Diffentiation 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
division capacity than ESCs (Illic and Polak, 2011). PSCs are multypotent cells that derive from perinatal extraembryonic tissues and share properties with ESCs and ASCs (Si et al., 2015). The latter, i.e., ASCs, are multypotent cells present in most adult tissues, where they play a key role in tissue regeneration (Nadig, 2009; Mahlia, 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 (Scureri 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 multypotent 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., 2013), 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 venlafaxine (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
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
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.

**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-Azacyline    | 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; HDPS, 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.

**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 al., 2015).
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             |
| 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 scaffold | 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 autologous BM-MSC combined with H2O2 and HC016 cells | 1-2/46 | 2017 | Ongoing | Spinal cord injury |
| NCT0308565/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 |
| 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 (1 year) | 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.
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 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 months. 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 (NCT03177398). Similar results have been found in other clinical trials (NCT02054208, NCT01547699).

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-Kosmann, 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 heterogeneity, 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 clinical 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 accessible perspective.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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