Mechanisms of Ageing and Development 181 (2019) 47–58

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Attenuation of frailty in older adults with mesenchymal stem cells

Victoria Florea[a], Luiza Bagno[a], Angela C. Rieger[a], Joshua M. Hare[a,b,*]

[a] Interdisciplinary Stem Cell Institute, University of Miami Miller School of Medicine, Miami, FL, United States
[b] Department of Medicine, University of Miami Miller School of Medicine, Miami, FL, United States

ARTICLE INFO

Keywords:
cell-based therapy
frailty
geriatrics
immunosenescence
inflammation
regenerative medicine

ABSTRACT

Aging frailty is a syndrome characterized by a progressive decline in health and clinical symptoms of exhaustion, weight loss, a feeling of slowing down, and a decrease in functional capacity. The biological substrate for frailty is sarcopenia, which is potentiated by chronic inflammation and depletion or impairment of endogenous precursor and stem cells. Current interventions focus on interdisciplinary approaches which include nutritional supplementation, physical exercise, and cognitive intervention. Clinical studies of these preventative approaches have shown inconsistent and modest benefits, further highlighting the unmet clinical need. A variety of pharmacologic and biologic therapies are currently being tested to treat aging. Cell-based therapy represents an attractive option that addresses the pathophysiology of the syndrome. Human allogeneic mesenchymal stem cells (MSCs) which possess immunomodulatory and tissue reparative properties, have been tested in Phase I and Phase II trials. These small early stage studies reveal that allogeneic MSCs administered to frail older adults are feasible to administer, safe and potentially efficacious, ameliorating signs and symptoms of frailty. These studies have formed the basis for larger ongoing trials. Here we review the pathobiology of frailty, and the potential for developing biological strategies to treat this important syndrome.

1. Introduction

Aging frailty is a geriatric syndrome that increases in incidence with advancing age, characterized by sarcopenia, inflammation, a loss of ability to perform activities of daily living and diminution of physical performance (Fried et al., 2001; Walston et al., 2017; Buckinx et al., 2015). Frailty currently represents a growing public health problem internationally, particularly in countries with populations of greatest longevity (Crimmins and Beltran-Sanchez, 2011). Frailty increases the risk for falls and is a major risk factor for mortality in individuals undergoing surgical procedures particularly cardiac and orthopedic (Partridge et al., 2012). Despite decades of research that have led to increasing recognition of the clinical features and pathophysiology of aging frailty, currently there are no approved medical or biological treatments that reduce the symptoms of frailty or that improve functional capacity, quality of life (QOL), or measures of health span in affected individuals (Walston et al., 2018). Here we review the syndrome of frailty and the concept of using cell-based therapy as a biologic candidate for this serious syndrome responsible for major morbidity and mortality in affected individuals (Fig. 1).

2. Identification of frailty

The word frailty from the Latin “fragilis” means “easily broken”. Frailty is a complex geriatric syndrome characterized by impaired performance and reduced functional reserve of multiple body systems, and is associated with increased risk for poor health outcomes including falls, hospitalization, disability, and mortality (Fried et al., 2001; Walston et al., 2017; Buckinx et al., 2015). There are several screening tools to identify patients at high risk of adverse clinical outcomes including prolonged recovery from illness, increased risk of falls, greater functional impairment leading to disability and dependency, and mortality.

Fried and colleagues in seminal work published in 2001 proposed a clinical phenotype of frailty validated in the Cardiovascular Health study as meeting three out of five phenotypic criteria: low grip strength, self-reported exhaustion, slowed walking speed, low physical activity, and unintentional weight loss (Fried et al., 2001). Each criterion is

Abbreviations: ACEI, angiotensin-converting enzyme inhibitors; ALS, amyotrophic lateral sclerosis; ALSFRS-R, ALS Functional Rating Scale–Revised; BM, bone marrow; BMMNC, bone marrow mononuclear cell; CRP, C-reactive peptide; CVD, cardiovascular disease; FEV1, forced expiratory volume in 1 s; HSCs, hematopoietic stem cells; IFN, interferon; IGF-1, insulin-like growth factor 1; IL, interleukin; M, million; MHC, major histocompatibility complex; MSC, mesenchymal stem cells; NO, nitric oxide; TE-SAEs, treatment-emergent serious adverse events; TGF-β, transforming growth factor-beta; TNF-α, tumor necrosis factor-alpha

* Corresponding author at: Interdisciplinary Stem Cell Institute, University of Miami Miller School of Medicine, Miami, FL 33136, United States.
E-mail address: JHare@med.miami.edu (J.M. Hare).

https://doi.org/10.1016/j.mad.2019.111120
Received 1 February 2019; Received in revised form 20 May 2019; Accepted 29 May 2019
Available online 07 June 2019
0047-6374/

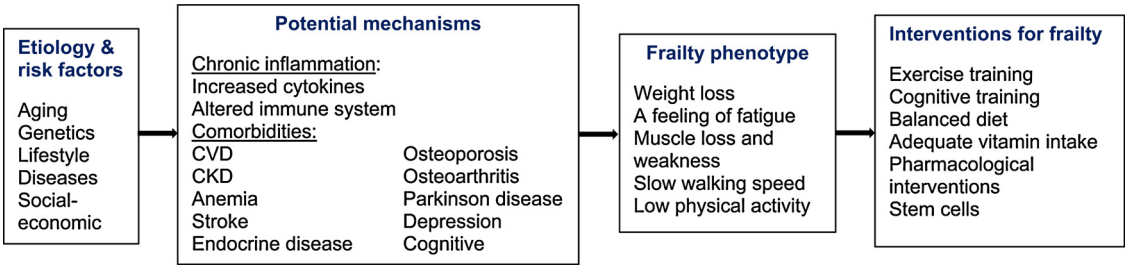

Fig. 1. Etiology/risk factors, potential mechanism, frailty phenotype and interventions for frailty.

categorized into a 3-level variable: robustness (normal), pre-frailty and frailty (Fried et al., 2001; Bandeen-Roche et al., 2006). The frailty phenotype does not require a preliminary clinical evaluation, and it helps to delineate frailty from disability and comorbidity. However, the frailty phenotype does not provide the biological understanding of decreased reserves, therefore it does not allow for the detection of frailty at the latent stage. An alternative tool for defining frailty is a risk index ("frailty index") validated in the Canadian Study of Health and Aging. The frailty index is a cumulative deficit model that includes disability, diseases, psychosocial risk factors, and physical and cognitive impairments (Rockwood and Mitnitski, 2011; Ensrud et al., 2008). The frailty score is measured by totaling the deficits of medical and psychological conditions, and it is composed of 80 items. Although calculation of the score is time consuming, it's informative for the continuous follow-up of a patient.

A clinically useful and validated tool to determine and grade the severity of frailty is the Canadian study of health and aging clinical frailty scale (CSHA) (Rockwood et al., 2005). This scale has been modified by other groups such as the Chinese-Canadian study of health and aging clinical frailty scale physician version (CSHA-CFS PV) (Chan et al., 2010). CSHA-CFS PV is a 7-point scale assigned after comprehensive geriatric assessments. The score ranges from 1 (very fit) to 7 (severely frail). The same group proposed a CSHA-CFS telephone version (CSHA-CFS TV) that allows the identification of potentially frail subjects with a convenient algorithm. A number of other instruments for frailty screening have been proposed, including FRAIL (Fatigue, Resistance, Ambulation, Illnesses, and Loss of weight) (International Academy of Nutrition and Aging) (Abellan van Kan et al., 2008), Frailty Instruments for Primary Care of the Survey of Health, Aging and Retirement in Europe (SHARE-FI) (Romero-Ortuno et al., 2010), the Groningen Frailty Indicator (Peters et al., 2012), Vulnerable Elder Survey-13 (Min et al., 2006), and the Tilburg Frailty Index (Gobbens et al., 2010). Importantly, frailty must be accepted as a clinical diagnosis and a clinical syndrome, facilitating appropriate management. The clinical diagnosis of aging frailty is recognized by the World Health Organization and there is an ICD-10 code for frailty.

Previous studies have demonstrated the link between frailty and cognitive performance, including perception speed, episodic memory, working memory, immediate memory, attention, verbal fluency and the clock drawing test (Fried et al., 2001; Buchman et al., 2008; Avila-Funes et al., 2009). Moreover, there is association between frailty and dementia, and increased prevalence of mortality in frail older individuals with cognitive impairment (Jacobs et al., 2011; Matusik et al., 2012; Cano et al., 2012). It was recently proposed that measurement of cognitive function should be added to physical performance for the definition of frailty. Cognitive impairment may lead to increased risk of acquiring individual components of frailty (Nyunt et al., 2017). For example, depression has been associated with hippocampal atrophy and subsequent cognitive impairments, and worsening of frailty (Panza et al., 2010; Mezuk et al., 2012, 2013; Lohman and Mezuk, 2013). Both frailty and cognitive impairment share many common risk factors and underlying mechanisms (Malmstrom and Morley, 2013; Kelaiditi et al., 2013), therefore a term of "cognitive frailty" has been introduced. Pathophysiological mechanisms such as oxidative damage and functional changes in the hippocampus and prefrontal cortex are important factors facilitating cognitive impairments (Bishop et al., 2010; Moreno-Jimenez et al., 2019). Structural and functional changes to microglial cells in the brain in combination with frailty may increase the risk of adverse outcomes (Clegg et al., 2013). Moreover, inflammatory markers such as CRP and IL-6 play important role in physical disability and neurodegenerative disease.

3. Underlying biological changes contributing to aging and frailty

There is an ongoing quest to understand the biological underpinnings of aging. In this regard, Lopez-Otin et al (Lopez-Otin et al., 2013) described nine cellular and molecular candidate hallmarks that contribute to the aging process. These include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication (Lopez-Otin et al., 2013). The biggest challenge is to define connections between these parameters and their contributions to aging, and then identify potential pharmaceutical or biological targets designed to reverse aging.

There are several environmental factors that augment or contribute to the development of frailty including chronic diseases, psychosocial status, and physiological perturbation. Frailty is associated with multiple comorbidities such as cardiovascular disease (CVD), endocrine diseases (diabetes mellitus), chronic obstructive pulmonary disease, chronic kidney disease, anemia, stroke, Parkinson disease, osteoporosis, osteoarthritis, depression, cognitive impairment, etc. (Liguori et al., 2018; Kojima, 2017; Kalyani et al., 2012). Moreover, previous studies have established links between chronic inflammation, autonomic nervous system lability, and energy metabolism dysregulation and frailty (Katayama et al., 2015; Michaud et al., 2013). Each of these pathological processes may accelerate the development of frailty.

Frailty is also associated with stem cell depletion and exhaustion. The stem cell function is characterized by decreased survival, proliferation, differentiation, and homing capacity. Cell-based therapy represents a promising approach to ameliorate and prevent development of frailty. MSCs are one of the most promising cell types used in regenerative medicine (Florea et al., 2017; Hare et al., 2017), since they are immunoprivileged (Hare et al., 2017). They possess antifibrotic, neoangiogenic, cardioprotective, and immunomodulatory effects (Uccelli et al., 2008; Ren et al., 2008; de Witte et al., 2018) mediated by contact-dependent intercellular interactions, the secretion of soluble factors, and through mitochondrial transfer (Liang et al., 2014). The results of two completed small early phase studies that used MSC infusion in frail individuals demonstrated safety and potential efficacy with improvements in physical activity, QOL, cognitive function, endothelial function, and levels of inflammatory biomarkers (Golpanian et al., 2017a; Tompkins et al., 2017a).

4. Aging-associated changes in the immune system

Age-related alterations in immune system function, a process termed immunosenescence, represent a major risk factor for increased susceptibility of older individual to disease. A major factor underlying immunosenescence is prolonged antigenic stimulation across life span (Gruver et al., 2007). The age-associated immunologic changes vary across older adults, and evidence suggests that there is an important association between degree of frailty and immune-competency (Fulop et al., 2010).

The functions of the innate-to-adaptive 'immune bridging' populations (natural Killer; invariant Natural Killer cells; and γδ T cells) appear to be better preserved with age than either the innate (neutrophils; monocytes/macrophages; myeloid-derived suppressor cells; and dendritic cells) or the adaptive (T cells - CD4 + and CD8 +; B cells) immune cells. Impaired neutrophil function is implicated in increased infectious disease risk that occurs in older as compared to younger (Jackaman et al., 2017). At the cellular level, neutrophil number and adhesive capabilities are unchanged with aging, while chemotactic responses and migratory function are reduced (Fulop et al., 2004; Sapey et al., 2014). In addition, some reports show that phagocytosis and production of reactive oxygen species by neutrophils are compromised with age (Tseng and Liu, 2014).

Frailty has also been associated with immune changes, including increased neutrophil:lymphocyte ratio and increased myeloid-derived suppressor cells (Elias et al., 2018). A change from a naïve to a memory lymphocyte phenotype also occurs with advancing age. This modification could explain the reduced ability of older adults to produce immune responses to antigens that they have not previously encountered (Lavy-Shahaf et al., 2014). In addition, there is a inversion of the CD4:CD8 ratio; the decline in CD4 + T cell numbers in older individuals is a marker of immunosenescence and predicts mortality (Hadrup et al., 2006).

Furthermore, aging is associated with increased levels of circulating cytokines and pro-inflammatory markers. In frailty, the immune phenotype is dysregulated due to chronic inflammation known as "inflammaging" a phenotype characterized by elevated levels of interleukin (IL)-1, IL-6, IL-8, IL-13, IL-18, C-reactive peptide (CRP), tumor necrosis factor-alpha (TNF-α), interferon (IFN)-α and IFN-β, transforming growth factor-β (TGF-β), and serum amyloid A (Mitnitski et al., 2015). In particular, cohort studies have indicated increased TNF-α and IL-6 levels as markers of frailty (Michaud et al., 2013). The chronic activation of Toll-like receptors, a consequence of the accumulation of reactive oxygen species during the aging process, also helps to trigger and intensify the inflammatory process (Cannizzo et al., 2011; Dewan et al., 2012).

5. Frailty, cardiovascular disease and endothelial dysfunction

The prevalence of CVD increases in individuals > 65 years of age, particularly in those > 80 years of age (Heidenreich et al., 2011). Approximately 50% of patients with CVD, such as coronary heart disease, angina, myocardial infarction, hypertension, heart failure (HF) with reduced ejection fraction, and HF with preserved ejection fraction develop frailty (Afilalo et al., 2009; Paneni et al., 2017). Moreover, frailty is a strong predictor of mortality in patients with CVD (Afilalo et al., 2009), as shown in a meta-analysis of nine studies that included 54,250 elderly patients (Fig. 2). As mentioned above, the pathophysiology of CVD and frailty are linked with perturbations in the inflammatory system (Michaud et al., 2013). Some inflammatory biomarkers, such as CRP, IL-6, factor VIII, and D-dimer, play a role in the pathogenesis of both processes (Tracy, 2003; Lang et al., 2010; Ershler and Keller, 2000).

Alterations in the structure and function of the cardiovascular system accompany aging, and contribute to increased risks of CVD development (Paneni et al., 2017; Newman et al., 2008). With aging,

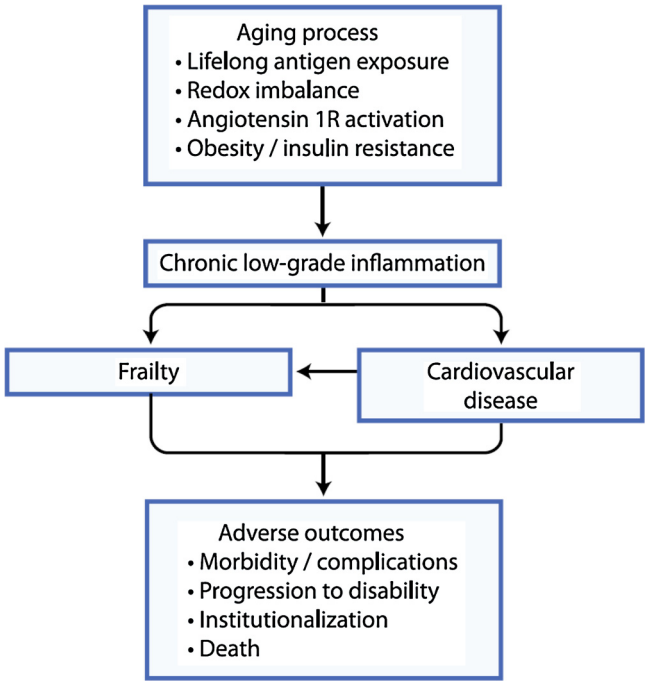

Fig. 2. Mechanistic interconnections between frailty and cardiovascular disease (CVD).

two main processes are involved in the phenotypic alteration of cardiovascular system: central arterial stiffness, due to increased collagen and decreased elastin, and endothelial dysfunction (Celermajer et al., 1994). The increased arterial stiffness elevates systolic pressure that leads to systolic hypertension, a major risk factor for CVD, which is difficult to control in elderly patients (Chobanian, 2007). Diminished bioavailability of nitric oxide (NO), a major mediator of vasodilatation, underlies age-dependent endothelial dysfunction (Taddei et al., 2001). Endothelial dysfunction includes reduced vasodilatory and antithrombotic properties, with an increase in oxidative stress and inflammatory cytokines (Delp et al., 2008) favoring atherogenesis and thrombosis, and predisposing to CVD (Lakatta, 2003). Accordingly, understanding the mechanisms by which age affects the cardiovascular system is crucial in order to prevent or attenuate progression of CVD in frail elderly individuals.

6. Frailty and skeletal muscle performance

Sarcopenia, dynapenia, and loss of strength with aging are important precursors to frailty (Wilson et al., 2017). Sarcopenia is characterized by muscular degeneration caused by the altered pro-inflammatory state, and is characterized by progressive and generalized loss of skeletal muscle mass and strength (Hubbard et al., 2009; Newman et al., 2001). Circulating pro-inflammatory molecules affect the repair and turnover of many tissues, including skeletal muscle (Franceschi and Campisi, 2014). Chronic inflammatory state is characterized by reduced synthesis and activity of insulin-like growth factor 1(IGF-1) that is crucial for muscle repair and maintenance of muscle integrity (Yakar and Adamo, 2012). IGF-1 activity is inhibited by high levels of IL-1, IL-6 and TNF-α. Importantly, high levels of IL-6 and low levels of IGF-1 correlate with lower muscle strength and power, and synergistically contribute to progressive disability and death (Cappola et al., 2003). Inflammation also impairs the uptake of long branched-chain amino acids, including leucine, isoleucine, tryptophan, serotonin, and methionine, that are essential for muscle anabolic processes (Moaddel et al., 2016). The preliminary results of the "BIOmarkers associated with Sarcopenia and Physical frailty in ElderRly pErsons" (BIOSPHERE) trial (Calvani et al., 2018a) showed that older adults with

physical frailty and sarcopenia had lower circulating levels of the essential amino acid methionine compared to control (Calvani et al., 2018b). Similarly, low plasma level of essential amino acids characterized the amino acid profile of severely frail Japanese older patients compared with non-frail subjects (Adachi et al., 2018).

7. Preclinical and clinical studies on frailty

Currently there are no proven interventions for the prevention and treatment of frailty. An interdisciplinary approach which includes nutritional modulation, cognitive intervention, and physical activity may attenuate the onset and progression of frailty (Bartali et al., 2006; Clegg et al., 2012; Theou et al., 2011; Pahor et al., 2018).

7.1. Non-pharmacological interventions for frailty

Nutritional modulation is one of the approaches to delay frailty. Nutritional interventions include identifying possible causes for reduced appetite, initiating oral supplementation, optimizing fluid intake, correcting metabolic acidosis, and promoting adequate vitamin D uptake.

Poor nutrient intake is associated with frailty in older adults (Bartali et al., 2006). An intake of ≤21 kcal/kg/day and a low intake of more than three nutrients (protein, vitamins D, E, and C, and folate) are related to exhaustion and decreased muscle strength, both of which are frailty criteria. After increasing energy intake, the feeling of exhaustion is reduced (Bartali et al., 2006). However, muscle strength does not improve, suggesting that the quality of the diet plays a major role in muscle strength (Bartali et al., 2006). It has been demonstrated that the optimal protein intake in older persons with sarcopenia is 1.0–1.2 g protein per kilogram body weight per day, and for those who are exercising the protein intake is > 1.2 g per kg body weight per day (Bauer et al., 2013). Additionally, protein supplementation enriched in essential amino acid leucine and vitamin D in older adults with sarcopenia at high risk for disability significantly increased muscle mass and improved chair-stand test, but not the handgrip strength and Short Physical Performance Battery scores (Bauer et al., 2015).

While poor nutrient intake is associated with frailty, caloric restriction without malnutrition ameliorates and reduces symptoms of frailty (Yamada et al., 2018). Yamada et al (Yamada et al., 2018) conducted a 30 year longitudinal study where biometric, physical activity, and metabolic data were used to evaluate frailty phenotype in a monkey model. Weakness, poor endurance, slowness, and low physical activity level were significantly greater in control compared with calorie restriction as was total incidence of frailty (Yamada et al., 2018). Moreover, caloric restriction improved muscle regenerative capacity by improving satellite cell function in the old skeletal muscle (Cerletti et al., 2012) and preservation of muscle mass and strength with aging (Colman et al., 2008).

Exercise is another therapeutic modality for frailty. Exercise training has been tested for its ability to slow the decline of muscle function in older adults in numerous trials. Improvements in muscle strength and mobility (Clegg et al., 2012; Theou et al., 2011) are among the most successful changes reported. Most of the studies concluded that resistance training can stabilize or increase muscle mass and delay the development of sarcopenia (Churchward-Venne et al., 2015). A randomized, placebo-controlled trial compared resistance exercise training, multinutrient supplementation, both interventions, or neither intervention (control group) in frail adults over a period of 10 weeks. There was a substantial increase in muscle strength, gait speed, stair climbing power, and spontaneous activity in the resistance exercise training group. However, the reported results were inconsistent and did not show a reduced incidence of sarcopenia due to the short follow-up period (Fiatarone et al., 1994).

Studies suggest that vitamin D is involved in the pathogenesis and management of frailty (Artaza-Artabe et al., 2016). Supplementation with vitamin D in older adults who are 25(OH) vitamin D deficient reduces falls (Murad et al., 2011), hip fractures (Bischoff-Ferrari et al., 2012), mortality (Rejnmark et al., 2012), and may improve skeletal muscle function (Muir and Montero-Odasso, 2011). A meta-analysis of 53 trials showed that vitamin D supplementation alone is unlikely to prevent fractures in older adults (Avenell et al., 2014), whereas supplementing vitamin D in combination with calcium can prevent hip or other types of fractures (Avenell et al., 2014). Conversely, another study demonstrated that daily doses of ≥800 IU of vitamin D alone can improve balance and skeletal muscle stregth (Muir and Montero-Odasso, 2011).

The FIT trial (Randomized Controlled Trial of Community-based Nutritional, Physical and Cognitive Training Intervention Programmes for At Risk Frail Elderly), compared the effects of 6-month-duration interventions with either nutritional supplementation, cognitive training, physical activity, the combination of treatments, or usual care control in pre-frail and frail older adults (Ng et al., 2015). Primary outcomes included frailty score, body mass index, knee extension strength, gait speed, energy/vitality, and physical activity levels. Secondary outcomes included activities of daily living dependency, hospitalization, and falls. Frailty score and status over 12 months were significantly improved in the 4 groups with interventions compared to control. Several physical frailty symptoms improved, including knee strength, physical activity, gait speed, and energy. However, none of the interventions improved the secondary outcomes, which included hospitalizations, falls, and performance of daily activities (Ng et al., 2015).

7.2. Pharmacological interventions for frailty

Pharmacological agents, including oral hypoglycemic agents (Laksmi et al., 2017), angiotensin-converting enzyme inhibitors (ACEI), selective androgen-receptor modulators (Narayanan et al., 2018), megestrol (an appetite stimulant) (Sullivan et al., 2007), testosterone (Emmelot-Vonk et al., 2008) have been investigated in frailty prevention and treatment, with controversial and inconclusive results.

Metformin is an antidiabetic drug that has been used to prevent frailty and ameliorate its progression (Laksmi et al., 2017; Wang et al., 2017). In a double blind randomized, placebo-controlled trial conducted on non-diabetic elderly pre-frail patients, metformin treatment significantly improved gait speed, but not handgrip strength (Laksmi et al., 2017). Another observational study found that metformin reduced the development of age-related comorbidity, including frailty-related diseases, CVD, cancer, and depression in older men with type 2 diabetes Wang et al., 2017). Finally, a recent meta-analysis revealed that metformin reduces all-cause mortality and ageing-related diseases independent of its effect of diabetes control (Campbell et al., 2017).

ACEI improve the structure and biochemical function of skeletal muscle (Schaufelberger et al., 1996), halt or slow the decline in muscle strength in elderly adults (Onder et al., 2002) and improve exercise capacity and maintain health-related QOL (Sumukadas et al., 2007).

Testosterone improves muscle strength but it also increases adverse cardiovascular and respiratory outcomes (Basaria et al., 2010). Importantly, testosterone undecanoate in combination with high calorie supplementation do not improve frailty scores (Theou et al., 2016). Likewise, IGF-1 therapy exhibits no beneficial effects on bone mineral density, fat mass, muscle strength, blood lipid parameters, and levels of postprandial glucose in postmenopausal women (Friedlander et al., 2001). Similarly, 2 years of supplementation with dehydroepiandrosterone sulfate in frail patients did not improve physical performance and QOL (Nair et al., 2006).

These studies suggest that ongoing testing of new strategies targeting some of the mechanisms involved in ageing are needed to prevent development of aged-related diseases, frailty and disability in older individuals.

8. Regenerative therapy for frailty

8.1. Stem cell depletion and exhaustion in aging

The decrease in stem cells residing within the niche and the lack of stem cell regenerative capacity, leading to an impaired ability to repair organs and tissues, is one of the main characteristics of aging (Carlson and Conboy, 2007), which can be exacerbated by comorbidities that lead to frailty. The stem cell function is compromised and is characterized by decreased survival, proliferation (Stenderup et al., 2003), differentiation, migration, and homing capacity (O'Driscoll et al., 2001).

Effects of aging have been studied in multiple cell lines, including hematopoietic stem cells (HSCs), MSCs, skeletal muscle stem (satellite) cells and intestinal epithelial stem cells. Aging of HSCs is associated with the accumulation of random DNA damage, cell cycle arrest, and telomere shortening (Rossi et al., 2007; Attema et al., 2009). These processes lead to decreased self-renewal, homing ability, and increased apoptosis with stress. Cellular senescence biomarker p16INK4a accumulates and modulates age-associated HSC function. Notably, the absence of p16INK4a improves engraftment and cell survival, and reduces mortality (Janzen et al., 2006). In contrast, p16INK4a + cells in thigh adipose tissue is associated with poor physical function in older women (Justice et al., 2018), suggesting that senescent cells might also play a role in the pathogenesis of sarcopenia.

BM-MSCs showed age-related decrease in proliferation and differentiation and increase in senescence. MSC depletion and impairment has been related to osteoporosis and decrease in fracture repair (Liu et al., 2015). Transplantation of young MSCs significantly slow the age-related loss of bone microstructure and density (Shen et al., 2011), and increases life span of aged rodents (Shen et al., 2011; Mansilla et al., 2016).

As mentioned above, sarcopenia, an underlying pathophysiologic substrate for frailty, has been linked to a significant decline in satellite cell regenerative capacity, which is critical for skeletal muscle repair following injury (Walston, 2015). Aging of satellite cells is characterized by a decline in stem cell numbers and functionality, and is associated with defects in self-renewing mechanisms, exhaustion by forced differentiation, apoptosis and senescence (Sousa-Victor et al., 2014, 2015). Lavasani et al. showed that administration of satellite cells from young healthy mice to progeroid mice promoted significant lifespan and healthspan extension. Moreover, it led to muscle regeneration and host neovascularization, suggesting that therapeutic effect is mediated by secreted factors that act systemically (Lavasani et al., 2012). Based on the preclinical data indicating the beneficial effects of cell-based therapy in age-related conditions, administration of allogeneic cells from healthy young adults may have therapeutic value for ameliorating and delaying aging-related function decline and improve the symptoms of frailty.

8.2. Immunomodulatory, anti-inflammatory, and antimicrobial effects of mesenchymal stem cells

Mesenchymal stem cells (MSCs) are multipotent progenitor cells characterized by self-renewal, production of clonal cell populations, and multilineage differentiation (Caplan, 1991). They lack the major histocompatibility complex (MHC) / human leukocyte antigen class II molecules making them suitable for allogeneic MSC therapy with no immunosuppression. In addition to their antifibrotic, neoangiogic, and cardioreparative properties, MSCs also possess immunomodulatory properties (Uccelli et al., 2008; Ren et al., 2008) and are under investigation as a potential immunotherapy (de Witte et al., 2018). These various MSCs effects can be mediated by contact-dependent intercellular interactions and the secretion of soluble factors such as growth factors, matrix proteins and cytokines, as well as through mitochondrial transfer and secretion of extracellular vesicles and exosomes (Fig. 3) (Liang et al., 2014).

MSCs influence the immune system in several ways. They play a role in regulating the innate immune response, by inhibiting maturation and antigen-presenting capacity of dendritic cells (Le Blanc and Davies, 2015; Jiang et al., 2005), and decreasing proliferation and cytotoxicity of natural killer cells (Spaggiari et al., 2008). They also suppress the adaptive immune response, by inhibiting both CD4+ helper, CD8+ cytotoxic T cell, and B cell proliferation and function. Additional immunomodulatory effects of MSCs exerted through direct intercellular contact include the induction of regulatory B-cells and T-cells, and deviation of monocyte/macrophage and dendritic cells to anti-inflammatory phenotype (de Witte et al., 2018). Pro-inflammatory cytokines present in the inflammatory environment stimulate the MSC immunosuppressive potential. MSCs reduce the expression of pro-inflammatory cytokine through paracrine mechanisms by sections of anti-inflammatory factors such as TGF-β, hepatocyte growth factor (HGF), NO, heme oxygenase (HO)-1, indoleamine 2,3-dioxygenase) and the expression of inhibitory co-stimulatory molecules such as TNF-related apoptosis-inducing ligand (TRAIL), programmed death ligand (PD-L1) that work together and influence the effector populations (Kyurkchiev et al., 2014). Moreover, recent data suggest that MSCs in the inflammatory environment induce phenotypic and functional changes in monocytes, which then modulate the adaptive immune cell compartment (de Witte et al., 2018; Braza et al., 2016). These immunoregulatory properties of MSCs provide new insight into potential treatments for tissue damage and inflammation.

In addition to the anti-inflammatory effects, antimicrobial effects are also attributed to MSCs (Alcayaga-Miranda et al., 2017; Mezey and Nemeth, 2015), including direct inhibitory effects on bacterial growth and indirect effects via secretion of immune-mediators that activate inflammatory cells. MSCs enhance the microbicidal activity of macrophages by promoting the transformation of naive macrophages into inflammatory M1 macrophages, without enhancing their antigen presenting cell function in response to a pathogenic insult (Vasandan et al., 2016). The conversion of already active M1 macrophages into anti-inflammatory M2 macrophages is also induced by MSCs, helping to resolve the hyper-inflammatory state (Vasandan et al., 2016). The indirect effects of MSCs on bacterial growth may be mediated by secretion of immune-mediators such as IL-6, IL-8, granulocyte-macrophage colony-stimulating factor and macrophage migration inhibitory factor that recruit neutrophils and enhance neutrophil antimicrobial activity (Brandau et al., 2014). Both, the MSCs and MSC-derived conditioned medium reduce the growth rate and survival of some pathogens such as Pseudomonas aeruginosa, *Staphylococcus aureus* and *Streptococcus pneumoniae* (Sutton et al., 2016). These properties of MSCs contribute to patient responses against invading pathogens, which may be relevant for preventing or treating frailty, which significantly exacerbates morbidity and mortality.

8.3. Mesenchymal stem cells for cardiovascular disease

Cell-based therapy is a novel approach with a potential to attenuate age-related CVD. For treating CVD, MSCs are one of the most promising cell types used in regenerative medicine (Florea et al., 2017; Hare et al., 2017). Several trials evaluated the effects of bone marrow (BM)-derived MSCs in patients with chronic heart disease (Heldman et al., 2014). The results demonstrated that MSCs administration improves LV structure and function, perfusion defects, functional capacity, as assessed by improved 6 min walk test (6MWT), QOL measured by Minnesota Living with Heart Failure Questionnaire score, and NYHA classification (Heldman et al., 2014; Mathiasen et al., 2015). The lack of MHC class II antigens and the ability of MSCs to secrete anti-inflammatory cytokines, underlies the absence of a major immune response following administration of allogeneic MSCs (Karantalis et al., 2015), and has generated great interest in using these cells for systemic and local application without the need for immunosuppression (Hare et al., 2017). The

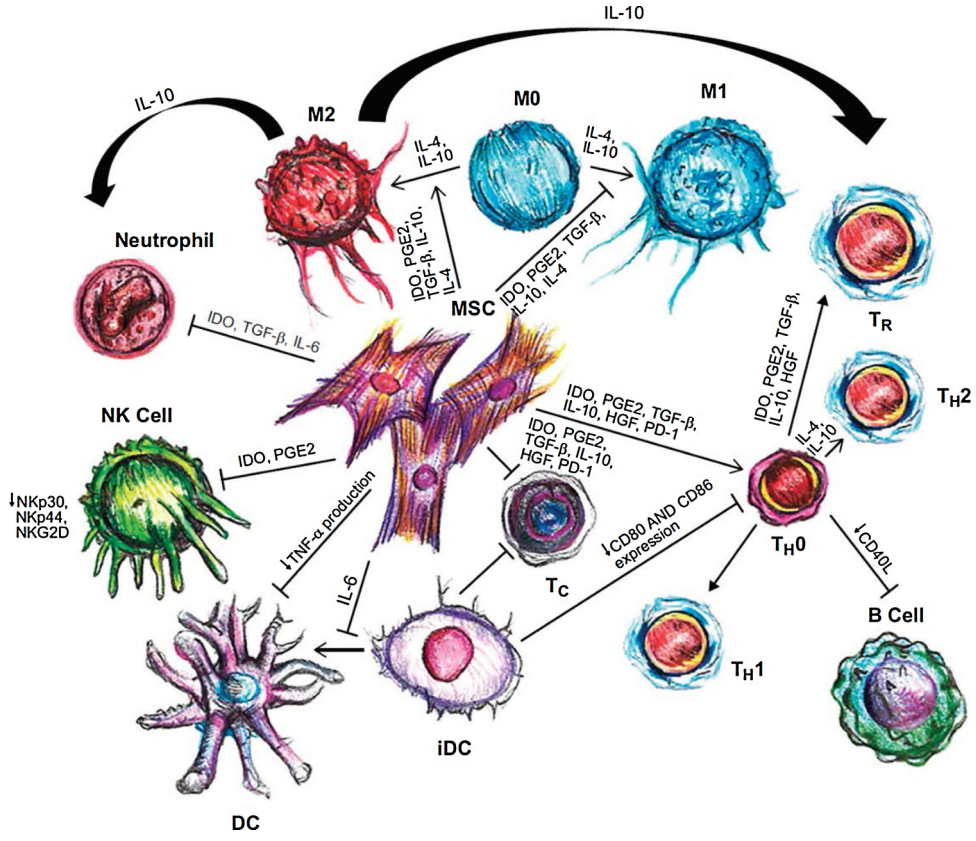

Fig. 3. Immune profile of mesenchymal stem cells (MSCs).

Table 1
Potential effects and mechanism of mesenchymal stem cells in patients with frailty.

Frailty symptoms	Potential MSC effects	Potential mechanisms
Unintentional weight loss	↓ chronic inflammation	↓ chronic inflammation (↓ TNF-α, ↓ CRP, ↓ IL-1, ↓ IL-6, ↑ TGF-β), ↓ onset of sarcopenia
A feeling of fatigue	↓ chronic inflammation, ↑ pulmonary function	↓ chronic inflammation (↓ TNF-α, ↓ CRP, ↓ IL-1, ↓ IL-6, ↑ TGF-β), ↑ endothelial function, ↑ pulmonary function (FEV1)
Muscle loss and weakness	↑ physical activity (six minute walk distance)	↑ skeletal muscle performance, ↑ cardiac function performance, ↓ onset of sarcopenia, ↑ endothelial function
Slow walking speed	↑ physical activity (six minute walk distance), ↑ pulmonary function	↑ skeletal muscle performance, ↑ cardiac function performance, ↑ pulmonary function (FEV1), ↑ endothelial function
Low levels of physical activity	↓ chronic inflammation, ↑ physical activity (six minute walk distance), ↑ quality of life	↓ chronic inflammation (↓ TNF-α, ↓ CRP, ↓ IL-1, ↓ IL-6, ↑ TGF-β), ↑ skeletal muscle performance, ↑ cognitive status

POSEIDON trial compared transendocardial stem cell injection of autologous vs. allogeneic MSCs in patients with ischemic cardiomyopathy. Both cell groups exhibited decreased myocardial remodeling and the cells were equally safe and produced an improvement in global and segmental LV contractility (Hare et al., 2012). In the recent POSEIDON DCM trial, transendocardial injection of allogeneic and autologous MSCs were compared in patients with non-ischemic dilated cardiomyopathy. The study demonstrated the safety of both cell types. Allogeneic MSC produced improvements in cardiac function, functional capacity and QOL, endothelial function, and pro-inflammatory and immune biomarkers. A post hoc analysis from the TRIDENT and POSEIDON DCM studies showed that unlike autologous BM-MSCs, allogeneic cells improved endothelial function measured by EPC proliferation and flow mediated vasodilation (Premer et al., 2015), two parameters that are impaired in HF and frailty. Taking into consideration that MSCs improve cardiac function, functional capacity, endothelial function and serum TNF-α in patients with heart disease, and CVD and frailty share the same pathophysiological processes, suggests that MSCs are a great candidate cell type for patients with frailty.

8.4. Novel therapeutic strategies for frailty

Currently, recommended therapies are limited to dietary modifications and exercise training. Given the increasing awareness of the underlying biology driving the process of frailty, there is an increased interest in treatments that have the potential to modify the aging process or to ameliorate factors that drive frailty. One particularly intriguing approach is to use cell-based therapy, with a lead candidate MSCs. MSCs have been shown to home to injury sites. They secrete bioactive molecules to reduce inflammation, induce tissue recovery, improve cardiovascular status in patients with HF, promote cellular repair, and possess immunomodulatory effects (Golpanian et al., 2016). Therefore, MSC administration in individuals with frailty support the idea of MSCs use to ameliorate or improve this syndrome (Table 1). To date there are two clinical trials that investigated the role of MSCs transplantation in frail older individuals.

The first study was the CRATUS (NCT02065245) phase I, open-label, dose escalation, pilot trial in which allogeneic BM-MSCs were administered to 15 patients to investigate the safety and efficacy of

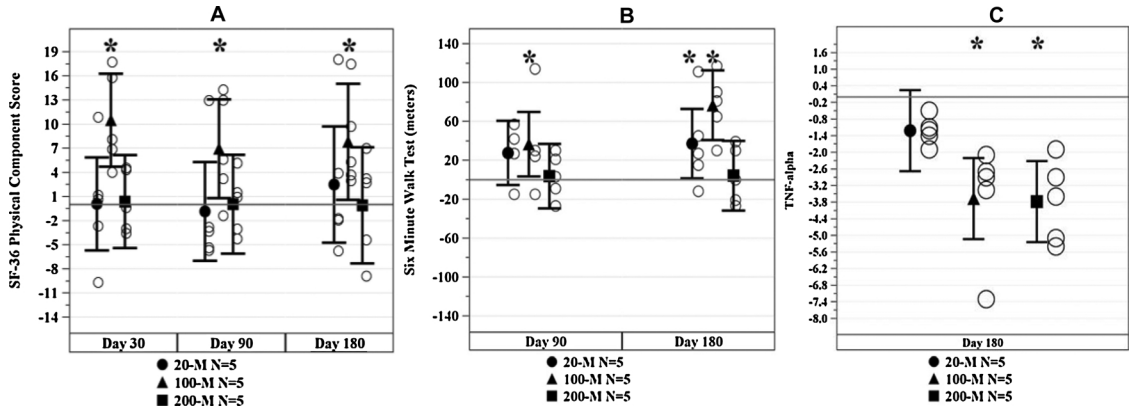

Fig. 4. Change in functional activity, quality of life and serum tumor necrosis factor alpha after administration of mesenchymal stem cells (MSCs) in frailty.

allogeneic MSCs for patients experiencing mild to moderate frailty (Golpanian et al., 2017b). The patients received either 50, 100, or 200 million (M) allogeneic MSCs via intravenous infusion (5 patients per group). The primary endpoint was safety assessed by the incidence of treatment-emergent serious adverse events (TE-SAEs) at 1 month post-MSC infusion. Additional safety was monitored by serious adverse events, adverse events, and clinical laboratory tests within 12 months post-infusion. The secondary outcomes were physical function assessments evaluated via 6MWT, handgrip strength, QOL evaluated via the SF-36 and EQ-5D questionnaires, patient-reported health status, and systemic inflammatory status measured at 3 and 6 months post-infusion. The MSC administration was well tolerated in frail individuals at all three doses. There were no TE-SAEs recorded, and no patient developed donor specific antibodies. The 6MWT, forced expiratory volume in 1 s (FEV1), cognitive status, as assessed by the Mini Mental State Examination (MMSE), and TNF-α improved at 6 months post-infusion. Additionally, QOL assessments demonstrated improvements in the physical components means scores of the SF-36 in the 100 M group. Subjects in the 100 M cell dose demonstrated the largest improvement at 3 and 6 months compared to baseline (Fig. 4) (Golpanian et al., 2017b).

The second CRATUS study was a phase II, randomized, double-blinded, placebo-controlled trial in which 30 frail individuals received either allogeneic MSCs at two doses (100 M or 200 M) or placebo (10 patients per group) (Tompkins et al., 2017a). The primary outcome was safety measured by TE-SAEs at 1-month post-infusion. The secondary outcomes were functional status and QOL assessments, and immune biomarkers measured at baseline and within 6 months post-infusion. No cell therapy related adverse effects were documented within 1 month post-infusion and throughout the duration of the study. There were no signs of adverse cardiopulmonary reaction following MSC administration. Short Physical Performance Battery (SPPB) total score and FEV1 were significantly improved in the 100 M cell group, without any changes in the 200 M and placebo groups. Moreover, the female sexual QOL measured by the sexual quality of life-female (SQOL-F) questionnaire improved in the 100 M group. Our findings show that 6MWT, a measure of frailty and a prognostic marker of mortality in older adults with heart failure (Boxer et al., 2010, 2008), improved in both trials in frail subjects following MSC administration in the 100 M group (Fig. 5). Additionally, both the 100 M and 200 M cell doses were effective in modulation of immunologic biomarkers. Reduction in the early T cell activation was noted in the 200 M group. There was a reduction in the late T-cell activation in the 100 M and 200 M groups compared to baseline. Finally, serum TNF-α levels decreased in the 100 M group at 6 months post-infusion (Tompkins et al., 2017a).

In summary, both clinical trials demonstrated safety and potential efficacy, suggesting that intravenous MSC administration is a promising and innovative approach for frailty. Importantly, the 100 M cell dose showed a maximal effect in measures of physical activity, QOL, and inflammatory biomarkers, which are important therapeutic assessments in the frailty syndrome. Although allogeneic MSCs hold a great promise as a cell therapy agent for frailty, the number of treated subjects in both studies was small, and there was no cell dose response relationship. Accordingly, larger and better powered trials are warranted to further define the effects of MSC infusion in individuals with signs and symptoms of aging frailty.

8.5. Role of repeated doses in cell therapy

Cell therapy approach demonstrated promising results for various comorbidities. However, the benefits are inconsistent and modest. One of the major reasons for the inconsistency and negative results could be a single cell dose administration and inadequate dose use. Given that the efficiency of cell engraftment is low, determination of optimum dose and effect of repeated cell dose administration is clinically relevant. Effect of repeated cell administration was investigated in preclinical and clinical studies using several cell types, including bone marrow mononuclear cells (BMMNCs), MSCs, and cardiac progenitor cells (CPCs) (Tang et al., 2018; Molavi et al., 2016; Oh et al., 2015, 2018; Tokita et al., 2016).

Effects of repeated cell transplantation were studied in patients with peripheral artery disease and critical limb ischemia (Molavi et al., 2016; Cobellis et al., 2008). A pilot randomized controlled study investigated the safety and efficacy of repeated BMMNC injection (every 3 weeks until the 4th injection) in comparison with a single cell injection in patients with critical limb ischemia. The study did not report any adverse effects and demonstrated that cell injections improved ankle-brachial index, visual analog scale, pain-free walking distance, Wagner stage, and reduced ulcer size. Although there was no difference in terms of clinical parameters between the groups, the pain-free walking distance improved in the group who received four injections of cells (Molavi et al., 2016).

Repeated intrathecal injections of autologous BM-MSCs demonstrated safety, feasibility, and therapeutic efficacy in amyotrophic lateral sclerosis (ALS) over 12 months (1 M cells per kg with a 26-day interval) (Oh et al., 2015, 2018). There was no difference in the incidence of AEs and SAEs between the groups, indicating that 2 repeated BM-MSC administration was safe. The ALS Functional Rating Scale–Revised (ALSFRS-R) and Appel ALS (AALS) scores were significantly improved in the MSC group compared to control group after 4- and 6-month follow-up. The MSC group showed decreased pro-inflammatory and increased anti-inflammatory cytokines. Despite ALSFRS-R improvement, there was no difference in long-term survival between groups Oh et al., 2018), suggesting that serial additional BM-MSC treatments after 6 months might improve long-term efficacy. Another study was designed to investigate the role of repeated intravenous

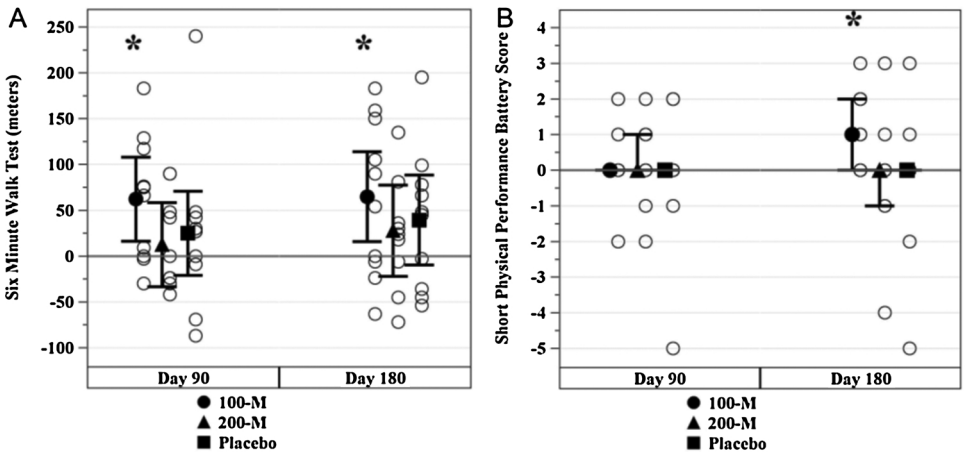

Fig. 5. Change in functional status after administration of mesenchymal stem cells (MSCs) in frailty.

infusions of human BM-MSCs in a liver failure experimental model (Miryounesi et al., 2013). Repeated administration of MSCs improved survival, liver fibrosis and necrosis compared with injection of the same number of MSCs in a single dose. This was associated with increased expression of the fibrogenic/fibrolytic related genes Col1a1, Timp1 and Mmp13 in the repeated transplant group (Miryounesi et al., 2013).

Bolli et al elegantly compared placebo control with single vs multiple infusions of CPCs in a rat MI model. Regional and global cardiac function improved with cumulative effects in the multiple infusions CPC group. Multiple infusions group also exhibited greater reduction in scar size, less collage and greater myocyte density in the risk region (Tokita et al., 2016). Importantly, infusion of a single, large dose of 36 M cells produced an initial improvement in cardiac function with no further improvements. In contrast, each of the 3 infusions of cells (12 M X 3) caused a greater improvement in left ventricular function compared to single, large dose. Additionally, repeated doses reduced collagen content and immune cell infiltration (Tang et al., 2018).

The role of chronic systemic inflammation is one of the main contributor to progression of frailty. The systemic anti-inflammatory processes are achieved after intravenous administration of MSCs. Although the short-term of anti-inflammatory effect is achieved after first cell infusion (Tompkins et al., 2017a; Tang et al., 2018; Oh et al., 2018), repeated cell administration over time will be required in order to sustain lower level of inflammatory biomarkers, and consequently improve the frailty phenotype. Important things to consider in the repeated cell administration approach are cell dose, time intervals between cell administration, and route of delivery. In some cases shorter interval between injections might cause a greater recovery and functional improvements (Yang et al., 2018). In summary, preliminary studies provide evidence that repeated injections are safe and potentially effective. Although the approach of multiple injections is feasible and well tolerated, future randomized, double-blind, large scale clinical trials are required to confirm the long-term safety and efficacy of the repeated cell administration.

8.6. Cell-based therapy and clinical challenges

Intravenous infusion of allogeneic MSC in patients with frailty demonstrated safety and potential efficacy. The cells improved physical activity, quality of life, cognitive status, and inflammatory markers. While previous experience of cell-based therapy for frailty have shown promise, challenges exist.

One of the challenges is to define optimal cell dose. One hundred million cell dose showed a maximal effect in most of the assessments in the CRATUS study (Tompkins et al., 2017a; Golpanian et al., 2017b). Importantly, the US Food and Drug Administration guidance for industry of investigational cellular therapy discussed the importance of cell dose optimization, and the establishment of a dose escalation schedule in preclinical and clinical studies. A variety of cell dose comparison clinical trials demonstrated an inverse dose response in various diseases. The POSEIDON trial randomized 30 patients to investigate three doses of autologous and allogeneic MSCs (20, 100, 200 million cells). The study demonstrated the greatest reduction in scar size, cardiac volumes, and increase in LVEF among patients who received the lowest cell dose of 20 million (Hare et al., 2012). In another dose-finding sub-study, the cardiac imaging analysis revealed an inverse relationship between the number of injections and improvements in LV volumes. Patients that received lower number of injections (≤ 20) demonstrated greater improvements in cardiac remodeling compared to those who received > 20 injections (Teerlink et al., 2017). Similarly, in the CRATUS dose escalation study that used 20, 100, and 200 million MSCs, the 100 million group improved physical activity, quality of life, cognitive status, and inflammatory biomarkers Golpanian et al., 2017b). In contrast, other studies showed a linear dose response, supporting that a larger dose is more efficacious (Quyyumi et al., 2011; Perin et al., 2015; Poglajen et al., 2014). The inconsistent responses could be affected by various confounding factors such as donor age, route of cell administration and timing of delivery.

Another challenge is that there is no direct evidence that cell-based therapy reverses sarcopenia or osteoporosis in frailty. In osteoporosis the number of stem cell decline. It is unclear whether infused stem cells can induce bone formation. Therefore, inclusion of bone density and a walking speed test assessments in future studies would be recommended.

The future of cell-based therapy for frailty is dependent on studies to better understand the effects of cell-based therapy on frailty. The ongoing phase IIb randomized, blinded, placebo-controlled trial (No. NCT03169231) is a trial that will include 150 subjects. It was designed to evaluate the safety and efficacy of allogeneic human MSC infusion in patients with aging frailty. Patients will receive either one of the doses of allogeneic MSCs (25 million, 50 million, 100 million, and 200 million) or placebo. The primary outcome is change from baseline in 6 min Walk Test (6MWT) compared to placebo at 180 days post-infusion. The secondary outcome assessments of the trial include: 1. change from baseline in physical function; 2. change in patient-reported outcome (PRO) measurement compared to placebo; and 3. change in serum TNF-a compared to placebo. These secondary measurements will also be made at 180 days following infusion.

9. Conclusions and future perspectives

Frailty associated with aging is recognized as a syndrome characterized by weight loss, poor exhaustion endurance, slowness, weakness, and low activity. Biologically, chronic inflammation and stem cell

depletion are important factors that contribute to frailty. On the bases of the data presented in the review, modulating chronic inflammation is a promising strategy to prevent and slow down not only frailty but also conditions associated with frailty. New biological treatments such as cell-based therapy that affects signaling pathways that regulate inflammation are needed to address the unmet clinical needs for individuals with frailty. Early interventions are warranted at the latent stage of frailty to reduce the burden of frailty progression, hospitalization, and mortality associated with frailty.

While MSC administration for frailty is a potential promising strategy, the results of early stage clinical trials warrant further evaluation in ongoing larger trials (Le Couteur et al., 2017). Larger studies with greater statistical power will offer the opportunity to define better the relationship between different measures of frailty in response to MSC infusion. Importantly, in the CRATUS study there was no dose escalation response, with peak efficacy observed with the 100 M cell dose. While previous studies have shown a reverse cell dose response (Tompkins et al., 2017b), it is important to evaluate several cell doses in frailty patients in a larger trial. Moreover, evaluation of repeated cell doses might produce better or no cumulative effects, thereby revealing whether recurrent infusions can amplify or sustain any potential clinical benefits.

In summary, the preliminary results of two small, early phase trials on MSCs for frailty support the idea that MSCs administration from young healthy adults might play an important role in improving human health span by improving the functions of multiple organs, including lung, heart, and immune system. While further studies are needed to determine the effects of MSCs transplantation in frailty, immunomodulatory and pro-regenerative effects are likely to contribute. MSC administration is a new and potentially promising tool for treating frailty in older adults, and larger randomized controlled trials are needed to test the effectiveness of this therapy for frailty.

Funding

JMH is supported by the National Institute of Health (NIH) grants, UM1 HL113460, 1R01 HL134558-01, 1R01 HL137355-01, as well as by the Starr, Soffer Family, and Marcus Foundations.

Acknowledgements

We thank Wayne Balkan, PhD, for his assistance in critical review and editing.

References

Abellan van Kan, G., Rolland, Y., Bergman, H., Morley, J.E., Kritchevsky, S.B., Vellas, B., 2008. The I.A.N.A Task Force on frailty assessment of older people in clinical practice. J. Nutr. Health Aging 12, 29–37.

Adachi, Y., Ono, N., Imaizumi, A., Muramatsu, T., Andou, T., Shimodaira, Y., Nagao, K., Kageyama, Y., Mori, M., Noguchi, Y., Hashizume, N., Nukada, H., 2018. Plasma amino acid profile in severely frail elderly patients in Japan. Int. J. Gerontol. 12, 290–293.

Afilalo, J., Karunananthan, S., Eisenberg, M.J., Alexander, K.P., Bergman, H., 2009. Role of frailty in patients with cardiovascular disease. Am. J. Cardiol. 103, 1616–1621.

Alcayaga-Miranda, F., Cuenca, J., Khoury, M., 2017. Antimicrobial activity of mesenchymal stem cells: current status and new perspectives of antimicrobial peptide-based therapies. Front. Immunol. 8, 339.

Artaza-Artabe, I., Saez-Lopez, P., Sanchez-Hernandez, N., Fernandez-Gutierrez, N., Malafarina, V., 2016. The relationship between nutrition and frailty: effects of protein intake, nutritional supplementation, vitamin D and exercise on muscle metabolism in the elderly. A systematic review. Maturitas 93, 89–99.

Attema, J.L., Pronk, C.J., Norddahl, G.L., Nygren, J.M., Bryder, D., 2009. Hematopoietic stem cell ageing is uncoupled from p16 INK4A-mediated senescence. Oncogene 28, 2238–2243.

Avenell, A., Mak, J.C., O'Connell, D., 2014. Vitamin D and vitamin D analogues for preventing fractures in post-menopausal women and older men. Cochrane Database Syst. Rev., CD000227.

Avila-Funes, J.A., Amieva, H., Barberger-Gateau, P., Le Goff, M., Raoux, N., Ritchie, K., Carriere, I., Tavernier, B., Tzourio, C., Gutierrez-Robledo, L.M., Dartigues, J.F., 2009. Cognitive impairment improves the predictive validity of the phenotype of frailty for adverse health outcomes: the three-city study. J. Am. Geriatr. Soc. 57, 453–461.

Bandeen-Roche, K., Xue, Q.L., Ferrucci, L., Walston, J., Guralnik, J.M., Chaves, P., Zeger, S.L., Fried, L.P., 2006. Phenotype of frailty: characterization in the women's health and aging studies. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 61, 262–266.

Bartali, B., Frongillo, E.A., Bandinelli, S., Lauretani, F., Semba, R.D., Fried, L.P., Ferrucci, L., 2006. Low nutrient intake is an essential component of frailty in older persons. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 61, 589–593.

Basaria, S., Coviello, A.D., Travison, T.G., Storer, T.W., Farwell, W.R., Jette, A.M., Eder, R., Tennstedt, S., Ulloor, J., Zhang, A., Choong, K., Lakshman, K.M., Mazer, N.A., Miciek, R., Krasnoff, J., Elmi, A., Knapp, P.E., Brooks, B., Appleman, E., Aggarwal, S., Bhasin, G., Hede-Brierley, L., Bhatia, A., Collins, L., LeBrasseur, N., Fiore, L.D., Bhasin, S., 2010. Adverse events associated with testosterone administration. N. Engl. J. Med. 363, 109–122.

Bauer, J., Biolo, G., Cederholm, T., Cesari, M., Cruz-Jentoft, A.J., Morley, J.E., Phillips, S., Sieber, C., Stehle, P., Teta, D., Visvanathan, R., Volpi, E., Boirie, Y., 2013. Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. J. Am. Med. Dir. Assoc. 14, 542–559.

Bauer, J.M., Verlaan, S., Bautmans, I., Brandt, K., Donini, L.M., Maggio, M., McMurdo, M.E., Mets, T., Seal, C., Wijers, S.L., Ceda, G.P., De Vito, G., Donders, G., Drey, M., Greig, C., Holmback, U., Narici, M., McPhee, J., Poggiogalle, E., Power, D., Scafoglieri, A., Schultz, R., Sieber, C.C., Cederholm, T., 2015. Effects of a vitamin D and leucine-enriched whey protein nutritional supplement on measures of sarcopenia in older adults, the PROVIDE study: a randomized, double-blind, placebo-controlled trial. J. Am. Med. Dir. Assoc. 16, 740–747.

Bischoff-Ferrari, H.A., Willett, W.C., Orav, E.J., Lips, P., Meunier, P.J., Lyons, R.A., Flicker, L., Wark, J., Jackson, R.D., Cauley, J.A., Meyer, H.E., Pfeifer, M., Sanders, K.M., Stahelin, H.B., Theiler, R., Dawson-Hughes, B., 2012. A pooled analysis of vitamin D dose requirements for fracture prevention. N. Engl. J. Med. 367, 40–49.

Bishop, N.A., Lu, T., Yankner, B.A., 2010. Neural mechanisms of ageing and cognitive decline. Nature 464, 529–535.

Boxer, R.S., Wang, Z., Walsh, S.J., Hager, D., Kenny, A.M., 2008. The utility of the 6-minute walk test as a measure of frailty in older adults with heart failure. Am. J. Geriatr. Cardiol. 17, 7–12.

Boxer, R., Kleppinger, A., Ahmad, A., Annis, K., Hager, D., Kenny, A., 2010. The 6-minute walk is associated with frailty and predicts mortality in older adults with heart failure. Congest. Heart Fail. 16, 208–213.

Brandau, S., Jakob, M., Bruderek, K., Bootz, F., Giebel, B., Radtke, S., Mauel, K., Jager, M., Flohe, S.B., Lang, S., 2014. Mesenchymal stem cells augment the anti-bacterial activity of neutrophil granulocytes. PLoS One 9, e106903.

Braza, F., Dirou, S., Forest, V., Sauzeau, V., Hassoun, D., Chesne, J., Cheminant-Muller, M.A., Sagan, C., Magnan, A., Lemarchand, P., 2016. Mesenchymal stem cells induce suppressive macrophages through phagocytosis in a mouse model of asthma. Stem Cells 34, 1836–1845.

Buchman, A.S., Schneider, J.A., Leurgans, S., Bennett, D.A., 2008. Physical frailty in older persons is associated with Alzheimer disease pathology. Neurology 71, 499–504.

Buckinx, F., Rolland, Y., Reginster, J.Y., Ricour, C., Petermans, J., Bruyere, O., 2015. Burden of frailty in the elderly population: perspectives for a public health challenge. Arch. Public Health 73, 19.

Calvani, R., Picca, A., Marini, F., Biancolillo, A., Cesari, M., Pesce, V., Lezza, A.M.S., Bossola, M., Leeuwenburgh, C., Bernabei, R., Landi, F., Marzetti, E., 2018a. The "BIOmarkers associated with Sarcopenia and PHysical frailty in EldeRly pErsons" (BIOSPHERE) study: rationale, design and methods. Eur. J. Intern. Med. 56, 19–25.

Calvani, R., Picca, A., Marini, F., Biancolillo, A., Gervasoni, J., Persichilli, S., Primiano, A., Coelho, H.J., Bossola, M., Urbani, A., Landi, F., Bernabei, R., Marzetti, E., 2018b. A distinct pattern of circulating amino acids characterizes older persons with physical frailty and Sarcopenia: results from the BIOSPHERE study. Nutrients 10.

Campbell, J.M., Bellman, S.M., Stephenson, M.D., Lisy, K., 2017. Metformin reduces all-cause mortality and diseases of ageing independent of its effect on diabetes control: a systematic review and meta-analysis. Ageing Res. Rev. 40, 31–44.

Cannizzo, E.S., Clement, C.C., Sahu, R., Follo, C., Santambrogio, L., 2011. Oxidative stress, inflamm-aging and immunosenescence. J. Proteomics 74, 2313–2323.

Cano, C., Samper-Ternent, R., Al Snih, S., Markides, K., Ottenbacher, K.J., 2012. Frailty and cognitive impairment as predictors of mortality in older Mexican Americans. J. Nutr. Health Aging 16, 142–147.

Caplan, A.I., 1991. Mesenchymal stem cells. J. Orthop. Res. 9, 641–650.

Cappola, A.R., Xue, Q.L., Ferrucci, L., Guralnik, J.M., Volpato, S., Fried, L.P., 2003. Insulin-like growth factor I and interleukin-6 contribute synergistically to disability and mortality in older women. J. Clin. Endocrinol. Metab. 88, 2019–2025.

Carlson, M.E., Conboy, I.M., 2007. Loss of stem cell regenerative capacity within aged niches. Aging Cell 6, 371–382.

Celermajer, D.S., Sorensen, K.E., Spiegelhalter, D.J., Georgakopoulos, D., Robinson, J., Deanfield, J.E., 1994. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. J. Am. Coll. Cardiol. 24, 471–476.

Cerletti, M., Jang, Y.C., Finley, L.W., Haigis, M.C., Wagers, A.J., 2012. Short-term calorie restriction enhances skeletal muscle stem cell function. Cell Stem Cell 10, 515–519.

Chan, D.C., Tsou, H.H., Chen, C.Y., Chen, C.Y., 2010. Validation of the Chinese-Canadian study of health and aging clinical frailty scale (CSHA-CFS) telephone version. Arch. Gerontol. Geriatr. 50, e74–80.

Chobanian, A.V., 2007. Clinical practice. Isolated systolic hypertension in the elderly. N. Engl. J. Med. 357, 789–796.

Churchward-Venne, T.A., Tieland, M., Verdijk, L.B., Leenders, M., Dirks, M.L., de Groot, L.C., van Loon, L.J., 2015. There are no nonresponders to resistance-type exercise training in older men and women. J. Am. Med. Dir. Assoc. 16, 400–411.

Clegg, A.P., Barber, S.E., Young, J.B., Forster, A., Iliffe, S.J., 2012. Do home-based exercise interventions improve outcomes for frail older people? Findings from a

systematic review. Rev. Clin. Gerontol. 22, 68–78.
Clegg, A., Young, J., Iliffe, S., Rikkert, M.O., Rockwood, K., 2013. Frailty in elderly people. Lancet 381, 752–762.
Cobellis, G., Silvestroni, A., Lillo, S., Sica, G., Botti, C., Maione, C., Schiavone, V., Rocco, S., Brando, G., Sica, V., 2008. Long-term effects of repeated autologous transplantation of bone marrow cells in patients affected by peripheral arterial disease. Bone Marrow Transplant. 42, 667–672.
Colman, R.J., Beasley, T.M., Allison, D.B., Weindruch, R., 2008. Attenuation of sarcopenia by dietary restriction in rhesus monkeys. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 63, 556–559.
Crimmins, E.M., Beltran-Sanchez, H., 2011. Mortality and morbidity trends: is there compression of morbidity? J. Gerontol. B Psychol. Sci. Soc. Sci. 66, 75–86.
de Witte, S.F.H., Luk, F., Sierra Parraga, J.M., Gargesha, M., Merino, A., Korevaar, S.S., Shankar, A.S., O'Flynn, L., Elliman, S.J., Roy, D., Betjes, M.G.H., Newsome, P.N., Baan, C.C., Hoogduijn, M.J., 2018. Immunomodulation by therapeutic mesenchymal stromal cells (MSC) is triggered through phagocytosis of MSC by monocytic cells. Stem Cells 36, 602–615.
Delp, M.D., Behnke, B.J., Spier, S.A., Wu, G., Muller-Delp, J.M., 2008. Ageing diminishes endothelium-dependent vasodilatation and tetrahydrobiopterin content in rat skeletal muscle arterioles. J. Physiol. 586, 1161–1168.
Dewan, S.K., Zheng, S.B., Xia, S.J., Bill, K., 2012. Senescent remodeling of the immune system and its contribution to the predisposition of the elderly to infections. Chin. Med. J. (Engl.) 125, 3325–3331.
Elias, R., Hartshorn, K., Rahma, O., Lin, N., Snyder-Cappione, J.E., 2018. Aging, immune senescence, and immunotherapy: a comprehensive review. Semin. Oncol. 45, 187–200.
Emmelot-Vonk, M.H., Verhaar, H.J., Nakhai Pour, H.R., Aleman, A., Lock, T.M., Bosch, J.L., Grobbee, D.E., van der Schouw, Y.T., 2008. Effect of testosterone supplementation on functional mobility, cognition, and other parameters in older men: a randomized controlled trial. Jama J. Am. Med. Assoc. 299, 39–52.
Ensrud, K.E., Ewing, S.K., Taylor, B.C., Fink, H.A., Cawthon, P.M., Stone, K.L., Hillier, T.A., Cauley, J.A., Hochberg, M.C., Rodondi, N., Tracy, J.K., Cummings, S.R., 2008. Comparison of 2 frailty indexes for prediction of falls, disability, fractures, and death in older women. Arch. Intern. Med. 168, 382–389.
Ershler, W.B., Keller, E.T., 2000. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annu. Rev. Med. 51, 245–270.
Fiatarone, M.A., O'Neill, E.F., Ryan, N.D., Clements, K.M., Solares, G.R., Nelson, M.E., Roberts, S.B., Kehayias, J.J., Lipsitz, L.A., Evans, W.J., 1994. Exercise training and nutritional supplementation for physical frailty in very elderly people. N. Engl. J. Med. 330, 1769–1775.
Florea, V., Rieger, A.C., DiFede, D.L., El-Khorazaty, J., Natsumeda, M., Banerjee, M.N., Tompkins, B.A., Khan, A., Schulman, I.H., Landin, A.M., Mushtaq, M., Golpanian, S., Lowery, M.H., Byrnes, J.J., Hendel, R.C., Cohen, M.G., Valasaki, K., Pujol, M.V., Ghersin, E., Miki, R., Delgado, C., Abuzeid, F., Vidro-Casiano, M., Saltzman, R.G., DaFonseca, D., Caceres, L.V., Ramdas, K.N., Mendizabal, A., Heldman, A.W., Mitrani, R.D., Hare, J.M., 2017. Dose comparison study of allogeneic mesenchymal stem cells in patients with ischemic cardiomyopathy (The TRIDENT study). Circ. Res. 121, 1279–1290.
Franceschi, C., Campisi, J., 2014. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J. Gerontol. A Biol. Sci. Med. Sci. 69 (Suppl. 1), S4–9.
Fried, L.P., Tangen, C.M., Walston, J., Newman, A.B., Hirsch, C., Gottdiener, J., Seeman, T., Tracy, R., Kop, W.J., Burke, G., McBurnie MA and Cardiovascular Health Study Collaborative Research G, 2001. Frailty in older adults: evidence for a phenotype. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 56, M146–56.
Friedlander, A.L., Butterfield, G.E., Moynihan, S., Grillo, J., Pollack, M., Holloway, L., Friedman, L., Yesavage, J., Matthias, D., Lee, S., Marcus, R., Hoffman, A.R., 2001. One year of insulin-like growth factor I treatment does not affect bone density, body composition, or psychological measures in postmenopausal women. J. Clin. Endocrinol. Metab. 86, 1496–1503.
Fulop, T., Larbi, A., Douziech, N., Fortin, C., Guerard, K.P., Lesur, O., Khalil, A., Dupuis, G., 2004. Signal transduction and functional changes in neutrophils with aging. Aging Cell 3, 217–226.
Fulop, T., Larbi, A., Witkowski, J.M., McElhaney, J., Loeb, M., Mitnitski, A., Pawelec, G., 2010. Aging, frailty and age-related diseases. Biogerontology 11, 547–563.
Gobbens, R.J., van Assen, M.A., Luijkx, K.G., Wijnen-Sponselee, M.T., Schols, J.M., 2010. Determinants of frailty. J. Am. Med. Dir. Assoc. 11, 356–364.
Golpanian, S., Wolf, A., Hatzistergos, K.E., Hare, J.M., 2016. Rebuilding the damaged heart: mesenchymal stem cells, cell-based therapy, and engineered heart tissue. Physiol. Rev. 96, 1127–1168.
Golpanian, S., DiFede, D.L., Khan, A., Schulman, I.H., Landin, A.M., Tompkins, B.A., Heldman, A.W., Miki, R., Goldstein, B.J., Mushtaq, M., Levis-Dusseau, S., Byrnes, J.J., Lowery, M., Natsumeda, M., Delgado, C., Saltzman, R., Vidro-Casiano, M., Pujol, M.V., Da Fonseca, M., Oliva Jr, A.A., Green, G., Premer, C., Medina, A., Valasaki, K., Florea, V., Anderson, E., El-Khorazaty, J., Mendizabal, A., Goldschmidt-Clermont, P.J., Hare, J.M., 2017a. Allogeneic human mesenchymal stem cell infusions for aging frailty. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 72 (11), 1505–1512. https://doi.org/10.1093/gerona/glx056.
Golpanian, S., DiFede, D.L., Khan, A., Schulman, I.H., Landin, A.M., Tompkins, B.A., Heldman, A.W., Miki, R., Goldstein, B.J., Mushtaq, M., Levis-Dusseau, S., Byrnes, J.J., Lowery, M., Natsumeda, M., Delgado, C., Saltzman, R., Vidro-Casiano, M., Pujol, M.V., Da Fonseca, M., Oliva Jr, A.A., Green, G., Premer, C., Medina, A., Valasaki, K., Florea, V., Anderson, E., El-Khorazaty, J., Mendizabal, A., Goldschmidt-Clermont, P.J., Hare, J.M., 2017b. Allogeneic human mesenchymal stem cell infusions for aging frailty. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 72, 1505–1512.
Gruver, A.L., Hudson, L.L., Sempowski, G.D., 2007. Immunosenescence of ageing. J. Pathol. 211, 144–156.
Hadrup, S.R., Strindhall, J., Kollgaard, T., Seremet, T., Johansson, B., Pawelec, G., thor Straten, P., Wikby, A., 2006. Longitudinal studies of clonally expanded CD8 T cells reveal a repertoire shrinkage predicting mortality and an increased number of dysfunctional cytomegalovirus-specific T cells in the very elderly. J. Immunol. 176, 2645–2653.
Hare, J.M., Fishman, J.E., Gerstenblith, G., DiFede Velazquez, D.L., Zambrano, J.P., Suncion, V.Y., Tracy, M., Ghersin, E., Johnston, P.V., Brinker, J.A., Breton, E., Davis-Sproul, J., Schulman, I.H., Byrnes, J., Mendizabal, A.M., Lowery, M.H., Rouy, D., Altman, P., Wong Po Foo, C., Ruiz, P., Amador, A., Da Silva, J., McNiece, I.K., Heldman, A.W., George, R., Lardo, A., 2012. Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. Jama J. Am. Med. Assoc. 308, 2369–2379.
Hare, J.M., DiFede, D.L., Rieger, A.C., Florea, V., Landin, A.M., El-Khorazaty, J., Khan, A., Mushtaq, M., Lowery, M.H., Byrnes, J.J., Hendel, R.C., Cohen, M.G., Alfonso, C.E., Valasaki, K., Pujol, M.V., Golpanian, S., Ghersin, E., Fishman, J.E., Pattany, P., Gomes, S.A., Delgado, C., Miki, R., Abuzeid, F., Vidro-Casiano, M., Premer, C., Medina, A., Porras, V., Hatzistergos, K.E., Anderson, E., Mendizabal, A., Mitrani, R., Heldman, A.W., 2017. Randomized comparison of allogeneic versus autologous mesenchymal stem cells for nonischemic dilated cardiomyopathy: POSEIDON-DCM trial. J. Am. Coll. Cardiol. 69, 526–537.
Heidenreich, P.A., Trogdon, J.G., Khavjou, O.A., Butler, J., Dracup, K., Ezekowitz, M.D., Finkelstein, E.A., Hong, Y., Johnston, S.C., Khera, A., Lloyd-Jones, D.M., Nelson, S.A., Nichol, G., Orenstein, D., Wilson, P.W., Woo, Y.J., American Heart Association Advocacy Coordinating C, Stroke C, Council on Cardiovascular R, Intervention, Council on Clinical C, Council on E, Prevention, Council on A, Thrombosis, Vascular B, Council on C, Critical C, Perioperative, Resuscitation, Council on Cardiovascular N, Council on the Kidney in Cardiovascular D, Council on Cardiovascular S, Anesthesia, Interdisciplinary Council on Quality of C and Outcomes R, 2011. Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. Circulation 123, 933–944.
Heldman, A.W., DiFede, D.L., Fishman, J.E., Zambrano, J.P., Trachtenberg, B.H., Karantalis, V., Mushtaq, M., Williams, A.R., Suncion, V.Y., McNiece, I.K., Ghersin, E., Soto, V., Lopera, G., Miki, R., Willens, H., Hendel, R., Mitrani, R., Pattany, P., Feigenbaum, G., Oskouei, B., Byrnes, J., Lowery, M.H., Sierra, J., Pujol, M.V., Delgado, C., Gonzalez, P.J., Rodriguez, J.E., Bagno, L.L., Rouy, D., Altman, P., Foo, C.W., da Silva, J., Anderson, E., Schwarz, R., Mendizabal, A., Hare, J.M., 2014. Transendocardial mesenchymal stem cells and mononuclear bone marrow cells for ischemic cardiomyopathy: the TAC-HFT randomized trial. Jama J. Am. Med. Assoc. 311, 62–73.
Hubbard, R.E., O'Mahony, M.S., Savva, G.M., Calver, B.L., Woodhouse, K.W., 2009. Inflammation and frailty measures in older people. J. Cell. Mol. Med. 13, 3103–3109.
Jackaman, C., Tomay, F., Duong, L., Abdol Razak, N.B., Pixley, F.J., Metharom, P., Nelson, D.J., 2017. Aging and cancer: the role of macrophages and neutrophils. Ageing Res. Rev. 36, 105–116.
Jacobs, J.M., Cohen, A., Ein-Mor, E., Maaravi, Y., Stessman, J., 2011. Frailty, cognitive impairment and mortality among the oldest old. J. Nutr. Health Aging 15, 678–682.
Janzen, V., Forkert, R., Fleming, H.E., Saito, Y., Waring, M.T., Dombkowski, D.M., Cheng, T., DePinho, R.A., Sharpless, N.E., Scadden, D.T., 2006. Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16INK4a. Nature 443, 421–426.
Jiang, X.X., Zhang, Y., Liu, B., Zhang, S.X., Wu, Y., Yu, X.D., Mao, N., 2005. Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood 105, 4120–4126.
Justice, J.N., Gregory, H., Tchkonia, T., LeBrasseur, N.K., Kirkland, J.L., Kritchevsky, S.B., Nicklas, B.J., 2018. Cellular senescence biomarker p16INK4a+ cell burden in thigh adipose is associated with poor physical function in older women. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 73, 939–945.
Kalyani, R.R., Varadhan, R., Weiss, C.O., Fried, L.P., Cappola, A.R., 2012. Frailty status and altered glucose-insulin dynamics. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 67, 1300–1306.
Karantalis, V., Schulman, I.H., Balkan, W., Hare, J.M., 2015. Allogeneic cell therapy: a new paradigm in therapeutics. Circ. Res. 116, 12–15.
Katayama, P.L., Dias, D.P., Silva, L.E., Virtuoso-Junior, J.S., Marocolo, M., 2015. Cardiac autonomic modulation in non-frail, pre-frail and frail elderly women: a pilot study. Aging Clin. Exp. Res. 27, 621–629.
Kelaiditi, E., Cesari, M., Canevelli, M., van Kan, G.A., Ousset, P.J., Gillette-Guyonnet, S., Ritz, P., Duveau, F., Soto, M.E., Provencher, V., Nourhashemi, F., Salva, A., Robert, P., Andrieu, S., Rolland, Y., Touchon, J., Fitten, J.L., Vellas, B., Iana/Iagg, 2013. Cognitive frailty: rational and definition from an (I.A.N.A./I.A.G.G.) international consensus group. J. Nutr. Health Aging 17, 726–734.
Kojima, G., 2017. Prevalence of frailty in end-stage renal disease: a systematic review and meta-analysis. Int. Urol. Nephrol. 49, 1989–1997.
Kyurkchiev, D., Bochev, I., Ivanova-Todorova, E., Mourdjeva, M., Oreshkova, T., Belemezova, K., Kyurkchiev, S., 2014. Secretion of immunoregulatory cytokines by mesenchymal stem cells. World J. Stem Cells 6, 552–570.
Lakatta, E.G., 2003. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: part III: cellular and molecular clues to heart and arterial aging. Circulation 107, 490–497.
Laksmi, P.W., Setiati, S., Tamin, T.Z., Soewondo, P., Rochmah, W., Nafrialdi, N., Prihartono, J., 2017. Effect of metformin on handgrip strength, gait speed, myostatin serum level, and health-related quality of life: a double blind randomized controlled trial among non-diabetic pre-frail elderly patients. Acta Med. Indones. 49, 118–127.
Lang, P.O., Mitchell, W.A., Lapenna, A., Pitts, D., Aspinall, R., 2010. Immunological pathogenesis of main age-related diseases and frailty: role of immunosenescence. Eur. Geriatr. Med. 1, 112–121.

Lavasani, M., Robinson, A.R., Lu, A., Song, M., Feduska, J.M., Ahani, B., Tilstra, J.S., Feldman, C.H., Robbins, P.D., Niedernhofer, L.J., Huard, J., 2012. Muscle-derived stem/progenitor cell dysfunction limits healthspan and lifespan in a murine progeria model. Nat. Commun. 3, 608.
Lavy-Shahaf, G.H., Averbuch, Helena, Amu, Dana, Ademokun, Sylvie, Alexander, Wu, Dunn-Walters, Yu-Chang, Choidi, Deborah, Mehr, Francesca, Ramit, 2014. Understanding Mechanisms of Immune System Aging: Immune System Cell Development and Antibody Repertoires.
Le Blanc, K., Davies, L.C., 2015. Mesenchymal stromal cells and the innate immune response. Immunol. Lett. 168, 140–146.
Le Couteur, D.G., Anderson, R.M., Newman, A.B., de Cabo, R., 2017. Stem cell transplantation for frailty. J. Gerontol. A Biol. Sci. Med. Sci. 72, 1503–1504.
Liang, X., Ding, Y., Zhang, Y., Tse, H.F., Lian, Q., 2014. Paracrine mechanisms of mesenchymal stem cell-based therapy: current status and perspectives. Cell Transplant. 23, 1045–1059.
Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., Abete, P., 2018. Oxidative stress, aging, and diseases. Clin. Interv. Aging 13, 757–772.
Liu, H., Xia, X., Li, B., 2015. Mesenchymal stem cell aging: mechanisms and influences on skeletal and non-skeletal tissues. Exp. Biol. Med. (Maywood) 240, 1099–1106.
Lohman, M.C., Mezuk, B., 2013. Frailty and depression: comorbidity in the context of imperfect measurement. J. Am. Geriatr. Soc. 61, 474.
Lopez-Otin, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2013. The hallmarks of aging. Cell 153, 1194–1217.
Malmstrom, T.K., Morley, J.E., 2013. The frail brain. J. Am. Med. Dir. Assoc. 14, 453–455.
Mansilla, E., Roque, G., Sosa, Y.E., Tarditti, A., Goya, R.G., 2016. A rat treated with mesenchymal stem cells lives to 44 months of age. Rejuvenation Res. 19, 318–321.
Mathiasen, A.B., Qayyum, A.A., Jørgensen, E., Helqvist, S., Fischer-Nielsen, A., Kofoed, K.F., Haack-Sørensen, M., Ekblond, A., Kastrup, J., 2015. Bone marrow-derived mesenchymal stromal cell treatment in patients with severe ischaemic heart failure: a randomized placebo-controlled trial (MSC-HF trial). Eur. Heart J. 36, 1744–1753.
Matusik, P., Tomaszewski, K., Chmielowska, K., Nowak, J., Nowak, W., Parnicka, A., Dubiel, M., Gasowski, J., Grodzicki, T., 2012. Severe frailty and cognitive impairment are related to higher mortality in 12-month follow-up of nursing home residents. Arch. Gerontol. Geriatr. 55, 22–24.
Mezey, E., Nemeth, K., 2015. Mesenchymal stem cells and infectious diseases: smarter than drugs. Immunol. Lett. 168, 208–214.
Mezuk, B., Edwards, L., Lohman, M., Choi, M., Lapane, K., 2012. Depression and frailty in later life: a synthetic review. Int. J. Geriatr. Psychiatry 27, 879–892.
Mezuk, B., Lohman, M., Dumenci, L., Lapane, K.L., 2013. Are depression and frailty overlapping syndromes in mid- and late-life? A latent variable analysis. Am. J. Geriatr. Psychiatry 21, 560–569.
Michaud, M., Balardy, L., Moulis, G., Gaudin, C., Peyrot, C., Vellas, B., Cesari, M., Nourhashemi, F., 2013. Proinflammatory cytokines, aging, and age-related diseases. J. Am. Med. Dir. Assoc. 14, 877–882.
Min, L.C., Elliott, M.N., Wenger, N.S., Saliba, D., 2006. Higher vulnerable elders survey scores predict death and functional decline in vulnerable older people. J. Am. Geriatr. Soc. 54, 507–511.
Miryounesi, M., Piryaei, A., Pournasr, B., Aghdami, N., Baharvand, H., 2013. Repeated versus single transplantation of mesenchymal stem cells in carbon tetrachloride-induced liver injury in mice. Cell Biol. Int. 37, 340–347.
Mitnitski, A., Collerton, J., Martin-Ruiz, C., Jagger, C., von Zglinicki, T., Rockwood, K., Kirkwood, T.B., 2015. Age-related frailty and its association with biological markers of ageing. BMC Med. 13, 161.
Moaddel, R., Fabbri, E., Khadeer, M.A., Carlson, O.D., Gonzalez-Freire, M., Zhang, P., Semba, R.D., Ferrucci, L., 2016. Plasma biomarkers of poor muscle quality in older men and women from the Baltimore longitudinal study of aging. J. Gerontol. A Biol. Sci. Med. Sci. 71, 1266–1272.
Molavi, B., Zafarghandi, M.R., Aminizadeh, E., Hosseini, S.E., Mirzayi, H., Arab, L., Baharvand, H., Aghdami, N., 2016. Safety and efficacy of repeated bone marrow mononuclear cell therapy in patients with critical limb ischemia in a pilot randomized controlled trial. Arch. Iran. Med. 19, 388–396.
Moreno-Jimenez, E.P., Flor-Garcia, M., Terreros-Roncal, J., Rabano, A., Cafini, F., Pallas-Bazarra, N., Avila, J., Llorens-Martin, M., 2019. Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. Nat. Med. 25, 554–560.
Muir, S.W., Montero-Odasso, M., 2011. Effect of vitamin D supplementation on muscle strength, gait and balance in older adults: a systematic review and meta-analysis. J. Am. Geriatr. Soc. 59, 2291–2300.
Murad, M.H., Elamin, K.B., Abu Elnour, N.O., Elamin, M.B., Alkatib, A.A., Fatourechi, M.M., Almandoz, J.P., Mullan, R.J., Lane, M.A., Liu, H., Erwin, P.J., Hensrud, D.D., Montori, V.M., 2011. Clinical review: the effect of vitamin D on falls: a systematic review and meta-analysis. J. Clin. Endocrinol. Metab. 96, 2997–3006.
Nair, K.S., Rizza, R.A., O'Brien, P., Dhatariya, K., Short, K.R., Nehra, A., Vittone, J.L., Klee, G.G., Basu, A., Basu, R., Cobelli, C., Toffolo, G., Dalla Man, C., Tindall, D.J., Melton 3rd, L.J., Smith, G.E., Khosla, S., Jensen, M.D., 2006. DHEA in elderly women and DHEA or testosterone in elderly men. N. Engl. J. Med. 355, 1647–1659.
Narayanan, R., Coss, C.C., Dalton, J.T., 2018. Development of selective androgen receptor modulators (SARMs). Mol. Cell. Endocrinol. 465, 134–142.
Newman, A.B., Yanez, D., Harris, T., Duxbury, A., Enright, P.L., Fried, L.P., Cardiovascular Study Research G, 2001. Weight change in old age and its association with mortality. J. Am. Geriatr. Soc. 49, 1309–1318.
Newman, A.B., Naydeck, B.L., Ives, D.G., Boudreau, R.M., Sutton-Tyrrell, K., O'Leary, D.H., Kuller, L.H., 2008. Coronary artery calcium, carotid artery wall thickness, and cardiovascular disease outcomes in adults 70 to 99 years old. Am. J. Cardiol. 101, 186–192.
Ng, T.P., Feng, L., Nyunt, M.S., Feng, L., Niti, M., Tan, B.Y., Chan, G., Khoo, S.A., Chan, S.M., Yap, P., Yap, K.B., 2015. Nutritional, physical, cognitive, and combination interventions and frailty reversal among older adults: a randomized controlled trial. Am. J. Med. 128, 1225–1236 e1.
Nyunt, M.S.Z., Soh, C.Y., Gao, Q., Gwee, X., Ling, A.S.L., Lim, W.S., Lee, T.S., Yap, P.L.K., Yap, K.B., Ng, T.P., 2017. Characterisation of physical frailty and associated physical and functional impairments in mild cognitive impairment. Front. Med. (Lausanne) 4, 230.
O'Driscoll, S.W., Saris, D.B., Ito, Y., Fitzimmons, J.S., 2001. The chondrogenic potential of periosteum decreases with age. J. Orthop. Res. 19, 95–103.
Oh, K.W., Moon, C., Kim, H.Y., Oh, S.I., Park, J., Lee, J.H., Chang, I.Y., Kim, K.S., Kim, S.H., 2015. Phase I trial of repeated intrathecal autologous bone marrow-derived mesenchymal stromal cells in amyotrophic lateral sclerosis. Stem Cells Transl. Med. 4, 590–597.
Oh, K.W., Noh, M.Y., Kwon, M.S., Kim, H.Y., Oh, S.I., Park, J., Kim, H.J., Ki, C.S., Kim, S.H., 2018. Repeated intrathecal mesenchymal stem cells for amyotrophic lateral sclerosis. Ann. Neurol. 84, 361–373.
Onder, G., Penninx, B.W., Balkrishnan, R., Fried, L.P., Chaves, P.H., Williamson, J., Carter, C., Di Bari, M., Guralnik, J.M., Pahor, M., 2002. Relation between use of angiotensin-converting enzyme inhibitors and muscle strength and physical function in older women: an observational study. Lancet 359, 926–930.
Pahor, M., Kritchevsky, S.B., Waters, D.L., Villareal, D.T., Morley, J., Hare, J.M., Vellas, B., 2018. Designing drug trials for frailty: ICFSR task force 2018. J. Frailty Aging 7, 150–154.
Paneni, F., Diaz Canestro, C., Libby, P., Luscher, T.F., Camici, G.G., 2017. The aging cardiovascular system: understanding it at the cellular and clinical levels. J. Am. Coll. Cardiol. 69, 1952–1967.
Panza, F., Frisardi, V., Capurso, C., D'Introno, A., Colacicco, A.M., Imbimbo, B.P., Santamato, A., Vendemiale, G., Seripa, D., Pilotto, A., Capurso, A., Solfrizzi, V., 2010. Late-life depression, mild cognitive impairment, and dementia: possible continuum? Am. J. Geriatr. Psychiatry 18, 98–116.
Partridge, J.S., Harari, D., Dhesi, J.K., 2012. Frailty in the older surgical patient: a review. Age Ageing 41, 142–147.
Perin, E.C., Borow, K.M., Silva, G.V., DeMaria, A.N., Marroquin, O.C., Huang, P.P., Traverse, J.H., Krum, H., Skerrett, D., Zheng, Y., Willerson, J.T., Itescu, S., Henry, T.D., 2015. A phase II dose-escalation study of allogeneic mesenchymal precursor cells in patients with ischemic or nonischemic heart failure. Circ. Res. 117, 576–584.
Peters, L.L., Boter, H., Buskens, E., Slaets, J.P., 2012. Measurement properties of the Groningen Frailty Indicator in home-dwelling and institutionalized elderly people. J. Am. Med. Dir. Assoc. 13, 546–551.
Poglajen, G., Sever, M., Cukjati, M., Cernelc, P., Knezevic, I., Zemljic, G., Haddad, F., Wu, J.C., Vrtovec, B., 2014. Effects of transendocardial CD34+ cell transplantation in patients with ischemic cardiomyopathy. Circ. Cardiovasc. Interv. 7, 552–559.
Premer, C., Blum, A., Bellio, M.A., Schulman, I.H., Hurwitz, B.E., Parker, M., Dermarkarian, C.R., DiFede, D.L., Balkan, W., Khan, A., Hare, J.M., 2015. Allogeneic mesenchymal stem cells restore endothelial function in heart failure by stimulating endothelial progenitor cells. EBioMedicine 2, 467–475.
Quyyumi, A.A., Waller, E.K., Murrow, J., Esteves, F., Galt, J., Oshinski, J., Lerakis, S., Sher, S., Vaughan, D., Perin, E., Willerson, J., Kereiakes, D., Gersh, B.J., Gregory, D., Werner, A., Moss, T., Chan, W.S., Preti, R., Pecora, A.L., 2011. CD34(+) cell infusion after ST elevation myocardial infarction is associated with improved perfusion and is dose dependent. Am. Heart J. 161, 98–105.
Rejnmark, L., Avenell, A., Masud, T., Anderson, F., Meyer, H.E., Sanders, K.M., Salovaara, K., Cooper, C., Smith, H.E., Jacobs, E.T., Torgerson, D., Jackson, R.D., Manson, J.E., Brixen, K., Mosekilde, L., Robbins, J.A., Francis, R.M., Abrahamsen, B., 2012. Vitamin D with calcium reduces mortality: patient level pooled analysis of 70,528 patients from eight major vitamin D trials. J. Clin. Endocrinol. Metab. 97, 2670–2681.
Ren, G., Zhang, L., Zhao, X., Xu, G., Zhang, Y., Roberts, A.I., Zhao, R.C., Shi, Y., 2008. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell 2, 141–150.
Rockwood, K., Mitnitski, A., 2011. Frailty defined by deficit accumulation and geriatric medicine defined by frailty. Clin. Geriatr. Med. 27, 17–26.
Rockwood, K., Song, X., MacKnight, C., Bergman, H., Hogan, D.B., McDowell, I., Mitnitski, A., 2005. A global clinical measure of fitness and frailty in elderly people. CMAJ 173, 489–495.
Romero-Ortuno, R., Walsh, C.D., Lawlor, B.A., Kenny, R.A., 2010. A frailty instrument for primary care: findings from the Survey of Health, Ageing and Retirement in Europe (SHARE). BMC Geriatr. 10, 57.
Rossi, D.J., Bryder, D., Seita, J., Nussenzweig, A., Hoeijmakers, J., Weissman, I.L., 2007. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature 447, 725–729.
Sapey, E., Greenwood, H., Walton, G., Mann, E., Love, A., Aaronson, N., Insall, R.H., Stockley, R.A., Lord, J.M., 2014. Phosphoinositide 3-kinase inhibition restores neutrophil accuracy in the elderly: toward targeted treatments for immunosenescence. Blood 123, 239–248.
Schaufelberger, M., Andersson, G., Eriksson, B.O., Grimby, G., Held, P., Swedberg, K., 1996. Skeletal muscle changes in patients with chronic heart failure before and after treatment with enalapril. Eur. Heart J. 17, 1678–1685.
Shen, J., Tsai, Y.T., Dimarco, N.M., Long, M.A., Sun, X., Tang, L., 2011. Transplantation of mesenchymal stem cells from young donors delays aging in mice. Sci. Rep. 1, 67.
Sousa-Victor, P., Gutarra, S., Garcia-Prat, L., Rodriguez-Ubreva, J., Ortet, L., Ruiz-Bonilla, V., Jardi, M., Ballestar, E., Gonzalez, S., Serrano, A.L., Perdiguero, E., Munoz-Canoves, P., 2014. Geriatric muscle stem cells switch reversible quiescence into senescence. Nature 506, 316–321.
Sousa-Victor, P., Garcia-Prat, L., Serrano, A.L., Perdiguero, E., Munoz-Canoves, P., 2015.

Muscle stem cell aging: regulation and rejuvenation. Trends Endocrinol. Metab. 26, 287–296.
Spaggiari, G.M., Capobianco, A., Abdelrazik, H., Becchetti, F., Mingari, M.C., Moretta, L., 2008. Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. Blood 111, 1327–1333.
Stenderup, K., Justesen, J., Clausen, C., Kassem, M., 2003. Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. Bone 33, 919–926.
Sullivan, D.H., Roberson, P.K., Smith, E.S., Price, J.A., Bopp, M.M., 2007. Effects of muscle strength training and megestrol acetate on strength, muscle mass, and function in frail older people. J. Am. Geriatr. Soc. 55, 20–28.
Sumukadas, D., Witham, M.D., Struthers, A.D., McMurdo, M.E., 2007. Effect of perindopril on physical function in elderly people with functional impairment: a randomized controlled trial. CMAJ 177, 867–874.
Sutton, M.T., Fletcher, D., Ghosh, S.K., Weinberg, A., van Heeckeren, R., Kaur, S., Sadeghi, Z., Hijaz, A., Reese, J., Lazarus, H.M., Lennon, D.P., Caplan, A.I., Bonfield, T.L., 2016. Antimicrobial properties of mesenchymal stem cells: therapeutic potential for cystic fibrosis infection, and treatment. Stem Cells Int. 2016, 5303048.
Taddei, S., Virdis, A., Ghiadoni, L., Salvetti, G., Bernini, G., Magagna, A., Salvetti, A., 2001. Age-related reduction of NO availability and oxidative stress in humans. Hypertension 38, 274–279.
Tang, X.L., Nakamura, S., Li, Q., Wysoczynski, M., Gumpert, A.M., Wu, W.J., Hunt, G., Stowers, H., Ou, Q., Bolli, R., 2018. Repeated administrations of cardiac progenitor cells are superior to a single administration of an equivalent cumulative dose. J. Am. Heart Assoc. 7.
Teerlink, J.R., Metra, M., Filippatos, G.S., Davison, B.A., Bartunek, J., Terzic, A., Gersh, B.J., Povsic, T.J., Henry, T.D., Alexandre, B., Homsy, C., Edwards, C., Seron, A., Wijns, W., Cotter, G., Investigators, C., 2017. Benefit of cardiopoietic mesenchymal stem cell therapy on left ventricular remodelling: results from the Congestive Heart Failure Cardiopoietic Regenerative Therapy (CHART-1) study. Eur. J. Heart Fail. 19, 1520–1529.
Theou, O., Stathokostas, L., Roland, K.P., Jakobi, J.M., Patterson, C., Vandervoort, A.A., Jones, G.R., 2011. The effectiveness of exercise interventions for the management of frailty: a systematic review. J. Aging Res. 2011, 569194.
Theou, O., Chapman, I., Wijeyaratne, L., Piantadosi, C., Lange, K., Naganathan, V., Hunter, P., Cameron, I.D., Rockwood, K., Visvanathan, R., 2016. Can an intervention with testosterone and nutritional supplement improve the frailty level of undernourished older people? J. Frailty Aging 5, 247–252.
Tokita, Y., Tang, X.L., Li, Q., Wysoczynski, M., Hong, K.U., Nakamura, S., Wu, W.J., Xie, W., Li, D., Hunt, G., Ou, Q., Stowers, H., Bolli, R., 2016. Repeated administrations of cardiac progenitor cells are markedly more effective than a single administration: a new paradigm in cell therapy. Circ. Res. 119, 635–651.
Tompkins, B.A., DiFede, D.L., Khan, A., Landin, A.M., Schulman, I.H., Pujol, M.V., Heldman, A.W., Miki, R., Goldschmidt-Clermont, P.J., Goldstein, B.J., Mushtaq, M., Levis-Dusseau, S., Byrnes, J.J., Lowery, M., Natsumeda, M., Delgado, C., Saltzman, R., Vidro-Casiano, M., Da Fonseca, M., Golpanian, S., Premer, C., Medina, A., Valasaki, K., Florea, V., Anderson, E., El-Khorazaty, J., Mendizabal, A., Green, G., Oliva, A.A., Hare, J.M., 2017a. Allogeneic mesenchymal stem cells ameliorate aging frailty: a phase II randomized, double-blind, placebo-controlled clinical trial. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 72, 1513–1522.
Tompkins, B.A., Rieger, A.C., Florea, V., Banerjee, M.N., Hare, J.M., 2017b. New insights into cell-based therapy for heart failure from the CHART-1 study. Eur. J. Heart Fail. 19, 1530–1533.
Tracy, R.P., 2003. Emerging relationships of inflammation, cardiovascular disease and chronic diseases of aging. Int. J. Obes. Relat. Metab. Disord. 27 (Suppl. 3), S29–34.
Tseng, C.W., Liu, G.Y., 2014. Expanding roles of neutrophils in aging hosts. Curr. Opin. Immunol. 29, 43–48.
Uccelli, A., Moretta, L., Pistoia, V., 2008. Mesenchymal stem cells in health and disease. Nat. Rev. Immunol. 8, 726–736.
Vasandan, A.B., Jahnavi, S., Shashank, C., Prasad, P., Kumar, A., Prasanna, S.J., 2016. Human Mesenchymal stem cells program macrophage plasticity by altering their metabolic status via a PGE2-dependent mechanism. Sci. Rep. 6, 38308.
Walston, J.D., 2015. Connecting age-related biological decline to frailty and late-life vulnerability. Nestle Nutr. Inst. Workshop Ser. 83, 1–10.
Walston, J., Robinson, T.N., Zieman, S., McFarland, F., Carpenter, C.R., Althoff, K.N., Andrew, M.K., Blaum, C.S., Brown, P.J., Buta, B., Ely, E.W., Ferrucci, L., High, K.P., Kritchevsky, S.B., Rockwood, K., Schmader, K.E., Sierra, F., Sink, K.M., Varadhan, R., Hurria, A., 2017. Integrating frailty research into the medical specialties-report from a U13 conference. J. Am. Geriatr. Soc. 65, 2134–2139.
Walston, J., Buta, B., Xue, Q.L., 2018. Frailty screening and interventions: considerations for clinical practice. Clin. Geriatr. Med. 34, 25–38.
Wang, C.P., Lorenzo, C., Habib, S.L., Jo, B., Espinoza, S.E., 2017. Differential effects of metformin on age related comorbidities in older men with type 2 diabetes. J. Diabetes Complications 31, 679–686.
Wilson, D., Jackson, T., Sapey, E., Lord, J.M., 2017. Frailty and sarcopenia: the potential role of an aged immune system. Ageing Res. Rev. 36, 1–10.
Yakar, S., Adamo, M.L., 2012. Insulin-like growth factor 1 physiology: lessons from mouse models. Endocrinol. Metab. Clin. North Am. 41, 231–247 v.
Yamada, Y., Kemnitz, J.W., Weindruch, R., Anderson, R.M., Schoeller, D.A., Colman, R.J., 2018. Caloric restriction and healthy life span: frail phenotype of nonhuman primates in the wisconsin national primate research center caloric restriction study. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 73, 273–278.
Yang, C., Wang, G., Ma, F., Yu, B., Chen, F., Yang, J., Feng, J., Wang, Q., 2018. Repeated injections of human umbilical cord blood-derived mesenchymal stem cells significantly promotes functional recovery in rabbits with spinal cord injury of two noncontinuous segments. Stem Cell Res. Ther. 9, 136.