

Regenerative capacity of autologous stem cell transplantation in elderly: a report of biomedical outcomes

The occurrence of chronic diseases such as neurological, metabolic and cardiovascular degenerative disorders increases with age. Cell therapy is an emerging approach to the treatment of these conditions. Of particular interest is the application of autologous stem cells because it eliminates post-transplantation immune rejection and there are less ethical concerns associated with their use. The regenerative capacity of stem cells harvested from elderly people is however controversial. In this review, we analyze if self-renewal potential, differentiation capability and expression of stemness genes in stem cells collected from elderly patients validate their application in clinical trials and examine the results.

First draft submitted: 15 April 2016; Accepted for publication: 12 January 2017; Published online: 21 February 2017

Keywords: adult stem cells • aging • autologous transplantation • clinical trial • elderly patients

Stem cells are found not only in embryonic or fetal tissues but also in all adult tissues in relatively high numbers. These cells are committed to tissue repair throughout adult life. Although the number of cells and their capabilities decrease over time, rich stem cell niches remain such as bone marrow and adipose tissue [1–5].

The observation that stem cells differentiate into several cell lineages reveals their potential for use in regenerative medicine. More importantly, stem cells harvested from adult tissue can be used for autologous transplantation and can also avoid immunological rejection. However, whether stem cells from elderly people have similar capabilities as those found in younger people is yet unknown.

Some studies suggest that elderly people have fewer stem cells and that they have lost their capacity for growth and differentiation *in vitro*. This review focuses on determining whether stem cells from elderly people have the same capabilities as those from younger

people of replacing those cells that are missing due to a degenerative disease.

Some evidence indicates that sufficient numbers of stem cells remain throughout adult life [6–8], providing an alternative for use in cell therapy.

Self-renewal capability

Self-renewal *in vitro* is one of the main stem cell characteristics that occur after harvest. Healthy adult stem cells grown *in vitro* have a high proliferation capacity. However, stem cells from elderly subjects show less proliferation potential [9]. Several studies have reported a decrease in the number of colony forming units in mesenchymal stem cell (MSC) cultures from donors aged ≥ 40 years [10–17]. *In vitro* doubling times are longer in cells taken from elderly patients than those from younger donors [14] and show a substantial decrease in proliferation rate [18,19].

Similar observations have been reported for lipoaspirate samples obtained from adipose tissue. *In vitro* doubling times dif-

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fer depending on donor age and are longer in those collected from older donors [7,8,11]. The relationship between the decrease in number and functionality of stem cells could be a consequence of the loss of proper environmental signals [20]. In addition, decreased telomere length and an increased rate of apoptosis and its signals have been reported in MSCs harvested from elderly donors [12,19].

In addition, the definition of MSCs requires the presence of specific cell membrane antigens, such as CD73, CD90, CD105 and a lack of CD45, CD14 or CD11b, CD79a and CD19, as well as human leukocyte antigen class II. This minimal criterion was established by the Tissue Stem Cell Committee of the International Society for Cellular Therapy [21]. Until now, flow cytometry has been performed on MSCs from younger and elderly patients to confirm the presence or absence of these specific stem cell markers [7,8]. The overall conclusions from these reports are that MSCs from elderly donors have less capability to grow.

Expression of stemness genes

Differential expression of stemness genes on MSCs from elderly donors may be ultimately responsible for the decline of the stem cell proliferation rate. Stemness genes characterized in bone marrow-derived stem cells from patients with amyotrophic lateral sclerosis (ALS) show decreased expression of two genes related with pluripotential for the transcription factors *OCT4* and *NANOG*. In addition, decreased expression of trophic factors, such as ANG, FGF-2, HGF, IGF-1, PIGF, SDF-1 α , TGF- β and VEGF, have been reported, whereas the expression of BDNF or ECGF does not decrease [17,22,23].

Similar observations have been reported for adipose-derived stem cells (ADSCs) from healthy patients aged from 50 to 60 years bearing the CD271⁺ membrane receptor and the expression of *NANOG*, *OCT4* and *SOX2* is significantly lower than that in younger patients [7].

However, Choumerianou *et al.* reported no difference in the expression of *NANOG* or *OCT4* between MSCs isolated from the bone marrow of children and those obtained from adults [24]. This observation was later confirmed by Siegel *et al.* in a study of *PRDM14* expression, which is another gene involved in cell pluripotency [17].

Despite these controversial reports, the general consensus supports that the expression of stemness genes is lower, but their activity is sufficient for growth and self-renewal.

In vitro differentiation

Several studies have shown that healthy adult stem cells grown under specific culture conditions will differenti-

ate into various cell lineages *in vitro*. For example, bone marrow aspirates taken from subjects aged from 19 to 57 years respond positively to induced differentiation into adipocytes, osteoblasts and chondroblasts *in vitro*, but some colonies display limited differentiation [25]. This result was interpreted as a loss of multilineage differentiation capability or restricted differentiation potential as a consequence of clonal cell isolation. Those authors did not analyze donor age, which could be responsible for the differences. The authors also reported decreased osteogenic differentiation potential of these cells during aging in humans. MSCs obtained from the vertebral bodies of the thoracic and lumbar spine differentiate *in vitro*, suggesting that the number of MSCs with osteogenic potential decreases substantially with age in humans [8,26]. In addition, an age-dependent decrease in proliferation and differentiation into osteoblasts has been reported due to an increase in the number of senescence-associated β -galactosidase-positive cells and apoptosis in MSCs [19]. Intrinsic age-related alterations in signaling responses to osteo-anabolic agents, such as parathyroid hormone (PTH), have been reported [27].

The bone-forming capacity is similar in cells obtained from younger and older donors. One hypothesis proposes that the senescence-associated decrease in bone formation is due to a defect in the bone microenvironment [12,13]. Chondrogenic differentiation is also controversial, as some studies have shown independent age-related responses or reduced capacity with age [6,18].

Bossolasco *et al.* reported a significant age-related diminished response of stem cells from patients with ALS aged from 37 to 78 years. In that report, MSCs obtained from the bone marrow showed a decreased response to *in vitro* adipogenic and osteoblastic induction. These authors also reported a fewer number of colony forming units but no differences in the clonogenic assays between patients with ALS and healthy donors aged from 26 to 38 years [28]. These results agree with the study by Ferrero *et al.*, who demonstrated that MSCs from healthy donors and patients with ALS (aged from 21 to 75 years) have similar osteogenic, adipogenic, chondrogenic and neurogenic differentiation potential [29]. However, another study reported that stem cells from patients with ALS (aged from 42 to 77 years) have dissimilar differentiation kinetics into the neuronal lineage but are still capable of responding to external factors and started to differentiate into a neurological lineage [22]. In contrast, the neuroectodermal differentiation potential of MSCs derived from elderly donors is completely lost [30]. ADSCs from 53 patients (aged from 50 to 89 years) revealed that elderly donors have many cells that retain a significant capacity to acquire the endothelial lineage [31].

Other stem cell sources, such as muscle-derived stem cells obtained from young (age 9 years) and old (age ≥ 60 years) humans, replicated 20- to 30-times *in vitro* and differentiated into different tissue lineages. These cells (satellite cells) are found in aged human skeletal muscle and are capable of regeneration [32–34].

Stem cells from elderly donors do not have as much pluripotential as cells from younger donors. Neverthe-

less, these cells are capable of self-renewal and differentiation into osteoblasts, chondroblasts, adipocytes and other cell lineages (Figure 1).

Clinical reports

Samples of pluripotent stem cells for autologous transplantation have been obtained from several tissues of differently aged donors. The most abundant and rela-

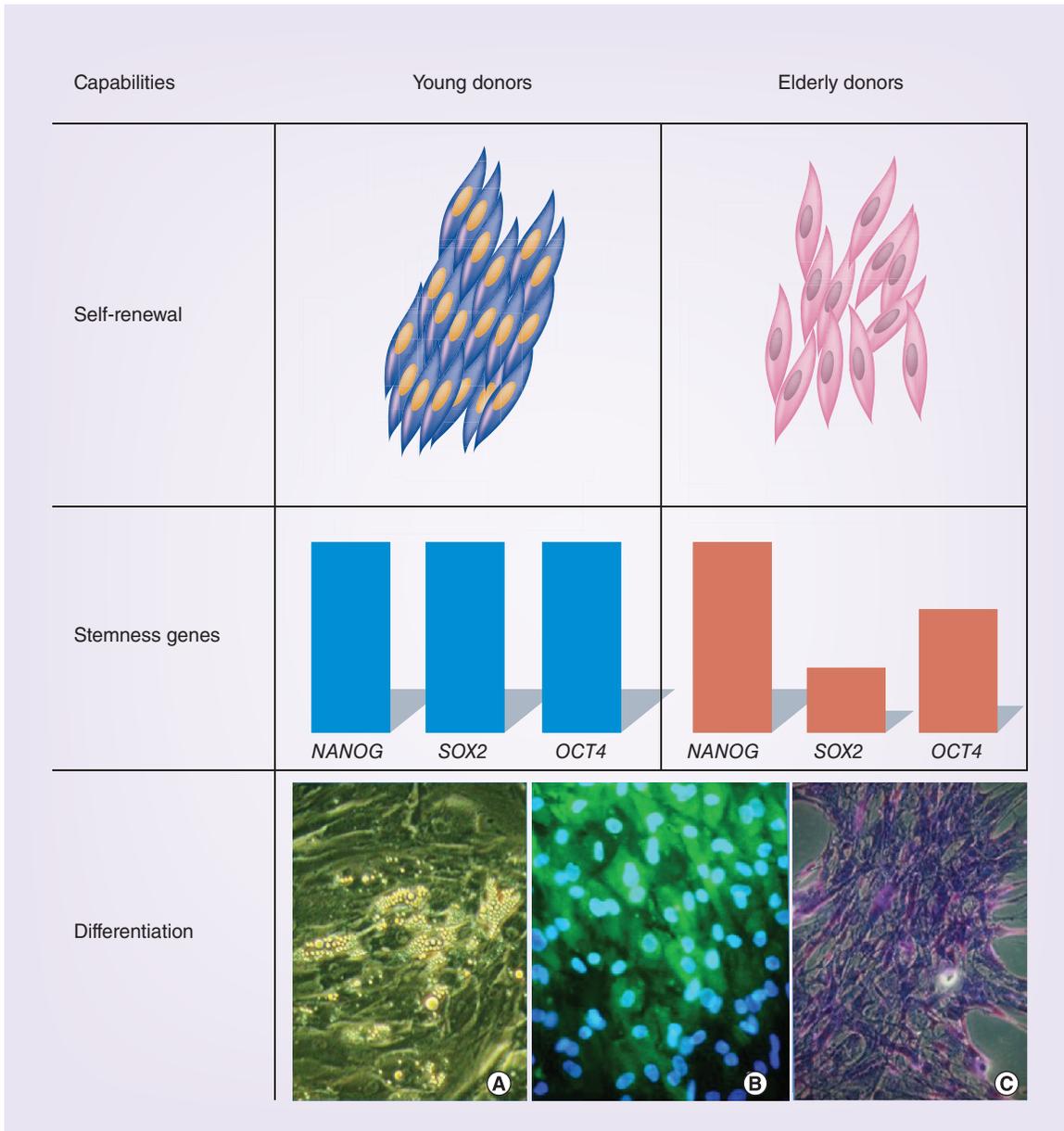


Figure 1. Comparisons of capabilities between mesenchymal stem cells obtained from young and old donors. Illustrative presentation of self-renewal capabilities: both cells present have the proliferative capacity, being high on cells from younger donators. Representative stemness genes expression: the expression of *OCT4* and *SOX2* decrease on cells from older donors. Differentiation: images of adipose-derived stem cells obtained in our laboratory from healthy donors and *in vitro* differentiate. Both sources of cells differentiate *in vitro* toward cells of mesodermal lineage. (A) Adipocytes showed intracellular lipid vacuoles. (B) Osteoblast showing osteocalcin (green) detected by immunofluorescent. (C) Chondroblast staining with Toluidine-blue for proteoglycans.

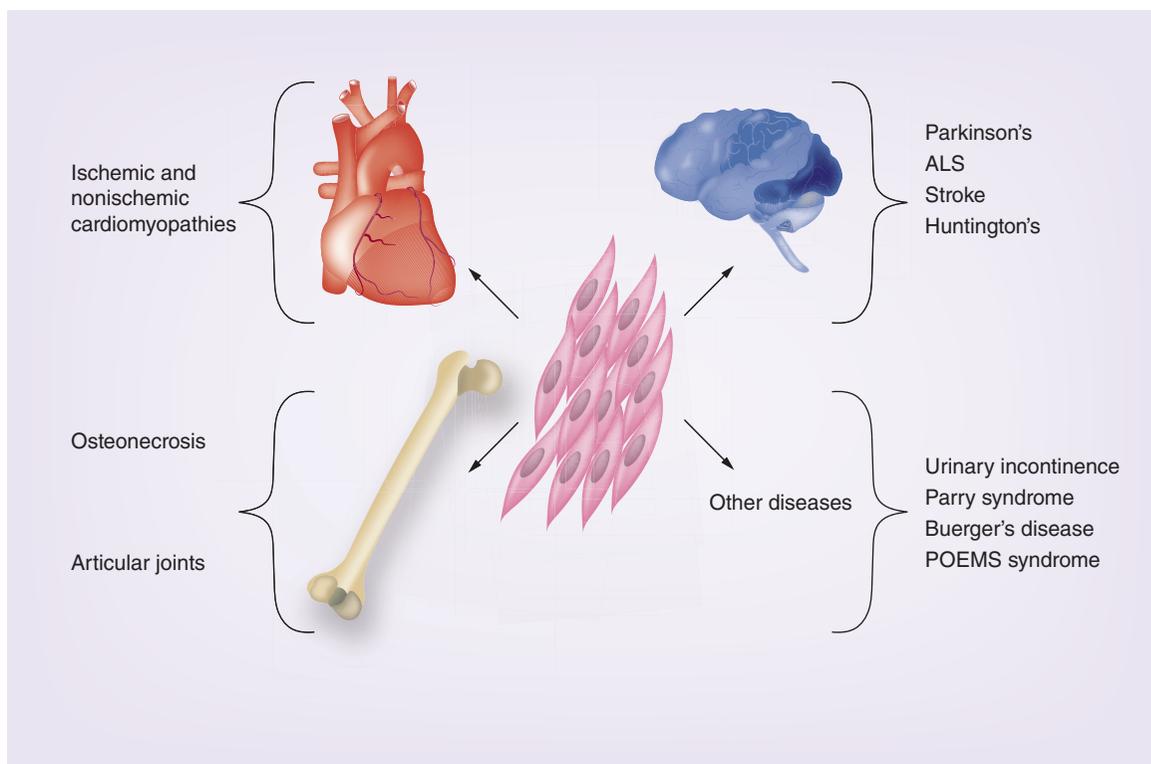


Figure 2. Clinical trials performed by autologous stem cells transplantation in older people. Degenerative diseases like cardiopathies and neuropathies, as well of loss of tissue or functionality, have shown some improve after transplantation.
 ALS: Amyotrophic lateral sclerosis; POEMS: Polyneuropathy, organomegaly, endocrinopathy, M-protein or monoclonal gammopathy and skin changes.

tively accessible sources for adult stem cells are bone marrow, peripheral blood and adipose tissue. Samples from elderly people have been obtained, applied to autologous transplantation and have improved some degenerative diseases (Figure 2).

Examples of autologous transplantation applications for neurodegenerative diseases include ALS, Parkinson's disease, multiple sclerosis and Huntington's disease.

Patients with ALS who received harvested stem cells from the bone marrow or peripheral blood showed improved quality of life and survival [35–43].

Intra-arterial autologous implantation into the posterior region of the circle of Willis resulted in improvements in Parkinson's disease severity in patients greater than 60 years old [44]. The MSCs were transplanted into the sublateral ventricular zone during stereotaxic surgery to demonstrate the safety of the procedure. No adverse events were detected and some disease improvement was reported [45,46].

Patients suffering from stroke have been treated with intravenous autologous transplantation of bone marrow mononuclear cells, but no beneficial effect of this treatment on stroke status outcome was reported. Nevertheless, the infusion-induced changes in the serum levels of GM-CSF, PDGF-BB and MMP-2 [47–50]. Patients

transplanted with bone marrow aspirated and cultured MSCs have significantly reduced the clinical and functional deficits [51–54]. Important differences between those trials were the type of cells applied; in the first case, all cells were mononuclear in the sample whereas in the second case, the MSCs were selected by culture.

MSC transplantation in patients suffering from multiple sclerosis results in an immediate immunomodulatory effect. Using the mononuclear fraction prevents disease progression and improves scores on the the Extended Disability Status Scale [55,56].

The beneficial effects of autologous cell transplantation have been reported in patients with neurodegenerative diseases, including those performed on elderly patients. The stem cell subpopulations selected for treatment may have improved the outcomes.

Patients with POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, M-protein or monoclonal gammopathy and skin changes) have improvements in forced expiratory volume and total lung capacity after receiving mononuclear cells [57,58] and nonhealing ulcers in patients with Buerger's disease treated with autologous bone marrow stem cells demonstrate accelerated healing [59]. Improvements in limb ischemia due to ischemic leg ulcers have also been reported [60–63].

Table 1. Clinical trials performed with autologous stem cells transplantation in several diseases.

Disease	Number of patients by clinical trial	Stem cells source	Application site	Stem cells applied	Result	Ref.
ALS	10, 67	Peripheral blood	Frontal motor cortex	MSCs CD133	Improve quality live and survival	[36,44]
ALS	30	Bone marrow	Intrathecal	Mononuclear fraction	Improve quality live	[37]
ALS	19	Bone marrow	Intrathecal	MSCs (culture)	Improve MSC migration	[38]
ALS	10	Bone marrow	Intrathecal	Mononuclear fraction	Immunomodulatory effects	[39]
ALS	11, 7	Bone marrow	Intraspinal infusion	Mononuclear fraction	Neurotrophic activity	[40,41]
ALS	10,19	Bone marrow	Spinal cord	MSCs (culture)	No adverse events. Safety	[42,43]
Parkinson's disease	50	Bone marrow	Intra-arterial	Mononuclear fraction	Improvement	[45]
Parkinson's disease	7	Bone marrow	Sublateral ventricular zone	MSCs	Improvement	[46]
Stroke	120, 11, 10, 20	Bone marrow	Intravenous infusion	Mononuclear cells	Safe. Improvement	[47,48,50–52]
Stroke	15	Peripheral blood	Corticospinal tract	CD34	Improvement	[53]
Sclerosis multiple	20	Bone marrow	Intravenously	Mononuclear cells	Improvement	[54]
POEMS	53, 59	Peripheral blood	Intravenous infusion	CD34	Improvement pulmonary morbidity	[57,58]
Buerger's disease	2	Bone marrow	Local intramuscular injection	CD34	Clinical benefit	[59]
Ischemic cardiomyopathy	12, 33	Right atrial appendage	Intracoronary	Cardiac stem cells (culture)	Improving LV systolic function	[65,68]
Ischemic cardiomyopathy	39, 42,12	Bone marrow	Infarcted myocardium zone	CD133	Improving LV systolic function	[66,67,73]
Ischemic cardiomyopathy	52, 56	Bone marrow	Intracoronary	Mononuclear cells	Improving LV systolic function	[69,70]
Osteonecrosis	48	Adipose stem cells	Percutaneous local injections	Stromal vascular fraction	Post-treatment improvement	[75]
Urinary incontinence	53, 3	Adipose stem cells	Submucosal space of the urethra	Stromal vascular fraction	Progressive improvement in urinary retention	[76]

ALS: Amyotrophic lateral sclerosis; LV: Left ventricle; MSC: Mesenchymal stem cell; POEMS: Polyneuropathy, organomegaly, endocrinopathy, M-protein or monoclonal gammopathy and skin change.

Numerous clinical trials have reported promising results using stem cells to revascularize patients with peripheral arterial disease [64].

Several clinical trials have been performed on ischemic and nonischemic cardiomyopathies in patients greater than 50 years old. The most promising among those trials included intracoronary infusion of mononuclear fractions of bone marrow-derived stem cells or MSCs from peripheral blood in patients suffering a myocardial infarction, in whom a moderate but significant improvement in left ventricular ejection volume was observed [65–72]. Transplantation of the specific stem cell fraction bearing CD133⁺, CD34⁺ or both antigens improves the quality of life after cell therapy in all cases [50,73,74]. There is no consensus about the best MSC subtype to treat ischemic heart disease [74]. Nevertheless, all studies in this area have reported improved cardiac function after autologous MSC transplantation.

Autologous ADSC transplantation has also been performed in elderly patients and autologous ADSCs have been injected safely into articular joints. Patients

with osteonecrosis show post-treatment improvements [75]. In addition, patients with urinary incontinence treated with ADSCs showed progressive improvement in urinary retention capacity [76]. Similarly, patients (>50 years old) suffering from an abdominal aortic aneurysm improved after autologous ADSC transplantation [77]. Applications of autologous MSCs to treat various diseases and the results are summarized in Table 1.

Discussion

Stem cells obtained from elderly patients retain pivotal membrane cells markers, such as CD73, CD90 and CD105, and have *in vitro* self-renewal and differentiation capabilities in adipocytes, osteoblasts and chondroblasts. In addition, stem cells from elderly patients express the transcription factors responsible for cell proliferation, such as *SOX2*, *NANOG* and *OCT4*. Some reports have indicated that these genes are expressed at lower levels in elderly subjects than stem cells obtained from younger donors. Nevertheless, the cells respond to induced differentiation as

Executive summary

Stem cells in elderly patients

- Frequently, life expectancy is accompanied by an increase of degenerative diseases.
- Cell therapy offers the opportunity to regenerate the lost function.
- Autologous transplantation eliminates immune rejection and ethical concerns.
- There are some skeptical issues about the regenerative capacity of stem cells from elderly people.

Self-renewal capabilities

- When people become older, their number of stem cells decreases.
- *In vitro*, stem cells proliferation rates decrease.
- The number of stem cells harvests enough for cell transplantation.

Stemness gene expression

- Stem cells from elderly people express pluripotential genes.
- Expression of *OCT4*, *NANOG* and *SOX2* genes declines.
- Nevertheless, these cells are capable of self-renewal.

In vitro differentiation

- Stem cells from elderly donors are capable of differentiating into adipocytes, osteoblasts and chondroblast lineage.
- Stem cells from elderly donors are capable of differentiating into neuronal lineage.

Clinical reports

- Several trials have been reported performing by autologous stem cells transplantation.
- In neurodegenerative diseases like amyotrophic lateral sclerosis, Parkinson's disease and Huntington's disease, autologous transplantations have shown an improvement of life quality.
- Autologous transplantation in patients suffering from myocardial infarction showed a significant improvement in the left ventricular ejection volume.
- Patients suffering from POEMS (polyneuropathy, organomegaly, endocrinopathy, M-protein or monoclonal gammopathy and skin changes) syndrome showed an improvement in the patient-forced expiratory volume and total lung capacity.
- Patient with Buerger's disease showed an acceleration in the healing process.
- Patients with urinary incontinence showing progressive improvement in urinary retention capacity.

Conclusion

- Stem cells from elderly donors are capable of self-renewal and differentiation *in vitro*.
- Stemness genes expression decreases, but it is enough for self-renewal.
- Autologous transplantation in elderly patients increases life span and life quality.

well as those obtained from younger donors. Several trials are currently being performed using autologous MSCs in elderly patients. Until more data are gathered indicating some beneficial effects, there is no consensus on the utility of stem cells as a gold standard treatment. Autologous bone marrow transplantation uses mobilized peripheral cells, ADSCs, mononuclear cells and their derived MSCs, or specific subpopulations, such as CD34⁺, CD133⁺ and CD271⁺ cells. The variability in the results may be due to the different sources of MSCs, different techniques used to obtain cells, time placed outside the body or harmful additives, among other reasons. An in-depth analysis of the results based on the specific characteristics of the cells used is needed, as well as their application route.

Conclusion

Stem cells from elderly donors have similarly capabilities to growth and differentiation as younger donors.

Future perspective

Results obtained to date suggest that autotransplantation of MSCs is an important emerging therapy

for degenerative diseases in elderly patients with the advantage of eliminating possible immune rejection from heterologous transplant, ethical concerns or other unknown effects from using transformed cells. MSCs are safe and have demonstrated improvements in some degenerative disease conditions. Results from clinical trials suggest hope for therapies of disease with no effective therapy today.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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