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Differentiation of Human Mesenchymal Stem Cells towards Neuronal Lineage: Clinical Trials in Nervous System Disorders

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Abstract

Mesenchymal stem cells (MSCs) have been proposed as an alternative therapy to be applied into several pathologies of the nervous system. These cells can be obtained from adipose tissue, umbilical cord blood and bone marrow, among other tissues, and have remarkable therapeutic properties. MSCs can be isolated with high yield, which adds to their ability to differentiate into non-mesodermal cell types including neuronal lineage both *in vivo* and *in vitro*. They are able to restore damaged neural tissue, thus being suitable for the treatment of neural injuries, and possess immunosuppressive activity, which may be useful for the treatment of neurological disorders of inflammatory etiology. Although the long-term safety of MSC-based therapies remains unclear, a large amount of both pre-clinical and clinical trials have shown functional improvements in animal models of nervous system diseases following transplantation of MSCs. In fact, there are several ongoing clinical trials evaluating the possible benefits this cell-based therapy could provide to patients with neurological damage, as well as their clinical limitations. In this review we focus on the potential of MSCs as a therapeutic tool to treat neurological disorders, summarizing the state of the art of this topic and the most recent clinical studies.

Key Words: Mesenchymal stem cells, Nervous system disorders, Cell-based therapy, Neuronal differentiation, Clinical trials

INTRODUCTION

Stem cells are a population of unspecialized cells with the ability to both self-renew and give rise to multiple cell types (Ahmadi *et al*., 2012). The stem cell niche consists of a heterogeneous cell population, extracellular matrix and different soluble factors, which together provide a suitable microenvironment (Ferroni *et al*., 2013) for the maintenance of these cells. Stem cells can be classified according to their differentiation capacity into: i) totipotent stem cells, which can generate all cell types of the organism, including embryonic and extraembryonic tissues (Sun and Ma, 2013); ii) pluripotent stem cells, which can differentiate into cell types from any of the three germ layers, thus being able to differentiate into all cells of the adult organism (Mahla, 2016); iii) multipotent stem cells, which can give rise to all cells derived from one specific lineage (Jopling *et al*., 2011); and finally, iv) unipotent stem cells, which can only generate one cell type (Jaenisch and Young, 2008).

Despite sharing common properties, stem cells exhibit different features when they originate from different sources (Kalladka and Muir, 2014). In fact, stem cells can be categorized as embryonic stem cells (ESCs), fetal stem cells (FSCs), perinatal stem cells (PSCs), adult stem cells (ASCs) and induced pluripotent stem cells (iPSCs). ESCs have the advantage of being pluripotent, although their use involves high risk of tumorigenicity and generates a great ethical controversy, since they derive from embryo at the blastocyst stage (Nadig, 2009). Interestingly, iPSCs have similar characteristics to ESCs, including pluripotency, but are generated from adult somatic cells by epigenetic reprogramming and thus they can be used without ethical debate (Salehi *et al*., 2016). FSCs are derived from fetal tissues, even though they exhibit a lower

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division capacity than ESCs (Ilic and Polak, 2011). PSCs are multipotent cells that derive from perinatal extraembryonic tissues and share properties with ESCs and ASCs (Si *et al*., 2015). The latter, i.e., ASCs, are multipotent cells present in most adult tissues, where they play a key role in tissue regeneration (Nadig, 2009; Mahla, 2016).

The exciting potential of stem cells in tissue regeneration and repair provides an alternative approach to cell-based therapies in various diseases, especially in those that affect the nervous system (NS) (Gage and Temple, 2013). In fact, stem cells may replace some non-functional cells in neurodegenerative disorders and NS lesions (Lunn *et al*., 2011). In this context, mesenchymal stem cells (MSCs), which are adult stem cells derived from the mesoderm and neuroectoderm (Ferroni *et al*., 2013), exhibit a high differentiation plasticity. These cells present some advantages compared to other stem cells such as neural stem cells (NSCs), namely lack of teratoma formation capacity since they derive from adult tissues and ability to migrate towards inflammatory foci through expression of chemokine receptors (Honczarenko *et al*., 2006; Wakao *et al*., 2012; Laroni *et al*., 2015; Frese *et al*., 2016). Moreover, the use of MSCs could avoid the toxicity of the immunosuppressive regimens used with NCS (Fu *et al*., 2008).

Previous review articles have discussed the MSCs ability to differentiate into neurons and their possible application in clinical trials (Scuteri *et al*., 2011; Ullah *et al*., 2015; Squillaro *et al*., 2016). This review summarizes the potential of MSCs in regenerative medicine applied to neurological disorders, and offers a comprehensive compilation of the most recent clinical studies that employ this type of cell therapy.

MESENCHYMAL STEM CELLS

MSCs are considered multipotent cells able to give rise to all cell types of mesodermal origin, including bone, cartilage and fat cells. However, their ability to differentiate into non-mesodermal cell types such as neurons has been widely reported (Drela *et al*., 2013; Salehi *et al*., 2016). Although these cells were isolated for the first time from bone marrow (Friedenstein *et al*., 1974), they reside in almost all tissues, with adipose tissue, placenta and umbilical cord being the most used MSC sources (Ferroni *et al*., 2013; Teixeira *et al*., 2013).

In recent decades, MSCs have been hailed as a therapeutic promise in regenerative medicine due to their accessibility and ease of *in vitro* expansion, reduced immunogenic properties (Salehi *et al*., 2016) and broad differentiation ability compared to other ASCs types (Drela *et al*., 2013; Ferroni *et al*., 2013). The International Society for Cellular Therapy (ISCT) established three minimal criteria for the correct identification of MSCs: i) plastic adherence; ii) positive expression of the CD73, CD90 and CD105 markers and negative for CD34, CD45, HLA-DR, CD14 or CD11B, CD79α or CD19; and iii) capacity of *in vitro* differentiation into adipocytes, chondrocytes and osteoblasts (Teixeira *et al*., 2013). Nevertheless, it has been reported that these stem cells may have variable biological features according to the source, the donor or the culture conditions (Han *et al*., 2017). In addition, the ability of MSCs to differentiate into cells of the neuronal lineage has been questioned. In fact, some stressful culture conditions can produce morphological changes and alter protein expression in MSCs without necessarily turning them into neurons. Among these conditions, serum deprivation, cell fusion or the addition of some components to differentiation media (e.g., dimethyl sulfoxide or β-mercaptoethanol) (Lu *et al*., 2004; Krabbe *et al*., 2005; Croft and Przyborski, 2006) have been assayed. However, more recent research has demonstrated successful and stable neuronal differentiation corroborated by multiple techniques both *in vitro* and *in vivo* (Mareschi *et al*., 2006; Zhang *et al*., 2006; Takeda and Xu, 2015).

MODULATION OF MESENCHYMAL STEM CELLS TO THE NEURONAL LINEAGE

Contrary to drug-based treatments, therapies employing living cells have the advantage of dynamically responding to a time-changing environment, rather than being focused on a single way of action (Kalladka and Muir, 2014). In order to treat neurological injuries with MSCs, these cells can be obtained from different sources such as the umbilical cord (HU-MSCs) (Hong *et al*., 2011), bone marrow (BM-MSCs) (Mahmood *et al*., 2003), amniotic fluid (Yan *et al*., 2013) or adipose tissue (AD-MSCs) (Gao *et al*., 2014a). However, no accurate studies comparing the functionality of MSCs according to their source have been conducted. Once implanted in the injured region, MSCs can exert the following therapeutical mechanisms: secretion of neurotrophic factors (Nagai *et al*., 2007), induction of neurogenesis and astroglial activation (Yoo *et al*., 2008), axon growth and enhancement of synaptic connections (Maltman *et al*., 2011), anti-apoptotic, immunomodulatory (Wang *et al*., 2012; Budoni *et al*., 2013) and antiinflammatory effects (Hawryluk *et al*., 2012), reduction of oxidative stress (Kemp *et al*., 2010; Chen *et al*., 2011), secretion of exosomes containing a wide range of bioactive molecules (Kim *et al*., 2012; Tomasoni *et al*., 2013), and expression of a great number of genes related to neuronal processes and transcription factors (Arboleda *et al*., 2011).

In general, transplanted MSCs are previously reprogrammed *in vitro* with the aim of improving their survival and accelerating their differentiation into nervous cells (Lu *et al*., 2001; Mahmood *et al*., 2002). *In vitro* reprogramming of MSCs towards neuronal lineage can be achieved through four different strategies (Fig. 1A): psychotropic drugs, small molecules, enriched media and epigenetic modifications. On the contrary, other research groups have transplanted MSCs not previously reprogrammed (Bhang *et al*., 2007), and even followed by the injection of growth factors in the treated zone (Liu *et al*., 2014).

It is known that some drugs, namely antidepressants and antipsychotics, can increase proliferation and differentiation rates of MSCs into neurons (Nakagawa, 2010). These drugs have proved to reverse gray matter loss and slow down the reduction in brain volume in patients with neurodegenerative disorders such as schizophrenia or depression. However, the mechanisms by which this occurs are not completely understood (Nasrallah *et al*., 2010), although it has been observed that inhibiting glycogen synthase kinase-3β (GSK-3β) plays a key role in the proliferation of hippocampal neuronal precursor cells (Morales-Garcia *et al*., 2012). Atypical antipsychotic drugs like risperidone, olanzapine and aripiprazole, and antidepressants like desvenlafaxine (Asokan *et al*., 2014) have proved to increase *in vitro* neurogenesis and neuron maturation in rat models, respectively. Additionally, the antidepressants imipramine, desipramine, fluoxetine and tianeptine have

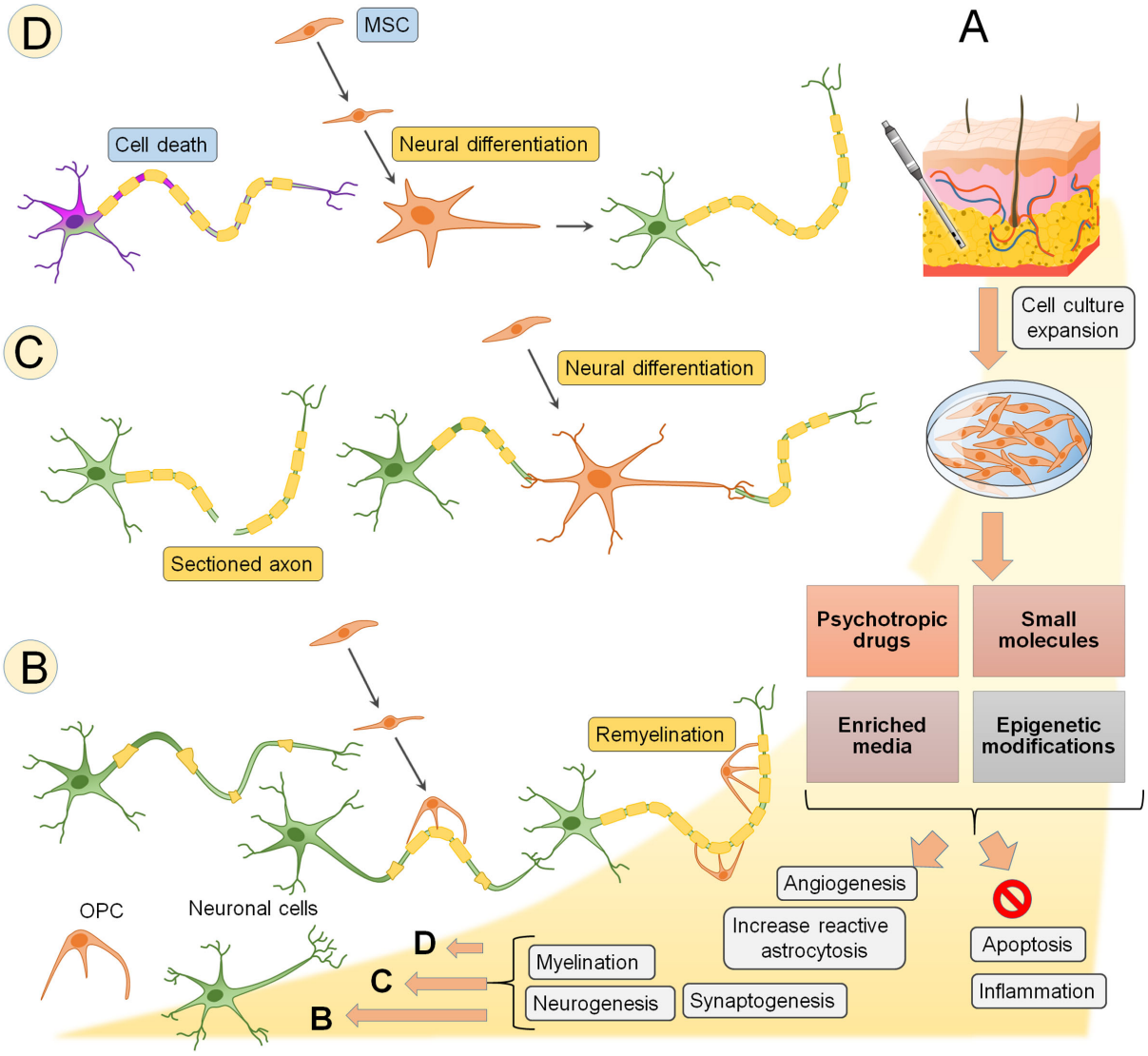

Fig. 1. Schematic process of the *in vitro* differentiation of mesenchymal stem cells (MSCs). Differentiation factors as drugs, small molecules, the enrichment of culture media and epigenetics changes (A) make different effects on MSC like increase reactive astrocytosis, synaptogenesis, neurogenesis, myelination, apoptosis inhibition and angiogenesis. In addition, inhibition apoptosis and inflamation are also induced. MSC may be applied to promote remyelination (i.e., multiple sclerosis) where the presence of MSC marks the sites where the oligodendrocyte progenitor cell (OPC) has to join to repair the lost myelin (B). Moreover, MSCs may be used to induce restitution of injured areas and damaged circuitry of the brain after stroke (C). Finally, therapeutic application of MSCs may represent a promising approach in the treatment of spinal cord injury (D).

shown improved differentiation efficiency of rat MSCs into neuron-like cells (Borkowska *et al*., 2015).

Small molecules are also emerging as cutting-edge tools in the pharmacotherapy of brain injuries. They can imitate the activity of endogenous proteins and modulate certain signaling pathways. Among these small molecules, a plethora of GSK-3β inhibitors stand out, particularly lithium, a mood stabilizer that accumulates in neurogenic brain regions and increases adult hippocampal neurogenesis (Boku *et al*., 2010; Zanni *et al*., 2017). Vitamin derivatives such as retinoic acid, known to induce differentiation of MSCs into neurogenic cells (Gao *et al*., 2014b; Halder *et al*., 2015), also play a role in the differentiation of neural progenitors. Finally, Alexanian *et al*. (2013) tested a combination of SMAD signaling inhibitors (SMAD1/3 and SMAD3/5/8) with chromatin modifying agents (trichostatin A and RG108) and modulators of cAMP levels, showing a high rate of BM-MSCs differentiation into neuron-like cells and an enhanced formation of synaptic-like structures. In addition, enrichment of culture media with growth factors and other inductor substances allow the *in vitro* differentiation of MSCs towards various cell types with morphological and functional differences (Zhu *et al*., 2009; Kajiyama *et al*., 2010; Ferro *et al*., 2011). Since the publication of the first neuronal differentiation medium for MSCs in 2000 (Woodbury *et al*., 2000), a number of media have been used for this purpose. Table 1 summarizes the substances most commonly employed and

Table 1. *In vitro* neuronal differentiation of MSC. Substances commonly used for neural differentiation and neuronal markers more frequently studied after the differentiation process

Compounds	Neuronal markers	Types of MSC	Ref.
EGF	CHAT, TH, TUB-III, MAP2	AD-MSC	Marei *et al*., 2018
Insulin	GFAP, TUB-III	AD-MSC	Ying *et al*., 2012
5-Azacytine	MAP2	AD-MSC	Zemel'ko *et al*., 2013
bFGF	GFAP, MAP2, MBP, nestin	UCSC	Rafieemehr *et al*., 2015
NGF	TUB-III, NF-M, PSD-95	UCSC	Jahan *et al*., 2017
SHH	Hb-9, Pax-6, NF, CHAT	UCSC	Yousef *et al*., 2017
cAMP	TUB-III, NSE, MAP2, GFAP	UCSC	Shahbazi *et al*., 2016
BDNF	Nestin, NSE, GFAP	BM-MSC	Liu *et al*., 2015
DMSO	NSE, GFAP	BM-MSC	Xu *et al*., 2016
Retinoic acid	NF	BM-MSC	Wang *et al*., 2013
BME	Nestin, NSE	BM-MSC	Shi *et al*., 2016
IBMX	TUB-III, GFAP, NF, NeuN, MAP2	BM-MSC / eMSC	Zemel'ko *et al*., 2014
BHA	MAP2, NSE, GFAP	BM-MSC	Mu *et al*., 2015
LIF	Nestin, TUB-III, GFAP, MBP, TH	hDPSC	Chun *et al*., 2016

AD-MSC, adipose-derived mesenchymal stem cells; BDNF, brain-derived neurotrophic factor; bFGF, basic fibroblast growth factor; BHA, butylated hydroxyanisole; BM-MSC, bone marrow-derived mesenchymal stem cells; BME, 2-mercaptoethanol; CHAT, choline O-acetyltransferase; DMSO, dimethyl sulfoxide; EGF, epidermal growth factor; eMSC, menstrual blood mesenchymal stem cells; GFAP, glial fibrillary acidic protein; Hb-9, homeobox gene 9; hDPSC, human dental pulp stem cells; IBMX, 3-isobutyl-1-methylxanthine; LIF, leukemia inhibitory factor; MAP2, microtubule-associated protein 2; MBP, myelin basic protein; MSC, mesenchymal stem cells; NCC, neural crest cells; NF, neurofilament; NGF, nerve growth factor; NSE, neuron-specific enolase; Pax-6, paired box protein 6; PSD-95, postsynaptic density protein 95; SHH, sonic hedgehog; TH, tyrosine hydroxylase; TUB-III, ß-tubulin III; UCSC, umbilical cord blood stem cells.

the neuronal markers most frequently studied to evaluate the differentiation efficiency of MSCs.

Epigenetic changes lead MSCs to a particular lineage by repressing genes related to the undifferentiated state and expressing those associated with differentiation (Teven *et al*., 2011; Herlofsen *et al*., 2013). A recent study stated that changes in the neuronal phenotype of MSCs can be a result of epigenetic modifications (Alexanian, 2015). Exposing MSCs to epigenetic modifiers and neuronal induction factors turns them into neuronal lineage-like cells, suggesting that cell plasticity can be handled by combining epigenetic modulating enzymes and specific signaling pathways. Nevertheless, the mechanisms by which MSCs undergo trans-differentiation are still unclear. Alexanian (2015) developed a protocol for neuronal differentiation based on testing various epigenetic modulators such as trichostatin (TSA), valproic acid (VPA), sodium butyrate, DNZep, RG108, 5-aza-dC, zebularine or BIX 01294, in combination with substances that promote iPSCs differentiation into neuronal lineage. These authors demonstrated that chromatin-modifying compounds increase the plasticity of already differentiated cells and make them suitable to respond to differentiation-inducing signals. Some studies have shown that the exposure of MSCs to VPA, a histone deacetylase inhibitor, leads to overexpression of specific markers of neural progenitors like GFAP and nestin (Dong *et al*., 2013). Fila-Danilow *et al*. (2017) confirmed that TSA and VPA affected the expression of neuronal lineage genes, and inhibited cell proliferation and neurospheres formation in a culture of rat MSCs.

Genetic modifications of MSCs can be an effective method to achieve a faster and more durable neuronal differentiation, either alone or in combination with differentiation media. Genetically modified MSCs have demonstrated the potential to secrete brain-derived neurotrophic factors, such as BDNF, VEGF, NGF, IGF-1 (Song *et al*., 2009; Gu *et al*., 2010; Wyse *et al*., 2014) and neurotrophins (Zhang *et al*., 2012), among others, and to serve as cellular vehicles for pro-drug gene therapy (Choi *et al*., 2012) to treat neurodegenerative disorders. The manipulation of MSCs towards a desired epigenetic status for their differentiation into the proper neuronal lineages could be the tool for the development of alternative cell therapies focused on neurodegenerative disorders.

CLINICAL TRIALS WITH MESENCHYMAL STEM CELLS FOR THE TREATMENT OF NERVOUS SYSTEM DISORDERS

The plasticity of MSCs has allowed the development of numerous clinical trials, many of which have not yet been completed (Table 2). However, data from the most recent ones show that the use of MSCs to treat NS pathologies does not imply adverse effects or structural changes. Furthermore, the increasing amount of positive results *in vivo* in phase I and II trials suggests that the transplantation of MSCs may lead to functional improvements and tissue regeneration.

After trauma, normal brain functioning may be disrupted, producing severe physical and emotional damage, which is known as traumatic brain injury (TBI). It has been shown that MSCs have potential applications in TBI to reduce brain damage and clinical sequelae. In fact, MSCs can reduce inflammation through immunosuppressive mechanisms and induce the secretion of growth factors that benefit neurons (Hasan *et al*., 2017). Two recent clinical trials, NCT02742857 and NCT02525432, use intrathecal and intravenous transplantation of MSCs to improve the prognosis of patients with TBI, although results are ongoing. Stroke occurs when a sudden interruption of blood flow takes place in the brain. Currently, TBI is one of the main objectives for the application of MSCs (Fig. 1B). The possibility that MSCs may be implanted into injury as self-renewing neuronal cells has been assayed (Maria Ferri *et*

Table 2. Clinical trials using MSCs for neural disorders

Identifier/ Country	Study	Phase/ Patients	Year	Safety and effectiveness	Disease
NCT02742857/ India	Intrathecal transplantation of bioactive peptides, MSC and transcranial laser stimulation	1/20	2017	Ongoing	Traumatic brain injury
NCT02525432/ USA	Intravenous transplantation of autologous BM-MSC	2/55	2017	Ongoing	Traumatic brain injury
NCT00875654/ France	Intravenous injection of autologous BM-MSC	2/31	2017	Ongoing	Stroke
NCT01287936/ USA	Transplantation of modified SB623 stem cells into ischemic, chronic and stable cerebrovascular accident	1-2/18	2016	Cells safe Improvement after 12 months	Stroke
NCT01310114/ USA	Intravenous administration of placenta-derived cells	2/44	2018	Ongoing	Ischemic stroke
NCT03356821/ Holland	Intranasal transplantation of BM-MSC in perinatal	1-2/10	2017	Ongoing	Arterial infarction
NCT01678534/ Spain	Transplantation of allogeneic AD-MSC	2/20	2017	Ongoing	Ischemic stroke
NCT01468064/ China	Transplantation of autologous BM-MSC and endothelial progenitors	1-2/20	2017	Ongoing	Ischemic stroke
NCT01922908/ USA	Transplantation of autologous BM-MSC	1-2/48	2017	Ongoing	Ischemic stroke
NCT03371329/ USA	Transplantation of BM-MSC	1/12	2017	Ongoing	Intracerebral hemorrhage
NCT02580019/ China	Transplantation of HU-MSC	2/2	2017	Ongoing	Ischemic stroke
NCT02378974 South Korea	Intravenous transplantation of HU-MSC	1-2/18	2017	Ongoing	Ischemic stroke
NCT03176498/ China	Intravenous transplant of HU-MSC	1-2/40	2017	Ongoing	Ischemic stroke (convalescence)
NCT03186456/ China	Transplantation of HU-MSC	1/40	2017	Ongoing	Ischemic stroke
NCT01716481/ South Korea	Intravenous transplantation of autologous MSC expanded with autologous serum	3/60	2017	Ongoing	Stroke
NCT01297413/ USA	Transplantation of allogeneic BM-MSC	1-2/38	2017	Ongoing	Ischemic stroke
NCT02849613/ France	Intravenous transplantation of allogeneic ADSC	2-3/400	2016	Ongoing	Stroke
NCT02165904/ Spain	Subarachnoid transfer of autologous BM-MSC	2/10	2017	No secondary effects (1 year) Functional improvements	Spinal cord injury
NCT02481440/ China	Transplantation of allogeneic umbilical cord-derived MSC	1-2/44	2018	Ongoing	Spinal cord injury
NCT01676441/ South Korea	Autologous MSC transplantation	2-3/32	2016	Ongoing	Spinal cord injury
NCT02574585/ Brazil	Transfer of autologous BM-MSC	2/40	2017	Ongoing	Spinal cord (lumbar) injury
NCT02574572/ Brazil	Transfer of autologous BM-MSC	1/10	2018	Ongoing	Spinal cord (cervical) injury
NCT02152657/ Brazil	Transfer of autologous MSC in spinal cord injuries by percutaneous puncture	5/1	2017	No publication available	Spinal cord injury
NCT02482194/ Pakistan	Intrathecal transfer of autologous BM-MSC	1/9	2016	No secondary effects (2 years)	Spinal cord injury
NCT02688049/ China	Combined treatment of MSC and NeuroRegen scaffold	1-2/30	2017	Ongoing	Spinal cord injury

Table 2. Continued 1

Identifier/ Country	Study	Phase/ Patients	Year	Safety and effectiveness	Disease
NCT02570932/ Spain	Intrathecal transfer of autologous BM-MSC	2/10	2017	Ongoing	Spinal cord injury
NCT01769872/ South Korea	AD-MSC transplantation	1-2/15	2016	No publication available	Spinal cord injury
NCT03003364 Spain	Intrathecal transfer of autologous WJ-MSC	1-2/10	2017	No publication available	Spinal cord injury
NCT02352077/ China	Transplantation of BM-MSC and NeuroRegen scaffold	1/30	2016	No secondary effects (1 year) Small sensorial improvements	Spinal cord injury
NCT02981576 Jordan	Comparison of AD- and BM-MSC transplantation	1-2/14	2017	Ongoing	Spinal cord injury
NCT02917291/ Spain	Transplantation of allogenic AD-MSC combined with H2O2 and HC016 cells	1-2/46	2017	Ongoing	Spinal cord injury
NCT03308565/ USA	AD-MSC transplantation in cerebrospinal fluid	1/10	2017	Ongoing	Spinal cord injury
NCT01909154/ Spain	Transfer of autologous BM-MSC	1/12	2017	No secondary effects Small sensorial improvements	Spinal cord injury
NCT01393977/ China	Differences between rehabilitation and transplantation	2/60	2017	No publication available	Spinal cord injury
NCT02881489/ Poland	Transplantation of autologous BM-MSC	1/30	2017	Ongoing	Spinal cord injury
NCT01873547/ China	Differences between rehabilitation and transplantation	3/300	2018	No publication available	Spinal cord injury
NCT01325103/ Brazil	Transplantation of autologous BM-MSC	1/20	2017	No secondary effects Neurological improvements	Spinal cord injury
NCT01377870/ Iran	Evaluation of the autologous MSC transplantation in multiple sclerosis	1-2/30	2018	No publication available	Multiple sclerosis
NCT01895439/ Jordan	Intrathecal administration of autologous BM-MSC	1-2/30	2017	No adverse secondary effects General improvement	Multiple sclerosis
NCT00813969/ USA	Autologous MSC transplantation	1/24	2016	No publication available	Multiple sclerosis
NCT01933802/ USA	Intrathecal administration of autologous MSC	1/20	2018	Minor secondary effects (24 hours) Improvements in muscular strength and bladder	Multiple sclerosis
NCT01606215/ United Kingdom	Transplantation of MSC	1-2/13	2016	No publication available	Multiple sclerosis
NCT01745783/ Spain	Intravenous transplantation of autologous BM-MSC	1-2/30	2018	No publication available	Multiple sclerosis
NCT02611167/ USA	Intravenous transplantation of allogeneic BM-MSC	1-2/20	2018	Ongoing	Parkinson
NCT01609283/ USA	Intrathecal transplantation of autologous MSC	1/27	2018	Ongoing	ALS

Table 2. Continued 2

Identifier/ Country	Study	Phase/ Patients	Year	Safety and effectiveness	Disease
NCT01759797/ Iran	Intravenous transplantation of autologous MSC	1/6	2016	No secondary effects	ALS
NCT02492516/ Iran	Intravenous transplantation of autologous AD-MSC	1/19	2016	No publication available	ALS
NCT02987413/ Brazil	Intrathecal transplantation of autologous MSC	1/3	2017	No secondary effects (1year)	ALS
NCT01771640/ Iran	Intrathecal transplantation of autologous MSC	1/8	2018	No secondary effects (6 months)	ALS
NCT02917681/ Brazil	Intrathecal transplantation of autologous MSC	1-2/28	2016	No secondary effects (10 months)	ALS
NCT02290886/ Spain	Intravenous transplantation of AD-MSC	1-2/40	2018	No secondary effects (10 months)	ALS
NCT03268603/ USA	Transplantation of autologous AD-MSC	2/60	2018	Ongoing	ALS
NCT03280056/ USA	Intrathecal transplantation of autologous BM-MSC	3/200	2018	Ongoing	ALS
NCT03296501 Poland	Intraspinal transplantation of AD-MSC	1/30	2017	Ongoing	ALS
NCT01777646/ Israel	Transplantation of autologous BM-MSC secreting neurotrophic factors	2/14	2018	No secondary effects (10 months)	ALS
NCT02600130/ USA	Intravenous transplantation of MSC versus placebo	1/30	2018	Ongoing	Alzheimer
NCT02833792/ USA	Transplantation of allogeneic MSC	2/40	2018	Ongoing	Alzheimer
NCT02054208/ South Korea	Transplantation of HU-MSC	1-2/45	2017	No secondary effects (2 years)	Alzheimer
NCT01547689/ China	Transplantation of HU-MSC	1-2/30	2016	No secondary effects (10 months)	Alzheimer
NCT03117738/ USA	Intravenous transplantation of autologous AD-MSC	1-2/60	2018	No secondary effects (3 years)	Alzheimer
NCT03172117/ South Korea	Transplantation of HU-MSC	1-2/45	2017	Ongoing	Alzheimer
NCT02672306/ China	Transplantation of HU-MSC	1-2/40	2018	Ongoing	Alzheimer
NCT02899091/ South Korea	Intravenous transplantation of MSC	1-2/24	2016	Ongoing	Alzheimer
NCT02315027/ USA	Intrathecal transplantation of autologous MSC	1/30	2017	No secondary effects (1 year)	SDS-MSA
NCT00911365/ South Korea	Transplantation of autologous MSC *versus* placebo	2/27	2017	No secondary effects	SDS-MSA
NCT03265444/ South Korea	Transplantation of BM-MSC	1/9	2018	Ongoing	MSA
NCT02855112/ Iran	Transplantation of allogeneic AD-MSC	1-2/10	2017	No secondary effects	SMA1
NCT02728115/ Brazil	Intravenous transplantation of autologous MSC	1/6	2017	Ongoing	Huntington's chorea
NCT03252535/ Brazil	Transplantation of MSC	2/35	2017	Ongoing	Huntington's chorea

AD-MSC, adipose tissue-derived mesenchymal stem cells; ALS, amyotrophic lateral sclerosis; BM-MSC, bone marrow-derived mesenchymal stem cells; HU-MSC, human umbilical mesenchymal stem cells; SDS-MSA, Shy-Drager syndrome (multiple system atrophy); SMA1, spinal muscular atrophy type 1 (Werdnig-Hoffman disease); WJ-MSC, Wharton's jelly-derived mesenchymal stem cells.

al., 2016). In fact, the use of the modified SB623 stem cells in this pathology improved the clinical evolution of the patient for at least one year. Numerous clinical trials are underway using BM-MSCs, HU-MSCs or placenta-derived cells transplanted by different methods to determine the utility of this therapy in stroke (Table 2).

On the other hand, spinal cord injury (SCI) triggers a severe loss of motor, sensory, and autonomic functions that lack proper treatment. The neurological deficit results from the direct trauma associated with a secondary injury characterized by local immune reaction, apoptosis of neurons, tissue atrophy with cavitation and glial scar formation (Fig. 1C). Recently, it has been demonstrated that MSCs promote the repair of spinal cord tissues in animal models, suggesting their potential clinical use (Qu and Zhang, 2017). A clinical trial using repeated doses of autologous BM-MSCs by subarachnoid route (NCT02165904) showed no secondary effects -at least after one year-, and functional improvements were reported. Some other studies showed similar results (NCT02352077, NCT01909154, NCT01325103).

Multiple sclerosis and amyotrophic lateral sclerosis (ALS) may be excellent candidates for MSCs-based therapies. The former is an inflammatory disease in which activated T cells induce axonal demyelination and neurological disability. The latter is a neurodegenerative disorder characterized by major alterations of the neuromuscular system with an unknown etiology, although the activity of the immune system is thought to play a relevant role. In both conditions, the therapeutic plasticity of MSCs may benefit the evolution and prognosis of the patients (Fig. 1D) (Ardeshiry Lajimi *et al*., 2013; Lewis and Suzuki, 2014). A recent clinical trial employing autologous BM-MSCs intrathecally followed by MSCs conditioned media to treat multiple sclerosis (NCT01895439) showed improvements in all the tests conducted (except for lesion volume), demonstrating the safety and feasibility of this treatment. A more recent study (NCT01933802) using the same cells and route of administration improved muscular strength and bladder function with minor secondary effects. In relation to ALS, a recent phase II study (NCT01777646) showed that MSCs induced the secretion of neurotrophic factors, and slowed its natural progression without adverse effects for at least 6 month. Similar results were observed in other studies employing MSCs from different sources and with different routes of administration (NCT01771640, NCT02290886 and NCT02987413).

Finally, MSCs are being tested in clinical trials in other NS disorders, such as Shy-Drager syndrome, Werdnig-Hoffman disease, Huntington's chorea and Alzheimer's disease (Table 2). Regarding Alzheimer's disease, the use of stem cells can be a hope. However, although some clinical studies support the safety and efficacy of this therapy, there is no unifying hypothesis for an underlying mechanism of action. The presence of neurotrophic factors, the activation of immunomodulatory molecules and the increase in the expression of synaptic proteins have been implicated (Bali *et al*., 2017). An ongoing phase II clinical trial with autologous AD-MSCs administered intravenously did not show adverse effects after 3 years (NCT03117738). Similar results have been found in other clinical trials (NCT02054208, NCT01547689).

In conclusion, different methods supporting and stimulating endogenous neurogenesis have shown significant beneficial effects on brain regeneration, e.g., improving functions lost by injury or disease. However, it seems that the efficiency of endogenous neurogenesis supported by extrinsic stimuli is not sufficient for the regeneration of large brain deficits.

DIFFICULTIES AND FUTURE PERSPECTIVES ON THE USAGE OF STEM CELLS IN THE CLINIC

The best pre-clinical results regarding the use of stem cells to treat nervous system diseases have been obtained with an early administration of cells following neuronal injury, aiming to inhibit the initial inflammatory response and to activate the immune cells. There is little evidence supporting significant benefits when cells are administered after one week (Woodcock and Morganti-Kossmann, 2013). Currently, the clinical usage of autologous MSCs is limited, mainly due to the difficulty of generating large amounts of cells to treat the patient in a short period of time. The high economic cost also constitutes a significant hindrance, especially when it comes to generating, processing and storing stem and progenitor cells before administering them to the patient. Other technical aspects that are worth considering before employing MSCs in the clinic include (Clement *et al*., 2017): i) difficulty of standardizing isolation protocols; ii) MSCs heterogeneicity, which affects their *in vitro* expansion; iii) long-term expansion of a limited number of clones with loss of multipotency; iv) choice of the route of administration, which can alter the distribution or localization of MSCs in the damaged tissue; v) dose and time of patient's follow-up; or vi) spontaneous transformation of MSCs in other cell types during the proliferative phase, including tumor cells. Although the main objective of these therapies is functional recovery, there are several unspecific effects that stem and progenitor cells can exert, like stabilization of the blood-brain barrier or brain edema in case of trauma. In order to ensure the safety and efficacy of the treatment, monitoring should be conducted by combining magnetic resonance imaging to show the brain injury and perfusion, the study of biological parameters such as systemic concentration of pro- and anti-inflammatory cytokines, and neurological tests that allow a comprehensive assessment of the neurological state of the patient.

In neuronal lineage-differentiated cells, not only the genome must be preserved, but also the epigenetic pattern must be carefully considered in order to certify the identity of cells after differentiation so as to guarantee that the cell type used at the beginning of the research is the same as the one transplanted into the patient. Neuronal stem cells can turn into neuronal tumor cells due to epigenetic and genetic modifications, first transforming differentiated cells into more primitive ones, and finally into tumor cells (Achanta *et al*., 2010). However, using autologous cell sources diminishes the risk of malignant transformation. Further studies are required to elucidate the mechanisms underlying stem cell transformation, which will significantly contribute to regulate cell destination and oncogenesis inhibition.

CONCLUSION

It is worth noting that, in spite of promising results from a large number of pre-clinical and clinial trials, MSCs therapies still have significant limitations. It is necessary to isolate and

culture homogeneous populations of MSCs, improve the differentiation efficiency and regeneration rate, establish an optimal transplantation methodology and ensure long-term biosecurity after transplantation. It is known that the process of neuronal differentiation is mostly controlled by changes in gene expression, and thus understanding the genetic behavior of MSCs during differentiation would help to create a more effective approach to cell therapy. To date, despite several methods to stimulate neurogenesis -with notable benefits for the patient- are known, their efficiency is not enough to repair the neuronal damage secondary to severe nervous system diseases. In order to restore the normal function of the damaged tissue, conventional therapeutic approaches may not be sufficient. In this regard, combining tissue engineering, pharmacology and classic rehabilitation could be the most promising strategy. Moreover, understanding the regeneration mechanisms of the nervous tissue will allow to coordinate and improve these therapies. New *in vivo* and *in vitro* studies are needed to identify the molecular interactions between the cell graft and the host, eventually leading to a better translation to the clinic from a responsible and accesible perspective.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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