Mesenchymal stem cells in neurological diseases

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Due to the limited capacity of the CNS for regeneration, more effective treatment of chronic degenerative and inflammatory neurological conditions, but also of acute neuronal damage from injuries or cerebrovascular diseases, could be only achieved, theoretically at least, by stem cells that may have the potential to either regenerate or to support the survival of the existing, partially damaged, cells. A small number of stem cells are found in the adult brain in very specific regions, but this intrinsic stem cell repertoire is rather small and does not contribute significantly to the repair of damaged tissues. Transplantation of stem cells has long been suggested as a possible logical approach for repair of the damaged nervous system. Embryonic cells carrying the pluripotent and self-renewal properties represent the prototype of stem cells, but there are additional somatic stem cells that may be harvested and expanded from various tissues during adult life, such as the mesenchymal stem cells (MSC), which offer several practical advantages for the clinical application. MSC can be obtained from every adult and there are effective culture protocols for their expansion to large numbers for clinical uses. They seem to carry fewer risks for malignancies and some initial indications of their short-term safety (upon system delivery), in clinical settings, exist in the literature. Therefore, in most of the registered clinical trials with stem cells, MSC is the primary stem cell population used. This review summarizes the rationale, the mechanisms and the worldwide clinical experience with MSC, in neurological and other diseases.

Keywords: clinical trials • immunomodulation • mesenchymal stem cells • neurological diseases • neuroprotection • stem cells • transdifferentiation

Stem cells as an option for treatment of inflammatory & degenerative neurological diseases

A logical approach for induction cell-protection or neuroregeneration seems to be with the external administration of stem cells, to promote the rebuilding of the affected tissues or to protect the partially affected cells (e.g., in the case of ischemic penumbra or partial inflammatory damage, such as multiple sclerosis [MS]) and prevent their complete degeneration. Such efforts using a plethora of stem cells have been the focus of regenerative medicine research during the last decade. Cell replacement therapy may theoretically aid in halting disease progression in degenerative CNS diseases, where pharmacological interventions are no longer effective or are unavailable.

Over the last few years, convincing evidence has accumulated showing the potential of various stem cell populations to induce regeneration in animal models of acute neuronal injury (such as following vascular events or acute traumatic injury) [1-3], inflammatory neurological autoimmune conditions [4-6], primary CNS degenerative diseases (such as Parkinson's disease [PD], Huntington's disease [HD],

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multiple system atrophy [MSA], amyotrophic lateral sclerosis [ALS] and Alzheimer's disease) and genetic diseases [7-15].

A review of the literature through the Pubmed website shows the phenomenal increase of published research papers in the field of stem cells during the last decade. The number of published papers related to stem cells rose from approximately 4000/year to more than 30,000/year, in the last 12 years. Specifically, the number of published research papers dealing with mesenchymal stem cells (MSC) rose from 200/year a decade ago, to more than 6000/year last year.

There are various types of stem cells. Embryonic stem cells (ESCs), are stem cells derived from the undifferentiated inner cell mass of the blastocyst; these cells are pluripotent, that is, they have the potential to differentiate into all cell types of the three germ layers: ectoderm, endoderm and mesoderm [16,17], and may therefore carry the potential for neural cell replacement [18]. Human ESC transplantation holds the risk of uncontrollable proliferation that may lead to cancer development, and it therefore does not provide a first line option for clinical applications.

Adult stem cells

The discovery of the existence of stem cells in various tissues (including the CNS), during adult life, expanded the horizons for clinical experimentation with these types of stem cells, without the above-mentioned risks [19-22]. Adult stem cells (ASCs) are found in several tissues in the body, such as the bone marrow (BM), the adipose tissues, the muscles and the umbilical cord. The state of these cells, either being committed or undifferentiated, depends on the tissue they reside in. Their main function is to maintain the steady state of the organs and possibly induce regeneration, upon injury of the host tissues [23-29]. Despite the potential of ASCs, ESC hold unique and preferable stem-cell properties as compared with ASC. Generally, ASC have less 'stemness' than ESC; ESC can produce almost every cell type, whereas the differentiation ability of ASC is usually limited towards the cells types of the niche where they are hosted. In addition, ESC are capable of unlimited division when placed in culture, whereas ASC do not hold such property (at least not to the same degree) [30,31].

In the last few years a new type of stem cell – the 'induced pluripotent stem cell' – became the focus of stem cell research [21,32]. Researchers were able to successfully reprogram mouse fibroblasts into cells that are very similar to embryonic stem cells, in terms of their differentiation potential, although they are generated from adult committed somatic cells [21,32].

Naturally, the first type of stem cells that seems to be relevant for neurodegenerative therapeutic approaches in neurological diseases is the neuronal type of ASCs, that is, the ASCs that reside in the CNS. Neural stem cells (NSCs) have the advantage of being naturally neuralized and there is no need for external manipulations to drive them to neuroectodermal commitment [20,33-36]. However, transplantation of fetal NSCs into adult brain tissue is coaxed with scientific and ethical hurdles. In addition, prolonged culturing of NSCs leads to a bias towards a glial differentiation pattern, at the expense of neuronal differentiation, which may significantly reduce the therapeutic potential of NSCs in diseases where neurodegeneration dominates and neuronal replacement is essential [37]. Additional problems associated with the possible clinical application of NSCs in neurological diseases include the difficulty in their isolation (fetuses are needed), the difficulty to produce large numbers of NSCs in cultures and the possible risk for - at least partial - rejection, upon transplantation.

Another source of ASCs, actually representing the greater pool of such stem cells, is the BM. The BM compartment contains mainly the hematopoietic stem cells, which constantly renew all the blood cells. An additional stem cell population residing in the adult BM, is that of the MSC. MSCs were shown to carry the ability to promote neuronal repair (through transdifferentiation or fusion with the existing cells), to protect damaged neuronal tissues (neuroprotection) and to downregulate the immune responses both *in vitro* and *in vivo* [38].

MSC

At the beginning of the 20th century, a reciprocal relation/interaction/collaboration between newly forming blood components and the mesoderm during embryogenesis was suggested. Maximow and colleagues, showed the importance of the marrow stromal tissue in the development and homeostasis of blood and hematopoietic tissues [39]. Later, Friedenstein et al. described for the first time that a population of stromal cells could be separated from BM bulks by adhesion to culture plastic dishes [40]. These cells were defined as fibroblastic with the ability to generate fibroblast colony-forming units [41]. In the beginning of 1990, Caplan and colleagues postulated that there is a subpopulation of the marrow stroma linked to mesenchymal tissue formation [42]. These cells were shown to give rise to mesodermal tissues as bone, fat and cartilage [19]. MSC do not carry specific cell markers; however the International Society for Cell Therapy defined MSC as cells negative for CD34, CD45, CD14 and HLA-DR, and positive for CD73, CD90 and CD105 [43].

Various studies have depicted two new roles of MSC: the ability to transdifferentiate into cells of endodermal

and ectodermal origin [44-46], including possible neural transdifferentiation [4,47-55] and immunomodulating properties [56]. Several studies have shown that under different culture conditions, whether by the use of growth factors or by chemical agents (or both), MSC can differentiate into neural, glial and astrocytic-like cells in vitro [4,48]. In addition, neurotrophic and neuroprotective effects of MSC were also documented by several in vitro and in vivo studies. MSC were found to express BDNF and NGF, which may support neuronal cell survival and induce nerve regeneration [57]. In in vitro studies, MSC were found to induce survival and neurite outgrowth in SH-5Y5Y neuroblastoma or other neuronal cell lines [58-60]. The second, newly discovered property of MSC was that of immunomodulation. MSC were found to suppress the proliferation and downregulate/manipulate the function of T and B lymphocytes as well as NK cells [61].

During recent years, the plasticity and transdifferentiation potential of MSC were also widely investigated, mainly in vitro. This potential might contribute to remyelination and myelin recovery in demyelinating disorders. In an animal model of induced focal demyelinated lesion of the spinal cord, intravenous (iv.) or intracerebral injection of MSC resulted in remyelination [62]. In a study by Inoue et al., mononuclear cells isolated from the BM were transplanted either intravenously or focally into rats with a demyelinated lesion of the spinal cord. Both routes of administration were shown to be effective in inducing remyelination [62]. Despite several studies suggesting the possible transdifferentiation potential of MSC into cells from the three germ layers [48,63], this issue is still debatable and controversial [64,65]. It has been suggested that the morphological changes and the positive immunoreactivity for neural markers in cultured MSC under chemical induction might be attributed to cellular toxicity, cell shrinkage and cytoskeletal changes [65]. Furthermore, it remains unclear whether these neural-like cells display any functional properties of neurons or glial cells. Additional doubts were raised regarding the in vivo transdifferentiation of MSC upon transplantation in animal models [66,67]. Due to this skepticism regarding the transdifferntiation potential of MSC and its role in tissue regeneration, the main mechanism of amelioration of tissue injury by MSC, is believed to be exerted through paracrine and endocrine mechanisms. A large number of cytokines and other immunoactive molecules are known to be secreted by MSC and seem to be involved in the immunomodulatory effects of MSC [61,68-70], including the regulation of the activity of lymphocytes, NK cells and macrophages [69]. MSC also produce angiogenic factors such as VEGF and SDF-1 [71], anti-apoptotic factors such as

IGF, VEGF, HGF and Akt-1 [68,72] and antioxidative factors such as superoxide dismutase [73]. The reported neurotrophic and neuroprotective effects of MSC may be attributed to the latter group of secreted factors, as discussed later.

In vitro neuroprotective features of MSC

Several studies suggested that the neuroprotective potential of MSC is mediated by the production of neurotrophic factors that support neuronal cell survival, induce endogenous cell proliferation and promote nerve fiber survival and even regeneration at sites of injury. For in vitro studies, several neural-like cell models (e.g., PC12 cells, dorsal root ganglion [DRG] cells, SH-SY5Y neuroblastoma cells) were utilized to depict the neuroprotective features of MSC. In a study by Scuteri et al., MSC obtained from adult Sprague-Dawley rats were co-cultured with DRG post-mitotic sensory neurons obtained from rat embryos at day E15 [74]. Co-cultures were maintained for 2 months. The co-culture with MSC allowed long-time survival and maturation of the DRG cells. The degree of survival and maturation of the DRG neurons was significantly lower when fibroblasts were used in the co-culture instead of MSC [74]. In a recent study by the same group it was found that MSC are able to prolong the survival of DRG neurons mainly by inhibiting proteolytic enzymes, and in particular the pathway of metalloproteinases, a group of proteins that are involved in many neuronal processes, including their survival [59]. Lu et al., used MSC isolated from adipose tissue to evaluate their neuroprotective potential on PC12 cells challenged with glutamate to cause excitotoxicityinduced apoptosis [60]. In this setting, MSC secreted neurotrophic factors including VEGF, HGF, BDNF and NGF. Addition of MSC-conditioned medium on the culture, had a protective effect on excitotoxicityinjured PC12 cells, as indicated by the increased cell viability, decreased number of TUNEL-staining positive nuclei and lowered caspase-3 activity [60]. In addition, co-culture of MSC with SH-SY5Y neuroblastoma cells enhanced the survival and neurite outgrowth of these cell lines [57]. In a study by Crigler et al., screening of cDNA library of human MSC was performed and revealed a high expression of transcripts encoding NGF and BDNF [57].

In vivo neuroprotective features of MSC

The indications that MSC may trans-differentiate into neural-like cells [4,47,48] and their ability to induce neurogenesis and neuroprotection [4,57,68], support the possibility that MSC-based therapy may be efficacious in the management of neurological diseases. In general, MSC seem to share with other types of stem cells the property of inducing and promoting a neuroprotective and neurotrophic environment, which was described by an elegant and pioneering study by Teng and colleagues [75]. In this study, the researchers utilized a scaffold seeded with NSC to promote functional recovery after spinal cord injury. The positive effect of the scaffold loaded with NSC on the neuronal repair and recovery was attributed to trophic effects of the NSC, rather than cellular replacement at the site of injury [75]. In the case of MSC, similar to the effects of other types of ASCs, the putative mechanisms of neuroprotection/neurorepair may include the production of neurotrophic factors that support neuronal cell survival, the induction of endogenous neuronal stem cells proliferation and the promotion of neuroregeneration at the site of injury [56]. These neuroprotective effects were evaluated in different animal models [56]. In a model of injured neurons of the optic tract in rats [76], it was found that MSC exert neuroprotection leading to improvement of the survival of a significant proportion of the axotomised retinal ganglion cells. The MSC used in this study were found to secrete immunomodulatory and neurotrophic factors including TGF-B, CNTF, BDNF and NT-4. In a model of induced focal demyelination of the spinal cord, iv. and intracerebral infusion of MSC resulted in remyelination [62]. In a model of stroke, the iv. administration of MSC resulted in improving functional recovery while reducing the apoptosis of cells in the injured tissue. Moreover, an increase in the expression of basic FGF and endogenous neurogenesis was observed [77]. In other studies, hippocampal administration of MSC in immunodeficient mice stimulated the proliferation, migration and differentiation of the endogenous NSCs, which survived as differentiated neural cells via their secretion of various trophic factors, including NGF, VEGF, CNTF and FGF-2 [78].

Although all of these and other studies presented potent and clear neuroprotective effects in different neurological disease models, researchers should take in to consideration the limits and questions still open regarding the effect of these cells in vivo, which may be very relevant for later stages with human use. There are still open questions regarding the survival and viability of the engrafted cells with the different injured tissues; the doses that should be used to get the maximum effect and how these cells act within the niche they are delivered to. In a study by Lepski and colleagues they evaluated the survival and neuronal differentiation of MSC administrated into the rodent brain [79]. They found that survival and differentiation of MSC is strongly dependent upon a permissive microenvironment. Identification of the proneurogenic factors present in the hippocampus could subsequently allow for the integration of stem cells into 'restricted' areas of the CNS [79]. Moreover, several studies indicate that ageing can affect the proliferation and differentiation capacities of MSC. It has been shown that long-term culture may result in senescence, loss of differentiation capacity and ultimate growth arrest [80–81]. It should be noted that different results concerning *in vivo* behavior of MSC can be attributed to species, gender and donor age of animals used in these studies, as well as differences in cell culture conditions.

In vitro immunomodulatory features of MSC

As reported previously and by our studies, MSC have important immunomodulating properties; they were found to suppress *in vitro* T- and B-cell functions, as well as NK cells [56]. MSC suppress the proliferation of both CD4+ and CD8+ T lymphocytes, as well as of NK cells, whereas they did not show an equal effect on the proliferation of B lymphocytes [84]. Although the exact mechanisms of the immunosuppressive effects of MSC are not yet fully clarified, two main mechanisms have been suggested:

- Humoral mechanisms, involving the production of soluble factors;
- Cell-to-cell contact dependent mechanisms [69,85].

Several soluble factors have been suggested to be involved, including TGF- β 1 [86], IFN- γ [84], indoleamine 2,3-dioxygenase (IDO) [87] and prostaglandin E2 [88].

In vivo immunomodulatory effects of MSC

The immunomodulatory and neuroprotective properties of MSC in vitro were confirmed by us and other groups mainly in the model of experimental autoimmune encephalomyelitis (EAE), an induced autoimmune inflammatory demyelinating paralytic disease that serves as an animal model of MS. The in vivo immunomodulatory effects of MSC were also documented in additional animal models, such as in GVHD models and other induced autoimmune diseases [89,90]. Based on their in vitro properties, one could assume that MSC may downregulate in vivo the autoimmune attack to myelin antigens in this model and possibly promote nervous tissue repair or neuroprotection. Zappia and colleagues demonstrated that the injection of syngeneic MSC, indeed ameliorated the clinical severity of the disease in a mouse model of acute monophasic EAE (induced in C57bl mice using MOG₃₅₋₅₅) and reduced demyelination and leukocytes infiltration of the CNS [5]. The findings were explained by the induction of T-cell anergy by MSC treatment. In the study by Zhang et al., it was shown that iv. administration of MSC could suppress the disease in a relapsing-remitting model of EAE induced in SJL mice [6]. MSC migrated into the CNS where they promoted

BDNF production and induced proliferation of a limited number of oligodendrocyte progenitors. Evidence of neuroprotection in EAE following MSC treatment was also shown by Chopp et al., accompanied by indications of in vivo neural differentiation of the transplanted cells [91]. Gerdoni et al. used in his study the relapsing-remitting model of EAE that was induced with PLP in SJL mice [92]. Intravenously treated mice with MSC had a milder disease and developed fewer relapses than the untreated control animals [92]. These results were coherent with histopathological findings that included decreased inflammatory infiltrates, and reduced demvelination and axonal loss in the brains of the treated mice. No evidence for in situ transdifferentation of the transplanted cells was documented [92]. In studies from our group, a model of chronic EAE (more reminiscent of human MS) was used and the effect of MSC transplantation via additional routes (both iv. and intraventricularly, directly into the brain and cerebrospinal fluid [CSF]), was evaluated. Although in previous studies the suggested mechanism of suppression of EAE following iv. injection of MSC was suggested to be that of induction of peripheral immunomodulation/anergy [5,92], in our experimental setting, we verified the advantages of direct injection of MSC into the ventricles of the brain, where they induced a more prominent reduction in infiltrating lesions, indicating an additional *in situ* immunomodulation. The peripheral immunomodulatory effects of MSC are likely equally important and the migratory ability of these cells to the lymph nodes and other lymphatic organs (when injected intravenously), shown in this work, argue in favor of such – additional – peripheral mechanisim. GFP-labeled MSC injected via the iv. route, migrated into the lymph nodes, spleen, lungs and brain. These findings are in agreement with previous studies regarding the biodistribution of iv.-injected MSC [93,94]. Long-term engraftment of the cells was evidenced and the injected cells were viable after 30-40 days of transplantation.

It is therefore logical to assume that the main immunomodulatory activity of MSC is exerted in the peripheral lymphoid organs where MSC migrate following iv. administration, inhibiting the homing of T cells in the CNS [4.5]. In addition to these peripheral effects, MSC migrating to the CNS following iv. and intracerebroventricular injection may also further modulate the local CNS autoimmune process, stimulate endogenous neurogenesis and protect neurons and oligodendrocytes, by similar paracrinic and neurotrophic mechanisms [4].

The above-discussed *in vivo* experiments in EAE utilized the model of autologous MSC transplantation. This setting is logically considered more convenient

for clinical transplantation since it does not hold any risks of rejection of the transplanted cells. However, in clinical reality, it is not always feasible that the patient can serve as a donor, due to his/her progressed medical condition. Moreover, if genetic factors are involved in the pathogenesis of MS, it would be preferable to avoid transplantation of stem cells carrying a putatively defective genome. Therefore, the possibility of allogeneic MSC transplantation (using MSC obtained from healthy donors) might be considered, especially since MSC were shown to 'escape' rejection by 'masking' parts of the immune response, such as the complement system [95]. Three main mechanisms contribute to this 'immune-privileged' status of MSC:

- MSC are hypoimmunogenic, often lacking MHC-II and costimulatory molecules expression [69];
- MSC prevent T-cell responses indirectly through modulation of the dendritic cells, and directly through downregulation of the NK, CD8+ and CD4+ T-cell functions [56];
- MSC induce a suppressive local microenvironment through the production of prostaglandins and IL-10, as well as by the expression of indoleamine 2,3,-dioxygenase, which depletes the local milieu of tryptophan [56].

A possible attractive explanation of the reported efficacy of allogeneic MSC-transplantation may involve a mechanism of a 'single hit' (probably immunomodulatory or neurotrophic, in its nature), directly following the injection of MSC, and before any putative rejection process may take place. In support of such a possibility come recent studies, which consistently reported that MSC induce significant beneficial clinical effects and potent immunomodulation and neuroprotection mediated by the production of neurotrophic factors and/or through the recruitment of local/intrinsic CNS precursor cells [96-99] or paracrinic mechanisms. Rafei and colleagues demonstrated that the suppressive effect of MSC on the encephalitogenicy of Th17 CD4-T cells was achieved through a metalloproteinasemediated paracrine proteolysis of CCL2, leading to an increase in the programed cell death, mediated by ligand-1 (PDL1) [97]. Indeed, others reported that interactions between PDL1 on MSC and PD1 on T cells are involved in the inhibition of T- [100] and B-cell proliferation [101], suggesting an interaction between MSC and lymphocytes that requires both cell contact and paracrine effects. Some of the immunomodulatory effects of MSC are species specific as indicated by the finding that IDO is involved in the immunosuppressive activity of human MSC and inducible NO synthase in that of mouse MSC [102].

Preclinical experience with MSC in neurological models

In recent years a lot of studies were conducted to evaluate the therapeutic potential of MSC in different animal models of different neurological diseases, including cerbrovascular diseases, neurodegenerative disease and others. In this section we present some of the experience and knowledge accumulated concerning several neurological diseases.

Cerebrovascular diseases

The use of MSC in different models of cerbrovascular diseases, especially stroke, has been documented in various studies in several models [3,77,103–105]. In a very recent study by Song and colleagues, the authors demonstrated that MSC transplantation has the potential to repair the ischemia-damaged neural networks and restor lost neuronal connection [106]. The recovered circuit activity contributed to the improved sensory motor function post-transplantation. In a model of intracerebral hemorrhage in rats, human MSC (derived from adipose tissue) were transplanted via femoral iv. administration [107]. The study demonstrated that the transplanted cells were detectable at the injured tissue. Functionally, the treated animals showed impressive improvement as evaluated by behavioral tests [107].

PD

Dezawa et al. succeeded at inducing the production of dopamine neurons derived from either rodent or human BM stromal cells, which were transplanted into the striatum of a PD model rat [108]. Transplanted rats demonstrated a substantial decrease in apomorphine-induced rotation behavior, and nonpharmacologic-behavior tests. In the grafted striatum, migration of the labeled MSC that expressed neurofilament and tyrosine hydroxylase (TH), was evidenced. Histopathological evaluation demonstrated the production of dopamine in the transplanted brains. In a recent study by Inden et al., a model of PD was generated in NOD/SCID mice using rotenone [109]. In this model, human MSC were transplanted by iv. delivery. Human nucleus (a specific marker of human cells) stained cells, were observed in the striatum of rotenone-treated mice transplanted with stem cells. These human nucleus-positive cells expressed the dopamine production enzyme, TH. In addition, α-synucleinpositive/TH-positive cells in the substantia nigra pars compacta decreased significantly following stem cell transplantation. Histopathological analysis also revealed that chronic exposure to rotenone decreased glial cell line-derived neurotrophic factor immunoreactivity and that each stem cells administration further enhanced this effect.

Several studies utilized 'engineered' MSC, which were enhanced to produce more neurotrophic factors such as BDNF, GDNF, VEGF and others, into models of PD [110-112]. In the study by Sadan et al., MSC were developed into cells (NTF-SC) producing and secreting high levels of factors such as BDNF and GDNF [110]. NTF-SC, were transplanted on the day of 6-OHDA administration, and amphetamine-induced rotations were measured as a primary behavior index [110]. The transplanted cells ameliorated amphetamine-induced rotations remarkably. Moreover, the transplantation inhibited dopamine depletion to a level of 72% of the contralateral striatum [110]. A histological assessment demonstrated that the cells induced regeneration in the damaged striatal dopaminergic nerve terminal network [110].

HD

Several recent studies evaluated the therapeutic potential of MSC in HD models [113-115]. In the study by Lin et al., HD mice that received human MSC transplantation demonstrated a significant improvement in motor function and increased survival [113]. Transplanted MSC survived, and induced neural proliferation and differentiation in the lesioned striatum. Moreover, the transplanted MSC showed indications of neural differentiation, neurotrophic and antiapoptotic effects [113]. Sadan et al. demonstrated that intrastriatal transplantation of neurotrophic factorsecreting human MSC improves motor function and extends the survival of R6/2 transgenic (HD) mice [114]. In another study, striatal transplants of MSC elicited behavioral and anatomical recovery in the quinolinic acid induced model of HD [115].

MSA

Experimental studies demonstrated that human MSC had a protective effect in animal models of MSA [13,14]. Recently, Stemberger and colleagues confirmed the neuroprotective effects of MSC in a transgenic mouse of MSA [116].

ALS

In a human SOD1 mutant mouse model, intrathecal (it.) administration of MSC ameliorated the decline of motor performance and induced neuroprotection [117]. Similar results were reported in a rat model of ALS [118]. In a recent study by Uccelli and colleagues, iv. MSC administration in mice expressing SOD1 carrying the G93A mutation (SOD1/G93A) found to improve survival and motor function [119]. This study evaluated several parameters post-transplantation including survival, motor abilities, histology, oxidative stress markers and [³H]D-aspartate release in the spinal cord. Both clinical evaluation and pathological findings support the efficacy of the transplanted MSC in this model of ALS [119].

MS

Pivotal studies with neuronal stem cells have shown a significant beneficial clinical effect in mice with experimental autoimmune EAE (the animal model of MS) [120,121]. Subsequently, ESCs and other types of ASCs were tested in various models of EAE, and especially MSC [4-6,122]. The additional scientific rationale for using MSC in EAE and MS derives from the reported strong immunomodulatory effects of these cells [4,5,123]. MSC injection, either iv. or into the CSF, strongly suppressed the clinical and pathological signs of EAE. Most importantly, remyelination was evident in these MSC-treated animals, accompanied by impressive neuroprotection [4,123]. These experiments were extensively described above.

Muscle diseases

Human embryonic stem cells, cultivated to enrich in mesenchymal precursors were shown able to differentiate into myotubes in vitro and regenerate a small proportion of the injured skeletal muscle in immunodeficient mice [124]. Other mesodermal progenitors isolated from differentiating ESCs, showed to induce activation of myogenic transcription factors in vitro and good differentiation in dystrophin fibers upon transplantation into dystrophic muscle. Injected mice also showed an improvement in the contractility force [125]. MSC have been also tested in acute and chronic muscle wastage, but results were controversial. Human MSC, injected into the tibialis anterior of mdx-mice, efficiently produced new, functional myofibers, without any sign of fusion [126]. Following BM transplantation in dystrophic mice, BM stromal cells were able to migrate and contribute to the formation of new muscle fibers

Clinical experience with MSC

Due to the above mentioned practical advantages of MSC, BM MSC are, to date, the most commonly used stem cell population in clinical trials, with the exception of hematopoietic stem cells (Table 1), especially regarding the treatment of neurological diseases. As these cells seem to be able to cross the blood-brain barrier, the need for invasive intracerebral surgery can be avoided in neurological diseases and, at least, the peripheral systemic administration has been proven a safe and efficient way for cell delivery in humans [127]. In a recent meta-analysis of clinical trials utilized intravascular delivery of MSC (intravenously or intra-arterially) testing immediate events (e.g., toxicity or fever),

organ system complications, infection, and long-term adverse events (e.g., death or malignancy) it was found that MSC administration is safe. The data revealed from randomized control trials did not detect an association between acute infusional toxicity, organ system complications, infections or deaths [127]. However, the extent to which MSC can be directed to a neural or other than mesodermal cellular fate either *ex vivo* or *in vivo* following transplantation is still a point of controversy.

Clinical grade production of human MSC

For clinical trials, isolated MSC should be produced according to good manufacturing practice. The culture process should be reproducible and efficient. According to guidelines of the International Society for Cell Therapy the minimal criteria to define human MSC are:

- MSC must be plastic-adherent when maintained in standard culture conditions using tissue culture flasks;
- More than 95% of the MSC population must express CD105, CD73 and CD90, as measured by flow cytometry. In addition, these cells should be negative for the CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA class II markers;
- The cells must be able to differentiate to osteoblasts, adipocytes and chondroblasts under *in vitro* differentiating conditions [43].

Safety is the major concern during the culture process as well as quality control of these cells. Several levels of quality control during the production of MSC are necessary. These should include various microbiological tests (including bacterial, viral and mycoplasma detection and LPS levels) and genetic-karyotype testing to exclude contaminations and genetic transformations or instability. The main source for MSC used for clinical trials is the BM compartment [128], but MSC can also be harvested by other tissues such as the adipose tissue and the umbilical cord [129]. The culture process is also an issue of major consideration, since the culture can be started from either unfractioned (whole BM) or fractioned cells (mononuclear fraction of BM after gradient density). The medium of choice for culturing is of equally high importance for the efficacy and safety of MSC. Generally, Dulbecco's Modified Eagle's medium or alpha-minimal essential medium are used for culturing with the addition of fetal bovine serum, fetal calf serum, human serum, plasma and platelets lysates, with the addition of growth factors such as FGF. The use of serum is one of the controversial parameters of the culture having an impact on the batchto-batch variability and the risks of contamination. The use of chemical-defined, xeno-free, serum-free medium may provide a preferable solution. The final product of MSC that will be used for transplantation should be

Table 1. List of some published trials with mesenchymal stem cells in various neurological diseases.												
Authors	Disease	Cell type	Route of administration	Results	Serious side effects	Ref.						
Koc et al.	Hurler syndrome, Metachromatic leukodystrophy	Allogenic MSC following BM transplant	iv.	No clinical improvement Improvement in NCV	None (related to MSC)	[153]						
Bang <i>et al</i> .	MCA CVA	Autologous MSC	iv.	Some clinical improvement	None	[132]						
Lee <i>et al.</i>	MSA	Autologous BM MSC	Intra-arterial and repeated iv.	Significant clinical and radiological improvement	Unknown	[143]						
Mazzini <i>et al</i> .	ALS	Autologous BM MSC	High thoracic spinal cord	Slowed disease progression	None	[165,166]						
Karussis <i>et al</i> .	ALS	Autologous BM MSC	it. and iv.	Stabilization	None	[144]						
Karussis <i>et al</i> .	MS	Autologous BM MSC	it. and iv.	Improvement and immunomodulatory effects	None	[144]						

ALS: Amyotrophic lateral scierosis; BM: Bone marrow; CVA: Cerebrovascular accident; it.: Intrathecal; IV.: Intravenous; MCA: Middle cerebral artery; MS: Multiple scierosis; MSA: Multiple system atrophy; MSC: Mesenchymal stem cells; NCV: Nerve conduction velocities.

> tested microbiologically to detect the presence of aerobic and anaerobic microbes, mycoplasma and endotoxin levels and genetically (karyotypic profile and stability) before the administration to patients. Viability of the cells should also be checked and be more than 80%.

> The lack of standardization for MSC isolation and culturing has delayed the progress in the field of MSC use in human diseases since the comparison of results from different laboratories was sometimes impossible. Any differences in the culture conditions might selectively favor the expansion of different subpopulation. Based on morphology, several distinct cell types can be distinguished: spindle-shaped fibroblast-like cells, large flat cells and small round-cell subpopulations [130]. The quality of preparations from different protocols vary and the cell products are therefore heterogeneous. The source and quality of the starting material, culture media, the use of animal serum, cytokines supplements, initial seeding cell density, number of passages upon culture and even type of cell culture dishes, all have a significant influence on the cell populations that are finally produced. Therefore, there is a need for the development of standardized cell culture reagents and products, common guidelines and standards (standard operating procedures) for MSC preparations and of molecular and cellular markers to define subpopulations with different potentials. Only by these standardizations can we truly evaluate the potential of MSC in the treatment of different human diseases.

Clinical experience with MSC in neurological diseases: pilot clinical trials

Cerebrovascular diseases

An open-label, observer-blinded trial evaluated the long-term (5 years) safety and efficacy of iv. MSC

transplantation in 85 patients with severe, middle cerebral artery territory ischemic stroke [131]. This study showed that the cumulative survival at 260 weeks was 72% in the MSC group and less than half (34%) in the control group. Significant side effects were not observed following MSC treatment. The occurrence of co-morbidities including seizures and recurrent vascular episodes did not differ between groups [132]. The follow up modified Rankin Scale in the control group score was decreased, whereas the number of patients with a low modified Rankin Scale (0–3) increased in the MSC group. The clinical improvement in the MSC group was associated with serum levels of SDF-1 and the degree of involvement of the subventricular region of the lateral ventricle [131].

Bang and colleagues transplanted autologous MSC in a Phase I/II clinical trial in 30 patients with middle cerebral artery cerebral infarcts [132]. An iv. infusion of autologous MSC was given to five patients and the rest served as control. No adverse cell-related effects were observed, and some clinical improvement was detected at 3, 6 and 12 months in the patients who received MSC, compared with the control population. Cumulatively, these human trials indicate the feasibility of stem cell therapy in stroke, but the currently existing clinical data are still limited and cannot provide consistent evidence of clinical efficacy [3,103,133].

PD & HD

The first clinical trials in PD were performed in the mid-1980s [134]. In total, close to 500 patients with PD and HD have been treated with neurosurgically implanted, fetal cell transplantation [135-140]. The results were highly variable and the clinical improvement not

sustained. Whilst open-label studies showed functional benefits, the double-blind trials failed to show significant benefit compared with placebo. Moreover, the graft–host connectivity was limited and there were cases of cyst formation and overgrowth [138]. The use of NSCs seems to be less attractive, since the number of dopamine neurons produced by them is relatively low [141,142]. There are no completed studies with MSC but some are underway (Table 2).

MSA

In a recently published study, the efficacy of autologous MSC in patients with MSA-cerebellar (MSA-C) type was evaluated [143]. In total, 33 patients with probable MSA-C received MSC (4×10^7 /injection) via intra-arterial and iv. routes, or placebo [143]. The MSC group had a smaller increase in total and part II UMSARS (unified MSA rating scale) scores compared with the placebo group after 1-year follow-up period. No serious adverse effects that were directly related to MSC treatment were found. However, intra-arterial infusion resulted in small ischemic lesions on MRI [143].

ALS

Mazzini et al. reported in a Phase I clinical trial the safety of MSC transplantation into the high thoracic spinal cord [165]. No transplant-related toxicity or adverse event was documented. Most of the patients had no change in disease progression and two patients showed a slower deterioration of vital capacity parameters and upper limb strength. In a more recent study from our group, 19 patients with ALS were treated with a combined it. and iv. injection of autologous MSC (in total 85 million cells per patient). No significant adverse events were reported and there was a stabilization of the disease during the 6 months of follow up [144]. An additional study is currently underway in our center, with the use of modified MSC enhanced to produce neurotrophic factors (NeuroOwnTM, Brainstorm Therapeutics Ltd; NY, USA) in 24 patients with ALS, using either the it. or the intramuscular route of administration. These early phase trials report promising initial results for MSC transplant, but safety and efficacy are still of concern.

MS

Phase I/II safety studies with MSC or BM-derived cells have been performed in MS [144–146]. Overall, MSC given intravenously or intrathecally were well tolerated, with some preliminary evidence of efficacy [144].

On the basis of the preclinical data from our studies and the cumulative data from other centers, an exploratory clinical trial with autologous BM-derived MSC in 15 patients with intractable MS, was initiated at Hadassah Medical Center (Jerusalem, Israel) [144,147]. In this trial, based on the data in EAE models (indicating two distinct mechanisms of action by the two different routes of MSC administration is most likely), a dual injection of it. (to access the CNS via the CSF) and iv. (to access the systemic circulation) was used to increase the potential therapeutic benefit. In some of the patients, MSC were tagged with the superparamagnetic iron oxide MRI contrast agent ferumoxides (FeridexTM) to track cell migration after local grafting.

Follow up of the patients for 6 months showed that the mean EDSS (disability) score of the transplanted MS patients improved from 6.7 ± 1.0 to 5.9 ± 1.6 . MRI visualized the MSC in the occipital horns of the ventricles, indicative of the possible migration of the labeled cells in the meninges, subarachnoid space and spinal cord. An increase in the proportion of CD4+, CD25+ regulatory T cells, a decrease in the proliferative responses of lymphocytes, and the expression of CD40+, CD83+, CD86+ and HLA-DR on myeloid dendritic cells was observed 24 h post-transplantation.

Since this was a pilot feasibility study, the most important finding was the satisfactory safety profile of autologous BM-derived stem cells administration in patients with MS. None of the patients experienced major adverse effects during the 6- to 25-months follow-up period. The follow-up MRI, 1 year after transplantation, did not show any unpredicted pathology or new activity of the disease. The experience accumulated with iv. administration of MSC in non-neurological diseases have also indicated safety of the procedure [148]. The study in our center (Hadassah Medical Center) also showed a tolerable short-term safety profile of the it. administration procedure of MSC at doses of up to 70 million cells per injection per patient. The it. approach, which was supported by the preclinical data from our group showing that this route of administration could induce superior neurotrophic and neuroprotective effects [4], may be more advantageous for cell-based therapies in neurological diseases, in which the areas of tissue damage are widespread throughout the neuroaxis, since it may increase the possibility of migration of the injected cells to the proximity of the CNS lesions. The injected cells may move with the CSF flow and have higher chances to reach the affected CNS areas. However, the most favorable route of stem cell administration in general and particularly MSC administration in patients with neurological diseases remains debatable.

In the above-described trial, MSC were labeled with iron particles for MRI analysis. Such labeling of MSC with the commercially used paramagnetic material, Feridex, was shown to be safe and had no negative effect on the functional (immunomodulatory and neurotrophic) properties of MSC [149]. It seems therefore that Feridex

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Table 2. List of registered trials with mesenchymal stem cells in various neurological diseases.											
NCT number	Country	Cell type	Phase	Route	Study design	Study results	Notes	Ref.			
Multiple sclerosis											
NCT01377870	Iran	BM-MSC	I/II	iv.	Safety/efficacy	_	Recruiting	[201]			
NCT00395200	UK	BM-MSC	I/II	iv.	Safety/efficacy	Safe Feasible Neuroprotection	Completed	[202]			
NCT01364246	USA	UCB-MSC	I/II	iv.	Safety/efficacy	_	Recruiting	[203]			
NCT00781872	Israel	BM-MSC	I/II	iv./it.	Safety/efficacy	Safe Feasible Immunomodulation	Active, not recruiting	[204]			
NCT01056471	Spain	AT-MSC	I/II	iv.	Safety/efficacy	-	Recruiting	[205]			
NCT01228266	Spain	BM-MSC	I/II	iv.	Safety/efficacy	-	Recruiting	[206]			
NCT00813969	USA	BM-MSC	Ι	iv.	Safety	_	Recruiting	[207]			
NCT01453764	Mexico	AD-MSC	I/II	iv.	Safety/efficacy	_	Recruiting	[208]			
Amyotrophic la	iteral sclerc	osis									
NCT01254539	Spain	BM-MSC	I/II	it.	Safety/efficacy	-	Recruiting	[209]			
NCT00855400	Spain	BM-MSC	I/II	is.	Safety/efficacy	Safe	Completed	[210]			
NCT01494480	China	UC-MSC	II	it.	Safety/efficacy	_	Not yet recruiting	[211]			
NCT01082653	USA	BM	Ι	it.	Safety/efficacy	Not published	Active, not recruiting	[212]			
NCT01142856	USA	BM-MSC	Ι	is.	Safety	_	Active, not recruiting	[213]			
NCT01363401	Korea	BM-MSC	I/II	it.	Safety/efficacy	_	Recruiting	[214]			
NCT01051882	Israel	Modified BM-MSC	I/II	im./it.	Safety/efficacy	-	Recruiting	[215]			
NCT00781872	Israel	BM-MSC	I/II	iv./it.	Safety/efficacy	Safe Feasible	Active, not recruiting	[204]			
Parkinson's dis	ease										
NCT00976430	India	BM-MSC	I/II	Surgical	Safety/efficacy	_	Ongoing, not recruiting	[216]			
NCT01446614	China	BM-MSC	I/II	iv.	Safety/efficacy	_	Recruiting	[217]			
NCT01453803	Mexico	AT-MSC	I/II	iv.	Safety/efficacy	_	Recruiting	[218]			
NCT00875654	France	BM-MSC	II	iv.	Safety	_	Recruiting	[219]			
NCT01501773	India	BM-MNC	II	iv.	Safety/efficacy/ feasibility	Not published	Completed	[220]			
NCT01389453	China	UCB-MSC	II	iv./it.	Safety/efficacy	_	Recruiting	[221]			
NCT01468064	China	BM-MSC/ EPC	I/II	iv.	Safety/efficacy	-	Recruiting	[222]			
NCT01461720	Malaysia	BM-MSC	II	iv.	Efficacy	_	Recruiting	[223]			
NCT00859014	USA	BM-MNC	Ι	iv.	Safety/efficacy	_	Recruiting	[224]			
NCT01297413	USA	BM-MSC	I/II	iv.	Safety/efficacy	_	Recruiting	[225]			
NCT01287936	USA	MSC	I/II	iv.	Safety/efficacy	_	Recruiting	[226]			
NCT01091701	Malaysia	BM-MSC	I/II	iv.	Safety/efficacy	_	Recruiting	[227]			
NCT00473057	Brazil	BM-MNC	Ι	iv.	Safety	Safe Feasible	Completed	[228]			
NCT01453829	Mexico	AT-MSC	I/II	iv.	Safety/efficacy	_	Recruiting	[229]			
NCT00768066	USA	BM-MSC/ BM	I/II	Transend- ocardial	Safety/efficacy	-	Recruiting	[230]			
AD: Alzheimer's dis	AD: Alzheimer's disease; AT: Adipose tissue-derived; BM: Bone marrow; EPC: Endothelial progenitor cells; im.: Intramuscular; is.: Intraspinal; it.: Intrathecal: iv.: Intrave-										

AD: Alzheimer's disease; AI: Adipose tissue-derived; BM: Bone marrow; EPC: Endothelial progenitor cells; im.: Intramuscular; is.: Intraspinal; it.: Intrathecal; iv.: Intravenous; MNC: Mononuclear cells; MSC: Mesenchymal stem cells; UC: Umbilical cord; UCB: Umbilical cord blood. may be used for the tracking of this type of stem cells in clinical applications, without compromising their major functional properties.

Additional clinical trials explored the safety, and therapeutic benefit of it. injection of ex vivo expanded autologous BM-derived mesenchymal stem cells in patients with advanced MS [146]. In the later study, assessment of the patients at 3–6 months revealed an improvement in EDSS score in 5/7, stabilization in 1/7, and worsening in only 1/7 patients. Vision and low contrast sensitivity testing at 3 months showed improvement in 5/6 and worsening in 1/6 patients. These preliminary results indicate additional (to the Hadassah trial) hints of clinical - but not radiological - efficacy and evidence of safety with no serious adverse events. A more recent Phase IIa study in ten patients with secondary progressive MS showed an improvement in visual acuity and visual evoked response latency, accompanied by an increase in optic nerve area, following iv. transplantation of autologous MSC [150]. Although, no significant effects on other visual parameters, retinal nerve fiber layer thickness, or optic nerve magnetization transfer ratio, were observed. This study provides a strong indication for induction of tissue repair with MSC transplantation, in humans.

Neurological diseases due to genetic defects

In the early 1990s, hematopoietic BM transplantations were tried in patients with lysosomal disorders [151,152]. Transplantation of allogeneic BM cells in Hurler syndrome was shown to halt progression of liver and heart abnormalities, but did not induce any beneficial effect on muscle manifestations of the disease. The transplantation was associated with a high incidence of graft failure and morbidity. The efficacy of these transplants is believed to be due to tissue infiltration of donor macrophages and transfer of enzymes into host cells by endocytosis [153]. Preclinical studies showed that in mice with acid sphingomyelinase deficit, MSC transplants delay the onset of neurological abnormalities and extend their lifespan [9,154,155]. Koc et al. infused allogeneic MSC in six patients suffering from Hurler syndrome and five with metachromatic leukodystrophy, following successful BM transplantation from HLA-identical siblings [153]. In four patients with metachromatic leukodystrophy, a significant improvement in nerve conduction velocities was observed but not accompanied by any apparent clinical change.

Muscle diseases

A single case study of a young Duchenne's muscular dystrophy patient, showed that 12 years after BM transplant, donor nuclei were shown to be fused in 0.5% of dystrophic myofibers [156]. In contrast, there were also several negative studies reporting a lack or incomplete muscle repair by MSC or hematopoietic stem cells [157]. In another study, 80% of BM-derived muscle-incorporated nuclei in the transplanted dystrophic mouse were found to be electrophysiologically 'silent' [158]. Hematopoietic cell transplantation alone resulted neither in any skeletal fiber regeneration nor in expression of dystrophin or other muscle genes [159].

Ongoing & in progress clinical trials with MSC in various neurological & other diseases

There are more than 200 ongoing clinical trials related to stem cells in neurological diseases currently registered on the NIH site. The neurological indications are extensive and include MS (n = 23), brain tumors (n = 52, almost all with autologous hematopoietic stem cell transplantation), stroke (n = 18), spinal injury (n = 11), ALS (n = 7), genetic-metabolic diseases and leukodystrophies (n = 15), and also PD, HD, Alzheimer's disease, MSA, CP, peripheral neuropathy, myastheniamyopathies, epilepsy and systemic autoimmune diseases with neurological complications. With the exception of hematopoietic stem cell transplantation, MSC is the most commonly used stem cell population in clinical trials (n = 56). These studies are currently in progress and are performed in countries all over the globe, including the USA, Australia, Canada, Israel, France, Germany, Italy, Ireland, Spain, Norway, UK, Brazil, Egypt, Turkey, Malaysia, India, Iran, Korea, China, Taiwan and Mexico (Table 2).

Unfortunately, in addition to these registered trials, there are numerous private stem cell 'centers' and companies that offer, upon payment of high fees, so-called 'stem cell treatments' (especially with MSC, which are easily obtained and expanded) in various neurological conditions. This is especially prominent in the developed countries where such medical procedures are almost entirely uncontrolled. The exact number of patients who have received such 'stem cell facilities' remains unknown, but is estimated to be several thousands. A recent case of catastrophic EAE, following 'MSC therapy' (of unknown quality), in such 'centers' [160,161], underlines the dangers of such uncontrolled use of stem cells in general and MSC particularly, and the need for the performance of stem cell therapies only under the strict, required, conditions, in well-organized scientific centers.

Conclusion & future perspective

A decade of intensive worldwide preclinical and clinical research has definitely moved forward our understanding of the place of stem cells in neurological diseases; however, the steps that were taken have still not clarified the picture.

Specifically for MSC, the undeniable evidence documented in animal models of MS and other neurological

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diseases, and in small clinical trials, have set a solid ground for the initiation of larger clinical trials testing the therapeutic efficacy of MSC in inflammatory and degenerative diseases of the CNS. The differences in trial design that led to varied outcomes in MSC transplantation for the treatment of GVHD [162], underline the need for harmonization of the protocols used. In addition, controlled studies using suitable clinical and surrogate markers (novel MRI and electrophysiological techniques) are needed to evidence neuronal regeneration and restoration of neurological dysfunction. Expert meetings led to consensus statements and the formulation of guidelines and a stable framework for the organization of multicenter clinical trials in MS and other neurological diseases (STEMS [163] and the International MSCT Study Group [164]). In the near

future, such controlled trials (some of which are currently underway), may provide the missing evidence of efficacy of MSC in neurological conditions, the mechanisms involved, the optimal administration route and dosage of the cells (or number of injections needed) and most importantly, provide long-term safety data.

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Executive summary

- Since damage in the CNS caused during the course of various neurological diseases is usually not reversible and endogenous regeneration is limited, new emerging neuroprotective and neurodegenerative methods aiming to preserve and/or restore neurological function are needed.
- Based on their neuroprotective and neurodegenerative potential, stem cells of various types (e.g., embryonic, adult or inducible) are candidates for such therapeutic approaches.
- Mesenchymal stem cells (MSC) are adult stem cells that were shown to possess neurotrophic/neuroprotective, but also immunomodulatory features and were effective in the treatment of the animal models of various neurological/ neuroimmunological diseases.
- The practical advantages of using MSC in human diseases include the relatively easy techniques to obtain them from adult patients and expand them in culture. However, there is great variability in the methods used for the culture of MSC and in the definition of the final product for injection; this underlines the essential need to standardize good manufacturing practice protocols for the isolation, culture and processing of MSC.
- The use of MSC in clinical practice is not associated with significant ethical hurdles. Early clinical trials with MSC showed the feasibility and safety of using MSC in human diseases.
- Despite the great steps made during the last years and the substantial accumulation of data concerning the mechanisms of action of MSC and the preliminary encouraging results in clinical trials, still much more is unknown than known concerning the application of MSC in the future routine clinical practice. Convincing evidence of efficacy of MSC-based treatment protocols in neurological diseases is still missing and the same holds for the identification of the optimal method of administration, the number of cells and the injection schedule.
- Long-term safety of treatment with MSC is an additional parameter that needs further investigation. Large controlled studies with MSC should, hopefully, provide these data in the next years.

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