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Transplanting Mesenchymal Stem Cells for Treatment of Ischemic Stroke

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Abstract

Stroke is a major disease that leads to high mortality and morbidity. Given the ageing population and the potential risk factors, the prevalence of stroke and socioeconomic burden associated with stroke are expected to increase. During the past decade, both prophylactic and therapeutic strategies for stroke have made significant progress. However, current therapies still cannot adequately improve the outcomes of stroke and may not apply to all patients. One of the significant advances in modern medicine is cell-derived neurovascular regeneration and neuronal repair. Progress in stem cell biology has greatly contributed to ameliorating stroke-related brain injuries in preclinical studies and demonstrated clinical potential in stroke treatment. Mesenchymal stem cells (MSCs) have the differentiating potential of chondrocytes, adipocytes, and osteoblasts, and they have the ability to transdifferentiate into endothelial cells, glial cells, and neurons. Due to their great plasticity, MSCs have drawn much attention from the scientific community. This review will focus on MSCs, stem cells widely utilized in current medical research, and evaluate their effect and potential of improving outcomes in ischemic stroke.

Keywords

Ischemic stroke, stem cell replacement, MSCs

Introduction

Stroke is a major disease with high mortality and morbidity. Given the currently ageing population and the potential risk factors, the prevalence of and socioeconomic burden associated with stroke are expected to increase[1]. During the past decade, both prophylactic and therapeutic strategies of stroke have made significant progress. However, the current therapies still cannot adequately improve the outcomes of the disease and may not apply to all patients[2]. For instance, ischemic stroke accounts for about 80% of all stroke events. Intravenous thrombolysis with recombinant tissue plasminogen activator (rtPA) added within 4.5 hours is the only FDA-approved remedy for treating acute ischemic stroke[3]. However, with the narrow time window, this treatment can only be applied to 5% or less of patients with ischemic stroke. Even with an efficient thrombolytic therapy, only 55 cases out of 1000 can survive with good prognosis[4]. Furthermore, 6% of tPA-treated ischemic patients will go under symptomatic intracerebral hemorrhage. Therefore, new therapeutic strategies with a wider time window and less hemorrhagic risk are highly needed. Cell-based remedies are emerging as ideal candidates for functional recovery in stroke patients[5]. Mesenchymal stem cells (MSCs) are the most commonly utilized stem cell in biological medical research and therefore will be the focus of this review.

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MSC Characteristics and Sources

In the late 1960s, Friedenstein et al. first discovered MSCs in the bone marrow stromal cells (BMSCs)[6]. Later MSCs were found to be capable of differentiating into mesenchymal cells, including adipocytes, cartilage producing chondrocytes as well as osteogenic osteoblasts[7]. Besides bone marrow, scientists have separated MSCs from many different types of tissues, such as Wharton's jelly (WJ) in the umbilical cord stromal cells (UMSCs), umbilical-cord blood, adipose-derived stromal cells (ADSCs) as well as dental tissues[8–11]. Further studies on MSCs differentiation have shown that these cells can differentiate into hepatocytes[12], cardiomyocytes[13], and neuron-like cells[14]. MSCs have become a promising type of cell for stem cell-based therapies as they exist in all kinds of readily available donor tissues, such as the tissue of pulp and adipose. However, a major issue in the broad study of MSCs is that comparison between different study groups is difficult. The research team usually has its own method of separating, extending and describing cells, resulting in different standards in defining the MSCs[8–10]. To begin addressing this issue, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposes set the minimum standards for defining human MSCs. First, MSCs must be plastic-adhered under standard culture conditions. Second, MSCs must express CD105, CD73, and CD90, lacking the expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules. Third, MSCs must differentiate into osteoblasts, adipocytes, and chondroblasts in vitro[15]. With the update of knowledge, the majority of MSCs do not express CD14 or CD11b, CD19 or CD79a, CD34, CD45, HLA-DR, while they express markers CD10, CD13, CD29, CD44, CD73, CD90, CD105, CD117, CD146, CD271, Stro-1 as well as stage-specific embryonic antigen-4 (SSEA-4)[16–19].

MSCs demonstrate a few properties that attract much research interest[20]. For example, they have the capability of differentiating into neurons, are easy to isolate and amplify from bone marrow, and have relatively low risk of immune rejection in allogeneic transplantation. There is much evidence from animal studies to show that MSC transplantation can reduce infarct volume, improve neurological function, and promote endogenous neurogenesis[21–23]. In this review, we will mainly focus on the underlying mechanisms by which MSCs exert neuroprotective effects after ischemic stroke in preclinical animal models and summarize the current clinical trials using MSCs in ischemic stroke.

Mechanisms of Action of MSCs in Ischemic Stroke

Mechanisms of action of MSCs are divided into two levels: a peripheral level that involves reducing the inflammation and immunomodulation, as well as a central level, which is affected by angiogenesis, astrocytes, neurogenesis, axons and oligodendrocytes.

Immunomodulation and Post-Stroke Inflammation

Although it is well accepted that immune response is important in the pathogenesis of ischemic stroke, the current knowledge on immune response in focal cerebral ischemia is far from sufficient[24]. The immune system becomes active in response to neuronal damage in the event of focal cerebral ischemia or transient ischemia. After stroke, immediate activation of innate immunity triggers inflammation[25]. Inflammatory mediators recruit more immune cells both in the central nervous system and from the periphery. The production of more inflammatory mediators will further activate the adaptive immunity[26]. Inflammation, regression of inflammation, and repair of nerve damage are key successions after stroke. Although the inflammatory response is initially beneficial for limiting and resolving ischemic stress, an unrestricted inflammatory response by the continuous infiltration of immune cells such as neutrophils, macrophages, natural killer (NK) cells and T cells, can cause significant damage to the penumbra after cerebral ischemic injury[27,28].

In vivo and in vitro examination showed that after studying hypoxic stroke neuronal cells and animal models of ischemic stroke, researchers found that MSCs reduced the expression of tumor necrosis factor-α and NF-kB through the vascular endothelial growth factor (VEGF) signaling as well as the interleukin-6 (IL-6) signaling[29]. The inhibition of NF-kB is associated with the anti-inflammatory and anti-apoptotic effects of MSCs[30]. Human umbilical cord blood MSCs remarkably attenuated the expression of IL-23 and IL-17 in infarcts and serum[31], reduced the infarct size, and alleviated neurological deficits in the middle cerebral artery occlusion (MCAO) model. MSCs secrete transforming growth factor-β (TGF-β) by attenuating the upregulation of monocyte chemoattractant protein-1 (MCP-1) as well as the penetration of $CD68^+$ immune cells via the compromised blood–brain barrier (BBB) to prevent the peripheral immune cells from exacerbating the inflammatory response in the ischemic rat brain[32]. In the ischemic stroke rat model, human MSCs reduced microglia activation, as indicated by lower expression of ED1 and Iba-1. They also attenuated astrogliosis, as indicated by lower GFAP level. These beneficial effects involved the noncanonical JAK-STAT signaling with unphosphorylated STAT3 in the immune cells[33].

Impact on Astrocyte, Microglia, Oligodendrocyte, and Axon

In the event of cerebral ischemia, astrocytes release neurotrophic factors and growth factors upon the simulation of MSCs administration. These factors include insulin-like growth factor 1 (IGF-1), VEGF, epidermal growth factor (EGF), and basic fibroblast growth factor (bFGF)[34]. Astrocytic apoptosis is attenuated and ischemic-induced

aquaporin-4 upregulation is normalized by MSCs, which contributes to maintaining BBB integrity after cerebral infarction[35]. MSCs reduce the TGFβ1 expressing in microglia/macrophages at the border of the ischemic area and promote down-regulation of the levels of plasminogen activator inhibitor 1 (PAI-1) in astroglia cells. After stroke, ingrown tPA and depressor PAI-1 are connected to neurite reconstruction[36].

Oligodendrocytes play an important role in the restoration after ischemia[37]. The oligodendrocyte precursor cells (OPCs) appear in the corpus callosum, corpus striatum and subventricular zone (SVZ) of adult mouse brain, differentiating into mature oligodendroglia cells (OLs). OLs are very sensitive to the ischemic stress since the white matter blood flow is lower than that in gray matter; the blood supply for deep white matter is even less[38]. Myelin sheaths in the form of mature oligodendrocytes are used to sprout axons in ischemic tissues. The transplantation of bone marrow (BM)-MSCs increased the number of oligodendrocyte progenitor cells in the ischemic hemisphere as well as the number of mature oligodendrocytes surrounding the lesion[39].

During the experimental stroke, BM-MSCs increased the axonal density in the surrounding area, which persisted for at least 1 year after stroke[40]. MSCs decrease the protein expression of reticulin (Rtn4 or Nogo) and induced neurocan (Ncan), an inhibitor of axonal growth[40]. According to Alder et al.[41], umbilical cord tissue-derived cells (hUTC) and MSCs of human secret the brain-derived neurotrophic factor (BDNF), leading to increased amount and size of main dendrites.

Increased Neurogenesis

Ischemic stroke injury causes a dramatic increase in the proliferation of NSCs, triggering gliogenesis and neurogenesis in SVZ and circumventricular organs (CVOs). Down the third and fourth ventricles, some niches of new stem cell are detected in the context of stroke. It is important that all niches share a common feature – rich in vasculature with high permeability[42]. The incremental post-stroke neurogenesis was observed in elderly patients[43]. Hypoxic preconditioning of transplanted BMSCs could promote their regenerative capability for the treatment of ischemic stroke[44]. BDNF-modified hBM-MSCs (MSCs-BDNF) promoted endogenous neurogenesis and functional recovery in MCAO rat models[45]. Systemic administration of exosomes released from mesenchymal stromal cells promoted endogenous neurogenesis after stroke in rats[46]. It was found that the ultrasound promoted neurogenesis when the mouse stroke models were exposed to 0.04 MHz ultrasound after hBM-MSCs injection[47].

Although post-stroke neurogenesis has been largely described, its role on restoration is still unknown[48]. The cellular therapy triggers the phosphatidylinositol-3-kinase (PI3 K)/Akt pathway in neural precursor cells, promoting cell survival, proliferation, and differentiation, as well as migration[49]. Upon the stimulation by BM-MSCs, brain parenchymal cells release neurotrophic factors, such as fibroblast growth factor and BDNF, to activate Akt/PI3 k pathway[50]. A study showed that transplanting hBMSCs into the ipsilateral brain parenchyma of MCAO rats could increase the expression of BDNF, neurotrophin-3 (NT-3) and VEGF in ischemic brain tissue, reduce infarct volume, and improve neurological function. Possibly, mechanisms for these beneficial effects were increased proliferation of neuronal progenitor cells in SVZ and in the subgranular zone (SGZ), accelerated migration of newborn neuroblasts to the ischemic border region (IBZ), diminished apoptosis, and increased differentiation of these cells into mature neurons[51]. Wharton's jelly (WJ-MSC) induced better neurogenesis via a paracrine mechanism, WJ-MSC expressed more genes involved in angiogenesis and neurogenesis, especially secretory factors[52].

Angiogenesis

Intravenous administration of BM-MSCs leads to releasing angiogenic growth factors as well as neurotrophic factors in time order, which includes angiogenin, hepatocyte growth factor (HGF), BDNF, fibroblast growth factor-2 (FGF-2), IGF-1, neutrophil activating protein 2 (NAP-2), and VEGF, to stimulate post-stroke neuronal growth and vascular formation[53]. These growth factors and neurotrophins all function in a paracrine or autocrine manner[54], which can regulate the cell differentiation, proliferation, and survival. In the peri-infarct area, some researchers found that the expression of these factors has been elevated by BM-MSCs, including stromal cell-derived factor-1 (SDF-1), BDNF, platelet-derived growth factor-AA (PDGF-AA), basic fibroblast growth factor, angiopoietin-2, CXCL-16, neutrophil-activating protein-2, and vascular endothelial growth factor receptor-3[55]. Furthermore, the expression of the axonal growth linked protein-43 (GAP-43) was also increased significantly in the brain tissues treated with BM-MSCs, while the axonal growth inhibitory protein ROCK II and NG2 were inhibited.

In the cerebral infarction region, the transplantation of BM-MSCs enhances the directed immigration and survival of neuroblasts as well[56]. The level of VEGF, phosphorylated ERK1/2 and RAF1 increases notably due to BM-MNC treatment, which also decreases the damage by white matter, stimulates angiogenesis, and facilitates a cognitive recovery in rats having bilateral common carotid arteries occlusion[56].

Other studies have compared various sources of MSCs based on biologically active molecular secretion, the effects of angiogenesis and functional recovery after experimental stroke, as well as different routes of administration. In the rat stroke model, BM-MSCs through intravenous and intra-arterial delivery improved brain perfusion and metastasis by the assessment of SPECT and PET, particularly in rats treated with arterial perfusion delivery. No micro-strokes

have been found after intra-arterial injection[57]. In another research, it was found that MSCs from adipose tissue (AD-MSCs) had the same effect as BM-MSCs in facilitating functional recovery, reducing necrosis and increasing neurogenesis, cell proliferation, and the markers of angiogenesis (e.g. VEGF) expression at 14 days after infarction in the model of rat stroke[58]. As to cellular MSCs-created exosomes post-stroke and traumatic brain injury, intravenous administration can improve functional recovery and enhance neurite reconstruction, angiogenesis as well as neurogenesis[46,59,60].

To improve growth factors delivery, some groups used transgenic stem cells of mesenchyme to overexpress growth factors that are known to trigger the survival as well as the differentiation of neurons. According to Van Velthoven et al.[61], the over-expressed BDNF on MSCs have an advantage over regular MSCs in the rat MCAO model in improving dyskinesia. Via intravenous or intracranial administration, Kurozumi et al[62] used MSCs transgene with fiber-mutant adenovirus vector having BDNF or GDNF (glial cell derived neurotrophic factor). Both decreased the infarct volume by 6% to 40% with equal efficacy. With herpes simplex virus type 1 (HSV-1) vector transgenic MSCs carrying VEGF, Miki et al.[63] found that it reduced infarct volume by 10% and improved functional deficits. Onda et al.[64] used an adenoviral vector-modified MSCs having angiopoietin 1, which reduced the infarct size by 30% and alleviated motor deficits.

Novel Mechanisms of MSCs

Several novel mechanisms of MSCs have been studied, for example, mitochondrial or exosomal transfer from transplanted MSCs or the use of gene therapy of MSCs. Currently, there are few research studies on mitochondrial or exosomal transfer from transplanted MSCs in ischemic stroke.

It has been shown that mitochondrial transfer can rescue stressed cells[65] and restore the loss of mitochondrial function in recipient cells[66]. Han et al.[67] found BM-MSCs rescued injured H9c2 cells via transferring mitochondria through tunneling nanotubes in an in vitro simulated ischemia/reperfusion model. A similar study showed that MSCs rescued injured endothelial cells in an in vitro ischemia-reperfusion model via tunneling nanotube like structure-mediated mitochondrial transfer[68]. Exosomes are microvesicles released by cells ranging from 40 to 100 nanometers[69]. They are produced by the endocytosis of cell membranes and the subsequent formation of intracellular vesicles, which are released into the extracellular space by exocytosis[69]. They exist in any biological fluid such as urine, cerebrospinal fluid, or blood. They are surrounded by a lipid bilayer. They contain nucleic acids such as messenger RNA (mRNA) and microRNA (miRNA) and other different molecules[70]. Thus, they represent a new kind of intercellular communication mechanism. There is growing evidence that exosomes play an important role in cell-cell communication[71]. MSCs also release exosomes, and data shows that exosomes released by MSCs mediate communication between the MSCs and other cells[72]. Xin et al.[60] suggested that exosomes from MSCs mediated the transfer of miR-133b to neurons and astrocytes that regulated gene expression, subsequently contributing to neurite remodeling and functional recovery following ischemic stroke. A review outlines the role of exosomes from MSCs in the recovery of ischemic stroke[73].

MSC-based gene therapy represents a novel potential therapeutic strategy for ischemic stroke in future. The current strategy based on cell therapy emphasizes the introduction of beneficial genes, which will improve the therapeutic ability of MSCs and have better homing efficiency. It has a wide range of implications in stem cell biology. The methods of MSC gene delivery include physical methods, chemical methods, and the use of viral vectors. Several physical methods such as nuclear transfection[74], electroporation[75], nanoparticle[76], and ultrasound transfection[77] were used to deliver the beneficial genes into MSCs. In addition to physical methods of gene delivery, several chemotherapeutics mediated by cationic lipids[78], calcium phosphates[79], cationic polymers[80], cationic polysaccharides[81], and cationic peptides[82] have been used for gene delivery. The main advantage of these non-virus-mediated (physical and chemical) gene delivery techniques is that they could be easily performed. However, the use of physical and chemical methods is limited due to low efficiency, not being suitable for transfection of large numbers of cells, and the use of chemical drugs possibly leading to higher concentrations of toxicity[83]. In addition, safety concerns have been considered, due to the non-degradable nature of certain polymers: for example, polyethylenimine (PEI) is a cationic non-degradable synthetic polymer, which is the most commonly used polymer for the development of nanocarriers for siRNA delivery, but at the expense of cytotoxicity, due to limited degradability[84]. Due to the limitations and disadvantages of the non-viral methods of gene delivery described above, some studies have used viral vectors to improve gene delivery. The viruses currently used as vectors are lentiviruses, adenoviruses, adeno-associated viruses, retroviruses, and baculoviruses[85]. Huang et al.[86] found that lentiviral vector-mediated BDNF gene-modified MSCs could enhance its therapeutic effect in ischemic stroke. Zhao et al.[87] introduced a novel strategy for combining transfer of MSCs and ex vivo HGF gene with multiple mutant herpes simplex virus type 1 vectors in a rat model of transient MCAO; the study showed that combination therapy was more effective than treatment with MSCs alone and might extend the treatment window from hyperacute to acute. At present, viral vector-based cytogenetic modification is widely used. However, low transduction efficiency and transgene potential limit its application in clinical trials.

Table 1. Mesenchymal Stem Cells Transplantation in Ischemic Stroke Clinical Trials.

NCT	Country	Phase	Cell Source/ Autologous or allogenicr	Doses/Single(S) or multiple(M)	Route	Time from stroke onset	Sample cases	Current status
00875654	France	II	MSCs/Autologous	ND/ND	IV	<6 weeks	30	Completed
01091701	Malaysia	I/II	MSCs/Allogenicr	2×10^6/S	IV	<10 days	ND	Withdrawn
01297413	USA	I/II	BMSCs/Allogenicr	$0.5–1.5 \times 10^6$/S	IV	>24 weeks	38	Not yet recruiting
01389453	China	II	UMSCs/Allogenicr	ND/M	IV/LP	1–2 weeks	100	Withdrawn
01461720	Malaysia	II	BMSCs/Autologous	ND/ND	IV	2–8 weeks	50	Unknown
01468064	China	I/II	BMSCs/Autologous	2.5×10^6/M	IV	<1 week	20	Recruiting
01678534	Spain	II	ADSCs/Allogenicr	1×10^6/S	IV	<2 weeks	20	Recruiting
01714167	China	I	BMSCs/Autologous	$2–4 \times 10^6$/ND	IC	3–60 months	30	Unknown
01716481	Korea	III	MSCs/Autologous	ND/ND	IV	<90 days	60	Recruiting
01849887	USA	I	BMSCs/Allogenicr	ND/ND	IV	ND	ND	Withdrawn
01922908	USA	I/II	BMSCs/Allogenicr	ND/S	IV	3–10 days	48	Not yet recruiting
02378974	Korea	I/II	UMSCs/Allogenicr	2×10^8/M	IV	<1 week	18	Not yet recruiting
02425670	India	II	BMSCs/Autologous	$3–50 \times 10^7$/ND	IV	7–30 days	120	completed
02564328	China	I	BMSCs/Autologous	ND/ND	IV	6–60 months	40	Recruiting
02580019	China	II	UMSCs/Allogenicr	2×10^7/S	IV	<12 weeks	2	Not yet recruiting
02849613	Europe	II/III	ADSCs/Allogenicr	ND/ND	IV	1–4 days	400	Recruiting
03176498	China	I/II	UMSCs/Allogenicr	ND/M	IV	ND	40	Not yet recruiting
03186456	China	I	UMSCs/Allogenicr	$0.5–1 \times 10^6$/M	IV	<2 weeks	40	Not yet recruiting
03356821	Netherlands	I/II	BMSCs/Allogenicr	5×10^7/S	Nasal	<7 days	10	Not yet recruiting

From information available at ClinicalTrials.gov, searched by 'MSCs' and 'ischemic stroke'. Table updated June 11, 2018.
IC: Intracerebral injection; IV: intravenous; LP: lumbar puncture; NCT: ClinicalTrials.gov identifier; ND: no data.

Safety in Preclinical Studies

Although using MSCs in animal stroke models was generally safe and had a significant effect on behavioral outcomes[88], some studies still showed side effects such as embolism[89], infection, and tumor formation[90]. Amyloid-β accumulation and calcium in the thalamus also appear[91]. Research on rat stroke models suggested that intra-arterial (IA) MSCs delivery is capable of reducing the flow of middle cerebral artery (MCA); however, this side effect appears to be dose-dependent. A dosage of 1 x 10^5 MSCs was shown to be the maximal tolerable dose of IA infusion, making no concessions to the blood flow of MCA. One study also showed that delivering MSCs at 24 hours after stroke significantly improved neurological function and reduced the infarct size at 1 month compared with control but delivering 1 hour after stroke did not confer such protective effects[92]. Wang et al. found that there was no standard dose for stem cell therapy currently associated with the route of administration and disease types. For intracerebral parenchymal transplantation, an excessively large transplant dose affected the nutrition of transplanted cells and could cause microemboli and vascular occlusion when administered intravascularly[93]. Although there is no uniform dose standard, dose control is very important in preventing embolism. Intravenous infusion is thought to be associated with embolization, and embolization can be reduced by intraperitoneal or other routes of transplantation[94].

Many safety problems have emerged with the intracerebral transplantation and interventional neuroradiography in acute stroke settings, such as maintain biological stability of the therapeutic product, larger MSCs doses can potentially affect organ perfusion, and the safety of allogeneic MSCs[88]. Another study showed that amyloid-β and calcium accumulation in the thalamus following intravenous injection of human bone marrow MSCs in MCAO model in rats, quantification of the area of the deposits showed a highly significant increase in amyloid-β and calcium deposition in the thalamus after infusion of MSCs at 48 hours after MCAO, there was a clear correlation between impaired forelimb performance on postoperative day 42 and amyloid-β and calcium accumulation in the thalamus[91]. MSC transplantation animal experiments found no obvious immune rejection. However, studies had shown that in vitro licensed WJ-MSCs did not improve experimental autoimmune encephalomyelitis in rats, due to increased immunogenicity resulting in rapid rejection[95].

Clinical Trials of MSC Transplantation

Cells derived from bone marrow displayed great prospects for safety and initial efficacy[96,97]. Some clinical tests in Phase I and Phase II have already begun, using cell populations originated from mesenchymal stem cells (Table 1). Early results revealed that intravenous injection of MSCs does not give raise to significant adverse effects but can improve functional measurements such as the Barthel Index (BI)[98], the National Institutes of Health Stroke Score (NIHSS) and the modified Rankin Scale (MRS)[96]. A long-term follow-up study of intravenous autologous mesenchymal stem cells transplantation in patients with ischemic stroke showed that no significant side effects were observed,

and the follow-up MRS score was decreased compared with the control group[99]. A meta-analysis from Lalu et al.[100] suggested that MSC therapy appeared safe, but there was a significant association between MSC and transient fever based on the current clinical trials, so further larger scale controlled clinical trials with rigorous reporting of adverse events were required to further define the safety profile of MSCs. Contradictory data shows that MSC injection may not improve the results of the function[101]. There was no significant difference in the BI score, MRS shift analysis, NIHSS score, or change in infarct volume at day 180 compared with the control group[101]. These studies used autologous MSCs which were expanded in culture before MSC transplantation[96–101]. Although no side effects of the products were reported, the cells were amplified in autologous serum, leading to faster cell expansion and reducing concern of heterogeneous contamination.

Conclusions and Future Prospects

There are many advantages of MSCs: they are easy to harvest, amplify and store for a long time; they can be quickly isolated with relative immune privileges; they can be managed in various manners; and their usage does not result in many ethical issues. However, so far, only the clinical trials of phase I and II have been reported, covering a small number of participants and a comparatively short duration of follow-up, while it stills lacks the larger-scale phase III clinical trials. Therefore, further research is needed to address the long-range safety and effect of therapy with MSCs[102].

Authors' Note

Wang and Tang contribute equally to this paper.

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