Elderly people have shown a rapid boost in Western populations parallelly to constant increase of life expectancy, even if not inevitably linked to an equivalent improvement of health (Lunenfeld and Stratton, 2013). Accordingly, ageing populations phenomenon is significantly associated with the onset of several chronic inflammatory diseases, described as age-related disease (ARD), including cardiovascular diseases (CVD), type 2 diabetes (T2D), osteoporosis, neurodegenerative diseases, and cancer (Edwards, 2012) (Fig. 1A). It rates that in 2030, among ARD, the CVD will result in 40% of all deaths and rank as the leading cause (Kirkwood, 2017; Jones et al., 2019). Consequently, the governments and scientific community are investigating fitting heath actions, including disease-prevention and health-promotion programs for targeting the major causes of morbidity and mortality in the aged population, and reducing the cost pressure related to ARD management and disability (Zolotor and Yorkery, 2019). In this context, promising appears the *emerging anti-ageing medicine*, a branch of medical science, (see Fig. 1B), which is getting a particular attention in the recent decades (Kirkland, 2013; Flatt et al., 2013; Lopreite and Mauro, 2017). The anti-ageing medicine intends to promote health-span and lifespan by employing specific nutritional and physical activity schemes, and applying biomedical interventions aimed at delaying or reversing the ageing process (Lemaître et al., 2015; da Costa et al., 2016; Lara et al., 2016). Furthermore, conventional and alternative medical specialties are used for designing an interconnected approach with the objective to achieve the best probable anti-ageing effect in individuals affected by ARD. Thereby, anti-ageing medicine is a holistic discipline based on the concept of disease as pathological condition affecting the entire body and not simply a part. Diverse organizations (e.g. the predominant is the

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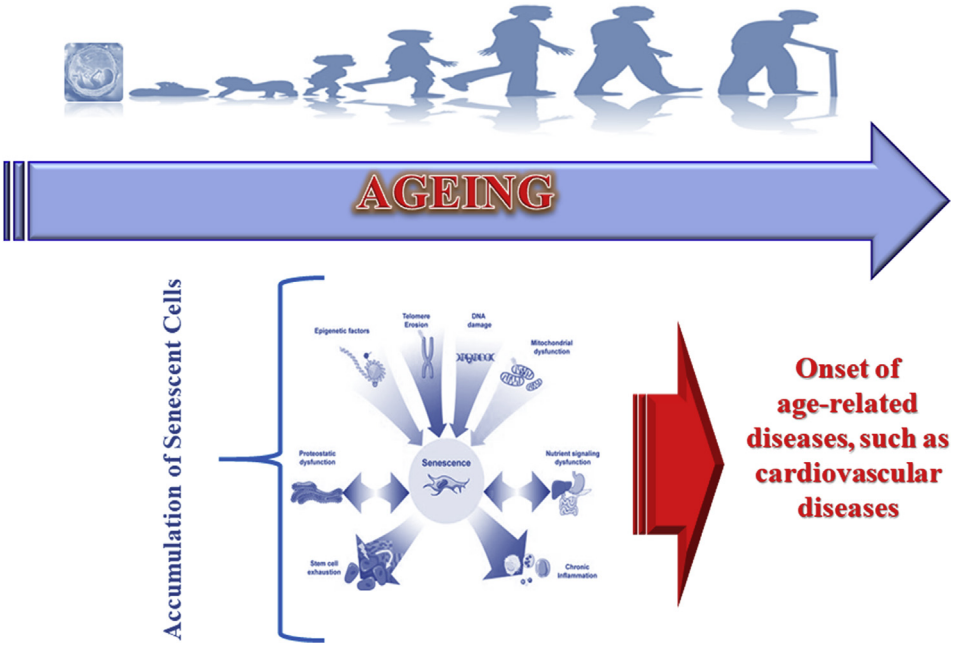

Fig. 1A. The strict relationship between aging and the onset of age-related diseases and the use of antiaging medicine as solution. With advancing time, the cells decrease their activity and show diverse alterations (i.e., including genomic instability, epigenetic deregulation, loss of proteostasis, mitochondrial dysfunction, telomere shortening, autophagy, impaired stress resistance and deregulated nutrient signaling). This results in the accumulation of damages that lead the cells in senescence/apoptosis and death. As result, their senescence or apoptosis occur, as well as the decline in their ability to be replaced because of exhaustion of stem cells. This determines an alteration of both homeostasis and function of tissues, organs, and systems, and the evocation of inflammation, fibrosis and progressively to the onset of age-related diseases.

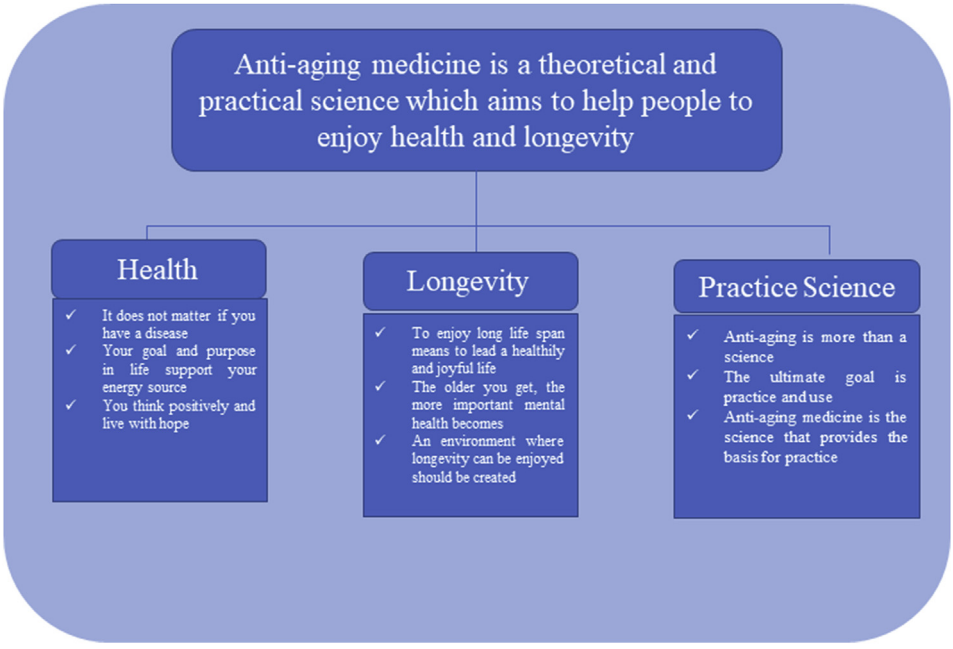

Fig. 1B. The antiaging medicine and its potential applications.

American Academy of anti-ageing medicine or A4M, not yet formally accredited by American Medical Association, https://www.a4m.com/) across the globe are proposing courses to physicians, having the interest in gathering a deep information on anti-ageing medicine and its potential intervention measures principally versus those with prevention character of diseases in order to reduce ARD prevalence and incidence and slow-down their estimated trend. Such results both in advances in the scientific knowledge and in creating an important platform of information for all the individuals of any age-range of a population, providing a novel perception about the most suitable life behaviours compatible to extend life span and tackling ageing. In addition, anti-ageing medicine offers a new idea on the ageing process, considering it a reversible phenomenon (Lemaître et al., 2015). Consequently, some recent theories propose that ageing occurs as consequence from other life processes, apparently having any specific function (Anton et al., 2005). This concept may appear extreme, but the idea, to manipulate ageing as other non-fundamental life's processes, increases. Consequently, age-associated senescence may appear as a complex of pathophysiological processes that could be prevented, delayed, or even reversed (Balistreri, 2018; Balistreri et al., 2020; Vaiserman et al., 2019). At present, novel biotechnologies, particularly including omics procedures (i.e., genomics, transcriptomics, proteomics, and metabolomics) have emergently applied in the research and potentially result capable to slow-down or postpone processes related to ageing, and consequently to be widely applied in anti-ageing medicine (Balistreri, 2018; Balistreri et al., 2020; Vaiserman et al., 2019). On the other hand, such

technologies have been used for identifying the molecular and cellular mechanisms related to ageing process, including genomic instability, epigenetic deregulation, loss of proteostasis, mitochondrial dysfunction, cellular senescence, exhaustion of stem cells, inflammation, telomere shortening, autophagy, impaired stress resistance and deregulated nutrient signaling (Balistreri, 2018; Balistreri et al., 2020; Vaiserman et al., 2019). This knowledge represents an initial point for developing innovative therapeutical strategies against the age-related functional decline and onset of pathological conditions of tissues, organs, and systems of an organism. Here, we report and discuss new concepts related to use of *senotherapeutics*.

1.1. Senescent cells as target for the treatment of ARD

Cellular senescence, and the consequent senescent cells (SC), are the typical features of ageing process, that arise from a complex number of cellular and molecular-induced processes (Olivieri et al., 2018; Childs et al., 2017). Mounting evidence reports such contribution of SC in determining the aged phenotype (Balistreri, 2018). However, the related mechanisms have not been fully elucidated, as well as the effective SC relationship with onset of human ARD. Certainly, a limiting factor, that has likely contributed to delay the detection of SC in humans, is the absence to date of standardized biomarkers of senescence *in vivo*. However, growing literature reports that SC induce harmful effects on the tissue microenvironment, by evocating the release of molecules acting as pathological facilitators or aggravators (amply quoted in Balistreri et al., 2013). Accordingly, SC contribute to ageing and ARD onset via the *senescence-associated secretory phenotype* (SASP), which consists in a variety of soluble factors, including proinflammatory mediators and matrix-degrading molecules. In tun, SASP contributes to evocate a state of chronic, systemic, low-grade inflammation, termed *inflammageing*, one of the principal risk factors related to the onset of the major ARDs. The percentage of the proinflammatory level necessary for achieving the condition over which diseases/disabilities arise, depends on genetic, environmental, and stochastic factors (Balistreri et al., 2013; Ovadya and Krizhanovsky, 2014). In turn, the age-related SC accumulation stimulates the immune system activation, and subsequently determines a consequent chronic immune condition that is closely related to a decreased SC clearance. Consequently, this continuous immune response and the related vicious circle generate inflammageing. Beyond immune cells and tissue cells, also adult stem cells from aged humans are also affected (mesenchymal stem cells included). Such evidences the capacity of a senescent milieu in reducing the stemness properties (Hall et al., 2016) or the differentiation capacity (Liu et al., 2017). At the same time, such underlines the actions and various characteristics of SASP in the different tissues.

Altogether, the observations above-described underline that the accumulation of SC in the tissues is responsible of inflammageing during ageing maintained by SASP itself (disseminating in blood circulation) and SASP mediated effects (Weilner et al., 2016a). Relevantly, SC are present in significant augments in all the tissues, organs, and systems where ARD occur. Precisely, they are abundant not only in tumors but also in degenerative diseases, indicating the key role of induced SC chronic inflammation in these illnesses (Prattichizzo et al., 2017; Weilner et al., 2016b). Accordingly, we recently studied the senescence of endothelial cells (ECs) and the consequent endothelial dysfunction as one of the main triggers involved not only in the onset and progression of CVDs but also of other ARDs like osteoporosis (Olivieri et al., 2016), since ECs are components of the stroma of all tissues and organs (Madonna et al., 2016; Regina et al., 2016; Prattichizzo et al., 2016; Kirkland and Tchkonia, 2017).

2. A new era in the field of geroscience: the *senotherapeutics*

The abovementioned remarks about SC reveal their key role in ARD and suggest them as potential target. Accordingly, Childs and coworkers (2017) have scrupulously reviewed such SC function in ageing and ARD and evidenced them as potential target for prolonging lifespan and health-span. Such evidence has started a new era in the field of geroscience thanks to developing the *senotherapeutics*, drugs affecting (in a large sense) the senescence process (Olivieri et al., 2016). Senotherapeutics currently comprises three therapeutic approaches:

I. molecules to selectively kill SC, defined *senolytics*;
II. compounds able in reducing evocated SC SASP, acting hence as SASP suppressors, or capable to change the senescent phenotype, called *senomorphics*;
III. inhibition of increase of the number of SC in the tissues.

The latter above reported is likely the oldest strategy. Accordingly, a lot of antioxidants have been demonstrated to postpone the senescence process *in vitro* (Bjelakovic et al., 2013). However, the application of these promising findings, *in vivo* models (i.e., mice models) by using traditional antioxidants (e.g., C and E vitamins) have been scarcely translated in emerging therapies (Bjelakovic et al., 2013). Furthermore, results from human cohort studies also evidence the incapacity of such compounds to stop the ARD, even if discordant data have been detected varying the molecules analyzed (Kirkland and Tchkonia, 2017a). In contrast, more interesting preclinical findings have been obtained using senolytics and SASP-suppressing compounds. The senolytics have arisen using large pharmacological screenings and differential gene expression studies. In addition, *survival pathways* have been detected in SC as senolytics (Kirkland et al., 2017b). Among these, five anti-apoptotic-pathways *(SCAPs)* have been discovered and successfully targeted, including Bcl-family proteins, PI3K-Akt, p53, ephrin-tyrosine kinases, HIF-1α, and HSP90 pathways (Kirkland et al., 2017). This area is rapidly growing, and the information offered by using new technologies, such as single-cell RNAseq, is permitting to provide more insights into the SC for accelerating the development of new senolytic drugs (Kirkland et al., 2017b). Remarkably, various pathological phenotypes related to ageing have been targeted with numerous senolytics in mice models, such as atherosclerosis. Until now seven classes of compounds with senolytic activity have been described. Among these, dasatinib, quercetin, BCL2 family inhibitors, forkhead box protein O4 (FOXO4)-interacting peptide blocking the link of FOXO4 with p53, have been recently reported. In addition, natural compounds, such as fisetin, a quercetin-related flavonoid, and piperlongumine also show senolytic or senomorphic action, as well as drugs used for clinical uses targeting the co-chaperone heat shock protein 90 (HSP90), have recently demonstrated as an innovative group of senolytics, evocating apoptosis of murine and human SC *in vitro* and able to expand healthspan *in vivo*. For the end evidence, the FDA-approved histone deacetylase inhibitor, panobinostat, has been considered as senotilic inducing apoptosis of tumor SC *in vitro*. Obviously, additional classes of potential senolytics will be detected thanks to emerging bioinformatic analyses and drug-screening approaches (Kirkland et al., 2017).

Concerning senomorphics, several classes have also been reported. Among these, inhibitors of IkB kinase (IKK) and nuclear factor (NF)-kB5, free radical scavengers and Janus kinase (JAK) pathway inhibitors, as well as rapamycin able to decrease SASP. Even fisetin has demonstrated to act with senomorphic impacts on some cell types and with senolytic effect on others, *in vitro*.

However, the major number of drugs have been successfully tested in mice, while have been demonstrated unfavorable for humans, inducing toxicity (e.g., chemotherapeutics and immunosuppressors). Thus, their translation to clinical trial testing should be restricted to certain conditions, while an appropriate compound for a whole-population treatment appears to be far to discover. At present, metformin appears as the most likely candidate for such use (quoted in Balistreri, 2018). Studies on model organisms have shown its ability to extend the lifespan (quoted in Balistreri, 2018), thanks to mechanisms largely debated, including: (a) the reduced insulin and IGF-1 signaling; (b) the inhibition of mTOR; (c)

reducing the levels of reactive oxygen species (ROS); (d) lowering inflammation, (e) reducing DNA damage, and (f) the activation of AMPK (quoted in Balistreri, 2018). Its effect on AMPK have however been mainly revealed.

3. Senotherapeutics and cardiovascular pathological conditions related to ageing

Evidence on clearance of SC from the arterial vessels with the aim to improve the typical age-related vascular phenotypes has led to suggest that senolytics have the potentiality to reduce cardiovascular pathological conditions associated with ageing (see Table 1). The tyrosine kinase inhibitor dasatinib and the flavonoid quercetin have been the first senolytic drugs to be described as able CVD treatments (Roos et al., 2016; Xu et al., 2018). However, adverse effects of some drugs have been recognized (Kirkland et al., 2017b). For instance, the drug navitoclax (ABT263) with senolytic activity commonly provokes neutropenia and thrombocytopenia. Senolytic treatments also show other issues. For example, they can neutralize SC by evocating potentially oncogenic mutations (Xu et al., 2018). However, mice treated with a combination of dasatinib and quercetin exhibited increased survival and health span. In addition, the treatment with dasatinib and quercetin has demonstrated to improve vasomotor function and to diminish aortic calcification in aged and hypercholesterolemic mice, respectively, significantly enhancing cardiac function in aged mice (Zhu et al., 2015; Roos et al., 2016). Furthermore, stimulation of cardiac progenitor cells in aged hearts and increased cardiomyocyte proliferative capacity have been described upon SC removal in aged mice, both using pharmacological approaches or in genetic senolytic models (Lewis-McDougall et al., 2019). Pharmacological and genetic senolytic models have, indeed, shown a connection between SC diminution, inhibition of heart fibrosis and improved cardiomyocyte proliferative expression profile (Anderson et al., 2019). Furthermore, senescent cardiomyocyte elimination via administration of ABT263 has been observed to improve myocardiac remodeling and the overall survival rate using a myocardial infarction mouse model (Walaszczyk et al., 2019). Accordingly, senolytics show the capacity to reverse phenotypic changes associated with ageing, via reversion of age-associated cardiac dysfunction and stimulating regenerative capacity, which bolsters senolysis as a potential approach of cardiovascular pathological conditions (Kirkland and Tchkonia, 2017a; Kirkland et al., 2017b). In addition, cardiac glycosides have been recently reported as senolytic compounds, having the strong potential as effective treatments against these conditions (Guerrero et al., 2019; Triana-Martínez et al., 2019). The HSP90 chaperone inhibitors have shown the same potential (Fuhrmann-Stroissnigg et al., 2017), including 17-DMAG able to improve atherosclerosis in mice (Lazaro et al., 2015), potentially due to its senolytic activity. Furthermore, 2-deoxy-D-glucose (2DG), a glucose analog able to inhibits ATP synthesis and determine cell cycle arrest and cell death, has been found to have senolytic action on senescent vascular smooth muscle cells. Precisely, 2DG potentially evocates an increased metabolic activity of SC, which may impact the progression of atherosclerosis (Gardner et al., 2015) (see Table 1).

However, the major number of senolytics have been clinically approved or are already in clinical trials for treating oncologic diseases, idiopathic pulmonary fibrosis, and chronic kidney disease (Mattison et al., 2014) studies. Concerning, senolytic drugs in the field of cardiovascular conditions, they have only been tested in animal models of disease, and clinical trials are currently awaited. In alternative via, some studies have recommended the suppressing of SC as another probable strategy for cardiovascular disorders. Accordingly, the activation of Sirtuin1 (SIRT1) signaling has been consistently described. Precisely, SIRT1 activation mediated by the polyphenol resveratrol has shown to inhibit both arterial wall inflammation and stiffening in nonhuman primates (Mattison et al., 2014). Similarly, SIRT1 specific activator SRT1720 reduces hypertension and arterial stiffness in mice (Xao et al., 2016) Important studies have discovered a down-regulated SIRT1 expression in vascular smooth cells of patients affected by abdominal aortic aneurysm, while SIRT1 activation induces inhibition of cell senescence and reduction of vascular inflammation (Chen et al., 2016). Accordingly, calorie restriction has been reported to be related to SIRT1 activation in vascular smooth cells and a reduced prevalence of abdominal aortic aneurysm

Table 1
Senotherapeutics, effects in cardiovascular system for counteracting pathological age-related conditions.

Drugs	Types	Effects	References
dasatinib and the flavonoid quercetin	senolytics	Increase of vasomotor function and reduction of aortic calcification in aged and hypercholesterolemic mice, respectively, significantly enhancing cardiac function in aged mice	Zhu et al. (2015) Roos et al. (2016)
navitoclax (ABT263)	senolytics	Increase of myocardiac remodeling and the overall survival rate using a myocardial infarction mouse model	Walaszczyk et al. (2019)
cardiac glycosides	senolytics	Reduction of age-associated cardiac dysfunction and stimulation of its regenerative capacity	Guerrero et al. (2019); Triana-Martínez et al. (2019)
HSP90 chaperone inhibitors, **such as 17-DMA**	senolytics	Improving of atherosclerosis in mice	Lazaro et al. (2015)
2-deoxy-D-glucose (2DG)	senolytics	Inhibiting of ATP synthesis and determine cell cycle arrest and cell death in senescent vascular smooth muscle cells, and retarding atherosclerosis progression	Gardner et al., 2015
SIRT1 activation mediated by the polyphenol resveratrol	inhibition of increase of the number of SC in the tissues	Inhibiting both arterial wall inflammation and stiffening in non-human primates	Mattison et al. (2014)
SIRT1 specific activator SRT1720	inhibition of increase of the number of SC in the tissues	Reducing hypertension and arterial stiffness in mice	Xao et al. (2016)
calorie restriction	inhibition of increase of the number of SC in the tissues	SIRT1 activation in vascular smooth cells and a reduced prevalence of abdominal aortic aneurysm	Liu et al. (2016)
pioglitazone	inhibition of increase of the number of SC in the tissues	stimulating telomerase activation and able to reduce the senescence of endothelial cells	Werner et al., 2011
rapamycin	senomorphics	determining an extension of mice lifespan, reduces senescence, and shows anti-atherosclerotic effects	Walters et al. (2016); Evangelisti et al. (2016)
statins	senomorphics	preventing SASP and regulating both the cell cycle and telomerase	Bennaceur et al. (2014)
polyphenols	senomorphics	antioxidant and anti-inflammatory effects	Mattison et al. (2014)
resveratrol	senomorphics	cell senescence suppressor of cardiovascular complications	Mattison et al. (2014)

(Liu et al., 2016). In addition, other related studies have also showed that the suppression of VSMC senescence is mediated by SIRT1 signaling pathways (van der Veer et al., 2007; Imai and Guarente, 2014). Other proposed clinical treatments are represented by pioglitazone stimulating telomerase activation and able to reduce the senescence of endothelial cells (Werner et al., 2011). Unconventional approaches to target SC are also being examined, including vaccines and other immune modulators, as well as toxin supply using SC-recognizing technologies. Metformin and rapamycin (sirolimus) inhibit SASP, reduce the pro-inflammatory milieu and the damage induced by activated SC (Kirkland and Tchkonia, 2017a) (see Table 1). Regarding rapamycin, it determines an extension of mice lifespan, reduces senescence, and shows anti-atherosclerotic effects (Walters et al., 2016; Evangelisti et al., 2016). Moreover, statins have been demonstrated to prevent SASP and regulate both the cell cycle and telomerase (Bennaceur et al., 2014).

Of note are the advances in the development of natural-based bioactive compounds with potential anti-senescence properties, called nutraceuticals (Nasri et al., 2014). For instance, polyphenols have anti-oxidant and anti-inflammatory effects, being possible senostatics by neutralizing pro-oxidant and pro-inflammatory signaling in SC (Gurau et al., 2018) Interestingly, another example is resveratrol, indicated as cell senescence suppressor of cardiovascular complications (Mattison et al., 2014).

Finally, mesenchymal stem cell (MSC)-based therapies are also reported to counteract senescence-associated cardiovascular conditions and complications. MSCs are multipotent cells with beneficial actions, such as multi-differentiation potential and low immunogenicity (Balistreri et al., 2020). Clinical trials on MSC transplantation are currently ongoing, indicating cardiac improvements in cases with heart failure secondary to ischemic cardiomyopathy (Balistreri et al., 2020).

Contemplating the whole collected evidence on the potential treatments for counteracting age-related cardiovascular conditions, limitations emerge, but a great hope is placed on clinical trials with senolytic agents. They, by evaluating their safety and efficacy, may lead to further progresses in developing appropriate patient drug administrations and offer effective treatments to inhibit the development of SC, associated pathological conditions and complications.

4. Conclusions

The development of senotherapies for delaying or stopping age-related cardiovascular conditions is of the great relevance, but currently shows diverse limitations in its effective clinical application. Probably, the integration of multi-omics approaches as a promising tool for their identification might be of help. This idea has derived from the consideration that current studies have been executed using individual omics types. This represents a limitation, because the separate omics evaluations enable the discovery of only a part of such molecules, reducing the possibility of identifying well-fitted therapies. The analysis of metabolomics, microbiomic, and nutrigenomic profiles are only just now emerging, and are leading to the identification of some promising molecules with anti-senescent effects (Scola et al., 2019). Executing all the omics analyses can certainly imply the detection of well-fitted therapies in humans, obtaining data from multidimensional levels. A further help in such research may also be derived from gender medicine that could add another crucial level in the differential management of cardiovascular pathologies associated with age and therapies according to gender. Today, gender represents one of the major challenges in cardiovascular management and therapies. Having panels of appropriate senotherapeutic molecules, for the various pathological conditions and for gender, could also facilitate the design of appropriate algorithms for their prevention. However, this field of research currently continues to be young, and consequently needs a major interest and more investigation, based on more accurate, standardized omics techniques. Nevertheless, the approaches and techniques developed can give a detailed idea of the information about detecting innovative treatments (Balistreri, 2018).

CRediT authorship contribution statement

Carmela Rita Balistreri: Writing – original draft, conceived, designed, wrote the review, based on recent literature evidence, prepared all the figures, contributed to scientific discussion and reviewed the manuscript. **Rosalinda Madonna:** All the authors read and approved the paper. **Peter Ferdinandy:** All the authors read and approved the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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