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Review

Application of mesenchymal stem cell therapy for aging frailty: from mechanisms to therapeutics

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Abstract

Aging frailty is a complex geriatric syndrome that becomes more prevalent with advancing age. It constitutes a major health problem due to frequent adverse outcomes. Frailty is characterized by disruption of physiological homeostasis and progressive decline of health status. Multiple factors contribute to development of frailty with advancing age, including genome instability, DNA damage, epigenetic alternations, stem cell exhaustion, among others. These interrelated factors comprehensively result in loss of tissue homeostasis and diminished reserve capacity in frailty. Therefore, the aged organism gradually represents symptoms of frailty with decline in physiological functions of organs. Notably, the brain, cardiovascular system, skeletal muscle, and endocrine system are intrinsically interrelated to frailty. The patients with frailty may display the diminished reserves capacity of organ systems. Due to the complex pathophysiology, no specific treatments have been approved for prevention of this syndrome. At such, effective strategies for intervening in pathogenic process to improve health status of frail patients are highly needed. Recent progress in cell-based therapy has greatly contributed to the amelioration of degenerative diseases related to age. Mesenchymal stem cells (MSCs) can exert regenerative effects and possess anti-inflammatory properties. Transplantation of MSCs represents as a promising therapeutic strategy to address the pathophysiologic problems of frail syndrome. Currently, MSC therapy have undergone the phase I and II trials in human subjects that have endorsed the safety and efficacy of MSCs for aging frailty. However, despite these positive results, caution is still needed with regard to potential to form tumors, and further large-scale studies are warranted to confirm the therapeutic efficacy of MSC therapy.

Key words: mesenchymal stem cells, aging frailty, aging, regenerative medicine, stem cell therapy

Introduction

The global population is aging rapidly due to an increase in life expectancy [1], so too has the increasing prevalence of aging frailty [2]. Frailty is an age-associated geriatric syndrome, defined as a state of increased physiological vulnerability to stressors due to multiple system dysregulation and reduced functional reserves [3]. Aging frailty is associated with functional limitations in daily living, which conferred the greater risk of poor health outcomes in the older population, such as mortality, disability, hospitalization and falls [4-6], alongside the increased healthcare costs which presents a major public health problem worldwide [7, 8]. Despite decades of research that have led to a growing understanding of biological alterations of frailty, the approved medical therapy that can effectively attenuate or reverse aging frailty is still not available [9]. To date, clinicians have attempted several interventions to improve and modify frailty status, including physical exercises (e.g., strengthening exercises), nutrition (e.g., protein and Vitamin D), and multidisciplinary interventions [9, 10], but the efficacy of these interventions for

protecting the frail patients against adverse outcomes is still controversial [11, 12]. Since frailty is one of the biggest threats to successful aging, a specific intervention that is expected to be effective to improve frailty status is highly needed. Currently, cell-based therapy is emerging as an innovative approach for several degenerative diseases. Mesenchymal stem cells (MSCs) represent as the ideal seeding cells for tissue engineering and regenerative medicine [13, 14]. To date, MSCs has become a promising candidate for intervening aging frailty. In this review, we mainly focused on the pathobiological process of aging frailty and summarized the roles and mechanisms of MSCs as the novel biologic agents used in the treatment of aging frailty. We also discussed the current status of MSCs utilized in clinical research as well as the challenge for successful clinical applications of MSC therapy.

Overview of Aging Frailty and its Pathophysiology

Aging frailty is a complex geriatric syndrome with multifactorial pathogenesis and declines in physiological reserves. Frail syndrome can lead to the reduced homeostatic capability to withstand stressors and increased vulnerabilities to environments, which account for the high risk of adverse events [2, 15, 16]. The overall prevalence of aging frailty in community worldwide is estimated to be between 5% and 20% [17-19]. The prevalence of frailty increases with age and women are more likely to be frail than men [18]. Aging frailty can be identified by two main models: physical frail phenotype and cumulative deficit index [2, 15]. According to the phenotypic model, frailty can be identified by the presence of at least three components: unintentional weight loss; self-reported exhaustion; weakness; slow walking speed and low physical performance [2]. It is characterized by diminished strength, endurance and reduced physiologic function, which increase an individual's vulnerability for developing increased dependency or death [20]. On the other hand, the deficit model describes frailty in terms of the accumulation of individual impairments that include comorbid diseases, symptoms, signs and disabilities, collectively referred to as deficits [15]. While these two instruments are different for evaluating frailty, both have received empirical validation.

With the process of aging, frailty may be caused by multiple causes and contributors, including genetic and environmental factors [21-24]. To be more specific, genome instability [25], DNA damage [26], epigenetic alternations [27], loss of proteostasis [28], oxidative stress [29], chronic inflammation [30], mitochondrial dysregulation [31], and stem cell exhaustion [24, 32] are involved in the progression of aging frailty. These hallmarks are interconnected and ultimately lead to cellular senescence. The senescent cells increase in multiple tissues with aging [33], and secrete a host of inflammatory cytokines, chemokines, growth factors and matrix remodeling proteases, collectively known as the senescence-associated secretory phenotypes (SASP), which lead to the chronic inflammation and age-related tissue deterioration [34, 35]. Moreover, senescence reduces the regenerative potential of stem cells pools and leads to endogenous stem cells exhaustion. The resident stem cells, including MSCs, HSCs (hematopoietic stem cells), neural stem cells (NSCs) and satellite cells undergo senescence during aging process, showing age-related decline in repopulation capacity and differentiation potential with reduced lifespan [36-39]. The reduced abilities of stem cells fail to maintain their proliferation capacity and differentiation potential [40]. Accordingly, the capacity to regenerate damaged tissues decline of regeneration upon damage decline, which results in the imbalance of tissue homeostasis after injury or stress [34, 41]. The sum of these integrative hallmarks produces the clinical phenotypes of the elderly with aging frailty, as seen in physiological loss of reserve and reduced organ function [42]. The disfunctions of brain, heart, muscle, and endocrine system are linked to aging and impaired homeostasis, which are believed to be involved in the development of frailty [16]. The multiple types of aging-related damages may constitute the major culprits of phenotypes of frailty, as the integrative consequence of stem cell exhaustion, diminished homeostasis, and organ repair [43]. In this regard, regenerative medicine and cellular therapy has been long proposed and examined clinically. As a promising candidate for tissue regeneration, MSCs have gathered great attention in the field of regenerative medicine. Transplantation of MSCs may serve as an innovative therapeutic approach for preventing and even reversing development of aging frailty [44, 45].

Basic Characteristics of MSCs

MSCs are the non-hematopoietic stem cells which exhibit spindle-shaped structure and plastic-adherent properties [46]. Originally isolated from bone marrow in 1968 [47], MSCs were successively found to exist in various tissues and can be easily harvested from multiple tissues, including adipose tissue, marrow spaces of long bone, skeletal muscle, synovial fluids, umbilical cord blood, placenta, and dental pulp [48-51]. As the multipotent progenitors, MSCs have displayed the ability to give rise to several different phenotypes, including osteocytes,

chondrocytes, adipocytes, fibroblast, and many others [52, 53]. However, MSCs exhibit heterogeneous features among their subpopulations regarding to their proliferation rate and secreted cytokines [54, 55]. In addition, the discrepancy of isolation and cultivation procedures between different laboratories also drives the development of standardized criteria for identifying unique populations of MSCs. In 2006, the International Society for Cellular Therapy (ISCT) has proposed the minimum criteria to define human MSCs [46]. According to ISCT, MSCs must be plastic-adherent and positive for specific surface makers, namely, CD73, CD90 and CD105 but be negative for CD14, CD19, CD34, CD45 and HLA-DR. More importantly, MSCs must be capable of differentiating into multilineage cell types *in vitro*. MSCs can migrate automatically toward injury areas and spontaneously differentiate into desired tissues to perform regenerative functions, which are described as tropism [48, 56]. The therapeutic effects of MSCs, including their anti-inflammatory and immunomodulatory abilities, are exerted via secretion of several cytokines and soluble factors and signaling pathway activation. MSCs had the low expression of MHC/HLA class I but do not express MHC/HLA class II, which can protect them from host immune detection. The biological property of immune evasion prolongs their persistence in the host and enhances their therapeutic effects [57]. To date, MSCs have been considered as one of the most promising stem cell types for cell therapy. MSCs are associated with unique capability of self-renewal and extensive potential of differentiation, which have generated great interest in the fields of regenerative medicine [58]. Multiple lines evidence have documented that the transplantation of MSCs can be utilized as a suitable therapeutic approach in the treatments of some intractable diseases, including traumatic brain injury [59] and spinal cord injury [60], cardiovascular diseases [61], stroke [62] and liver diseases [63]. The specific characteristics, along with the therapeutic benefits of MSCs support the potential use of MSCs in future therapies for aging frailty.

MSC Therapy for the Attenuation of Aging Frailty

Aging Brain

Frailty is a state of increased vulnerability to stressor events due to multimorbidity and multiple impairments in different systems. Aging brain or frail brain would lead to central nervous system impairments with cognitive decline, which play a crucial role in the development of physical frailty [64, 65]. More importantly, the deterioration of brain is associated with gait impairments, which is considered as an important contributor to frailty [66].

Neuroprotective Effects of MSCs

In aging process, almost all the brains undergo characteristic changes, including brain atrophy, loss of neurons and synapse connections. These age-related changes are responsible for the decline in neuronal activity and synaptic dysfunction that linked to neurodegeneration [67]. The effects of transplanted MSCs have been documented *in vivo* and vitro experiments in several studies, which have shown that MSCs could promote neurogenesis and improve neurological state [68, 69]. Intravenous infused MSCs can cross the blood-brain barrier (BBB), which is an essential prerequisite for proper efficacy [70-72]. Then intravenous injected MSCs can migrate to the injured regions and differentiate into neuron-like-cells via secreting various neurotrophic factors, such as nerve growth factor (NGF), vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF2). These secretomes are released from non-genetically modified MSCs, playing a significant role in inducing neuronal differentiation and increasing survival rates after injury [73, 74]. Likewise, administration of MSCs via intracerebral and intrathecal routes also showed positive results of neuronal regeneration promoted by MSCs in animal models [75, 76]. Moreover, microglia and astrocytes in aging brain become senescent and express the senescence-associated secretory phenotype; several inflammatory cytokines are secreted to maintain state of low-grade inflammation that play a significant role in natural aging and neurodegeneration [77]. MSCs possess anti-inflammatory properties adding to their neuro-protective effects. A great number of studies have showed that transplanted MSCs could reduce the levels of pro-inflammatory cytokines [78], or promote macrophages to polarize into the anti-inflammatory M2 phenotype [79]. The anti-inflammatory effects are conducted through secreting multiple cytokines, including IL-10 and transforming growth factor-β (TGF-β) [80]. At such, the anti-inflammatory microenvironments induced by transplanted MSCs help promote neurogenesis and prevent neural degeneration [78, 81]. Ameliorating cognitive decline may be a promising approach to prevent brain frailty. There are several altered proteins in the aged brains. The presence of amyloid-β, neurofibrillary tangles, Lewy bodies, the causative factors of neuro-degenerative diseases, such as AD, may contribute to deterioration of brain [66, 82, 83]. Inspiringly, MSCs administration has been documented to reduce plaque deposition, restore microglial function and increase synaptic and dendritic stability in animal

models of AD [68, 69]. To date, substantive preclinical studies are underway to provide positive results, and MSC-based therapy carries promise to reverse the deterioration of brain, which has become a potential therapeutic approach for the amelioration of aging frailty (Figure 2).

Cardiovascular Risk

The cardiovascular diseases and aging frailty often coexist. Growing evidence has showed that cardiovascular diseases including myocardial infarction, atrial fibrillation and chronic heart failure are associated with the increased high incidence of aging frailty [84-86]. Cardiovascular diseases could give rise to physical disability and frailty through impaired muscle function [85, 87]. The interplay between cardiovascular diseases and frailty may provide a novel therapeutic strategy in the interventions of frailty.

Cardioprotective Effects of MSCs

In the aging process, aging is associated with the gradual loss of biological functions, resulting in the increased cardiac vulnerability to cardiovascular dysfunction. The cardiac senescence is reflected by decreased cardiac performance and progressive cardiac structural remodeling. The various phenotypic changes in functions and structures of heart, including cardiomyocyte hypertrophy and apoptosis [88], interstitial fibrosis [89], comprehensively account for the decreased cardiac function, which may eventually lead to the progression of cardiovascular diseases in the aging populations. Several preclinical studies have demonstrated that MSCs could exert cardio-protective effects and promote cardiac functions through different mechanisms. MSCs could migrate to the injured zone and differentiate into endothelial cells and cardiomyocyte-like cells to promote neovascularization and cardiac functions, which can effectively offer repair in the sites of damaged myocardium. It has been found that MSCs exert many therapeutic functions through paracrine effects [90, 91]. MSCs can produce multiple cytokines and angiogenic factors released directly in soluble form or in extracellular vesicles and exosomes, playing a role

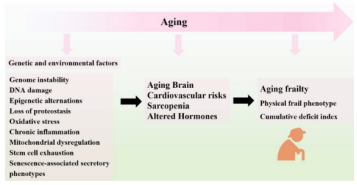

Figure 1. Overview of Aging Frailty and its Pathophysiology. In the process of aging, several genetic and environmental factors gradually result in the loss of tissue homeostasis and organ dysfunction, ultimately leading to the progression of frailty in the aged organism.

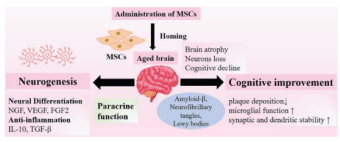

Figure 2. Neuroprotective Effects of MSCs on Aging Brain. Administration of MSCs have shown therapeutic potential for the treatment of age- related brain dysfunction. The neuroprotective effects of MSCs include promoting neurogenesis, neural differentiation and anti-inflammation, and these effects are mainly associated with paracrine functions. In addition, MSCs could improve cognitive functions through reducing plaque deposition and enhancing synaptic stability. Abbr: NGF: nerve growth factor; VEGF: vascular endothelial growth factor; FGF2: fibroblast growth factor 2; IL-2: interleukin-2; TGF-β: transforming growth factor-β.

in improving cardiac functions after damage [92]. The left ventricular ejection fraction (LVEF) is a significant parameter for evaluation of cardiac function, which would become deteriorated subsequently after ischemic events. It has been shown that LVEF can be successfully preserved in the MSCs treated group as compared to the control group in the animal model with ischemic myocardium [93]. The positive results have been further confirmed in clinical trials that transplantation of MSCs could significantly attenuate adverse ventricular remodeling and improve LVEF in patients with heart failure [94, 95]. Furthermore, many other studies have demonstrated MSC therapy can be capable of reducing the infract size and promoting cardiac hemodynamics in mice with ischemic myocardium [96]. Current evidence shows that MSCs could persist for 4 weeks after transplantation, predominantly in the border zone of infarcted myocardium, whereas few MSCs were detected in the normal cardiac tissues [97].

It has been well recognized that fibroblast could replace cardiomyocytes after injury, which cause myocardial remodeling and fibrotic scarring. The anti-fibrotic molecule, TNF-α-induced protein 6 (TNAIP6) is secreted by MSCs to decrease the damage to the heart and fibrosis. MSCs suppress the excessive inflammatory responses caused by cardiomyocyte cells injury and subsequent fibrosis [98]. In addition, MSCs attenuate arrhythmia by improving impulse conduction in the model of myocardial infarction [99]. Taken together, this novel approach of MSCs transplantation can ameliorate cardiovascular symptoms via several mechanisms, including angiogenesis, repair of the injured tissue, and reduction of infarct size as well as regulation of cardiac structural remodeling, which has a great potential to be applied in the regenerative medicine to improve the treatment of aging frailty [100] (Figure 3).

Sarcopenia

Sarcopenia is an age-related disease with the progressive loss of muscle mass and strength [101, 102]. The declines in skeletal mass and function pose significant risks for adverse outcomes including mortality, disability and falls among older adults [103-105]. The identification of sarcopenia is based on the co-occurrence of low muscle mass as well as slow gait speed or weak handgrip strength as measures of low muscle function [106]. Sarcopenia has been considered as an important component of frailty syndrome and the pathway through which the frail condition can be intervened or reversed [107].

Protective Effects of MSCs on Muscles

The interventions that can alleviate sarcopenia may be an important approach to improve or reverse frailty status. It has been showed that MSCs could attenuate sarcopenia via increasing skeletal muscle weight and myofiber cross-sectional area in animal models of sarcopenia [108]. The physical performance including muscle strength as well as endurance were significantly enhanced. MSCs also inhibit apoptosis of muscles and suppress expressions of chronic inflammatory cytokines, which may explain the improvement of skeletal muscle strength and function after transplantation of MSCs. In addition, MSCs have capability to activate resident skeletal muscle stem cells, which lead to myogenesis and differentiation of muscle tissues [109]. The positive results provide novel insights into sarcopenia intervention, suggesting a potential role for MSC therapy in aging frailty (Figure 4).

Altered Hormones

Advance in age leads to the disruption of endocrine system and imbalance of metabolic homeostasis, which may result in the breakdown of adaptation process in response to stresses [110]. The alternations in hormonal networks and abnormal hormonal excesses or deficits during aging can be translated in clinical scenarios that promote the pathogenesis of frailty and diseases [111]. As age-related disruption of the endocrine system is considered as a fundamental event in the pathogenesis of frailty, the efficacious strategies that can promote metabolism are needed.

Therapeutic Effects of MSCs on Hormones

Accumulating evidence shows that adverse ageing profiles and frailty are related to the alternations in hormonal networks [110-112]. Age-related frailty is a common problem in older adults, as a result of the imbalance between the anabolic and catabolic hormones. The circulating anabolic hormones, including insulin-like growth factor (IGFs), growth hormone, and sex hormones, are important in maintaining healthy body compositions and organ functions. However, there is an overall decline in the amounts of hormones with age. For instance, the decreased levels of testosterone could lead to hypogonadism and reduced muscle mass. Researchers have documented that MSCs transplantation could recover the levels of testosterone back to normal through paracrine functions [113]. Notably, growth hormone and IGF-1 also decrease with aging, the insufficient hormones result in body composition parameters with elevated fat mass and reduced lean mass [110, 114]. MSCs exerting beneficial paracrine effects are well recognized. It has been found that MSCs are capable

of secreting multiple growth factors and cytokines, promoting regeneration of Leydig cells and many surrounding cells [113, 115]. In addition, MSCs can develop and differentiate into Leydig cells in the adult testis [116].

In addition to the deficiency of hormones, decreased sensitivity of tissues to actions of hormone take place in the elderly. Notably, insulin resistance develops with age, which is a state of poor sensitivity of peripheral tissues to insulin [117]. Insulin resistance may lead to metabolic disorders and accelerate decline in muscle strength and function that give rise to frailty [118, 119]. The roles of aging endocrine system in the development of frailty and as a target for interventions of frailty are investigated. The chronic inflammation is an important determiner of insulin resistance [120], so the protective role of MSCs in improving insulin sensitivity via suppressing the inflammatory activity has been focused. Preclinical study showed that MSCs after transplantation could significantly promote the response of target organs to insulin [121]. The therapeutic effect of MSCs may be attributed to regulation of immune process and systemic inflammation [122]. Numerous data have reported that MSC-based therapy can attenuate insulin resistance and improve beta cell function via inhibiting the production of inflammatory cytokines (e.g., IL-1β, Il-18, TNF-α) [123]. MSCs play a pivotal role in reducing the number of CD3+ and CD4+ T lymphocytes, which initiate the inflammatory process in the organism [122]. Given the therapeutic potential of MSCs on delineating the age-related alterations of hormones, MSC-based therapy may be a very promising candidate for promoting quality of life in the elderly population (Figure 5).

Clinical Transplantation of MSCs in Patients with Aging Frailty

While current evidence sheds a promising light for the stem cell-based therapy, data related to frailty is still limited in clinical settings [124, 125]. Aging FRailTy via IntravenoUS Delivery (CRATUS) went through the phase I and II stages. The phase I trial was a nonrandomized, dose-escalation study, which has reported the beneficial effects after transplantation of BM-derived MSCs in patients with aging frailty [124]. In that study, a total of 15 eligible patients were enrolled to receive the intravenous infusion of MSCs with the dose: 20-million, 100-million, 200-million,

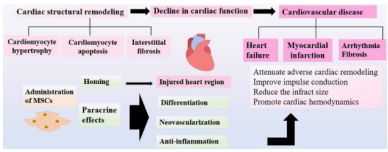

Figure 3. Cardioprotective Effects of MSCs. With advancing age, heart often develop decreased cardiac performance. The progressive cardiac structural remodeling results in low cardiac function and cardiovascular diseases that may contribute to aging frailty. After administration, MSCs home to the injured regions, where MSCs differentiate into endothelial cells and cardiomyocyte-like cells to promote neovascularization and cardiac functions. MSCs can suppress the excessive inflammatory responses and subsequent fibrosis via paracrine functions. MSC therapy show positive results by improving the prognosis of cardiovascular diseases.

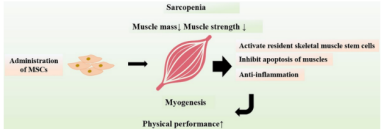

Figure 4. Protective Effects of MSCs on Muscles. Sarcopenia is a major contributor to frailty in the elderly. Transplanted MSCs can exert protective effects on muscles, including inhibition of muscles apoptosis and regulation of chronic inflammatory as well as activation of resident skeletal muscle stem cells. Administration of MSCs can promote myogenesis and improve physical performance.

respectively (5 patients in each group). Inspiringly, all patients in the treatment groups had increased 6-minute walk distance at 3 months and 6 months. The levels of inflammatory cytokine, TNF-α decreased at 6 months. Among the three groups, 100-million cell-dose group showed the best performance in the improvement of 6-minute walk distance, cognitive status and physical function. With regard to the safety of MSCs administration, no treatment-emergent serious adverse events occurred within 1-month post infusion. All patients could tolerate the doses of MSCs infused well. One death was reported at 258 days after infusion in the 200-million group which was determined to be irrelevant to MSCs transplantation. This study above-mentioned was succeeded by the randomized, double-blinded, and placebo-controlled, stage II of CRATUS study [125]. In the consecutive study, a total of 30 patients with aging frailty were randomized into 100-million, 200-million, and placebo groups. The results showed that immunologic improvement was seen in both the treatment groups. Notably, patients in the 100-million group performed better than that in the 200 million with improved 6-minute walk distance, short physical performance, forced expiratory volume in 1 second and decreased serum TNF-α levels from baseline to 6 months. More importantly, this study documented that intravenous administration of MSCs was safe, which did not incur any treatment-related serious adverse events for 12 months post infusion. Intriguingly, the consecutive two trials confirm that 100-million cells represent the superior dose level compared to 200-millon cells, yet the mechanism underlying the inverse dose relationship cannot be sufficiently explained [125]. A plausible explanation may be associated with deleterious effects of higher doses on cell retention, survival, or performance. Despite the positive findings, these two trials are preliminary and require larger RCTs to yield more convincing conclusions.

In recent years, failure of MSCs to improve clinical outcome have been frequently encountered [126, 127], partially due to variability in culture methodologies [128], and poor survival of MSCs after transplantation [129]. The effect of MSCs largely depends on their capabilities to migrate, adhere, engraft to the injured site. Notably, the freshly isolated cells cultured in presence of specific cytokines or hypoxic conditions have higher engraftment efficiency [130]. Furthermore, aggregate culture conditions used for MSC production may improve secretory capacity [128]. The use of different MSC derivatives, such as extracellular vesicles and exosomes, may be more effective and preferable than the use of MSCs. There is still a long way to go before considering MSCs as an ideal clinical tool for aging frailty.

Challenges for Clinical Application of MSC Therapy

Efficacy

MSCs have the distinct advantages of rapid expansion, multi-lineage differentiation and potent ability of secreting tropic and immunomodulatory cytokines. For years, transplantation of MSCs has evolved as the promising therapeutic strategy for regenerative medicine and tissue engineering [131].

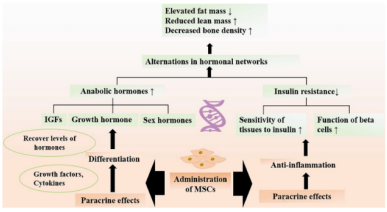

Figure 5. Therapeutic Effects of MSCs on Abnormal Hormones. Age-related alternation in hormonal networks include the decline in levels of circulating anabolic hormones and insulin resistance, which are associated with development of frailty. Administration of MSCs can increase the levels of anabolic hormones through parscrine functions and improve insulin sensitivity by regulating immune response. MSC therapy attenuate the age-related structural and functional changes of muscle and bone, thereby promoting the quality of life among the older adults.

However, there are major limits to MSCs utilization. The allogeneic MSCs derived from different donors display different biological properties. Aged MSCs tend to exhibit the cellular senescence associated phenotypes, including the enhanced senescence-associated β-galactosidase activity, decreased stemness of stem cells, increased p16 expression, and apoptosis of cells as well as telomere attrition [132, 133]. With telomeres shortening, aged MSCs gradually cease to proliferate after a certain number of cell divisions. The proliferation and differentiation potential of MSCs progressively decline with age of donor and passage number of MSCs cultured *in vitro* [134, 135]. Cellular senescence impairs the self-renewal and differentiation potential of MSCs, which limit their therapeutic effects [136]. The replicative senescence of MSCs significantly limits their expansion to the large quantity necessary for clinical applications that need hundreds of millions of MSCs for per treatment [137]. Moreover, there are limits for autologous MSC applications. It is difficult to obtain sufficient amount of healthy MSCs from patients with some systemic diseases. Additionally, the process of autologous extraction is time-consuming, which is difficult to be utilized for the acute treatment of life-threatening diseases [131]. Other concerns regarding the efficacy of MSCs are their persistence after transplantation. These issues need to be addressed prior to widespread clinical application to enhance the efficacy of MSC therapy.

Safety Concerns

MSCs are emerging as the promising sources of cell-based therapy due to their pluripotency and ease of expansion. However, ethical issues regarding to security remain inadequately addressed. It has been noted that long-term MSC expansion *in vitro* can lead to chromosomal abnormalities [138, 139], which may induce tumors *in vivo* [140]. In the tumor micro-environment, MSCs possess immunosuppressive effects, which promote the progression of tumors [141, 142]. MSCs show the potential to differentiate into multiple tissues, such as bone and cartilage, so the unwanted differentiation of transplanted MSCs may promote tumor growth [143]. Furthermore, it is well accepted that angiogenesis exerts an important role in invasion and metastasis of tumors. MSCs can differentiate into vascular endothelial cells, secreting several growth factors including VEGF and PDGF (platelet-derived growth factor), which promote tumor angiogenesis and invasive behavior [144]. MSCs also involve in the tumor invasion and metastasis known as epithelial to mesenchymal transition (EMT), a process driving tumor cells to lose polarity and acquire invasive phenotype [145, 146]. In this regard, the tumorigenic potential of MSCs may become a major safety concern for the use of MSCs in clinical practice. MSC-based therapy may be a double-edged sword; the application of MSCs in clinical setting should be evaluated cautiously due to security concerns. Of note, as paracrine effect of MSCs plays a pivotal role, the bioactive secretions of MSCs have good efficacy and safety. For instance, extracellular vesicles, exosomes, and cytokines can avoid the risk of genetic instability and potential malignant transformation may be developed as a safe and effective agent in the regenerative medicine.

Conclusions and Future Perspectives

Frailty syndrome is a nonspecific state of increased vulnerability to stressors and is much more common in the old populations. Frailty is strongly associated with adverse outcomes, which may place a heavy burden on society in the coming years. As there is no specific approved treatment for frail patients, deeper understanding of the biological mechanisms of aging frailty to explore effective interventions is of great significance. Notably, multiple pathologic changes develop with age, aside from DNA damage and chronic inflammation that may contribute to aging frailty, endogenous stem cell exhaustion may be involved in the process of aging frailty. The frail patients may display the disruption of physiological homeostasis with decline in functions of several organs.

MSCs are emerging as the ideal sources of cells to solve the multi-organ problems. MSCs have potent self-renewal and differentiation capability. They are easy to be harvested from many tissues and can engraft to injured sites. In addition, the immune privileged state and anti-inflammatory property make MSC-based therapy as a promising tool in systemic applications. Current evidence has showed that MSCs could ameliorate status of frailty by promoting the functions of multiple important organs, including brain, muscles, heart, and endocrine system. To date, allo-hMSCs had undergone the phase I/II trials in which the safety and efficacy of MSC-based therapy for aging frailty were initially demonstrated. MSCs could attenuate symptoms of frail patients and no treatment-related serious adverse event was reported.

Transplantation of MSCs has generated great interests in regenerative medicine. However, the disputes arise regarding lack of efficacy as well as tumorigenic potential of MSCs on basis of current evidence. Although many findings shed a new light on MSC-based therapy for aging frailty, the scales and numbers of current clinical trials remain small, much further studies are warranted to elucidate if such therapeutic strategy could be safe and effective on

regenerative medicine. The underlying mechanism of MSCs transplantation for the intervention of aging frailty should also be investigated.

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Competing Interests

The authors have declared that no competing interest exists.

References

1. Mathers CD, Stevens GA, Boerma T, White RA, Tobias MI. Causes of international increases in older age life expectancy. Lancet. 2015; 385: 540-8.
2. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci. 2001; 56: M146-56.
3. Chen X, Mao G, Leng SX. Frailty syndrome: an overview. Clin Interv Aging. 2014; 9: 433-41.
4. Li X, Ploner A, Karlsson IK, Liu X, Magnusson PKE, Pedersen NL, et al. The frailty index is a predictor of cause-specific mortality independent of familial effects from midlife onwards: a large cohort study. BMC Med. 2019; 17: 94.
5. Brummel NE, Bell SP, Girard TD, Pandharipande PP, Jackson JC, Morandi A, et al. Frailty and Subsequent Disability and Mortality among Patients with Critical Illness. Am J Respir Crit Care Med. 2017; 196: 64-72.
6. Duarte GP, Santos JLF, Lebrão ML, Duarte YAO. Relationship of falls among the elderly and frailty components. Rev Bras Epidemiol. 2019; 21Suppl 02: e180017.
7. Buckinx F, Rolland Y, Reginster JY, Ricour C, Petermans J, Bruyere O. Burden of frailty in the elderly population: perspectives for a public health challenge. Arch Public Health. 2015; 73: 19.
8. Hoogendijk EO, Afilalo J, Ensrud KE, Kowal P, Onder G, Fried LP. Frailty: implications for clinical practice and public health. Lancet. 2019; 394: 1365-75.
9. Walston J, Buta B, Xue QL. Frailty Screening and Interventions: Considerations for Clinical Practice. Clin Geriatr Med. 2018; 34: 25-38.
10. Pahor M, Kritchevsky SB, Waters DL, Villareal DT, Morley J, Hare JM, et al. Designing Drug Trials for Frailty: ICFSR Task Force 2018. J Frailty Aging. 2018; 7: 150-4.
11. Van der Elst M, Schoenmakers B, Duppen D, Lamboite D, Fret B, Vaes B, et al. Interventions for frail community-dwelling older adults have no significant effect on adverse outcomes: a systematic review and meta-analysis. BMC Geriatr. 2018; 18: 249.
12. Li CM, Chen CY, Li CY, Wang WD, Wu SC. The effectiveness of a comprehensive geriatric assessment intervention program for frailty in community-dwelling older people: a randomized, controlled trial. Arch Gerontol Geriatr. 2010; 50 Suppl 1: S39-42.
13. Saler M, Caliogna L, Botta L, Benazzo F, Riva P, Gastaldi G. hASC and DFAT, Multipotent Stem Cells for Regenerative Medicine: A Comparison of Their Potential Differentiation *In vitro*. Int J Mol Sci. 2017; 18.
14. Fu X, Liu G, Halim A, Ju Y, Luo Q, Song AG. Mesenchymal Stem Cell Migration and Tissue Repair. Cells. 2019; 8.
15. Rockwood K, Mitnitski A. Frailty in relation to the accumulation of deficits. J Gerontol A Biol Sci Med Sci. 2007; 62: 722-7.
16. Clegg A, Young J, Iliffe S, Rikkert MO, Rockwood K. Frailty in elderly people. Lancet. 2013; 381: 752-62.
17. Saum KU, Dieffenbach AK, Müller H, Holleczek B, Hauer K, Brenner H. Frailty prevalence and 10-year survival in community-dwelling older adults: results from the ESTHER cohort study. Eur J Epidemiol. 2014; 29: 171-9.
18. Collard RM, Boter H, Schoevers RA, Oude Voshaar RC. Prevalence of frailty in community-dwelling older persons: a systematic review. J Am Geriatr Soc. 2012; 60: 1487-92.
19. Andrade JM, Duarte YAO, Alves LC, Andrade FCD, Souza Junior PRB, Lima-Costa MF, et al. Frailty profile in Brazilian older adults: ELSI-Brazil. Rev Saude Publica. 2018; 52Suppl 2: 17s.
20. Morley JE, Vellas B, van Kan GA, Anker SD, Bauer JM, Bernabei R, et al. Frailty consensus: a call to action. J Am Med Dir Assoc. 2013; 14: 392-7.
21. Viña J, Tarazona-Santabalbina FJ, Pérez-Ros P, Martínez-Arnau FM, Borras C, Olaso-Gonzalez G, et al. Biology of frailty: Modulation of ageing genes and its importance to prevent age-associated loss of function. Mol Aspects Med. 2016; 50: 88-100.
22. Bektas A, Schurman SH, Sen R, Ferrucci L. Aging, inflammation and the environment. Exp Gerontol. 2018; 105: 10-8.
23. Dato S, Montesanto A, Lagani V, Jeune B, Christensen K, Passarino G. Frailty phenotypes in the elderly based on cluster analysis: a longitudinal study of two Danish cohorts. Evidence for a genetic influence on frailty. Age (Dordr). 2012; 34: 571-82.
24. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013; 153: 1194-217.
25. Seo AY, Leeuwenburgh C. The Role of Genome Instability in Frailty: Mitochondria versus Nucleus. Nestle Nutr Inst Workshop Ser. 2015; 83: 19-27.
26. Puscedda I, Farrell CJ, Di Pierro AM, Jani E, Herrmann W, Herrmann M. The role of telomeres and vitamin D in cellular aging and age-related diseases. Clin Chem Lab Med. 2015; 53: 1661-78.
27. Booth LN, Brunet A. The Aging Epigenome. Mol Cell. 2016; 62: 728-44.
28. Koga H, Kaushik S, Cuervo AM. Protein homeostasis and aging: The importance of exquisite quality control. Ageing Res Rev. 2011; 10: 205-15.
29. Alvarez-Satta M, Berna-Erro A, Carrasco-Garcia E, Alberro A, Saenz-Antoñanzas A, Vergara I, et al. Relevance of oxidative stress and inflammation in frailty based on human studies and mouse models. Aging (Albany N Y). 2020; 12: 9982-99.
30. Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. Nat Rev Cardiol. 2018; 15: 505-22.
31. Gomez-Cabrera MC, Sanchis-Gomar F, Garcia-Valles R, Pareja-Galeano H, Gambini J, Borras C, et al. Mitochondria as sources and targets of damage in cellular aging. Clin Chem Lab Med. 2012; 50: 1287-95.
32. Sun XL, Hao QK, Tang BJ, Xiao C, Ge ML, Dong BR. Frailty and Rejuvenation with Stem Cells: Therapeutic Opportunities and Clinical Challenges. Rejuvenation Res. 2019; 22: 484-97.
33. Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM. Cellular senescence in aging primates. Science. 2006; 311: 1257.
34. Lefebvre NK, Tchkonia T, Kirkland JL. Cellular Senescence and the Biology of Aging, Disease, and Frailty. Nestle Nutr Inst Workshop Ser. 2015; 83: 11-8.
35. Tchkonia T, Zhu Y, van Deursen J, Campisi J, Kirkland JL. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. J Clin Invest. 2013; 123: 966-72.
36. Yu KR, Kang KS. Aging-related genes in mesenchymal stem cells: a mini-review. Gerontology. 2013; 59: 557-63.
37. Wang MJ, Chen J, Chen F, Liu Q, Sun Y, Yan C, et al. Rejuvenating Strategies of Tissue-specific Stem Cells for Healthy Aging. Aging Dis. 2019; 10: 871-82.
38. Warren LA, Rossi DJ. Stem cells and aging in the hematopoietic system. Mech Ageing Dev. 2009; 130: 46-53.
39. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature. 2005; 433: 760-4.
40. Jones DL, Rando TA. Emerging models and paradigms for stem cell ageing. Nat Cell Biol. 2011; 13: 506-12.
41. Auman JR, Watson MM, Hagland HR, Sereide K. Molecular and biological hallmarks of ageing. Br J Surg. 2016; 103: e29-46.
42. Schultz MB, Sinclair DA. When stem cells grow old: phenotypes and mechanisms of stem cell aging. Development. 2016; 143: 3-14.
43. Golpanian S, DiFede DL, Pujol MV, Lowery MH, Levis-Dusseau S, Goldstein BJ, et al. Rationale and design of the allogeneiC human mesenchymal stem cells (hMSC) in patients with aging fRAilTy via intravenoUS delivery (CRATUS) study: A phase I/II, randomized, blinded and placebo controlled trial to evaluate the safety and potential efficacy of allogeneic human mesenchymal stem cell infusion in patients with aging frailty. Oncotarget. 2016; 7: 11899-912.
44. Raggi C, Berardi AC. Mesenchymal stem cells, aging and regenerative medicine. Muscles Ligaments Tendons J. 2012; 2: 239-42.
45. Schulman IH, Balkan W, Hare JM. Mesenchymal Stem Cell Therapy for Aging Frailty. Front Nutr. 2018; 5: 108.
46. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006; 8: 315-7.
47. Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet. 1970; 3: 393-403.
48. Samsonraj RM, Raghunath M, Nurcombe V, Hui JH, van Wijnen AJ, Cool SM. Concise Review: Multifaceted Characterization of Human Mesenchymal Stem Cells for Use in Regenerative Medicine. Stem Cells Transl Med. 2017; 6: 2173-85.
49. Rodriguez AM, Elabd C, Amri EZ, Ailhaud G, Dani C. The human adipose tissue is a source of multipotent stem cells. Biochimie. 2005; 87: 125-8.
50. Zhang X, Hirai M, Cantero S, Ciubotariu R, Dobrila L, Hirsh A, et al. Isolation and characterization of mesenchymal stem cells from human umbilical cord blood: reevaluation of critical factors for successful isolation and high ability to proliferate and differentiate to chondrocytes as compared to mesenchymal stem cells from bone marrow and adipose tissue. J Cell Biochem. 2011; 112: 1206-18.

51. Sinclair K, Yerkovich ST, Chambers DC. Mesenchymal stem cells and the lung. Respirology. 2013; 18: 397-411.
52. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999; 284: 143-7.
53. Cook D, Genever P. Regulation of mesenchymal stem cell differentiation. Adv Exp Med Biol. 2013; 786: 213-29.
54. Majore I, Moretti P, Hass R, Kasper C. Identification of subpopulations in mesenchymal stem cell-like cultures from human umbilical cord. Cell Commun Signal. 2009; 7: 6.
55. Riekstina U, Cakstina I, Parfejevs V, Hoogduijn M, Jankovskis G, Muiznieks I, et al. Embryonic stem cell marker expression pattern in human mesenchymal stem cells derived from bone marrow, adipose tissue, heart and dermis. Stem Cell Rev Rep. 2009; 5: 378-86.
56. Nitsche F, Müller C, Lukomska B, Jolkkonen J, Deten A, Boltze J. Concise Review: MSC Adhesion Cascade-Insights into Homing and Transendothelial Migration. Stem Cells. 2017; 35: 1446-60.
57. Ryan JM, Barry FP, Murphy JM, Mahon BP. Mesenchymal stem cells avoid allogeneic rejection. J Inflamm (Lond). 2005; 2: 8.
58. Squillaro T, Peluso G, Galderisi U. Clinical Trials With Mesenchymal Stem Cells: An Update. Cell Transplant. 2016; 25: 829-48.
59. Das M, Mayilsamy K, Mohapatra SS, Mohapatra S. Mesenchymal stem cell therapy for the treatment of traumatic brain injury: progress and prospects. Rev Neurosci. 2019; 30: 839-55.
60. Mukhamedshina Y, Shulman I, Ogurcov S, Kostronikov A, Zakirova E, Akhmetzyanova E, et al. Mesenchymal Stem Cell Therapy for Spinal Cord Contusion: A Comparative Study on Small and Large Animal Models. Biomolecules. 2019; 9.
61. Goradel NH, Hour FG, Negahdari B, Malekshahi ZV, Hashemzehi M, Masoudifar A, et al. Stem Cell Therapy: A New Therapeutic Option for Cardiovascular Diseases. J Cell Biochem. 2018; 119: 95-104.
62. Doeppner TR, Hermann DM. Mesenchymal stem cells in the treatment of ischemic stroke: progress and possibilities. Stem Cells Cloning. 2010; 3: 157-63.
63. Mahmood A, Seetharaman R, Kshatriya P, Patel D, Srivastava AS. Stem Cell Transplant for Advanced Stage Liver Disorders: Current Scenario and Future Prospects. Curr Med Chem. 2019.
64. Malmstrom TK, Morley JE. The frail brain. J Am Med Dir Assoc. 2013; 14: 453-5.
65. Robertson DA, Savva GM, Kenny RA. Frailty and cognitive impairment--a review of the evidence and causal mechanisms. Ageing research reviews. 2013; 12: 840-51.
66. Schneider JA, Li JL, Li Y, Wilson RS, Kordower JH, Bennett DA. Substantia nigra tangles are related to gait impairment in older persons. Ann Neurol. 2006; 59: 166-73.
67. Boisvert MM, Erikson GA, Shokhirev MN, Allen NJ. The Aging Astrocyte Transcriptome from Multiple Regions of the Mouse Brain. Cell Rep. 2018; 22: 269-85.
68. Lo Furno D, Mannino G, Giuffrida R. Functional role of mesenchymal stem cells in the treatment of chronic neurodegenerative diseases. J Cell Physiol. 2018; 233: 3982-99.
69. Duncan T, Valenzuela M. Alzheimer's disease, dementia, and stem cell therapy. Stem Cell Res Ther. 2017; 8: 111.
70. Ren C, Guingab-Cagmat J, Kobeissy F, Zoltewicz S, Mondello S, Gao M, et al. A neuroproteomic and systems biology analysis of rat brain post intracerebral hemorrhagic stroke. Brain Res Bull. 2014; 102: 46-56.
71. Matsushita T, Kibayashi T, Katayama T, Yamashita Y, Suzuki S, Kawamata J, et al. Mesenchymal stem cells transmigrate across brain microvascular endothelial cell monolayers through transiently formed inter-endothelial gaps. Neurosci Lett. 2011; 502: 41-5.
72. Liu L, Eckert MA, Riazifar H, Kang DK, Agalliu D, Zhao W. From blood to the brain: can systemically transplanted mesenchymal stem cells cross the blood-brain barrier? Stem Cells Int. 2013; 2013: 435093.
73. Munoz JR, Stoutenger BR, Robinson AP, Spees JL, Prockop DJ. Human stem/progenitor cells from bone marrow promote neurogenesis of endogenous neural stem cells in the hippocampus of mice. Proc Natl Acad Sci U S A. 2005; 102: 18171-6.
74. Tan B, Liao Z, Wei X, He Y, Wei G, Johnstone BH, et al. AMP-activated kinase mediates adipose stem cell-stimulated neuritogenesis of PC12 cells. Neuroscience. 2011; 181: 40-7.
75. Bao X, Wei J, Feng M, Lu S, Li G, Dou W, et al. Transplantation of human bone marrow-derived mesenchymal stem cells promotes behavioral recovery and endogenous neurogenesis after cerebral ischemia in rats. Brain Res. 2011; 1367: 103-13.
76. Ruan GP, Han YB, Wang TH, Xing ZG, Zhu XB, Yao X, et al. Comparative study among three different methods of bone marrow mesenchymal stem cell transplantation following cerebral infarction in rats. Neurol Res. 2013; 35: 212-20.
77. Pizza V, Agresta A, D'Acunto CW, Festa M, Capasso A. Neuroinflamm-aging and neurodegenerative diseases: an overview. CNS Neurol Disord Drug Targets. 2011; 10: 671-34.
78. Lee HJ, Lee JK, Lee H, Carter JE, Chang JW, Oh W, et al. Human umbilical cord blood-derived mesenchymal stem cells improve neuropathology and cognitive impairment in an Alzheimer's disease mouse model through modulation of neuroinflammation. Neurobiol Aging. 2012; 33: 588-602.
79. Kudlik G, Hegyi B, Csibrala Á, Monostori É, Buday L, Uher F. Mesenchymal stem cells promote macrophage polarization toward M2b-like cells. Exp Cell Res. 2016; 348: 36-45.
80. Kyurkchiev D, Bochev I, Ivanova-Todorova E, Mourdjeva M, Oreshkova T, Belemezova K, et al. Secretion of immunoregulatory cytokines by mesenchymal stem cells. World J Stem Cells. 2014; 6: 552-70.
81. Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R. Mesenchymal Stem Cell Secretome: Toward Cell-Free Therapeutic Strategies in Regenerative Medicine. Int J Mol Sci. 2017; 18.
82. Elobeid A, Libard S, Leino M, Popova SN, Alafuzoff I. Altered Proteins in the Aging Brain. J Neuropathol Exp Neurol. 2016; 75: 316-25.
83. Wyss-Coray T. Ageing, neurodegeneration and brain rejuvenation. Nature. 2016; 539: 180-6.
84. Polidoro A, Stefanelli F, Ciacciarelli M, Pacelli A, Di Sanzo D, Alessandri C. Frailty in patients affected by atrial fibrillation. Arch Gerontol Geriatr. 2013; 57: 325-7.
85. Stewart R. Cardiovascular Disease and Frailty: What Are the Mechanistic Links? Clin Chem. 2019; 65: 80-6.
86. Newman AB, Gottdiener JS, McBurnie MA, Hirsch CH, Kop WJ, Tracy R, et al. Associations of subclinical cardiovascular disease with frailty. The journals of gerontology Series A, Biological sciences and medical sciences. 2001; 56: M158-M66.
87. Strandberg TE, Pitkälä KH, Tilvis RS, O'Neill D, Erkinjuntti TJ. Geriatric syndromes--vascular disorders? Ann Med. 2013; 45: 265-73.
88. Oka T, Komuro I. Molecular mechanisms underlying the transition of cardiac hypertrophy to heart failure. Circ J. 2008; 72 Suppl A: A13-6.
89. Creemers EE, Pinto YM. Molecular mechanisms that control interstitial fibrosis in the pressure-overloaded heart. Cardiovasc Res. 2011; 89: 265-72.
90. Zhang J, Wu Y, Chen A, Zhao Q. Mesenchymal stem cells promote cardiac muscle repair via enhanced neovascularization. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. 2015; 35: 1219-29.
91. Ward MR, Abadeh A, Connelly KA. Concise Review: Rational Use of Mesenchymal Stem Cells in the Treatment of Ischemic Heart Disease. Stem Cells Transl Med. 2018; 7: 543-50.
92. Gallina C, Turinetto V, Giachino C. A New Paradigm in Cardiac Regeneration: The Mesenchymal Stem Cell Secretome. Stem Cells Int. 2015; 2015: 765846.
93. Makkar RR, Price MJ, Lill M, Frantzen M, Takizawa K, Kleisli T, et al. Intramyocardial injection of allogenic bone marrow-derived mesenchymal stem cells without immunosuppression preserves cardiac function in a porcine model of myocardial infarction. J Cardiovasc Pharmacol Ther. 2005; 10: 225-33.
94. Bartolucci J, Verdugo FJ, González PL, Larrea RE, Abarzua E, Goset C, et al. Safety and Efficacy of the Intravenous Infusion of Umbilical Cord Mesenchymal Stem Cells in Patients With Heart Failure: A Phase 1/2 Randomized Controlled Trial (RIMECARD Trial [Randomized Clinical Trial of Intravenous Infusion Umbilical Cord Mesenchymal Stem Cells on Cardiopathy]). Circ Res. 2017; 121: 1192-204.
95. Lalu MM, Mazzarello S, Zlepnig J, Dong YYR, Montroy J, McIntyre L, et al. Safety and Efficacy of Adult Stem Cell Therapy for Acute Myocardial Infarction and Ischemic Heart Failure (SafeCell Heart): A Systematic Review and Meta-Analysis. Stem Cells Transl Med. 2018; 7: 857-66.
96. Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, Anversa P. Transplanted adult bone marrow cells repair myocardial infarcts in mice. Ann N Y Acad Sci. 2001; 938: 221-30.
97. Nagaya N, Fujii T, Iwase T, Ohgushi H, Itoh T, Uematsu M, et al. Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis. Am J Physiol Heart Circ Physiol. 2004; 287: H2670-6.
98. Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, et al. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell. 2009; 5: 54-63.
99. Panda NC, Zuckerman ST, Mesubi OO, Rosenbaum DS, Penn MS, Donahue JK, et al. Improved conduction and increased cell retention in healed MI using mesenchymal stem cells suspended in alginate hydrogel. J Interv Card Electrophysiol. 2014; 41: 117-27.
100. Yan CW, Lee SH. Enhancement of Functionality and Therapeutic Efficacy of Cell-Based Therapy Using Mesenchymal Stem Cells for Cardiovascular Disease. Int J Mol Sci. 2019; 20: 982.
101. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. Age Ageing. 2010; 39: 412-23.
102. Rosenberg IH. Sarcopenia: origins and clinical relevance. The Journal of nutrition. 1997; 127: 990S-1S.
103. Landi F, Cruz-Jentoft AJ, Liperoti R, Russo A, Giovannini S, Tosato M, et al. Sarcopenia and mortality risk in frail older persons aged 80 years and older: results from ilSIRENTE study. Age Ageing. 2013; 42: 203-9.
104. Vetrano DL, Landi F, Volpato S, Corsonello A, Meloni E, Bernabei R, et al. Association of sarcopenia with short- and long-term mortality in older adults admitted to acute care wards: results from the CRIME study. The journals of gerontology Series A, Biological sciences and medical sciences. 2014; 69: 1154-61.

Frailty and Rejuvenation with Stem Cells: Therapeutic Opportunities and Clinical Challenges

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Abstract

Frailty, one appealing target for improving successful aging of the elderly population, is a common clinical syndrome based on the accumulation of multisystemic function declines and the increase in susceptibility to stressors during biological aging. The age-dependent senescence, the frailty-related stem cell depletion, chronic inflammation, imbalance of immune homeostasis, and the reduction of multipotent stem cells collectively suggest the rational hypothesis that it is possible to (partially) cure frailty with stem cells. This systematic review has included all of the human trials of stem cell therapy for frailty from the main electronic databases and printed materials and screened the closely related reviews themed on the mechanisms of aging, frailty, and stem cells, to provide more insights in stem cell strategies for frailty, one promising method to recover health from a frail status. To date, a total of four trials about this subject have been registered on clinicaltrials.gov. The use of mesenchymal stem cells (MSCs), doses of 100 million cells, single peripheral intravenous infusion, follow-up periods of 6–12 months, and a focus primarily on safety and secondarily on efficacy are common characteristics of these studies. We conclude that intravenous infusion of allogenic MSCs is safe, well tolerated, and preliminarily effective clinically. More preclinical experiments and clinical trials are warranted to precisely elucidate the mechanism, safety, and efficacy of frailty stem cell therapy.

Introduction

According to the 2018 Aging and Health report by the World Health Organization,[1] by 2050, there will be an estimated population aged 60 years or more of 2 billion people, accounting for 22% of the whole population, nearly double that in 2015 (900 million, 9%). A longer life brings with it opportunities for society as a whole in many ways, which are heavily dependent on the person's health and successful aging. However, the cumulative decline in physical and mental capacity currently disturbing many old people brings negative implications to the added years.[1] Hence, almost all countries are forced to face the challenge of ensuring that their social system and health system are ready for this demographic change. Under these circumstances, frailty, the most problematic manifestation of aging[2] and one of the major parts of Comprehensive Geriatric Syndrome, is intensively associated with physical and mental function declines and deserves public attention.

An appropriate target to promote successful aging: frailty

Frailty is an independent clinical definition, different from both comorbidity (one of its etiology factors) and disability (one of its adverse outcomes).[3] Frailty has recently been defined by a professional global task force as a progressive systematic decline of physiological reserves and an increase of vulnerability to minor stressors.[4,5] The main clinical manifestations of frailty present as unintentional weight loss, exhaustion, weakness, slow walking speed, and low physical activity. These symptoms, which are named Fried phenotypes,[3] are more comprehensively presented as the Frailty Index (FI),[6] taking into account multidimensional cumulative deficits, which are closely related with adverse clinical events, such as falls, fractures, disability, and mortality[3,4,6]

Several major tools are used to assess frailty[5]: (1) the Fried Phenotype Criteria and its rapid screen form[7]; (2) the FI and the Clinical Frailty Scale of Rockwood and Mitnitski, which is concerned with poly-morbidities; and (3) mixed models, such as the Frailty Criteria of the International Nutrition Society, the Study of the Osteoporotic Fractures Index, the Tilburg Frailty Indicator, and the Edmonton Frailty Scale. Among a world of measurements, currently, the most internationally well-accepted assessments are Fried's criteria and the FI. The FI and the Edmonton Frailty Scale are superior to other methods when predicting death.[2,8,9]

Based on a representative review (n = 61,500) and a cohort study (n = 16019),[10,11] the general prevalence of frailty, which increases with age and is higher in women than in men, is 11.0%–14.9% among the population over 65 years and 40% among people over 80. According to the consensus, the prevalence of frailty in community dwellings was ~3.5%–27% in the Asia-Pacific region, which was comparable to that in Europe and America.[5] The steadily aging population base and a series of interlinked clinical

frailty-related events collectively impose a heavy burden on the public health cost worldwide. Frail participants had an average total health cost of €2,476/year and prefrail participants of €2,056/year, which is approximately twice as high as that of the nonfrail (€1,217/year) in Spain.[12] This situation received particular attention in Asia, where the elderly who urgently need health care are often unable to access enough publicly funded health care services.[13]

To decrease the cumulative vulnerability and dependence of the older population, which cause complicated demographic, health-related, and social problems, frailty can thus be selected as an appropriate target that we must urgently deal with. Therefore, our aim is to develop a good understanding of the potential mechanisms and the efficacy of matching therapy. Nevertheless, the optimal preventions and treatments are still poorly explored, and there are no specific, effective, and pathophysiology reversing strategies for the treatment of frailty.[4,14]

Biological aging combined with stressors: the driving force of frailty

Biological aging is natural and involves a gradual decline of physiological reserves; nevertheless, in frailty, this process is accelerated and concomitant with falling homeostasis.[2,12] Among all of the aging symptoms elucidated by López-Otín et al.,[15,16] stem cell exhaustion and altered intercellular communication are likely the ultimate characteristics contributing to the clinical manifestations of aging-related frailty.[17–19] However, there is uncertainty regarding the precise level and the kind of these aging characteristics[15,16] as they integrate with the accelerating cumulative decline of physiological reserves observed in aging-related frailty.[2] Simply put, the age-related pathophysiology mechanism, combined with inner and outer stressors, which drive frailty, calls for a convenient regenerative strategy that is more effective than current therapeutic methods.[8,20–23] Therefore, much attention has recently been focused on stem cell strategies, which possess promising potential.

Matched therapy strategy of frailty: stem cells

Embryonic stem cells (ESCs) and adult stem cells are two main categories of stem cells, along with the embryonic-like inducible pluripotent stem cells (iPSCs) derived from different somatic cells by activating the “Yamanaka factors” Oct4, Sox2, Klf4, and Myc (“OSKM”).[24,25] Mesenchymal stem cells (MSCs), one subset of adult stem cells, have several advantages in frailty therapy: the wide autologous or allogenic sources of acquisition (bone marrow, adipose tissue, umbilical cord or cord blood, placenta, and peripheral blood)[19,26] and the therapeutic properties of migration to inflammation and injury sites, differentiation into various tissue-specific precursor cells, secretion of trophic bioactive compounds, and mediation of immunomodulatory effects.[24,27] There

is, clearly, an opportunity to now apply stem cell strategies for the age-related and stressor-involved clinical condition of frailty for the aging population.

Although the subject of stem cell therapy for frailty has been considered by some leading research teams globally,[19,24,28] few human trials are registered at clinicaltrials.gov at present,[29,30] which act as the potential new landmarks of frailty therapy. To date, there is little agreement on frailty stem cell therapy,[19,24,28] thus calling for more insights into this promising approach. The question how the aging process and relatively minor stressor events combine to build the foundation for frailty and why stem cell therapy is the favorable approach for treating aging-related frailty are the issues addressed in this systematic review.

Methods

Given the contradiction between the significance of frailty stem cell therapy and the limited numbers of human trials directly adopting stem cells as intervention to treat frailty, the search strategy was not just rigorously confined to randomized clinical trials of stem cell-based frailty therapy, but also included leading reviews elaborating on the stem cell function decline in frailty during biological aging and the promising potentials of stem cells in frailty treatment.

Search strategy

With inclusion and exclusion criteria prespecified as below, we identified recent publications reporting the advance of frailty, mainly addressing the pathophysiological mechanism and potential targets for stem cells, and all of the publications on stem cell treatments for frailty, by searching several main electronic databases (EMBASE, All EMBASE REVIEWS, MEDLINE, and Cochrane CENTRAL from Ovid SP; PUBMED; OpenGrey; CBM) and clinicaltrials.gov (November 22, 2018; in English and Chinese), using the key search strategy “stem cell AND frail,” with a series of Boolean operators. Two individuals carried out the database searches and screened abstracts or full texts independently; a third author resolved the disagreements. The relevant bibliographies were screened to further identify valuable publications.

Inclusion and exclusion criteria

Mainly, we included the studies in which the frailty patients were directly treated with allogeneic or autologous stem cells of different sources and in which the safety and efficacy were compared with the control counterparts treated with placebo (or not). Due to the low number of frailty stem cell trials completed presently and the high significance of this promising novel strategy of stem cells to treat frailty, some other types of important literature were also searched and screened as independent parts

(not shown). We excluded studies that did not directly use stem cells as an intervention to treat frailty, such as the transplantation of hematopoietic stem cells for diseases that include leukemia.

Data extraction

The following items were extracted by two individuals independently from each included study and registered clinical trial: reference details (title and date); condition and interventions (stem cell type, dose, delivery route, and frequency); aims and characteristics (study type, phase, and study design); recipients (age and sex); and main outcome measures. When safety and efficacy tests were performed serially, we schemed to extract the data at the different time points in the safety part but only extracted data for the final time point in the efficacy part, for acute and chronic adverse reactions were both indispensable for the safety assessment. For missing or incomplete data, we requested them from the authors or else estimated numerical values by digital ruler software. The flowchart is shown in Figure 1.

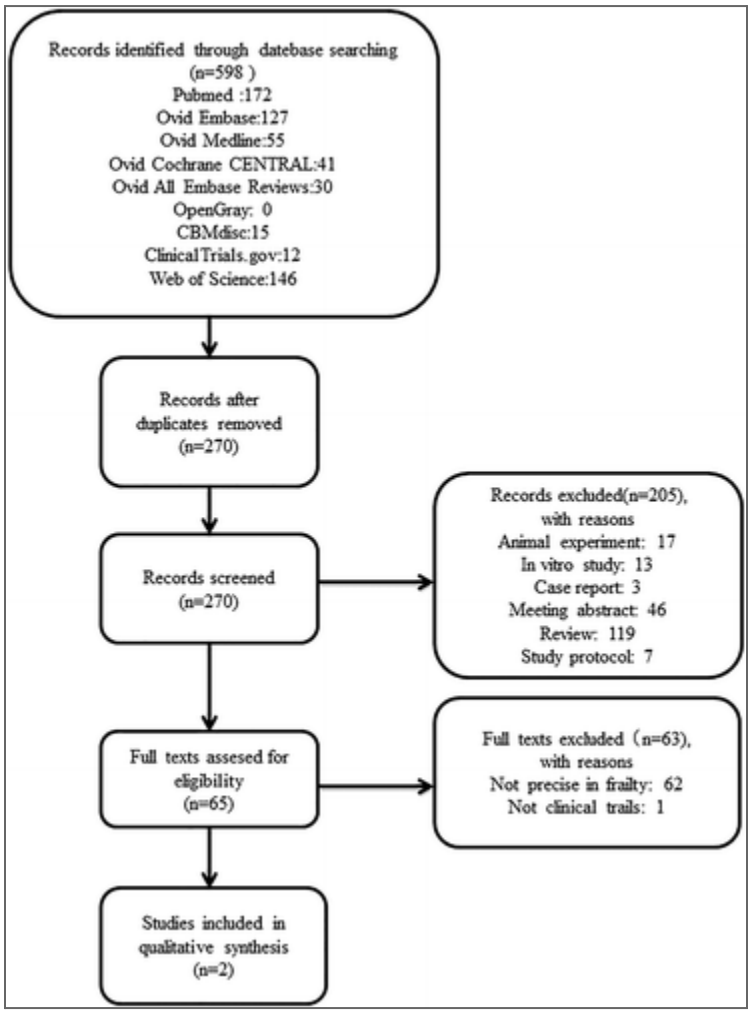

FIG. 1. Flowchart of the literature retrieving and screening.

Results

The design characteristics of the included allogenic bone marrow derived MSC studies for frailty

Regarding the one human trial of the only two original articles included so far, this project was launched as a phase I/II, randomized, blinded, and placebo-controlled clinical trial (No. NCT02065245) and was named as CRATUS (the Greek god symbolizing power and strength)[24,29–31] in 2014. It was estimated to be completed in 2020. The project is under the charge of the team of Joshua M. Hare of the Interdisciplinary Stem Cell Institute at the University of Miami Miller School of Medicine and their commercial collaborator EMMES Corporation. The primary objective is to determine the safety of different doses of allogenic bone marrow mesenchymal stem cells (Allo-BMMSCs) and the tolerability of cell infusion; the secondary objective is to explore the potential treatment efficacy in improving frailty.

The Allo-BMMSCs, a U.S. FDA-regulated drug product, were derived from bone marrow of eligible male or female donors aged 20–45 years, cultured and amplified *in vitro*, and then identified by measuring the gene expression of white blood cell RNA.[31] Patients of both sexes, aged 60 to 95 years, with a score of 4–7 on the Canadian Clinical Frailty Scale (apparently vulnerable to severely frail) and a score of less than or equal to 24 on the Mini Mental State Examination (MMSE) were taken as eligible subjects. In total, 65 participants were enrolled. Groups treated with 20, 100, or 200 million cells (5 patients per group) and groups treated with 100 or 200 million cells or placebo (10 patients per group) were formed for the pilot safety phase and for randomized phase trials. All of the cell intervention subjects received single peripheral intravenous infusion of Allo-BMMSCs with a total volume of 80 mL at an average speed of 2 mL/min, so the total infusion time was 40 minutes.[14,29–31] Within the 12-month follow-up period, primary outcomes include any incidence, mainly in the first 30 days postinfusion, expressed as treatment-emergent serious adverse events (TE-SAEs), such as death, stroke, hospitalization for worsening dyspnea, nonfatal pulmonary embolism, and clinically significant serum chemistry and hematology test abnormalities. Secondary outcomes include indicators for physical function, quality of life, exercise, change in ejection fraction, and inflammatory markers, assessed at 3 and 6 months postinfusion.[29–31] The details of the study are shown in Table 1.

Activity CHAMPS questionnaire, reduced activity Community Healthy Activities Model Program for Seniors questionnaire; Allo-BMMSCs, allogenic bone marrow mesenchymal stem cells of 20–45 year donors; CBC, complete blood cell count; CRP, C-reactive protein; EF, ejection fraction; EQ-5D, EuroQol five dimensions questionnaire; FEV1, forced expiratory volume in 1 second; IIEF, International Index of Erectile Dysfunction; IL-6,

interleukin 6; LAEs, related long-term adverse events; 4MGST, 4-m gait speed test; 6MWT, 6-minute walk distance test; MCS, Mental Component Score; MFI, exhaustion-multidimensional fatigue inventory; MMSE, Mini-Mental State Examination; PCS, Physical Component Score; SF-36, 36-Item Short Form Health Survey; SPPB score, short physical performance battery score; SQOL-F, Sexual Quality of Life-Female Questionnaires; TE-SAEs, treatment emergent-serious adverse events, defined as the composite of death, nonfatal pulmonary embolism, stroke, hospitalization for worsening dyspnea; TNF-α, tumor necrosis factor-α; WBCs, white blood cells.

The comprehensive analysis of the results of enrolled phase I/II clinical trials

According to outcomes from the only two published studies of stem cell trials for frailty,[29,30] all 15 patients of the pilot phase and 30 patients of the randomized phase actually had scores of 4–6 on the Clinical Frailty Scale, so the basal degree of frailty ranged between “moderate” and “vulnerable,” and no severely frail patients were enrolled. The average age of subjects was 78.4 ± 4.7 in the pilot study, 75.5 ± 7.3 in the randomized phase, and 76.0 ± 6.7 in the whole study. Among the 45 subjects, nearly all were of the Caucasian race, and no participants of Hispanic or Latino ethnicity were included.

Comparing all 45 patients who underwent cell infusion and the control counterparts for the main safety evaluation, 2 patients died 8 months postinfusion and in 4 patients donor-specific reactions occurred, as observed by calculated panel reactive antibodies, which, however, were unrelated events or had no clinical significance. Notably, no patients demonstrated adverse signs of cardiopulmonary reactions after the infusion, and the basic clinical hematology and chemistry tests were stable during the entire study period.

As regards the efficacy, remarkably, 100-million cell doses exhibited a more effective reaction than the 20- and 200 cell doses, and the level of tumor necrosis factor-α (TNF-α), an important biomarker closely associated with inflammation and immunity, had significantly decreased at 6 months in all cell treatment groups. However, examination of other physical indices, cardiopulmonary function, quality of life, and biomarker levels, such as the 6-minute walk distance test (6MWT), exhaustion-multidimensional fatigue inventory (MFI), and C-reactive protein (CRP) levels, did not give consistent results and/or did not show statistical significance between groups.

In summary, the allogenic MSC intervention for frailty is safe and well tolerated with no TE-SAEs and no significant immune reactions throughout the whole duration of the study. In addition, single peripheral infusion of allogenic MSCs preliminarily proved efficacy.

Overview of worldwide ongoing human trials of stem cell therapy for frailty

Systematic analysis of all the ongoing and completed clinical trials applying stem cells of multiple types to treat frailty provides an overview of the progress in this novel field.

A total of four human trials, Nos. NCT01501461, NCT02065245, NCT02982915, and NCT03169231, were registered on clinicaltrials.gov between 2011 and 2017. The first one, No. NCT01501461, was registered in 2011 by Zuniga et al. of the Instituto de Medicina Regenerativa and the Ageless Regenerative Institute in Mexico, but it was withdrawn in 2018 because the company was dissolved. The other three trials, including No. NCT02065245 (analyzed above), are ongoing (Table 2).

Trials ID	Start year	Status	Phase	Condition or disease	Age/sex	Enrollment number	Intervention	Cells dose/patient	Delivery	Follow-up time frame	Primary outcome indicators	Secondary outcome indicators
NCT01501461	2011	Withdrawn	Not applicable (open-label, nonrandomized)	Frailty syndrome	55–90 years/all	0	Auto-ADSCs	Not applicable	Single intravenous injection	1. 3 months 2. 6 months	1. Improvement in PPT 2. Number of Aes 3. Improved	Exercise capacity

											BC/BD 4. Quality of life	
NCT02982915	2016	Recruiting	Phase I/II (double blind, randomized)	Aging frailty	65–90 years/all	43	Allo-BMMSCs; co-injecting with fluzone high dose vaccine	2×10^7; 1×10^8	Single peripheral intravenous infusion	1. 1 and 4 weeks postvaccination 2. 30 days 3. 6 months 4. 12 months	1. Incidence of TE-SAEs 2. Adaptive immunity 3. Primary B cell response	1. Postvaccination changes 2. Changes of biomarkers 3. Different decline rate from aging frailty 4. Death from any cause
NCT03169231	2017	Recruiting	Phase IIb (double blind, randomized	Aging frailty	70–85 years/all	120	Allo-BMMSCs	2.5×10^7; 5×10^7; 1×10^8;	Single peripheral intraven	6 months	6MWT	1. Physical function capacity

, parallel assignment)			**Placebo.**	**ous infusion**			**using PROMIS-PF-SF 20a 2. Serum TNF-α**

AEs, adverse events; Auto-ADSCs, autologous adipose-derived stem cells; BC/BD, body composition/bone density; PROMIS-PF-SF 20a, Patient-Reported Outcome Measurement-Physical Function-Short Form 20a; TE-SAEs, treatment-emergent serious adverse events.

The trial Nos. NCT02982915 and NCT03169231 are both multicenter, randomized, blinded, and placebo-controlled clinical studies launched by the company Longeveron LLC in 2016 and 2017, respectively, which are also the trials of Joshua Hare. Both studies adopt the same cell product, derived from allogenic human bone marrow and named Longeveron MSCs (LMSCs), as co-treatment or independent treatment strategies. The study No. NCT02982915 is a phase I/II trial to test the safety and efficacy of LMSCs for improving the vaccine immune response. A total of 43 subjects, of both sexes, aged 65 to 90 years, and having scores of 4 to 7 on the Canadian Frailty Scale and a distance of >200 and <400 m on the 6MWT, were enrolled. In the pilot phase, three cohort groups, A, B, and C, were arranged to receive an infusion of 20–100 million LMSCs, followed by an intramuscular injection of Fluzone High Dose Vaccine at 1–4 weeks postinfusion. Groups A and B corresponded to the patients who had received LMSCs in the pilot phase. In the randomized phase, two groups (10 patients each) received a single infusion of 100 million LMSCs or placebo. The trial No. NCT03169231, a phase IIb study conducted in 11 medical centers in California and Florida, includes 120 subjects and is a follow-up study on that of Hare et al. in Miami (No. NCT02065245). The objective is to assess the safety of LMSC intervention and its efficacy in improving physical function (mobility and tolerance) and TNF-α levels. The enrollment criteria are more narrowly defined,[4] for example, age of 70 to 85 years, a score of 5 (mildly frail) or 6 (moderately frail) on the Clinical Frailty Scale, a distance of >200 and <400 m on the 6MWT, and a serum TNF-α level >2.5 pg/mL. Three treatment groups (doses of 25, 100, and 200 million LMSCs) and one placebo group were

arranged in parallel and followed up for 180 days postinfusion. The details and a comparison of ongoing trials are illustrated in Figure 2.

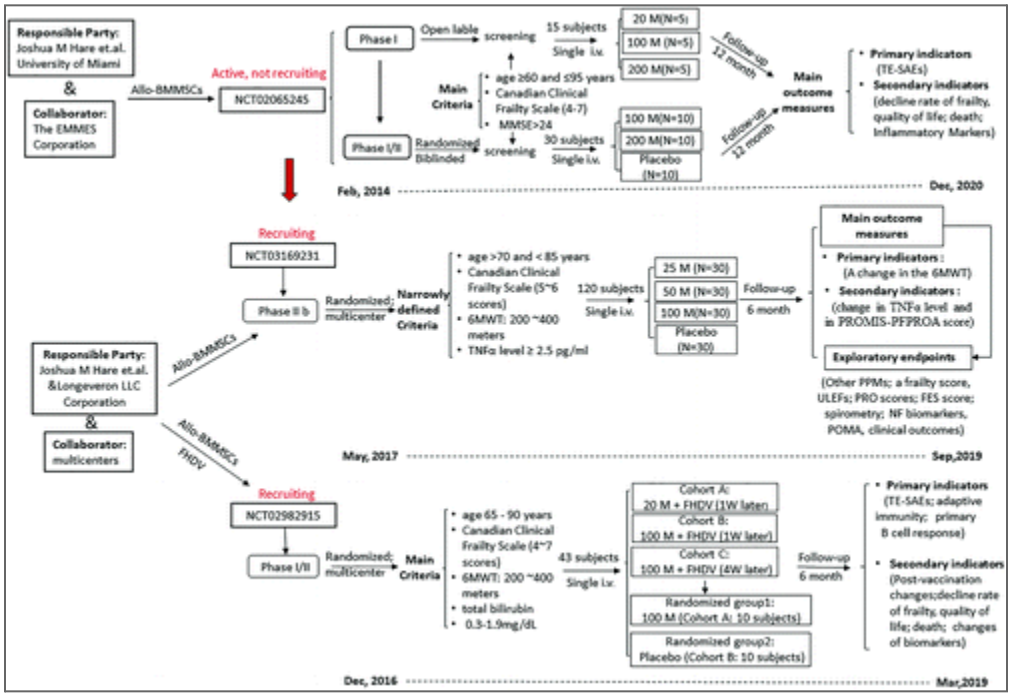

FIG. 2. Current worldwide ongoing clinical trials of stem cells for frailty. This flowchart shows the main schemed work of the three total ongoing clinical trials (NCT01501461withdrawn); the NCT03169231 is the next-step trial of NCT02065245, mainly to assess the safety and efficacy of Longeveron Mesenchymal Stem Cells, with more narrowly defined criteria. Allo-BMMSCs, allogenic bone marrow mesenchymal stem cells; FES, falls efficacy scale score; FHDV, fluzone high dose vaccine; M, million cells; 6MWT, 6-minute walk distance test; MMSE, Mini-Mental State Examination; N, number of patient; NF, neuroinflammatory biomarkers; POMA, Performance Oriented Mobility Assessment; PPMs, physical performance measures; PROMIS-PFPROA score, PROMIS-Physical Function Patient Reported Outcome Assessment; PRO scores, patient-report outcome scores; Single i.v., single peripheral intravenous infusion; TNF-α, tumor necrosis factor-α; ULEFs, upper and lower extremity function; W, week. It is originally produced on basis of data from clinicaltrials.gov. Color images are available online.

Discussion

People are living longer. Frailty has become a public priority, as the global population is aging at an accelerating speed,[4] and it is a major contributor to disability, dependence, and death, and it reduces health and successful aging.[1]

Complicated mechanism of frailty

For aging-related frailty, the complicated underlying mechanism, involving genetic, epigenetic, and environmental factors,[32–34] has not been clearly elucidated yet.[4,28,35] Nevertheless, it is generally agreed upon that the underlying mechanism of frailty intertwines with an accelerated aging process[2,6,16] and is influenced by stressors, such as damaged cells, pro-inflammatory macromolecules, toxic metabolites, pathogenic microbes, and social dysfunction (Fig. 3).

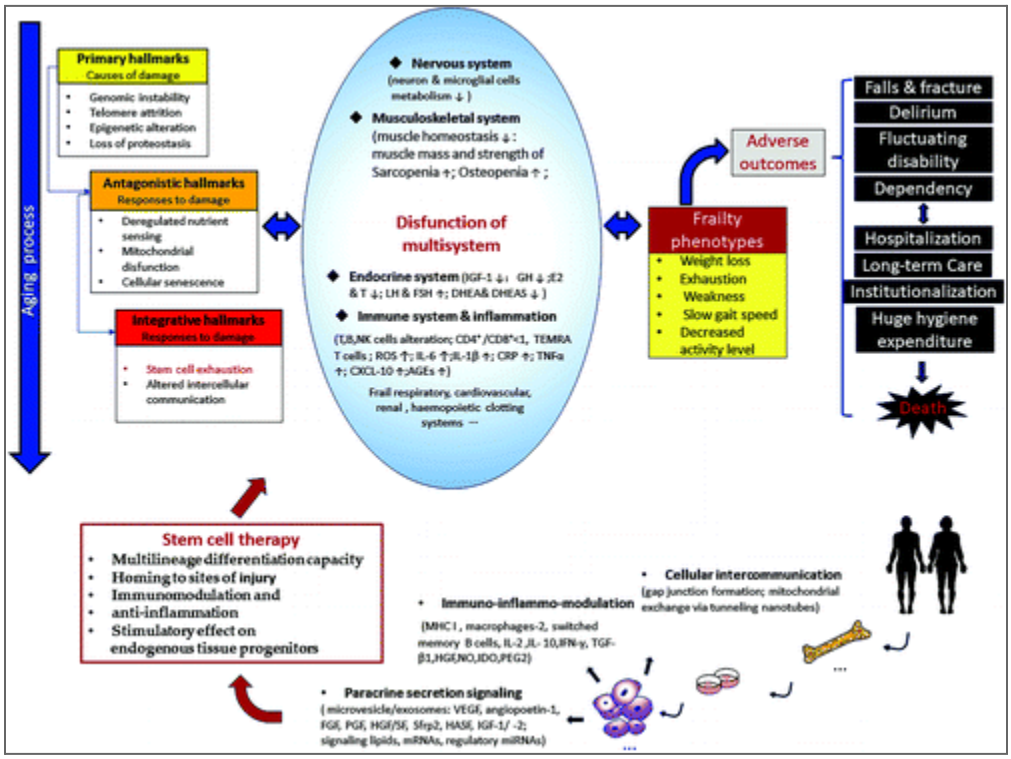

FIG. 3. Main relationships among aging frailty and stem cell therapy. This figure illustrates the underlying mechanism, main clinical phenotypes and adverse outcomes of frailty which intertwined with biological aging, and the appealing potentials of stem cells to treating frailty. AGEs, advanced glycation end products; CRP, C-reactive peptide; CXCL-10, CXC chemokine ligand-10; DHEA & DHEAS, dehydroepiandrosterone and DHEA sulfate; E2 & T, estradiol and testosterone; FGF, fibroblast growth factor; GH, growth hormone; HASF, hypoxic-induced Akt-regulated stem cell factor; HGF, hepatocyte growth factor; HGF/SF, hepatocyte growth factor/scatter factor; IDO, indoleamine 2,3-dioxygenase; IFN-γ, interferon-gamma; IGF-1, insulin-like growth factor-1; IGF-1/-2, insulin-like growth factor-1/-2; IL-6, interleukin 6; IL-1β, interleukin beta-1; IL-2, interleukin-2; IL-10, interleukin-10; LH & FSH, luteinizing hormone and follicle stimulating hormone; MHC I, major histocompatibility complex class I; NK cells, natural killer cells; NO, nitric oxide; PEG2, prostaglandin E2; PGF, placental growth factor; ROS, reactive

oxygen species; Sfrp2, secreted frizzled-related protein 2; TEMRA T cells, antigen experienced CD8+ T cells re-expressing the naive marker CD45RA; TGF-β1, transforming growth factor-beta1; VEGF, vascular endothelial growth factor. It is based on the literature of López-Otín et al.; Clegg et al.; Fried et al.; Schulman et al.; Tompkins et al.; Larrick et al.[2,3,14,16,19,28] Color images are available online.

Stem cell depletion and exhaustion is one of the ultimate culprits in aging and frailty,[16,30,36] as it compromises endogenous rejuvenation of the physiological reserve in aging-related frailty.[14,16,28] All adult stem cells lose function over time, for instance, those in stem cell compartments of hematopoietic tissue,[37] forebrain, bone, and muscle fibers.[38] Satellite cells, or skeletal muscle stem cells, are impaired and lost in aging muscle, causing the main frailty phenotypes of losing muscle mass and strength.[39] Circulating osteogenic progenitor cells, surrogates of the mesenchymal repository in the body, decrease with age, and the stem cell properties also decrease, both facilitating frailty.[40] Besides, BMMSCs from old animals show decreased expression levels of multiple genes related to cellular maturation and migration. Further proof obtained from experiments with interleukin-10 (IL-10), IL-1 receptor antagonist (IL-1RN), inducible nitric oxide synthase (iNOS), transforming growth factor (TGF)-β3, matrix metalloproteinase-9 (MMP9) (after blocking TNF receptor 2 [TNFR2]), and interferon gamma receptor 1 (IFNGR1) in BMMSCs suggests that the downregulation of special receptors in BMMSCs compromises their protective properties and contributes to the functional attrition of these cells.[41]

Inflammation is also a core mechanism behind frailty.[30,42,43] Changes in several kinds of inflammasome or pro-inflammatory pathways are highly important. The overactivation of the NF-κB pathway and the NLRP3 inflammasome leads to an increased production and release of inflammatory cytokines, such as IL-1β, TNF-α, and interferons.[44,45] The activation of NF-κB in the microenvironment of the hypothalamus triggered by inflammatory and stress responses results in a reduced production of gonadotropin releasing hormone (GnRH), which facilitates frailty-associated changes, such as muscle weakness, osteopenia and bone fragility, and reduction of neurogenesis.[46] Besides, the sirtuin pathway can modulate inflammatory responses. SIRT1, SIRT2, and SIRT6 downregulate the inflammatory activation by deacetylating NF-κB subunits and repressing the transcription of inflammation-related genes.[47] Many inflammatory mediators are independently correlated with frailty, such as CRP, IL-6, TNF-α, and CXC chemokine ligand-10 (CXCL-10).[48] The increased IL-6 and TNF-α levels can individually or collectively decrease muscle mass and strength, facilitating the development of sarcopenia.[49] High levels of CRP, IL-6, and TNF-α are even independent predictors of

mortality.[14,50] Noteworthily, some anti-inflammatory factors were reported to be reduced in frailty, such as vitamin C, E, α-tocopherol, and total thiol levels.[51,52]

Except the two core observations mentioned above, the declining physiology reserve and compromised capacity of rejuvenation in frailty also unfolds as a consequence of changes in other aspects. The frailty-associated functional impairment of the immune system has been well documented.[53,54] Immunosenescence manifests as a decline in the clearing of infectious agents, senescent cells, and infected or even malignant cells,[16] which aggravates the aging and frailty phenotypes. The activity of T cells is impaired, as indicated by the decrease in the CD4:CD8 ratio,[55] an indicator for infection.[56] In a microenvironment with high TNF-α levels, the function of B cells is compromised, which leads to a shift to subsets of dysfunctional and exhausted B cells rather than memory B cells.[57,58] Moreover, the association of oxidative stress biomarkers, including malondialdehyde (MDA), paraoxonase-1 (PON-1), lipoprotein phospholipase A2 (LpPLA2), 4-hydroxy-2,3-nonenal (HNE), derivate of reactive oxygen metabolites (d-ROM), oxidized glutathione/glutathione (GSSG/GSH), isoprostanes, protein carbonylation, and 8-hydroxy-20-deoxyguanosine, with frailty was assayed.[51,52,59] Higher levels of hematological fibrinogen VIII and D-dimer lead to fatigue and increase the risk of venous thromboembolism compared to nonfrail people, even after adjusting for cardiovascular diseases (CVDs) and diabetes.[60,61] In addition, in the endocrine system, serum hormones, such as testosterone and dehydroepiandrosterone (DHEA), 25(OH) vitamin D, growth hormone, insulin-like growth factor-1 (IGF-1), and ghrelin, are closely related with frailty.[62–64] The main effect of testosterone is in activating protein synthesis. The testosterone and its higher affinity form, dihydrotestosterone, can upregulate the expression of muscle-specific genes and increase muscle strength through the Wnt/β-catenin signaling pathway.[60,61] The situation of frailty becomes worse when more than two synthetic hormones are lacking, especially when coupled with vitamin B12 deficiency and/or celiac disease.[65]

At the organismal level, the multidimensional and multifactorial mechanism that causes frailty phenotypes or syndromes manifests as unintentional weight loss (especially the lean body mass), declining strength and endurance, slower gait speeds, reduced balance, less activity, and impaired cognition and social function.[14,24,33] Among them, the loss of muscle mass and strength, sarcopenia, and cognition impairment play large roles in frailty syndrome.[66,67] Interestingly, body weight can sometimes increase in frailty. This is because fat mass increases and muscle mass decreases with aging, leading to sarcopenic obesity.[68] Clinically, the lower skeletal muscle index, lower hip bone mineral density, and larger waist circumference can raise the risk of osteoporosis, fall, and fracture in frail people.[69] Timed walk and grip strength can act as predictors of

mild cognitive impairment, as cognition impairment disturbs both gait speed and grip strength.[33] Frailty and delirium seem distinct geriatric syndromes, with frailty being a chronic condition and delirium an acute change of cognition. Frailty may predispose patients to delirium, and delirium disturbs the recovery of frailty from stressors, predicting a negative prognosis.[70] Besides, an independent impact of depression on frailty has been proposed.[71]

Promising stem cell strategy for frailty

As no standard and effective treatment for frailty patients exists, the repletion of multipotent stem cells is an appealing strategy to rejuvenate the multifactorial dysfunction in frailty. As an important step in conducting any stem cell therapy is an appropriate choice of cell sources, various types of stem cells are exploited, such as ESCs[72] (highly undifferentiated and pluripotent), MSCs[73,74] (easily available and low immunogenicity), and iPSCs[75,76] (possessing pluripotency to differentiation). Meanwhile, it is considered that limbal stem cells are basically matured and endothelial progenitor cells are favored for their special properties of perivascular reparation, which are needed in regenerative medicine.[77] Among them, the distinctive advantages of MSCs of low immunogenicity, relatively abundant sources, easy isolation and expansion,[72] multilineage differentiation, secretion of immunomodulatory and anti-inflammatory factors, and stimulation of endogenous progenitors[4,14,19,28] make MSCs an attractive candidate strategy for frailty treatment.[28]

MSCs secrete a variety of factors and this can be regulated by the microenvironment.[78] TGF-β and IL-10 are relatively well studied.[16] MSCs modulate TGF-β to activate the STAT6 pathway in response to IL-4 signaling.[79] TGF can regulate immunity by facilitating the increase of T regulatory cells (Treg) and the decrease of $CD4_+$ and $CD8_+$ T cells and T helper 1 (Th1) cells.[80] MSCs secrete IL-10 by directly interacting with T cells to inhibit the production of pro-inflammatory cytokines by macrophages, which modulates anti-inflammatory and immunoregulatory actions. In addition, MSCs can release extracellular vesicles (exosomes or microvesicles), which contain cytokines and growth factors, including vascular endothelial growth factor, hepatocyte growth factor/scatter factor,[81] fibroblast growth factor, IGF-1 and IGF-2, and placental growth factor,[14] and some other signaling lipids, mRNAs, and miRNAs.[82]

In recent years, clinical and preclinical investigations applying stem cells have made considerable progress in the treatment of a wide spectrum of diseases of the elderly population, most of which are closely interrelated with frailty and contribute to adverse outcomes. MSCs secrete paracrine factors, exosomes, and small extracellular vesicles, reduce inflammatory factors, and activate the resident cells after injury.[14,19,24,28] It has

been shown that MSCs promote the proliferation, differentiation, and migration of resident stem cells to prevent cardiomyocyte apoptosis, reducing fibrosis after myocardial infarction by modulating secreted frizzled-related protein 2, IGF-1 hypoxia-induced Akt-regulated stem cell factor,[83,84] and the proteins, peptides, and miRNAs secreted in/on exosomes and extracellular vesicles. The outcomes of many CVDs were improved by MSCs, for example, myocardial infarction[85] and nonischemic[86] and ischemic cardiomyopathy.[81] It is likely that these beneficial effects are mainly mediated by the secreting function, especially the paracrine system,[87] and secondarily by the direct cellular contact, such as the formation of gap junctions through tunneling nanotubes.[28,88] These hypotheses, however, remain to be verified. As the 6MWT, an important physical function assessment tool that was originally developed for assessing cardiac and pulmonary disorders,[29,30] it can support the proof for the potential benefits of MSCs in the treatment of frailty.[14] Therapeutic effects of stem cells have also been shown in Parkinson's disease,[89] amyotrophic lateral sclerosis,[73] chronic obstructive pulmonary disease,[90] idiopathic pulmonary interstitial fibrosis,[91] diabetes,[73] lupus,[92] traumatic brain and spinal cord injury,[74] stroke,[93] and atherosclerosis.[94,95] These indirectly suggest the feasibility of the application of stem cells in frailty treatment.

Current challenges in stem cell therapy for frailty

For frailty, the paucity of relevant acknowledged animal models and the lack of clinical standard diagnosis, outcome measures, and reliable, validated, and sensitive biomarkers pose barriers to the preclinical and clinical research.[4] Therapeutic interventions to ameliorate the signs and symptoms of aging-related frailty mainly focus on resistance exercise regimes, the Mediterranean diet,[96] and protein, caloric, vitamin D,[5,97] and hormonal supplementation,[97–99] which, independently or in combination, have made some progress.[100] However, there are no effective and special treatment strategies for frailty so far.[4,14]

Challenges exist, although preclinical and clinical evidence collectively predict a promising future of the stem cell approach for frailty. Current human trials show preliminary efficacy, but many outcome items are variable.[29,30] Inspiringly, a phase IIb human trial, including 120 subjects, is ongoing to compensate this (No. NCT03169231). However, there are no solid data that provide evidence that sarcopenia or osteoporosis could be reversed by stem cell therapy, which are both closely related with frailty. In osteoporosis, the number of BMMSCs declines. It is uncertain whether infused stem cells differentiate into osteoblasts and induce bone formation, for that the transplanted stem cells do not migrate to bone surfaces, do not show long-term engraftment, and disproportionally facilitate adipogenesis instead of osteogenesis.[101,102] Thus, genetically modified stem cells, for example, iPSCs, were proposed as an alternative approach.[102]

For sarcopenia, exogenous stem cells, such as satellite cells,[103] muscle-derived stem cells,[104] perivascular stem cells,[105] ESCs, and iPSCs,[75] were used to promote the regeneration of skeletal myofiber. However, limited success has been reached so far. There may be reasons like that the satellite cells are generally quiescent in adult skeletal muscle[106,107] and a small contribution is made by them even in a circumstance of a large hypertrophy of the skeletal muscle.[108] Besides, cell deliverability and *in vitro* expansion are issues that require attention.[109] Interestingly, a cohort study,[110] in which 998 hematopoietic cell transplantation (HCT) survivors and 297 matched siblings were examined, found that frailty increased the risk of mortality by 2.76 times, even after adjusting for predictors, but the young adult HCT survivors were 8.4-fold more likely to be frail at old age than their siblings. These findings appear to suggest that hematopoietic cell therapy can not only not ameliorate frailty but also exacerbate the situation. Reasons may include the following.[110] First, this study was not an interventional trial directly investigating the efficacy of stem cell therapy on frailty. The included subjects should be comparable between intervention and control groups, that is, the participants receiving HCT and controls should have the same underlying disease. Second, HCT injured normal tissues, which intensified the susceptibility of the ill fragile body and eased the development of frailty when confronted with harmful factors compared with their siblings. Third, hematopoietic cells mainly differentiate into blood cells, including red blood cells, white cells, and platelets, but the comparatively limited potency compared to other types of stem cells compromises their application when applied to cure illnesses other than diseases of the blood system, such as frailty.

We would like to mention some limitations of our review. There are few studies about stem cell therapy for frailty, both in animal models and clinical trials, so in this systematic review we could not conduct a deep meta-analysis. With the aging global population and the promising exploration of stem cells, numerous studies about this interesting subject are expected to follow. In addition, stem cells are a biological therapeutic strategy, but the stability and oncogenicity require consistent long-term verification. Third, our main focus was aging-related frailty, and the prevalence of frailty in young adults was not taken into account. Younger people increasingly tend to suffer from frailty, and future investigations should take this into account as well.

The major strength of the present systematic review is that it elaborates on the relationships of aging-related frailty and stem cell therapy from a holistic and logistic perspective.

Conclusion

Frailty urgently requires attention. Stem cell therapy for frailty possesses great potential. Currently, although there still are challenges, single peripheral intravenous infusion of allogenic MSCs is proved to be safe, well tolerated, and effective in modulating immunity and inflammation, and it preliminarily shows a tendency to improve physical functions and quality of life. Finally, many other human trials on this subject will explore the depth and breadth of this novel cell-based frailty treatment.

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Research Paper

Cell therapy with intravascular administration of mesenchymal stromal cells continues to appear safe: An updated systematic review and meta-analysis

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ABSTRACT

Background: Characterization of the mesenchymal stromal cell (MSC) safety profile is important as this novel therapy continues to be evaluated in clinical trials for various inflammatory conditions. Due to an increase in published randomized controlled trials (RCTs) from 2012–2019, we performed an updated systematic review to further characterize the MSC safety profile.

Methods: MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials and Web of Science (to May 2018) were searched. RCTs that compared intravascular delivery of MSCs to controls in adult populations were included. Pre-specified adverse events were grouped according to: (1) immediate, (2) infection, (3) thrombotic/embolic, and (4) longer-term events (mortality, malignancy). Adverse events were pooled and meta-analyzed by fitting inverse-variance binary random effects models. Primary and secondary clinical efficacy endpoints were summarized descriptively.

Findings: 7473 citations were reviewed and 55 studies met inclusion criteria (n = 2696 patients). MSCs as compared to controls were associated with an increased risk of fever (Relative Risk (RR) = 2·48, 95% Confidence Interval (CI) = 1·27–4·86; I^2 = 0%), but not non-fever acute infusional toxicity, infection, thrombotic/embolic events, death, or malignancy (RR = 1·16, 0·99, 1·14, 0·78, 0·93; 95% CI = 0·70–1·91, 0·81–1·21, 0·67–1·95, 0·65–0·94, 0·60–1·45; I^2 = 0%, 0%, 0%, 0%, 0%). No included trials were ended prematurely due to safety concerns.

Interpretations: MSC therapy continues to exhibit a favourable safety profile. Future trials should continue to strengthen study rigor, reporting of MSC characterization, and adverse events.

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1. Introduction

Mesenchymal stromal cells (mesenchymal stem cells; MSCs) are multipotent stem cells that can be isolated from many adult tissues (e.g. bone marrow, adipose tissue). First described in 1974 [1], they have recently received attention in a number of different clinical fields for their potential therapeutic effects. While often described as 'adult stem cells', MSCs have limited cellular differentiation ability as compared to other types of stem cells. Pre-clinical evidence suggests that MSCs exert their beneficial effects primarily through immunomodulatory and paracrine mechanisms. MSCs target sites of inflammation and secrete bioactive molecules [2] and there is a growing body of literature demonstrating the efficacy of MSC therapy in a variety of pre-clinical models, including acute lung injury [3,4], sepsis [5] and acute myocardial infarction [6]. Indeed, evidence of the immune-modulatory ability of MSC therapy in pre-clinical models has led to interest in the possible therapeutic role for MSCs in a variety of acute and chronic inflammatory conditions.

To date, several small clinical trials have investigated the efficacy and safety of MSCs for a variety of conditions including chronic heart failure, acute myocardial infarction, hematological malignancies, graft versus host disease and acute respiratory distress syndrome. While the results of some trials suggest benefit, larger trials with clinically important endpoints are needed before more definitive conclusions can be drawn. Thus, as more and more patients are being asked to participate in the studies, the safety of MSC therapy is of increasing importance and any risk of adverse events could represent a significant barrier to their successful translation into clinical practice. These potential risks include neoplastic potential due to MSCs' proliferative capacity, susceptibility to infection given their immunomodulatory effects, embolism of the cells, zoonoses associated with cell culture reagents, and acute or chronic immunogenicity of the cells themselves [7]. A previous systematic review published by our group in 2012 included eight randomized controlled trials (RCTs) (n = 369 patients) and identified fever as the only adverse event that was significantly associated with MSC therapy [8]. Given the increase in published RCTs and patients enrolled in MSC trials since that time, we decided to conduct and update our systematic review to further characterize the safety profile of MSC-based therapy and descriptively summarize primary and secondary efficacy outcomes in MSC RCTs.

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Research in context

Evidence before this study

Several small clinical trials have investigated the efficacy and safety of MSCs in diseases, including chronic heart failure, acute myocardial infarction, hematological malignancies, graft versus host disease and the acute respiratory distress syndrome, and found some benefit with MSC therapy compared to controls. A previous systematic review examined the safety of intravascular administration of MSC therapy in heterogeneous adult patient populations. The review included eight RCTs and identified fever as the only adverse event that was significantly associated with MSC therapy. Since that publication in 2012, several reviews of MSC efficacy and or safety have included safety as part of the review objective. However, only one review included a detailed and systematic examination of the efficacy and safety of intravascular MSC administration that was limited to acute myocardial infarction and ischemic heart failure conditions and found no association between MSC therapy and acute adverse events (less than 24 h after study treatment); however MSC therapy compared to controls was associated with delayed neurological events.

Added value of this study

In our updated systematic review that now includes over 40 additional RCTs and over 2000 additional patients, aside from fever, we continue to detect no significant reported safety signals associated with MSC treatment.

Implications of all the available evidence

Our findings suggest that with the accumulation RCT evidence, the administration of MSCs continues to appear safe. The findings from our review should provide additional assurance to researchers, clinicians, health regulators and patients and families that, with this updated evidence, the administration of MSCs continues to appear safe. Future trials should continue to strengthen study rigor, reporting of MSC characterization and functionality, and adverse events as clinical indications as well as manufacturing processes evolve and second generation MSC products make their way to clinical trials.

2. Methods

The methods of this systematic review and meta-analysis are similar to our previously published review [8] with a few modifications; these are the addition of key words in our search strategy to capture placenta derived MSC trials, the inclusion of only randomized controlled trials, a focus on reporting adverse events that were pre-specified and that are potentially relevant to MSC administration, the addition of one additional pre-specified adverse event category (thrombotic and thromboembolic events) and one additional sub group analysis according placental MSCs, documentation of all reported serious adverse events and their relatedness to study treatment (in the MSC or control group), pooling of pre-specified adverse event estimates according to relative risks and 95% confidence intervals, and a descriptive summary of primary and secondary efficacy outcomes in the included RCTs. This report follows the PRISMA guidelines (complete checklist can be found in Appendix 2) [9] and because our review is an update of a previously published review, no protocol was registered.

2.1. Search strategy and selection criteria

We conducted electronic searches of Ovid MEDLINE (1950 to April 2019), EMBASE (1980 to April 2019) and Cochrane Central Register of Controlled Trials (April 2019). Given the non-standard terminology associated with MSCs, a number of terms were used (Appendix 1, search strategy). ClinicalTrials.gov was searched for ongoing or recently completed trials. Abstracts and proceedings from clinical conferences were identified and searched using Web of Science (April 2019). Bibliographies of retrieved articles and relevant reviews were manually searched. All searches were performed without any language restrictions; if included, any non-English studies were subsequently translated for data extraction.

We included RCTs that examined the intravascular (venous and arterial) administration of MSCs compared to a control group that did not receive MSCs in adult populations. We excluded studies that exclusively used non-intravascular routes of administration (e.g. injection into a joint), ex vivo differentiated MSCs, or MSCs co-administered with other experimental cells or treatments.

Study screening and selection, data extraction and risk of bias assessments were all performed in duplicate by three independent reviewers (DW, MT, ED) using standardized forms.

2.2. Data analysis

Data were extracted under the following subheadings using a standardized spreadsheet: RCT characteristics and patient populations, MSC preparation and administration, assessment of risk of bias, and primary (safety) and secondary (efficacy) outcome measures. We recorded primary and secondary efficacy endpoints as reported in the RCTs. We contacted authors via email correspondence when data relevant to our systematic review was not reported in the included studies.

Safety was examined according to pre-specified incident adverse events according to the following categories: (1) immediate events (i.e., fever and non-fever acute infusional toxicity that occurred within 24 h of study drug administration) that captured the potential for MSCs to embolize or cause hypersensitivity reactions, (2) infection events that occurred at any time post-infusion because MSCs are known to immune-modulate in pre-clinical models, (3) thrombotic

or thrombo-embolic events because MSCs can express or secrete tissue factor and other coagulation proteins [10–14] and therefore there is a theoretical risk of activation of coagulation and consequent adverse clinical events (i.e. deep venous thrombosis, pulmonary embolism, arterial thrombosis etc.), and (4) longer-term events including death and malignancy, the latter of which was captured due to the theoretical risk that MSCs could engraft.

Adverse event data were extracted based on the longest follow-up point. Adverse event data from RCTs with more than one MSC study arm (ex: dose escalation trial) were combined into one MSC study group. Meta-analyses for each pre-specified adverse event category was performed using OpenMetaAnalyst (for Windows 7). Data were analyzed using DerSimonian-Laird random effects models with a correction factor of 0.5 added to both arms for studies with 0 counts. Pooled events were described using Relative Risks (RR) and 95% confidence intervals (95% CI).

For all pre-specified adverse events we documented whether the events were reported as serious and if they were related to the study treatment (in either the MSC group or control group); we also captured other serious adverse events that were not pre-specified in our review and their reported relatedness to the study treatment. Finally, we captured the number of studies that were aborted pre-maturely due to safety concerns.

We used the CONSORT approach to harm reporting as a guide to capture the quality of adverse event reporting [15]. Specifically, we examined whether the reported approach to monitoring/recording adverse events (*a priori* plan to monitor events, types of events, frequency, and follow-up duration for events) were defined in the methods sections of the included studies.

Data related to MSC characterization as defined by the Dominici criteria were also recorded [16]. These included MSC cell source and origin, tri-lineage differentiation potential, cell surface markers, and cell morphology and adherence to plastic. We also described measures of MSC production (MSC viability, MSC potency, culture medium, and cryopreservation technique) because these measures could potentially impact both therapeutic efficacy and safety.

Heterogeneity between RCTs was evaluated using the I^2 as well as the P-value from X^2 test. Sub group analyses for each pre-specified adverse event category were planned according to the individual patient populations (cardiovascular, neurological, hematological/oncological, endocrine, renal, liver, respiratory, infectious, immune-deficient/inflammatory, other), MSC characteristics (type, origin, source), and MSC preparation (fresh versus cryopreserved, xenogeneic versus xeno-free culture media). No adjustments for multiple comparisons were made for these sub group analyses as they were considered exploratory. A post-hoc sensitivity analysis of the pre-specified adverse event pooled estimates that excluded studies published in abstract form only was also conducted to evaluate the robustness of the study findings. The secondary efficacy outcomes were not pooled but rather summarized descriptively in table format for the reader.

RCTs that met inclusion criteria were assessed for risk of bias according to the Cochrane Collaboration methods [17].

2.3. Role of funding source

The funders had no role in the analysis, interpretation of the study results, or drafting of the manuscript. The authors independently designed the study, collected data, had access to the raw data, did the statistical analysis, and were responsible for the decision to submit for publication.

3. Results

Our search identified 7473 unique titles and 55 RCTs met inclusion criteria (see Fig. 1 for PRISMA flow diagram). All 55 RCTs were included for review (n = 2696 patients) [18–66] (Table 1); six of the 55 RCTs were published in abstract form only [19,45,61,64,65,67]. Included RCTs were conducted in 12 different countries and 20 (36·4%) were multi-center [20–23,26,27,29,38,40,46,47,49,55,62–64,66,68–70]. Sample sizes ranged from nine to 135 patients (49·9 ± 31·3, mean ± standard deviation). The follow-up period ranged from one day to 60 months (14·2 ± 13·5, mean ± standard deviation). Thirteen (23·6%) reported funding from a for-profit manufacturer of MSCs (i.e. Osiris Therapeutics, Inc., FCB-Pharmacell Company Limited, Celgene Cellular Therapeutics, etc.) [20–23,27,29,49,52,55,56,65,69,70].

Patient populations were diverse and included cardiovascular (12 trials, n = 612 patients) [21,24–27,29,37,40,42,44,49,58], neurological (10 trials, n = 242 patients) [30–32,36,45,48,57,62,65,69], renal (three trials, n = 177 patients) [55,63,67], liver (seven trials, n = 404 patients) [35,43,47,53,54,59,66], respiratory (three trials, n = 134 patients) [18,23,68] and endocrine diseases (four trials, n = 169 patients) [22,28,39,50], hematological/oncological malignancies (five trials, n = 318 patients) [33,34,41,46,71], immune deficient or inflammatory conditions (nine trials, n = 544 patients) [20,38,51,52,60,61,64,70,72], general frailty (one trial, n = 30 patients) [56], and severe sepsis in severely neutropenic patients with hematologic malignancies (one trial, n = 30 patients) [19].

With respect to MSC preparation and administration, of the 55 included RCTs, 31 (56·4%) examined bone marrow [19,21–27,29–34,36,39,41–43,45,47,53,55–59,61,66,68,71], 16 (29·1%) umbilical cord [28,35,38,40,44,46,48–52,54,60,63,65,72], four (7·3%) adipose-derived MSCs [18,20,62,64], two (3·6%) placenta-derived cells [69,70]; and in two RCT (3·6%) the source of MSCs was unclear [37,67]. See Supplementary Table 1 for expanded detail. Twenty (36·4%) RCTs used autologous MSCs [24–26,29–32,36,37,39,42,43,45,47,57–59,61,62,66], 29 (52·7%) used third party unmatched allogeneic MSCs [18–23,27,28,35,38,40,44,46,48–56, 60,63–65], and four (7·3%) used allogeneic MSCs from matched donors [33,34,41,67]. Two (3·6%) RCTs used placenta-derived mesenchymal-like cells [69,70] and one (1·8%) RCT used mesenchymal precursor cells (MPC) rather than MSCs [22]. Twelve (21·8%) RCTs cultured the MSCs in a xeno-free medium [21,27,28,39,41,49,50,52,55,58,59,71], whereas the remainder either used a xenogenic product (40·0%, n = 22) [18,22,23,25,26,29–35,42,46,47,53,56,57,60,62,66,68] or did not report the medium used (38·1%, n = 21) [19,20,24,36–38,40,43–45,48,51,54,61,63–65,67,69,70,72]. Fifteen (27·3%) RCTs cryopreserved MSCs prior to administration [19–23,27,30,39,52,53,55,56,60,69,70], 32 (58·2%) used fresh MSCs [18,24–26,28,29,31–35,38,40,42–44,46-51,54,57–59,62-64,66,68,72], two (3·6%) used both a fresh and cryopreserved product [41,71] and in six (10·9%) it was unclear [36,37,45,61,65,67]. One trial that used both a fresh and cryopreserved product (1·8%) [71] and five of the 32 RCTs that used fresh MSCs (9·1%) used a cryopreserved cell product that was thawed and cultured prior to injection for a fresh cell product [18,47,49,68,72]. Of the 22 RCTs that reported cryopreserving their product, 14 (25·6%) used dimethyl sulfoxide as the cryoprotectant solution at a concentration of 10% or less [18,19,21–23,27,41,47,52,55,56,60,68,71]; the type cryoprotectant was unclear for the eight other RCTs (14·5%). Seven (12·7%) of the included RCTs reported all three Dominici criteria for MSC characterization [20,21,40, 41,68,71,72]. Twenty-nine (52·7%) RCTs reported on cell viability [20–23,25,26,29–33,37,39,40,43,47–49,52,54-58,62,68,71,72] and eight (14·5%) reported on a measure of MSC potency [20,22,23,25, 29,47,62,68].

A description and frequency of the pre-specified incident adverse events defined in our systematic review (infusional toxicity: fever and non-fever, infection, thrombotic or thromboembolic events, death and malignancy) for each included RCT is provided in Supplementary Table 2 and a summary of pooled data presented as forrest plots for each pre-specified adverse event category are summarized in Figs. 2A–F.

With respect to the occurrence immediate adverse events, a total of 19 RCTs (n = 880 patients) reported on fever infusional toxicity [20,31,32,35–38,43,47–51,57,59,60,63,71,72]. In the pooled analysis, the risk of fever was significantly greater in the MSC group as compared to the control group (Relative Risk (RR) =2·48, 95% Confidence Interval (CI) = 1·27–4·86, I^2 = 0%; see Fig. 2a). Pooled analysis of reported non-fever infusional toxicity events in a total of 32 RCTs (n = 1525 patients) however did not reveal any significant increase in risk for the MSC as compared to the control group (RR = 1·16, 95% CI = 0·70–1·91, I^2 = 0%; see Fig. 2b) [18,21–23,25–27,29,30,33,34,37,39,40,44,46,48–54,56–60,63,68-70].

A total of 27 RCTs (n = 1315 patients) reported on infection [19–23,27,30–34,38–40,47,50,52–57,62,63,69,70,72]. In the pooled analysis, there was no significant increase in the risk of infection for the MSC as compared to the control group (RR =0·99, 95% CI = 0·81–1·21, I^2 = 0%; see Fig. 2c).

The occurrence of thrombotic or thrombo-embolic events were reported in a total of 24 RCTs (n = 1112 patients) [20,21,26,29,32,33,37,40,50,56,58,60,63,66,69,70]. In the pooled analysis there was no significant increase in the risk of thrombotic/thrombo-embolic events for MSCs as compared to the control group (RR = 1·14, 95% CI = 0·67–1·95, I^2 = 0%; see Fig. 2d).

A total of 40 (n = 1991 patients) and 19 (n = 1015 patients) RCTs reported on death [18–21,23–27,29,31–37,40–42,44–46,48-53,55,56, 58,59,62,63,66,68–71] and malignancy and or ectopic tissue formation respectively [20,27,31–34,38–41,44,47,49,53, 55–57,70,71]. In the pooled analysis, the risk of death was significantly lower for the MSC group as compared to the control group (RR = 0·78, 95% CI = 0·65–0·94, I^2 = 0%; see Fig. 2e). There was no significant increase found in the risk of malignancy or ectopic tissue formation for the MSC as compared to the control group (RR = 0·93, 95% CI = 0·60–1·45, I^2 = 0%; see Fig. 2f).

The results of the risk of bias assessment found that only six (10·9%) RCTs fulfilled all six criteria for low risk of bias (Table 2) [23,27,30,40,49,62]. Nine (16·4%) RCTs met five of six primary criteria [21,28,32,46,50,52,56,68,69]. The allocation lists were concealed in 24 (43·6%) [21–23,27,28,30–32,39,40,46,47,49,50,52,53,55,56,59,60,62,63,68,69]; 21 (38·1%) were double blinded [18,21,23,27,28,30,40,46,49–52,55–57,62,64,65,68–70] and three (5·5%) had an open label intervention but blinded outcome measures [29,32,54]. In terms of other potential sources of bias, 35 (63·6%) of the RCTs were registered with either clinicaltrials.gov or their own regional registration program [18–23,26,29,30,35,38–41,43,46–49,51–57,60,62–66,68,70,72]. Thirty-eight (69·0%) RCTs did not report an *a priori* sample size calculation or provide a rationale for the sample size [18,19,21, 24–28,31–39,41,42,44,45,48–52,54,57–59,61,64–67,69,71,72].

Sub-groups were meta-analyzed for the six pre-specified adverse event outcome categories and are summarized in Supplementary Table 3. Briefly, the risk of fever related acute infusional toxicity in the MSC group was increased in the neurological and immune/inflammatory populations, when unmatched allogeneic and autologous, bone marrow, umbilical, or fresh MSCs were administered, and when the MSC culture medium was xenogenic or unclear. The risk of

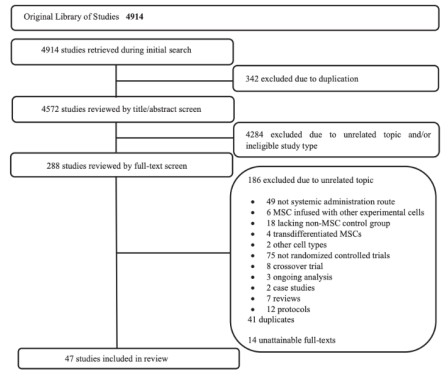

Fig. 1. Literature search and study inclusion. MSC= mesenchymal stem cell; *Figure is reflective of search data from 2012 to present and does not include search data from the prior publication, including the 8 RCTs previously included.

Table 1
Characteristics of included RCTs.

Source	Country	Patient Population (Sample Size)	Single-center vs multi-center (Number of centers)	Follow Up Duration (months)	Intervention	Control Comparison	Patients Evaluated (n (% male))		Age (years ± SD)	
							T	C	T	C
Cardiovascular										
Chen et al., 2004	PRC	Acute myocardial infarction (69)	Single-center	6	Autologous BM-MSCs	Saline, IC	34 (94)	35 (97)	58 ± 7	57 ± 5
Chen et al., 2006	PRC	Ischemic heart failure (45)	Single-center	12	Autologous BM-MSCs	Maximal medical therapy	22 (88)	23 (92)	59 ± 7	57 ± 7
Chullikana et al., 2015	India	Acute ST-elevation myocardial infarction (20)	Multi-center (4)	24	Unmatched allogeneic BM-MSCs	Multiple electrolytes (Plasma-lyte A), IV	10 (100)	10 (80)	47·3 ± 12·1	47·8 ± 6·5
Gao et al., 2013	PRC	Acute ST-elevation myocardial infarction (43)	Multi-center (4)	24	Autologous BM-MSCs	Routine therapy	21 (100)	22 (86)	55·0 ± 1·6	58·6 ± 2·5
Gao et al., 2015	PRC	Acute ST-elevation myocardial infarction (116)	Multi-center (11)	18	Unmatched allogeneic UC-MSCs	Saline with heparin, IC	58 (95)	58 (88)	57·3 ± 1·3	56·7 ± 1·7
Hare et al., 2009	USA	Acute myocardial infarction (53)	Multi-center (10)	6	Unmatched allogeneic BM-MSCs	Vehicle, IV	34 (82)	19 (79)	59 ± 12	55 ± 10
Lee et al., 2014	ROK	Acute myocardial infarction (58)	Multi-center (3)	6	Autologous BM-MSCs	Standard treatment	30 (90)	28 (89)	53·9 ± 10·5	54·2 ± 7·7
Wang et al., 2006[**]	PRC	Idiopathic dilated cardiomyopathy (24)	Single-center	6	Autologous MSCs	Saline, IC	12 (75)	12 (67)	54 ± 11	58 ± 11
Wang et al., 2014	PRC	Acute myocardial infarction (58)	Single-center	6	Autologous BM-MSCs	Saline, IC	28 (68)	30 (53)	58·0 ± 10·2	56·1 ± 9·8
Zhao et al., 2015	PRC	Chronic systolic heart failure (59)	Single-center	6	Unmatched allogeneic UC-MSCs	No IC injection, only drug therapy alone	30 (80)	29 (66)	52·9 ± 16·3	53·2 ± 11·5
Bartolucci et al., 2016/ 2017	Chile	Stable heart failure (30)	Multi-center (2)	12	Unmatched allogeneic UC-MSCs	Placebo NR	15 (80·0)	15 (93·3)	57·33 ± 10·05	57·20 ± 11·64
Xiao et al., 2017	PRC	Dilated cardiomyopathy (37)	Single-center	12	Autologous BM-MSCs	Placebo (saline)	17 (70·6)	20 (70·0)	51·6 ± 12·2	54·4 ± 11·6
Neurological										
Ibrahim et al., 2016[*]	Malaysia	Acute middle cerebral artery stroke (17)	NR	12	Autologous BM-MSCs	Standard treatment	NR	NR	NR	NR
Lee et al., 2008	ROK	Multiple system atrophy (29)	Single-center	12	Autologous BM-MSCs	NR	11 (73)	18 (67)	58 ± 7	57 ± 7
Lee et al., 2010	ROK	Ischemic stroke (52)	Single-center	60	Autologous BM-MSCs	Rehabilitation alone	16 (50)	36 (72)	64 ± 12	65 ± 15
Lee et al., 2012	ROK	Multiple system atrophy (31)	Single-center	12	Autologous BM-MSCs	Saline, IV and IA	14 (65) [****]	17 (63) [****]	56·1 ± 8·9 [****]	55·8 ± 6·1 [****]
Xie et al., 2007[***]	PRC	Spinal cord injury (24)	Single-center	3	Autologous BM-MSCs	Rehabilitation alone	11 (81)	13 (77)	(18–49)	(21–53)
Xie et al., 2016	PRC	Encephalopathy (22)	Single-center	6	Unmatched allogeneic UC-MSCs	Saline, IV	12 (67)	10 (60)	58·0 ± 7·4	63·3 ± 6·11
Fernandez et al., 2018	Spain	Secondary progressive multiple sclerosis (30)	Multi-center (2)	12	Autologous Adipose-MSCs	Placebo (Ringer's lactate)	10 (40) [****] 9 (22) [****]	11 (27) [****]	44·8 ± 8·0 [****] 47·8 ± 9·7 [****]	46·3 ± 8·9 [****]
Tsang et al., 2017	PRC	Chronic stroke/vegetative state (9)	Single-center	Day of or day after	Autologous BM-MSCs	Placebo (5% normal human albumin)	5 (40)	4 (75)	52·8 (48–56)	51·5 (41–59)
Kim et al., 2018[*]	ROK	Cerebral infarction (12)	Single-center	6	Unmatched allogeneic UC-MSCS	Placebo	8 (NR)	4 (NR)	NR	NR
Lublin et al., 2014	USA/Canada	Multiple sclerosis (16)	Multi-center (8)	12	Placenta-derived mesenchymal-like cells	Placebo	6 (33) 6 (17)	4 (50)	52·5 (41–58) 47·5 (36–56)	47·5 (40–52)

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Table 1 (Continued)

Source	Country	Patient Population (Sample Size)	Single-center vs multi-center (Number of centers)	Follow Up Duration (months)	Intervention	Control Comparison	Patients Evaluated (n (% male))		Age (years ± SD)	
							T	C	T	C
Oncological/Hematological										
Gao et al., 2016	PRC	Stem cell transplantation for hematologic malignancy (124)	Multi-center (5)	51 (24–70)	Unmatched allogeneic UC-MSCs	Saline, IV	62 (47)	62 (48)	NR	NR
Liu et al., 2011	PRC	Stem cell transplantation for leukemia (55)	Single-center	24	Matched allogeneic BM-MSCs	Stem cell transplant alone	27 (74)	28 (68)	30 (14–46)	31.5 (12–48)
Ning et al., 2008	PRC	Stem cell transplantation for hematologic malignancy (25)	Single-center	36	Matched BM-MSCS	Stem cell transplant alone	10 (90)	15 (87)	36 ± 11	39 ± 12
Kuzmina et al., 2012	Russia	Recipients of allogeneic bone marrow transplants for hematological malig-	Single-center	32	Unmatched BM-MSCs	Standard aGVHD prophylaxis	19 (42)	18 (39)	34 (20–63)	29 (19–60)

Table 1 (Continued)

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Table 1 (Continued)

Source	Country	Patient Population (Sample Size)	Single-center vs multi-center (Number of centers)	Follow Up Duration (months)	Intervention	Control Comparison	Patients Evaluated (n (% male))		Age (years ± SD)	
							T	C	T	C
Oncological/Hematological										
Gao et al., 2016	PRC	Stem cell transplantation for hematologic malignancy (124)	Multi-center (5)	51 (24–70)	Unmatched allogeneic UC-MSCs	Saline, IV	62 (47)	62 (48)	NR	NR
Liu et al., 2011	PRC	Stem cell transplantation for leukemia (55)	Single-center	24	Matched allogeneic BM-MSCs	Stem cell transplant alone	27 (74)	28 (68)	30 (14–46)	31.5 (12–48)
Ning et al., 2008	PRC	Stem cell transplantation for hematologic malignancy (25)	Single-center	36	Matched BM-MSCS	Stem cell transplant alone	10 (90)	15 (87)	36 ± 11	39 ± 12
Kuzmina et al., 2012	Russia	Recipients of allogeneic bone marrow transplants for hematological malignancies (37)	Single-center	32	Unmatched BM-MSCs	Standard aGVHD prophylaxis	19 (42)	18 (39)	34 (20–63)	29 (19–60)
Shipounova et al., 2014	Russia	Recipients of allogeneic bone marrow transplants for hematological malignancies (77)	Single-center	60	Matched BM-MSCS	Standard aGVHD prophylaxis	39 (NR)	38 (NR)	NR	NR
Endocrine										
Carlsson et al., 2015	Sweden	Type 1 diabetes mellitus (18)	Single-center	12	Autologous BM-MSCs	Insulin-only treatment	9 (89)	9 (56)	24 ± 2	27 ± 2
Hu et al., 2013	PRC	Type 1 diabetes mellitus (29)	Single-center	24	Unmatched allogeneic UC-MSCs	Saline, IV	15 (60)	14 (57)	17·6 ± 8·7	18·2 ± 7·9
Hu et al., 2016	PRC	Type 2 diabetes mellitus (61)	Single-center	36	Unmatched allogeneic UC-MSCs	Saline, IV	31 (55)	30 (53)	52·43 ± 4·88	53·21 ± 8·22
Skyler et al., 2015	USA	Type 2 diabetes mellitus (61)	Multi-center (18)	3	Unmatched allogeneic BM-MPCs	Saline, IV	15 (67) 15 (60) 15 (60)	**16 (75)**	57·7 ± 8·2 55·3 ± 11·4 57·2 ± 6·6	**58·7 ± 7·3**
Renal disease										
Swaminathan et al., 2018	USA	Patients undergoing cardiac surgery using cardiopulmonary bypass, who developed acute kidney insufficiency (135)	Multi-center (27)	3	Unmatched allogeneic BM-MSCs	Placebo	67 (65·7)	68 (82·4)	65·6 ± 11·9	67·0 ± 9·9
Korotkov et al., 2018[*]	Belarus	Renal transplantation (NR)	Single-center	7 days	Matched allogeneic MSCs	Standard treatment	NR	NR	NR	NR
Sun et al., 2018	PRC	Renal allograft (42)	Multi-center (3)	12	Unmatched allogeneic UC-MSCs	Standard treatment	21 (67)	21 (52)	40·8 ± 9·2	47·1 ± 10·2
Liver disease										
Suk et al., 2016	ROK	Alcohol-related liver cirrhosis (68)	Multi-center (12)	12	Autologous BM-MSCs	Standard treatment	21 (83) [***] 23 (89) [***]	24 (94) [***]	53·1 ± 8·7 [***] 54·4 ± 7·9 [***]	53·7 ± 8·2 [***]
Shi et al., 2012	PRC	Acute-on-chronic liver failure (43)	Single-center	18	Unmatched allogeneic UC-MSCs	Placebo (saline)	24 (83)	19 (79)	40 (24–59)	45 (26–62)
Salama et al., 2014	Egypt	Post-HCV end-stage liver disease (40)	Multi-center (2)	6	Autologous BM-MSCs	Antiviral therapy (no hepatic artery infusion)	20 (85)	20 (80)	50·27 ± 6·05	50·90 ± 7·23
Xu et al., 2014	PRC	Hepatitis B virus-related liver cirrhosis (56)	Single-center	6	Autologous BM-MSCs	Standard care	27 (65) [***]	29 (58) [***]	44 ± 12 [***]	45 ± 10 [***]
Lin et al., 2017	PRC	Hepatitis B virus-related acute-on-chronic liver failure (110)	Single-center	6	Unmatched allogeneic BM-MSCS	Standard treatment	56 (91·1)	54 (98·2)	40 ± 9.9	42.8 ± 8.4
Zhang et al., 2017	PRC	Liver fibrosis induced by hepatolenticular degeneration (60)	Single-center	3	Autologous BM-MSCs	Standard treatment	30 (53·3)	30 (56·7)	30·98 ± 11·25	32·1 ± 10·36
Shi et al., 2017	PRC	First cadaveric liver transplantation (27)	Single-center	6	Unmatched allogeneic UC-MSCs	Standard treatment	14 (92·9)	13 (92·3)	57 ± 12	55 ± 11

(continued on next page)

Table 1 (Continued)

Source	Country	Patient Population (Sample Size)	Single-center vs multi-center (Number of centers)	Follow Up Duration (months)	Intervention	Control Comparison	Patients Evaluated (n (% male))		Age (years ± SD)	
							T	C	T	C
Respiratory										
Weiss et al., 2013	USA	Moderate to severe chronic obstructive pulmonary disease (62)	Multi-center (6)	24	Unmatched allogeneic BM-MSCs	Vehicle solution, IV	30 (60)	32 (56)	68·1 ± 7·54	64·1 ± 8·76
Zheng et al., 2014	PRC	Acute respiratory distress syndrome (12)	Single-center	1	Unmatched allogeneic adipose-MSCs	Saline, IV	6 (100)	6 (83)	66·7 ± 20·4	69·8 ± 9·1
Matthay et al., 2018	USA	Acute respiratory distress syndrome (60)	Multi-center (5)	2	Unmatched allogeneic BM-MSCs	Plasma-Lyte A, IV	40 (58)	20 (50)	55 (17)	55 (20)
Infectious										
Galstian et al., 2015/ 2016[*]	Russia	Patients with severe neutropenia and severe sep-	Single-center	3	Unmatched allogeneic BM-MSC's	Standard treatment	15 (43) [*****]	15 (54) [*****]	48 (30–75) [*****]	55 (33–81) [*****]

Table 1 (Continued)

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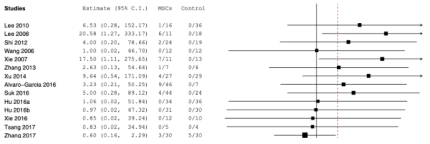
Zheng et al., 2014	PRC	Acute respiratory distress syndrome (12)	Single-center	1	Unmatched allogeneic adipose-MSCs	Saline, IV	6 (100)	6 (83)	66·7 ± 20·4	69·8 ± 9·1
Matthay et al., 2018	USA	Acute respiratory distress syndrome (60)	Multi-center (5)	2	Unmatched allogeneic BM-MSCs	Plasma-Lyte A, IV	40 (58)	20 (50)	55 (17)	55 (20)
Infectious										
Galstian et al., 2015/ 2016*	Russia	Patients with severe neutropenia and severe sepsis (30)	Single-center	3	Unmatched allogeneic BM-MSCs	Standard treatment	15 (43) *****	15 (54) *****	48 (30–75) *****	55 (33–81) *****
Immune-deficient/auto-immune/inflammatory										
Alvaro-Garcia et al., 2016	Spain	Refractory rheumatoid arthritis (53)	Multi-center (18)	6	Unmatched allogeneic adipose-MSCs	Lactated Ringer's solution, IV	20 (10) 20 (10) 6 (0)	7 (14)	54·15 ± 7·79 57·40 ± 11·01 50·33 ± 15·62	58·43 ± 14·25
Zhang et al., 2013	PRC	HIV-1 infection (13)	Multi-center (NR)	12	Unmatched allogeneic UC-MSCs	Saline, IV	7 (71)	6 (83)	30 (26–49)	38 (19–55)
Hu et al., 2016	PRC	Ulcerative colitis (70)	Single-center	24	Unmatched allogeneic UC-MSCs	Saline, IV and IA	34 (62)	36 (61)	42·9 ± 23·1	43·7 ± 28·7
Deng et al., 2017	PRC	Systemic lupus erythematosus (18)	Single-center	12	Unmatched allogeneic UC-MSCs	Placebo	12 (8)	6 (0)	29 ± 10	29 ± 7
Zhang et al., 2018	PRC	Crohn's disease (82)	Single-center	12	Unmatched allogeneic UC-MSCs	Standard treatment	41 (58.5)	41 (63.4)	34·3 (21–44)	32·7 (20–41)
Arruro et al., 2017*	Columbia	Crohn's disease (26)	Single-center	6	Autologous BM-MSCs	Standard treatment	NR	NR	NR	NR
Panes et al., 2017*	Spain	Crohn's disease (131)	Multi-center (NR)	12	Unmatched allogeneic adipose-MSCs	Placebo	70 (NR)	61 (NR)	NR	NR
Yang et al., 2018	PRC	Rheumatoid arthritis (105)	Single-center	11	Unmatched allogeneic UC-MSCs	Placebo	28 (25) 24 (21)	53 (19)	50·7 51·2	49·8
Melmed et al., 2015	USA	Crohn's disease (46)	Multi-center (NR)	24	Placenta-derived mesenchymal-like cells	Placebo	8 (53) 5 (33)	7 (44)	35·3 ± 14·0 36·2 ± 11·6	36·5 ± 7·3
Other										
Tompkins et al., 2017	USA	Frailty (30)	Single-center	12	Unmatched allogeneic BM-MSCs	Placebo	10 (60)	10 (60)	75·0 ± 7·4	75·3 ± 6·8

PRC= People's Republic of China; USA= United States of America; ROK= Republic of Korea; T= treatment; C= control; BM= bone marrow; UC= Umbilical cord; MSCs= mesenchymal stromal cells; MPCs= mesenchymal precursor cells; IA= intra-arterial; IC= intracoronary; IV= intravenous; NR= not reported.
* Abstract form only.
** Foreign language text only.
*** Age and gender demographic data reported only for patients that completed trial follow-up.
**** Age and gender demographic data reported for all patients randomized, not necessarily infused with study treatment.
***** Age and gender demographic data reported only in earlier abstract, not updated to current abstract.



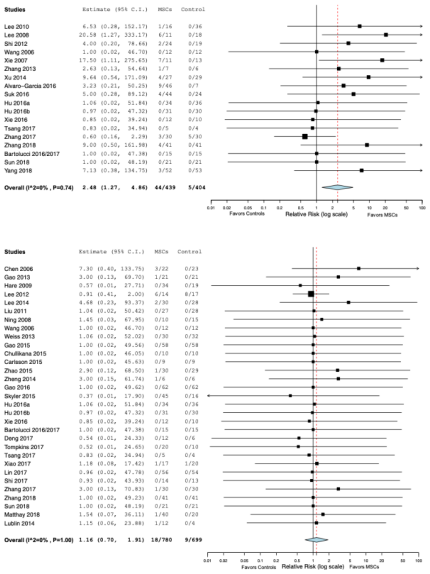
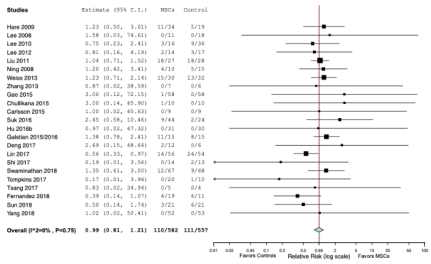


Fig. 2. a) Infusional toxicity: Fever. b) Infusional toxicity: Non-fever. c) Infection. d) Thrombotic/thrombo-embolic events. e) Mortality. f) Malignancy or ectopic tissue formation. RR, relative risk; CI, confidence interval; MSC, mesenchymal stem cell.



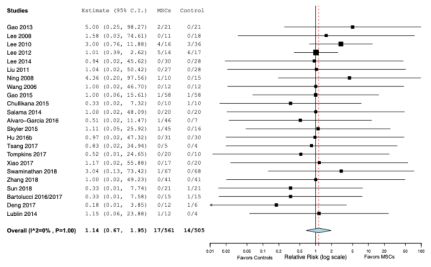
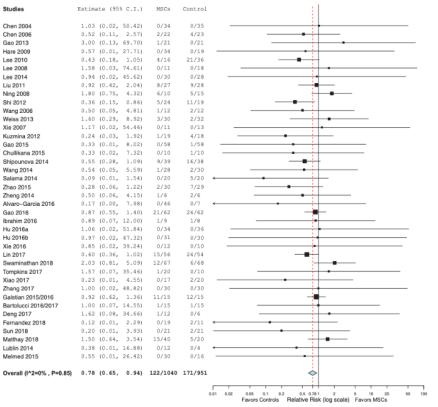


Fig. 2. *Continued*



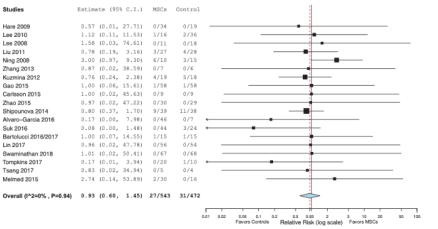
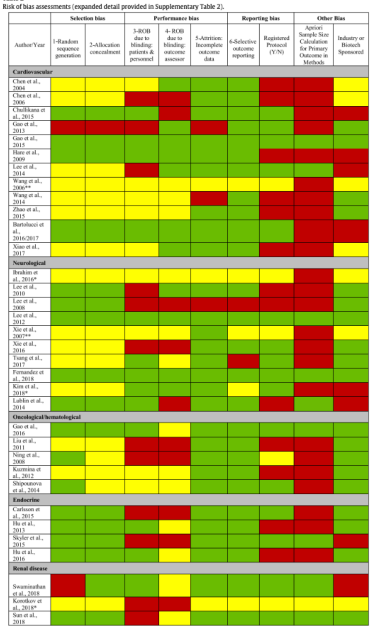


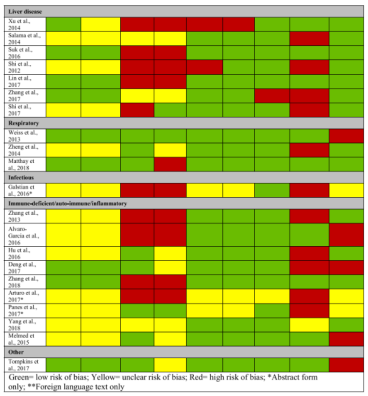
Fig. 2. *Continued*

Table 2
Risk of bias assessments (expanded detail provided in Supplementary Table 2).



Author/Year	Selection bias		Performance bias			Reporting bias		Other Bias	
	1-Random sequence generation	2-Allocation concealment	3-BOB due to blinding: patients & personnel	4- BOB due to blinding: outcome assessor	5-Attrition: Incomplete outcome data	6-Selective outcome reporting	Registered Protocol (Y/N)	Apriori Sample Size Calculation for Primary Outcome in Methods	Industry or Biotech Sponsored
Cardiovascular									
Chen et al., 2004									
Chen et al., 2006									
Chullikana et al., 2015									
Gao et al., 2001									
Gao et al., 2012									
Hare et al., 2009									
Lee et al., 2014									
Wang et al., 2006**									
Wang et al., 2014									
Zhao et al., 2015									
Bartolucci et al., 2016/2017									
Xiao et al., 2017									
Neurological									
Ibrahim et al., 2014*									
Lee et al., 2010									
Lee et al., 2008									
Lee et al., 2012									
Xie et al., 2007**									
Xie et al., 2016									
Tsang et al., 2017									
Fernandez et al., 2018									
Kim et al., 2018*									
Lublin et al., 2014									
Oncological/hematological									
Guo et al., 2016									
Liu et al., 2011									
Ning et al., 2008									
Kazmina et al., 2012									
Shipounova et al., 2014									
Endocrine									
Carlsson et al., 2015									
Hu et al., 2013									
Skyler et al., 2015									
Hu et al., 2016									
Renal disease									
Swaminathan et al., 2018									
Kunskov et al., 2018*									
Sun et al., 2018									

(continued)



Liver disease										
Xu et al., 2014										
Salama et al., 2014										
Suk et al., 2016										
Shi et al., 2012										
Lin et al., 2017										
Zhang et al., 2017										
Shi et al., 2017										
Respiratory										
Weiss et al., 2013										
Zheng et al., 2014										
Matthay et al., 2014										
Infectious										
Galstian et al., 2019*										
Immunodeficient/auto-immune/inflammatory										
Zhang et al., 2013										
Alvaro-Gracia et al., 2017										
Hu et al., 2018										
Dang et al., 2017										
Zhang et al., 2018										
Amaro et al., 2017*										
Panes et al., 2017*										
Yang et al., 2018										
Mehmet et al., 2015										
Other										
Tompkins et al., 2017										

Green= low risk of bias; Yellow= unclear risk of bias; Red= high risk of bias; *Abstract form only; **Foreign language text only

Table 3
Comparison of 2012 versus 2019 Safety Outcomes and Quality of Safety Reporting Findings.

Safety Outcomes	2012 SafeCell SR		2018 SafeCell SR Update	
	# of RCTs*	Findings (RR, 95% CI)	# of RCTS*	Findings (RR, 95% CI)
Infusional toxicity- non-fever	4/8	2·01 (0·34–11·77)	32/55	1·16 (0·70–1·91)
Infusional toxicity- fever	4/8	9·28 (2·02–42·71)	19/55	2·48 (1·27–4·86)
Infection	4/8	1·09 (0·61–1·94)	27/55	0·99 (0·81–1·21)
Malignancy or ectopic tissue formation	4/8	2·21 (0·85–5·74)	19/55	0·93 (0·60–1·45)
Mortality	8/8	1·22 (0·71–2·10)	40/55	0·78 (0·65–0·94)
Thrombotic or embolic events	4/8**	2·71 (0·86–8·48)	24/55	1·14 (0·67–1·95)
Quality of Safety Reporting	# of RCTs*	Findings (X)	# of RCTs*	Findings (X)
A priori plan to monitor adverse events	3/8	37·5%	43/55	78·2%

* That reported the adverse event.
** Original review did not report on this outcome, but event rate is inserted for comparison purposes.

death was significantly reduced in the MSC group in three clinical populations (cardiovascular, neurological, and liver disease), and with autologous, umbilical and freshly cultured MSCs. The sensitivity analysis which excluded RCTs published in abstract form only did not affect the strength or direction of the pre-specified pooled adverse event estimates (see Table 4).

A description of all reported serious adverse events (pre-specified and not pre-specified in our review) and their relatedness to study treatment in the MSC or control group is also provided in Supplementary Table 4. Of all reported serious adverse events (SAEs), a total of 1 SAE in the control group (ventricular tachycardia post-infusion in a trial that administered study drug intravenously) [21] and 3 SAEs in the MSC group (treatment related fever [47], in-stent thrombosis with death and acute coronary artery occlusion [26], the latter two of which were also associated with intra-coronary injection of study drug) were considered related to study treatment. Four other SAEs in the MSC group (grade 1 anaphylactoid reaction [69], gastric ulcer perforation [70], hypersensitivity reaction [70], and anal cancer [70]) were also judged to be possibly related to study treatment [70]. None of the included RCTs were ended prematurely due to safety concerns.

Table 4
Post-hoc sensitivity analysis of Safety Outcomes Reporting Findings in all studies versus only full-text publications.

Safety Outcomes	All studies		Only full-text publications	
	# of RCTs*	Findings (RR, 95% CI)	# of RCTs*	Findings (RR, 95% CI)
Infection	23/55	0·99 (0·81–1·21)	22/55	0·94 (0·76–1·17)
Mortality	40/55	0·78 (0·65–0·94)	38/40	0·74 (0·60–0·92)

* That reported the adverse event.

A total of 43 (78·2%) of the 55 RCTs reported an *a priori* plan to monitor safety [18,20–23,26–35,38–40,42–44,46,47,49–51,53,55–60,62,63,65,67–72]; 20 (36·4%) of the RCTs also reported an *a priori* plan to monitor for expected adverse events to be monitored [26,29–32,35,38–40,42,46,49,50,53,55–58,63,68] (see Supplementary Table 4). Forty-five (81·8%) RCTs provided an *a priori* description of follow-up frequency for adverse events [18,20–35,38–40,42–44,47–53,55–60,62,63,65–70,72].

When comparing the pre-specified adverse event profile from 2012 compared to our updated systematic review, the risk of fever is the safety outcome that remains significantly associated with MSC administration (see Table 3). There now appears to be a reduction in the risk of death in association with MSC therapy. In comparison to 2012, our updated review found that more of the included RCTs reported an *a priori* plan to monitor for the occurrence of adverse events (37·5% versus 78·2%, respectively).

Thirty-five (63·6%) of the 55 RCTs included at least one efficacy outcome as a primary endpoint [21,24–26,28–31,35,36,38,39,41–47,49,52–55,57–61,63,64,66,67,70,71] where the remaining RCTs focused on safety alone. Of the 36 RCTs that reported efficacy outcomes, 23 (41·8%) found that MSCs were efficacious in at least one of the primary efficacy outcomes [24,28–31,35,38,43,44,46,47,49,52–54,58–60,64,66,67,70,71]. A more detailed description of each RCT's primary and secondary endpoints and their respective findings is provided in Supplementary Table 5.

4. Discussion

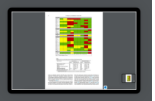
In our updated systematic review that now includes over 40 additional RCTs and over 2000 additional patients, we continue to detect no associations between MSC treatment and the development of non-fever acute infusional toxicity, infection, or malignancy, nor did we detect associations between MSC treatment and the development of thrombotic or thrombo-embolic events. There does continue to be a significant association between MSC administration and reported fever. However of the 19 RCTs (n = 880 patients) that reported on fever, only six were reported as serious, albeit all in MSC treated patients. In contrast, with an increase in the number of RCTs and patients in our updated review, the risk of death is now significantly reduced in the MSC as compared to the control group. In our updated review we also found that the approach to safety reporting was improved as many more authors reported an *a priori* plan to monitor for safety (78·2% versus 35·5%) and none of the trials were ended pre-maturely due to safety concerns. The findings of our updated review should provide additional assurance to researchers, clinicians, regulators, and patients and families that the administration of MSCs continues to appear safe.

Our systematic review will require future updates as scientists continue to unravel the multitude of mechanisms of actions associated with the cells, as the sources and origins of MSCs expand, and the manufacturing process and the development of second generation MSC products evolve. To illustrate, recent in vitro, pre-clinical, and clinical data has found that MSCs can express or increase secretion of proteins associated with coagulation (ex: tissue factor, thrombin anti-thrombin complexes) and with reports of thromboses [10–14]. Depending on the clinical population this potential procoagulant effect could result in a beneficial or harmful clinical effect. In our updated review, we began to address this concern with the inclusion of thrombotic/thromboembolic events as a pre-specified adverse event category. Our findings suggest that these incident events reported in the included RCTs are rare (31 events in 24 RCTs and 1112 patients studied), were reported in both study groups (n = 17 and 14 in the MSC and control groups respectively) and were not significantly associated with administration of MSCs. Although a significant association was not detected, it is likely that these events will be rare and as such we encourage investigators to *a priori* plan to monitor and report on these events to enable the detection of future thrombotic safety signals.

In contrast to our review from 2012, we found that safety reporting was improved in that more investigators reported an *a priori* plan to monitor for adverse events (78·2% versus 35·5% respectively). Serious adverse events that were reported as related to or as possibly related to study treatment (either in the MSC or control group) (n = 8 out of 2634 patients studied) were very rare. This could be because these events are indeed rare or because it can be challenging if not impossible to attribute an event to study treatment, especially when the event does not occur during or shortly after completion of the infusion. To address this challenge in adverse event reporting, we sought to capture and synthesize pre-specified adverse events and any other SAE, irregardless of relatedness to study treatment in each of the RCTs. Even using this approach, safety signals other than fever generation were not detected.

A significant impediment to understanding whether MSCs are efficacious and safe relates to the quality of trial design and transparent reporting. Of the 55 included RCTs, only six trials met all six criteria for low risk of bias whereas none of the RCTs from the 2012 review met all six criteria. Although an improvement from 2012, it is important for investigators to address these risk of bias elements at the design phase of these clinical trials to maximize the internal validity of their research findings. With regard to MSC characterization, only seven trials reported on all three Dominici criteria [16] which aim to provide minimal and standardized criteria to define a MSC. Furthermore, only 29 of the included trials (52·7%) reported some measure of MSC viability during the manufacturing process and even fewer (n = 8, 14·5%) reported on a measure of MSC potency or functionality. We strongly assert that it is critical for investigators to transparently report on MSC characteristics, potency and viability in order to help readers, researchers, health regulators, and the community to better understand why a given trial may have succeeded or failed to meet study endpoints and with the ultimate aim to help move the field forward.

Our systematic review has several strengths. We included a transparent search strategy, pre-defined a set of adverse events that were clinically relevant to MSC administration, and reported on all SAEs that were and were not identified as part of our *a priori* event categories irregardless of relatedness to study treatment to provide the most comprehensive and up to date evaluation of the safety profile of MSC therapy. Our review also has limitations. Six of the RCTs were published in abstract form only and as such contained limited information to populate in our review. However, we included these trials



so that the readership is aware of them and can further evaluate the efficacy and safety of study results when the full trials are published. Furthermore, the strength or direction of our pooled apriori adverse outcome estimates were not influenced by removal of studies that were published in abstract form only. As in our 2012 review, we pooled incident adverse events from RCTs from diverse adult clinical populations, MSC characteristics and MSC manufacturing in an effort to obtain signals for harm. However, to begin to address this diversity and due to the increased number of included RCTs in this review, we conducted several *a prior* derived sub group analyses to examine for heterogeneity in our *a priori* derived adverse event estimates and acknowledged that these analyses should be considered hypothesis generating. Only a few of the included trials (10·9%) met all six low risk of bias criteria which threatens the internal validity of the study findings from the perspectives of both safety and efficacy and we strongly encourage investigators to address these biases at the design stage and during the conduct of these RCTs. Finally, pooling efficacy outcomes for all of the included RCTs was not feasible within the scope of this safety review. However, in an attempt to provide some measure of efficacy information for the readership, we summarized the primary and secondary efficacy endpoints and associated results descriptively in Supplementary Table 5.

In conclusion, our review provides a systematic examination for incident adverse events related to the use of MSCs. Aside from fever, we did not identify any significant reported safety signals. Results from our systematic review provide further assurance to readers, investigators, health regulators, and our patients and communities that, with this updated evidence, MSC therapy continues to appear safe.

Declaration of Competing Interests

LM reports grants from CIHR, OIRM and SCN during the conduct of this study. DJS and SHJM reports affiliations with Northern Therapeutics Inc., outside the submitted work. BH reports prior honoraria from Cornerstone Research Group for the provision of methodologic advice related to systematic review and meta-analysis, outside the submitted work. KRW reported grants for CIHR, outside the submitted work. No other authors have any affiliations to report in relation to this study.

Acknowledgments

Our systematic review was funded by the Ontario Research Fund, the Ontario Institute for Regenerative Medicine and the Stem Cell Network; none of whom were involved in study design, in the collection and interpretation of data, in the writing of the report, nor in the decision to submit the paper for publication. Our research team would like to thank Ms. Risa Shorr (Medical Information Specialist) for her assistance with building and conducting the electronic search strategy. We would also like to thank Ms. Elham Sabri for her statistical expertise and Ms. Marnie Gordon for the administrative assistance that she provided.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2019.100249.

Appendix 1. Search strategy

Medline
Database: Ovid MEDLINE(R) ALL (1946 to September 23, 2019)
Search Strategy:

1. exp Mesenchymal Stem Cells/ (33,741)
2. exp Mesenchymal Stem Cell Transplantation/ (10,675)
3. exp Multipotent Stem Cells/ (36,140)
4. exp Mesenchymal Stromal Cells/ (33,741)
5. (mesenchymal adj3 (stem or stroma$1 or progenitor*) and cell $1).tw. (47,264)
6. (mesenchymal adj2 (stem or stromal or progenitor or multipotent or bone marrow or adipose or placenta*)).tw. (47,000)
7. (MSC or MSCs or ADMSC or ADMSCs or BM-MSC or BM-MSCs or BMD-MSC or BMD-MSCs or BMDMSC or BMDMSCs).tw. (29,267)
8. ((multipotent or multi-potent) adj3 (stroma$1 cell$1 or stem cell$1)).tw. (4521)
9. marrow stroma$1 cell$1.tw. (6975)
10. (colony-forming unit fibroblast* or CFU-F$1).tw. (844)
11. Mesoderm/cy (5710)
12. or/1-11 (71,482)
13. (ae or to or po or co).fs. (3,812,354)
14. (safe or safety).ti,ab. (723,468)
15. side effect$.ti,ab. (236,090)
16. ((adverse or undesirable or harm* or serious or toxic) adj3 (effect* or reaction* or event* or outcome*)).ti,ab. (494,272)
17. exp product surveillance, postmarketing/ (14,736)
18. exp adverse drug reaction reporting systems/ (7274)
19. exp clinical trials, phase iv/ (289)
20. exp poisoning/ (154,008)
21. exp substance-related disorders/ (269,073)
22. exp drug toxicity/ (111,624)
23. exp abnormalities, drug induced/ (14,457)
24. exp drug monitoring/ (19,962)
25. exp drug hypersensitivity/ (44,888)
26. (toxicity or complication* or noxious or tolerability or hypersensitivity or abnormal*).ti,ab. (1,919,833)
27. exp Postoperative Complications/ (525,174)
28. exp Intraoperative Complications/ (51,079)
29. or/13-28 (6,186,659)
30. 12 and 29 (10,413)
31. randomized controlled trial.pt. (489,730)
32. controlled clinical trial.pt. (93,263)
33. randomized.ab. (454,901)
34. placebo.ab. (200,750)
35. drug therapy.fs. (2,140,942)
36. randomly.ab. (318,366)
37. trial.ab. (476,933)
38. groups.ab. (1,955,339)
39. (clinical trial* or multicenter study).pt. (747,085)
40. or/31-39 (4,746,525)
41. exp animals/ not humans/ (4,617,450)
42. 40 not 41 (4,135,721)
43. 30 and 42 (1815)
44. **limit 43 to yr="2012 -Current" (1346)**

Cochrane

Search Name: McIntyre-Lauralynn-MSCS-Safety_2019-09-25
Date Run: 25/09/2019 19:04:03
Comment:
ID Search Hits

1. MeSH descriptor: [Mesenchymal Stem Cells] explode all trees 97
2. MeSH descriptor: [Mesenchymal Stem Cell Transplantation] explode all trees 183
3. MeSH descriptor: [Multipotent Stem Cells] explode all trees 99