

Concise Review: Mesenchymal Stem Cells in Neurodegenerative Diseases

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Key Words. Mesenchymal stem cells • Neurodegenerative diseases • Neurotrophic factors • Immunomodulation • Neurogenesis

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ABSTRACT

Stem cell-based therapies for neurodegenerative diseases aim at halting clinical deterioration by regeneration and by providing local support for damaged tissue. Mesenchymal stem cells (MSCs) hold great potential for cell therapy as they can be efficiently derived from adult tissue, ex vivo expanded in culture and safely transplanted autologously. MSCs were also shown to be able to differentiate toward neural fates and to secrete a broad range of factors able to promote nervous tissue maintenance and repair. Moreover, upon transplantation, MSCs were shown capable of homing toward lesioned areas, implying their potential use as vehicles for therapeutic agents administration. Indeed, various advantageous effects were reported following human MSCs transplantation into rodent models of neurodegenerative diseases, such as neurotrophic factor-mediated protection, enhanced neurogenesis, modulation of inflammation, and abnormal protein aggregate clearance. Per journal style, most nonstandard abbreviations must be used at least two times in the abstract to be retained; NTF was used once and thus has been deleted. Recent studies have also used ex vivo manipulation for enhanced expression of potentially favorable factors, by so exploiting the homing capacity of MSCs for effective expression at the lesion site. Here, we will summarize current advancements in MSCs-based therapies for neurodegenerative diseases. We will examine the roles of central mechanisms suggested to mediate the beneficial effects of MSCs-based therapy and consider the augmentation of these mechanisms for superior clinical outcomes in rodent models of neurodegeneration as well as in clinical trials. STEM CELLS 2017; 00:000–000

SIGNIFICANCE STATEMENT

Mesenchymal stem cells (MSCs) hold great potential as source for cell-based therapy for neurodegenerative diseases. In this review, we summarize recent progress using human MSC transplantation into rodent models of neurodegeneration. We examine the molecular mechanisms mediating disease amelioration through this approach and emphasize the potential of ex vivo manipulation for these cells before transplantation.

INTRODUCTION

Neurodegenerative diseases involve progressive decline in neuronal function, brain atrophy, and often involve abnormal deposition of proteins. Although various neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and multiple system atrophy (MSA) occur in different brain regions and display different etiology, cumulative data suggest common cellular and molecular mechanisms.

Although there are immense efforts for the development of therapies for neurodegenerative diseases throughout the last several decades, effective therapeutic agents are still of need. This lack may result from several challenges: First, although multiple cellular and molecular mechanisms were

shown to be involved in the etiology of these diseases, the cause of neuronal death still remains obscure, and no single molecular pathway was shown able to modulate disease progression. Second, for most of these diseases, early diagnosis is impeded due to the absence of efficient biomarkers. Third, progressed neurodegeneration often involves secondary effects such as chronic inflammation, requiring adjustment of treatment. Finally, drugs administered into the central nervous system (CNS) should be able to cross the brain–blood barrier (BBB), as well as to target specific cell types within different CNS regions, requiring efficient vectors able to carry therapeutic agents toward their target sites.

Mesenchymal stem cells (MSCs) are adult multipotent progenitors derived from various adult tissues and are capable of self-renewal in vitro [1]. MSCs are defined by their spindle-shaped

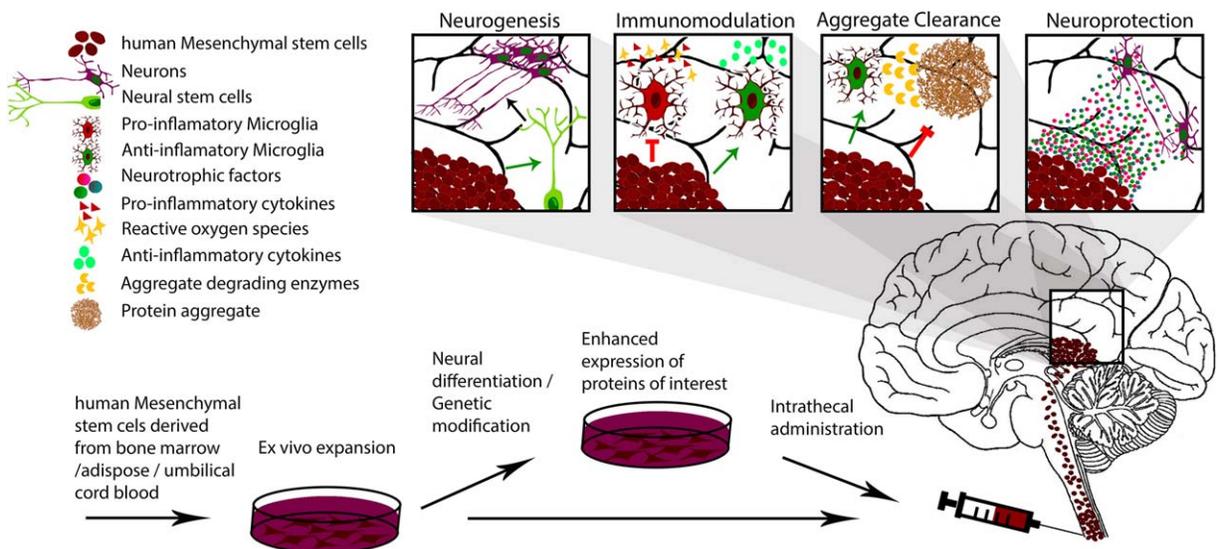


Figure 1. Human mesenchymal stem cell (hMSC)-based therapy for neurodegeneration. Shown is a scheme describing major stages in hMSCs-based therapy: derivation from adult tissue, ex vivo expansion and manipulation, administration into the spinal canal, and migration toward lesioned area. Enlarged areas show the molecular mechanisms shown to contribute to amelioration of neurodegenerative diseases upon MSCs transplantation.

morphology, their ability to adhere to tissue culture plastic, and their unique expression of cluster of differentiation cell surface molecules [2]. Upon growth in culture, MSCs are able to greatly expand while retaining their multipotent potential. This allows the generation of high cell quantities, setting these cells as highly efficient source for cell-based therapy. Also, MSCs were safely used for autologous transplantation, and exhibited no toxicity or tumorigenicity following transplantation into rodents or human patients [3, 4]. Moreover, upon transplantation, MSCs possess the capability to migrate toward neural lesions, presumably due to attraction by chemokines [5, 6]. Finally, paracrine secretion from these cells offer broad clinical potential by regulating immunomodulation, angiogenesis, apoptosis, oxidative stress, cell-differentiation, extra-cellular matrix composition, and more (reviewed in ref. 7).

Multiple reports over the last decade showed improvement in various models of neurodegenerative diseases or acute brain insults following MSCs transplantation in multiple rodent models. MSCs transplantation often improved survival rates, declined pathology, and rescued cognitive function decline. However, the exact mechanism by which MSCs exert their function remains debatable, as several mechanisms have been offered, such as neuroprotection by secretion of neurotrophic factors (NTFs), induction of neurogenesis, modulation of inflammation, and prevention of misfolded protein aggregation (illustrated in Fig. 1). As the majority of these diseases display complex etiology, it seems that multiple beneficial roles of MSCs are able to target different aspects of diseases. However, understanding the molecular mechanisms by which these cells exert their function could facilitate the generation of advanced MSCs-based therapies for neurodegeneration.

In this review, we will present the advances in human MSCs (hMSCs)-based therapies in rodent models of neurodegenerative diseases such as AD, PD, ALS, and MSA as well as in acute models of stroke. We will also review outcomes from human clinical trials using this therapeutic approach and will discuss the limitations that hamper the therapeutic potential of these cells. We will focus on the different mechanisms

enhanced following treatment with these cells (as summarized in Table 1) and will describe the methods used to improve therapeutic efficacy of the hMSCs (as summarized in Table 2).

INDUCTION OF NEURONAL REGENERATION

Neurodegeneration is mainly characterized by progressive neuronal loss. However, various neurodegenerative diseases exhibit unique neuronal pathologies. PD involves the loss of dopaminergic neurons in the Substantia Nigra (SN). ALS involves the degeneration of motor neurons (MNs) in the brainstem and spinal cord. AD entails global neuronal loss in the cerebral cortex and hippocampus, and HD is characterized by degeneration of projection neurons in the dorsal striatum [8]. As the molecular mechanisms driving neuronal pathology in these diseases remains elusive, functional recovery by regeneration of damaged tissue is regarded a major therapy strategy.

Neuronal Differentiation of MSCs

The discovery that MSCs derived from both mouse and human origins can be manipulated to differentiate into functional neurons [9–11] encouraged the use of MSCs-derived neuronal cell types for the replacement of damaged neural tissue. Emerging protocols have used specific culture conditions for ex vivo differentiation of human MSCs into dopamine-secreting [12, 13] and acetylcholine (ACh)-secreting [14, 15] neuronal-like cells. Alternatively, superior similarity to original neuronal subtypes was reported through the genetic modification-mediated transdifferentiation of human bone marrow-derived MSCs (hBM-MSCs) using ectopic expression of neuronal subtype-specific transcription factors [16, 17]. Although few studies have shown functional recovery in brain injury upon transplantation of MSCs-derived neuronal cells into murine brains [17–20], it is not clear whether the observed recovery was indeed due to functional integration

Table 1. Naive human mesenchymal stem cells in murine models of neurodegeneration

Authors	Cell source	Model animal	Suggested mechanism	Clinical improvement
Alzheimer's [61]	Umbilical cord blood	Aβ-inoculated mice	Reduced glia activation, oxidative stress, and apoptosis	Improved learning/memory performance
Alzheimer's [36]	Umbilical cord blood	APP/PS1 mice	Reduced Aβ and p-tau deposition	Improved spatial learning and memory decline
Alzheimer's [89]	Umbilical cord blood	APP/PS1 mice	Modulation of microglia activation sICAM-1 mediated upregulation of neprilysin	Increased neuronal survival in vitro
Alzheimer's [93]	Not mentioned	Aβ-inoculated mice	Induced Aβ clearance	Enhanced hippocampal neurons survival
Alzheimer's [37]	Not mentioned	Aβ-inoculated mice	Induction of autophagy Induced Aβ clearance	Improved working memory
Parkinson's [34]	Bone marrow	6-OHDA mice	Enhanced endogenous neurogenesis NTF secretion	Enhanced dopaminergic neurons survival
Parkinson's [36]	Bone marrow	MPTP-induced mice	Enhanced endogenous neurogenesis Enhance EGFR expression	Enhanced dopaminergic neurons survival
Parkinson's [94]	Bone marrow	MPTP-induced mice	Enhanced endogenous neurogenesis Induction of autophagy	Enhanced dopaminergic neurons survival
Parkinson's [63]	Bone marrow	α-Synuclein-inoculated mice	Induced α-synuclein clearance	Increased neuronal survival
Stroke [35]	Bone marrow	Acute ischemia rats	Modulation of microglia activation MSCs IL-4 secretion Induced α-synuclein clearance	Reduced infarct volume Functional recovery in NSS
Stroke [65]	stable bone marrow derived B10 line	Acute ischemia rats	NTF secretion Enhanced endogenous neurogenesis Reduced apoptosis	Reduced infarct volume
Stroke [71]	Adipose	Acute ischemia rats	Reduced microglia activation Reduced NF-κB signaling BBB integrity maintenance	Enhanced neuronal survival Improved functional behavior
Stroke [70]	Bone marrow	LPS induced rats	Reduced neutrophil infiltration Reduced endothelial vasculature damage BBB integrity maintenance	Enhanced neuronal survival
MS [41]	Bone marrow	EAE mice	Reduced neutrophil infiltration modulation of microglia activation NGF mediated axonal protection	Reduced mortality Reduced disease severity Reduced neuronal loss Deceleration in disease progression
MS [73]	Bone marrow	EAE mice	Reduced immune cell infiltration Induced Th2 immune response	Reduced disease severity Reduced axonal loss
MS [74]	Adipose	EAE mice	Enhanced oligodendrogenesis Decreased spinal cord inflammation Decreased demyelination Induced Th2 immune response	Reduced disease severity Reduced axonal loss
MS [75]	Bone marrow	EAE mice	Neurotrophic factor secretion Enhanced oligodendrogenesis Reduced demyelination	Decreased white matter lesions Reduced disease severity Reduced disease severity
MS [78]	Placenta	EAE mice	Neuroprotection Immunomodulation mediated by reduced TSG6 expression	Reduced disease severity
MS [77]	Decidua	EAE mice	Decreased inflammatory infiltration Immunomodulation	Reduced disease severity

Table 1. Continued

	Authors	Cell source	Model animal	Suggested mechanism	Clinical improvement
MS	[76]	Adipose	EAE rats	Decreased immune cell infiltration HLA-G mediated Immunomodulation	Reduced disease severity Amelioration of axonal loss Enhanced vascular congestion Enhanced neuronal survival
Inflammation	[70]	Bone marrow	LPS induced rats	BBB integrity maintenance Reduced neutrophil infiltration modulation of microglia activation	
ALS	[60]	Bone marrow	SOD1 female mice	Reduced astrogliosis and microglia activation	Sustained survival of motor neurons Improved motor functions
ASD	[33]	Bone marrow	BLBP mice	NTF secretion Enhanced endogenous neurogenesis	Reduced stereotypical behavior Reduced cognitive rigidity Improved social behavior

Abbreviations: BBB, brain-blood barrier; EAE, experimental autoimmune encephalomyelitis; HLA-G, human leukocyte antigen G; 6-OHDA, 6-hydroxydopamine; IL-4, interleukin 4; LPS, lipopolysaccharide; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MSC, mesenchymal stem cells; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; NGF, nerve growth factor; NSS, neurological Severity Score; NTF, neurotrophic factors; sICAM-1, soluble intracellular adhesion molecule-1; TSG6, tumor necrosis factor-inducible gene 6.

of MSCs-derived neuronal cells into excising neuronal networks. Moreover, the ability of MSCs to differentiate into fully functional neurons was not proven, as their transdifferentiation capacity and electrophysiological functionality remains under debate (reviewed in refs. 21, 22). These challenges, as well as the difficulty in establishing sufficient amount of cells for transplantation, discouraged further advancement of this strategy.

Induction of Endogenous Neurogenesis

An alternative approach for functional regeneration after neuronal loss is to encourage endogenous neural stem cells in the brain to generate the appropriate neurons. In mice, sub-ventricular zone (SVZ) cell proliferation and neuronal differentiation are enhanced following stroke, providing evidence that the adult brain has the capacity to replace damaged cells using endogenous precursors [23]. Impaired neurogenesis, on the other hand, was associated with several neurodegenerative diseases [24], suggesting that inducing neurogenesis can be used as a therapy. Identification of factors that enhance endogenous neurogenesis in acute insult or chronic neurodegeneration was suggested as an effective therapeutic approach.

NTFs are secreted proteins that regulate multiple aspects of neural cell functions and are widely known to play central roles during brain development, homeostasis, and neurodegeneration [25]. In particular, multiple neurotrophic factors (NTFs) have been implicated in induction of neurogenesis in the adult SVZ. Brain-derived NTF (BDNF) and vascular endothelial growth factor (VEGF) administration into the lateral ventricles of adult rats was shown to increase the generation of new neurons [26]. The enhancement of neurogenesis by enriched environment was shown to be dependent on BDNF [27] and VEGF was shown to mediate exercise-induced neurogenesis [28]. Also glial cell line-derived NTF (GDNF), fibroblast growth factor 2 (FGF2), and neurotrophin-3 (NT-3) were shown to have roles in enhancing adult neurogenesis [29, 30].

Munoz and colleagues has shown that (hBM-MSCs) injected into the dentate gyrus of healthy mice were able to promote proliferation and differentiation of neural stem cells [31]. This effect was attributed to the elevated secretion of NTFs such as nerve growth factor (NGF), VEGF, ciliary NTF (CNTF) and FGF2 from transplanted cells. We showed that hBM-MSCs injected into the SVZ and sub-granular zone (SGZ) of healthy mice housed in enriched cages, promoted neurogenesis in the SVZ but not the SGZ associated with elevated BDNF secretion [32]. Notably, human nuclei staining analysis confirmed that neural progeny was derived from endogenous progenitors rather than from the transplanted cells.

The induction of neurogenesis by hMSCs administration was shown beneficial in several models of neurological diseases. We have recently shown a beneficial effect of hBM-MSCs in a BTBR mouse model of autism spectrum disorder. hBM-MSCs transplantation into these mice, who typically show reduced hippocampal neurogenesis and BDNF secretion, resulted in elevation of these properties along with improved cognitive functions [33]. Dramatic improvement in cognitive functions and social behavior were demonstrated 3 weeks following transplantation, suggesting the role of NTFs secretion-mediated induced neurogenesis in this model. Induced neurogenesis following hBM-MSCs transplantation was also reported in a 6-hydroxydopamine (6-OHDA) mouse model of PD, where grafted cells were shown to secrete

BDNF 23 days after transplantation [34]. In a rat model of cerebral ischemia, we also observed enhanced neurogenesis that was associated with induced levels of BDNF, NT-3, and VEGF were reported following transplantation, suggesting that neurogenesis was mediated by NTFs secretion [35].

Park et al. showed that hBM-MSCs administration enhanced the expression of epidermal growth factor receptor (EGFR) and enhanced neurogenesis in the SVZ and the SN in an 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD. As EGFR is known to enhance neural progenitor cell (NPC) proliferation, the authors suggested that the neurogenic activity of hBM-MSCs was indeed mediated through EGFR signaling [36].

WNT signaling is known to be involved in proliferation capacity of NPCs. Beta-amyloid ($A\beta$) exposure of murine NPCs was shown to decrease their proliferation capacity as well as the expression level of the WNT components β -catenin and NGN1. Coculture of NPCs with hMSCs significantly rescued the expression of these genes and enhanced proliferation and neuronal differentiation. Importantly, hMSCs-mediated enhancement of neurogenesis was dependant on WNT signaling activation. hMSCs transplantation into $A\beta$ -treated mice showed enhanced differentiation of NPCs in the hippocampus into mature neurons [37].

Together, these findings show that paracrine secretion from hMSCs results with enhanced neurogenic capacity and subsequent functional improvement. However, we cannot exclude the possibility of direct effect of NTF and other factors on various cellular processes such as homeostasis, reduction in oxidative stress, modulation of inflammation, and neuronal survival. As the modulation of neurogenesis is widely accepted as a key mechanism regulating neurodegeneration, the induction of neurogenesis by cell therapy using hMSCs holds great potential for future therapy (reviewed in ref. 25).

ENHANCED NTFs SECRETION

In addition to their role in promoting neurogenesis, ectopic expression of NTFs is known to improve neuronal survival following acute and chronic brain damage, providing a potential strategy for the treatment of various neurodegenerative diseases [38–40]. Functional improvement in murine models of neurodegeneration following hMSCs transplantation is frequently attributed to enhanced levels of NTFs in the brain, providing neuroprotection, reduction in oxidative stress, induced neurogenesis, and modulation of the inflammatory response [32–36, 41–45]. hBM-MSCs were shown to express neural genes and were suggested to be predisposed to differentiate into neural cell fates [46]. We sought to harness this potential to generate hBM-MSCs-derived, NTFs secreting, astrocytic-like cells. We developed a novel protocol for ex vivo differentiation to astrocytic-like cells termed NTFs-secreting MSCs (MSC-NTFs). MSC-NTFs show elevated secretion of BDNF, GDNF, and insulin-like growth factor 1 as well as elevated expression of astrocytic markers such as glial fibrillary acidic protein and glutamine synthetase.

MSC-NTFs were shown to evoke improved clinical outcomes compared with naïve hBM-MSCs in several rodent models of neurodegeneration. Upon MSC-NTFs transplantation into the striatum of 6-OHDA model of PD, rats show improved behavior in the amphetamine-induced rotations test, higher preservation of the tyrosine-hydroxylase-positive area in the striatum

and reduced dopamine depletion when compared with naïve hBM-MSCs [47]. Notably, conditioned media from MSC-NTFs was shown to protect neuroblastoma cell line against 6-OHDA neurotoxicity. This NTF-induced protection was also shown in a quinolinic acid model of neurotoxicity [48].

Conditioned media from MSC-NTFs was also shown to promote neuronal protection against oxidative stress and to inhibit proliferation of immune cells in response to multiple sclerosis (MS) related antigens [49]. Additionally, MSC-NTFs were shown to promote survival of retinal ganglion cells after optic nerve injury [50], to improve motor functions and to inhibit neuromuscular junction degeneration in a rat model of sciatic nerve injury [51], suggesting an enhanced neuroprotective effect.

The neuroprotective and neurogenic capacity of MSC-NTFs in these models, has encouraged the use of MSC-NTFs for autologous transplantation in patients with ALS. To date, a phase I/II and a phase 2a clinical trials for the treatment of ALS patients with MSC-NTFs was conducted by Brainstorm[®] Cell Therapeutics, and succeeded in meeting safety and efficacy criteria (See MSCs Therapy in Human Clinical Trials section).

Alternative ex vivo approach uses genetic manipulation of isolated hBM-MSCs to enhance the expression of NTFs. Using this strategy, hBM-MSCs over-expressing GDNF were shown to promote recovery in a 6-OHDA rat model of PD, resulting in reduction of amphetamine-induced rotations and rejuvenation of dopamine fibers [52]. Transplantation of hBM-MSCs over-expressing GDNF and VEGF were also used in a mutant Superoxide dismutase 1 (*SOD1*) rat model of ALS and showed improved neuro-muscular junction innervation and improved MN survival [53]. Similarly, transplantation of hBM-MSCs over-expressing BDNF promoted neurogenesis, improved behavioral scores, and increased lifespan in mice models of HD [54].

In summary, these lines of research validate the important role of NTFs in restoring homeostasis and halting degeneration, but also highlight the capability of hMSCs to serve as vectors for ectopic expression of beneficial factors through paracrine secretion as a therapeutic approach for nervous system disorders.

IMMUNOMODULATION AND NEUROINFLAMMATION

Neuroinflammation refers to a variety of chronic, proinflammatory, immune system-mediated processes, mainly associated with neurodegenerative diseases. Cumulative evidence suggests that inflammation plays a major role in the progression of several neurodegenerative diseases. Post-mortem AD brains exhibit activated microglia and astrocytes as well as positive staining for multiple anti-inflammatory chemokines and cytokines [55]. PD entails enhanced microglia activation, astrogliosis, and lymphocyte infiltration [55] as well as increased levels of proinflammatory cytokines in the blood and cerebrospinal fluid (CSF) [56]. Also in ALS, accumulation of activated microglia and macrophages was shown next to degenerating areas, along with multiple proinflammatory compounds and upregulation of the proinflammatory cytochrome C oxidases 1 and 2 (COX-1 and COX-2) [57]. Moreover, many inflammation-related compounds have detrimental roles on neurogenesis, consequently hampering endogenous tissue repair mechanisms [58]. Therefore, the modulation of the immune response toward an anti-inflammatory state emerges

Table 2. Ex vivo manipulated human mesenchymal stem cells in murine models of neurodegeneration

	Authors	Source	Ex vivo manipulation	Model animal	Suggested mechanism	Clinical improvement
Parkinson's	[18]	Bone marrow	NICD transduction and NTF induction	6-OHDA rats	GDNF secretion	Decreased apomorphine induced rotations
Parkinson's	[5]	Bone marrow	MSC-NTF	6-OHDA rats	NTF secretion	Decreased amphetamine induced rotations
Parkinson's	[52]	Bone marrow	NICD and GDNF transduction	6-OHDA rats	GDNF secretion	Reduced dopamine depletion
Stroke	[17]	Bone marrow	Neuronal differentiation by NGN1 overexpression	Acute Ischemia rats	Enhanced endogenous neurogenesis	Enhanced striatal regeneration
MS	[51]	Bone marrow	MSC-NTF	EAE mice	Anti inflammation	Decreased amphetamine induced rotations
MS	[79]	Adipose	IL-4 transduction	EAE mice	NTF secretion	Dopamine-fibers rejuvenation
MS	[80]	Adipose	IL-10 transduction	EAE mice	NTF secretion	Improved motor recovery
ALS	[53]	Bone marrow	GDNF and VEGF transduction	SOD1 rats	NTF secretion	Reduced ischemic core
Huntington	[48]	Bone marrow	MSC-NTF	QA-induced rats	NTF secretion	Increased survival
Huntington	[54]	Bone marrow	BDNF transduction	YAC128 and R6/2 mice	BDNF secretion	Delayed symptom onset
Schiatric nerve injury	[51]	Bone marrow	MSC-NTF	Schiatric nerve crushed rats	Neurotrophic factor secretion	Reduced disease severity
Optic nerve injury	[50]	Bone marrow	MSC-NTF	Optic nerve transectioned rats	NTF secretion	Reduced disease severity

Abbreviations: EAE, experimental autoimmune encephalomyelitis; GDNF, glial cell line-derived neurotrophic factor; 6-OHDA, 6-hydroxydopamine; MSC-NTFs, neurotrophic factor-secreting mesenchymal stem cells; NICD, notch intracellular domain; NGN1, neurogenin 1; NMIJ, neuromuscular junction; NTF, neurotrophic factors; QA, quinolinic acid; VEGF, vascular endothelial growth factor.

as a potential disease-modifying therapeutic strategy for neurodegeneration [59].

The inflammatory response in the CNS, unlike the rest of the body, is primarily mediated by the activation of microglia cells, specialized macrophages of the nervous system. Under physiological conditions, deactivated microglia support tissue homeostasis through production of neurotrophic and anti-inflammatory factors [55]. Upon activation, following exposure to pathogen or brain injury, activated microglia migrate along a chemotactic gradient, recruit circulating immune cells that infiltrate into the CNS through the BBB. Microglia cells are also able to perform phagocytosis and mediate neuroinflammation through secretion of proinflammatory cytokines, chemokines, and reactive oxygen species [58].

Ultimately, enhanced proinflammatory activation of microglia cells can result in chronic inflammation and accelerated neuronal death through oxidative stress and apoptosis. Several studies suggested that hMSCs hold various immunomodulatory roles, mainly through the manipulation of microglia-mediated neuroinflammation. hBM-MSCs transplantation into SOD1 female mice decreased microglial activation and astrogliosis which was associated with improved behavioral score [60]. In an acute model of AD, human umbilical cord blood-derived MSCs (hUCB-MSCs) also reduced the levels of microglial and astrocytic activation as well as apoptosis [61]. Following work showed that familial AD model APP/PS1 mice exhibit substantially increased levels of the proinflammatory cytokines tumor necrosis factor (TNF)- α and interleukin (IL)-1 β when compared with wild-type mice and that the expression of these factors is significantly reduced following hUCB-MSCs transplantation. Alternatively, the expression of the anti-inflammatory markers IL-4, AMCase, YM-1, and Arg-1 was elevated following transplantation indicating a switch in microglial activation from a proinflammatory state to an anti-inflammatory [62].

It was demonstrated that hBM-MSCs promote secretion of IL-4 from microglia cells and stimulated α -synuclein clearance in a PD mouse model [63]. A 1-year follow-up clinical trial using these cell for the treatment of MSA revealed a higher IL-4 expression and a reduction in the α -synuclein levels in the CSF of hBM-MSCs-treated patients when compared with placebo group [63, 64] (See MSCs Therapy in Human Clinical Trials section). Importantly, hBM-MSCs-treated patients showed a lesser decrease in cerebral glucose metabolism and grey matter density as well as decreased deterioration of cognition when compared with placebo group. Finally, recent in vitro and in vivo analyses have indicated the roles of other molecular pathways, such as nuclear factor kappa-light-chain-enhancer of activated B cells signaling [65] and sphingosine-1-phosphate signaling [66] in hMSCs mediated of modulation of inflammation.

hMSCs were also shown to maintain BBB integrity. Following neuroinflammation, the BBB is disrupted in a process involving morphogenic and paracrine changes in astrocytes and endothelial cells, allowing increased penetrability and neutrophil infiltration [67–69]. Park et al. showed that hBM-MSCs were able to restore BBB integrity in lipopolysaccharide-induced rats, resulting in reduced neutrophil infiltration and enhanced neuronal survival. The authors suggested that improved BBB integrity was due to morphogenic changes in astrocytes and endothelial cells as well as

anti-inflammatory modulation of microglia [70]. Similarly, Chung et al. reported that human adipose derived MSCs (hA-MSCs) transplantation following transient global cerebral ischemia in rats, minimized BBB disruption and neutrophil infiltration induced by ischemia and improved endothelial vasculature [71]. Together, these reports demonstrate another therapeutic advance for hMSCs following acute injury or chronic inflammation.

Immunomodulation and MS

MS is a chronic, autoimmune, and neurodegenerative disease of the CNS, in which immune cells, predominantly autoreactive CD4+ T-helper cells, infiltrate into the CNS and promote an inflammatory response, resulting in myelin injury and axonal loss [72]. Similarly to microglia directed therapy, current treatment strategies for MS commonly aim at modulating the immune response, through a shift from a proinflammatory response, mediated by cytokines secreted by T-helper 1 (Th1) cells, to an anti-inflammatory response, mediated by cytokines secreted by T-helper 2 (Th2) cells [72].

hMSCs were widely evaluated for the treatment of MS, through transplantation into the experimental autoimmune encephalomyelitis (EAE) mice model of MS. Following intravenous transplantation into rodents, hMSCs migrate from the blood stream into the CNS and localize to white matter demyelination sites, and to peripheral lymph organs and consequently induce EAE amelioration [41, 73–75]. Repeated reports ascribed this amelioration to a modulation of the immune response. Using hBM-MSCs, Bai et al. showed a decrease in leukocyte infiltration into the CNS and enhanced a Th2 cytokine profile, but also enhanced oligodendrogenesis, suggesting that hBM-MSCs affect immunomodulation but also enhanced neuronal repair [73]. Similar results showing a Th2 cytokine shift and enhanced oligodendrogenesis were reported using hA-MSCs [74, 76] and using human decidua-derived MSCs (hD-MSCs) [77]. hA-MSCs were also shown to express the immunosuppressive gene *human leukocyte antigen G (HLA-G)* [76] and to induce the secretion of the anti-inflammatory protein TNF- α -stimulated gene/protein 6 following culture with inflammatory cytokines [78]. Payne et al. tested the effect of hA-MSCs ex vivo modified to over-express the anti-inflammatory ILs-4 and -10 in EAE mice. In both cases, they observed disease attenuation and enhanced Th2 anti-inflammatory response, confirming the significance of immunomodulation in this model [79, 80].

hBM-MSCs-mediated improvement in MS was also attributed to NTFs secretion. Zhang et al. showed that EAE clinical score is correlated with decreased NGF expression, both rescued upon hBM-MSCs transplantation [41]. We previously showed that MSC-NTFs (discussed earlier) injected intracerebroventricularly (ICV) into EAE mice were able to ameliorate motor functions and suppress EAE in mice to a greater extent than naïve hBM-MSCs, suggesting neurotrophic-mediated disease amelioration [49].

Finally, hBM-MSCs differentiated into neural progenitor fate shown both enhanced immune-regulatory properties and NTFs secretion [81]. Multiple intrathecal injections of these cells into EAE mice reduced immune cell infiltration into the CNS and enhanced endogenous neural progenitor proliferation [81] and clinical trials using these cells are ongoing (ClinicalTrials.gov identifier: NCT01933802).

PROTEIN AGGREGATE CLEARANCE

The abnormal aggregation of proteins and formation of inclusion bodies is a major hallmark of neurodegenerative diseases [82], and protein aggregation is known to be a major factor in neurodegeneration onset. PD involves intraneuronal formation of inclusions in the SN termed Lewy bodies, mostly composed of the α -synuclein protein and triplication of the *SNCA* gene encoding α -synuclein was found to cause PD [83]. HD is characterized by expansion of CAG repeats in the N-terminus of the huntingtin gene, resulting in protein polyglutamination, leading to formation of fibers with β -sheet structure and aggregation. It was shown that the number of CAG repeats within the Huntingtin gene linearly correlates with early HD onset [84]. AD is associated with both extracellular amyloid plaques mostly containing the A β peptide, and intracellular neurofibrillary tangles containing the phosphorylated tau protein. According to the amyloid cascade hypothesis [85], the formation of amyloid plaques is essential for AD pathology. ALS involves the intraneuronal formation of inclusions, mostly in spinal MNs, containing TAR DNA-binding protein 43 (TDP-43), FUS and SOD1 proteins. Moreover, various prion diseases such as Creutzfeldt-Jakob disease and Gerstmann-Sträussler-Scheinker disease involve both intra- and extracellular deposition of abnormally folded prion proteins. Therefore, reducing abnormal protein aggregates by preventing aggregation as well as by imposed clearance of aggregating proteins is a main strategy for neurodegenerative disease therapy.

Microglia and Proteolytic Enzyme Secretion

As the main constituent of extracellular plaques in AD, A β levels are thought to dictate AD progression. Enhanced expression of A β -degrading enzymes such as insulin-degrading enzyme or neprilysin in AD mice models resulted in reduced A β burden and premature lethality rescue [86]. Moreover, the secretion of A β -degrading enzymes from microglia was shown to be affected by age [87] and regulated by ApoE [88], the two major risk factors for AD. Recent coculture experiments shown that soluble intracellular adhesion molecule-1 secreted by hUCB-MSCs induced the secretion of neprilysin from microglia [89]. Transplantation of these cells into APP/PS1 mice also shown induced neprilysin expression, alongside with reduced A β plaques in the hippocampus, demonstrating a role for hMSCs in enhancing the cells endogenous proteolytic machinery [89]. A phase I/II clinical trial based on these results is currently recruiting patients (ClinicalTrials.gov identifier: NCT02054208).

Autophagy

Autophagy is a cellular pathway involved in protein and organelle degradation through formation of autophagic vacuoles that fuse with lysosomes [90]. Expectedly, autophagy was shown to play a key role in aggregate clearance in several neurodegenerative disease models [91]. Knock-out of the essential autophagy gene *Atg7* in mice was reported to cause accumulation of poly-ubiquitinated proteins, substantial neuronal loss and behavioral defects [92]. Shin et al. reported that ICV administration of hBM-MSCs into A β -inoculated mice increased the survival of hippocampal neurons and reduced the levels of A β . A β clearance was attributed to the recovery

of the cells inherent clearance machinery through the autophagy-lysosomal pathway [93], as seen by formation of autophagic vacuoles and induction of the expression of the autophagy initiator BECN1. This group has also shown that hBM-MSCs-mediated activation of autophagy improved viability and reduced α -synuclein accumulation in the midbrains of MPTP-treated mouse model of PD [94]. Together, these findings indicate that hBM-MSCs are able to induce protein aggregate clearance through an increase in autophagy.

MSCs THERAPY IN HUMAN CLINICAL TRIALS

Along with the advancements in murine models of neurodegeneration, hMSCs are widely examined as therapeutic agents in human clinical trials aiming to ameliorate neurodegeneration and acute brain injury as well as various other pathologies. To date, over 700 clinical trials using hMSCs are listed in clinicaltrials.gov, out of which more than 40 have reached phase III. hMSCs were reported safe for administration to the CNS through intravenous and intrathecal transplantation and were studied in the context of multiple neurodegenerative diseases and acute brain injuries. In this section, we will review current results using hMSCs-based therapy in human clinical trials (summarized in Table 3).

Substantial progress have been done using hMSCs for the treatment of MS. Li et al. demonstrated that hUCB-MSCs derived from healthy donors and administered into MS patients were able to ameliorate disease symptoms and reduce relapse occurrence. The immunomodulatory beneficial outcome was evident as a shift in peripheral blood cytokine expression toward a Th2 response [95]. Immunomodulation was also recorded following hBM-MSCs transplantation in MS and ALS patients [96]. Peripheral blood monocyte analysis 24 hours after transplantation revealed an increase in the proportion of immunosuppressive CD4⁺ CD25⁺ regulatory T cells and a decrease in the proportion of proinflammatory myeloid dendritic cells. Expanded disability status scale scores of hBM-MSCs-treated MS patients showed functional improvement 6 months following transplantation, while ALS functional rating scale (ALS-FRS) scores in ALS patients remained stable throughout this period, indicating immunomodulatory-mediated clinical potential for hBM-MSCs. A work by Mohajeri et al. has also reported a similar immunomodulatory effect following autologous transplantation of hBM-MSCs in six MS patients [97]. Six months following transplantation, all patients demonstrated clinical stability. They have also demonstrated a significant upregulation in the expression of the transcription factor FoxP3 in blood mononuclear cells. As Foxp3 is known as a marker of immunosuppressive CD4⁺ CD25⁺ regulatory T cells, this study further indicate the anti-inflammatory role of hBM-MSCs [98]. In another clinical study, Connick et al. administered autologous hBM-MSCs intravenously to 10 patients with secondary progressive MS with clinical evidence of optic nerve involvement. After hBM-MSCs treatments, patients showed improved visual acuity and an increase in optic nerve area, providing evidence that hBM-MSCs treatment can affect disease score in progressive MS [99].

hBM-MSCs were also beneficial following autologous injection into ALS patients. Mazzini et al. autologously administered hMSCs into the spinal cord in seven ALS patients and

Table 3. Human mesenchymal stem cells in human clinical trials

	Authors	Cell source	Administration	Number of patients enrolled	Clinical outcome
MS	[95]	Allogenic, Umbilical cord blood	Intravenous injection	13 in treatment group, 10 in control group	Improved functional performance Reduced relapse occurrence Enhanced Th2 response
MS and ALS	[98]	Autologous, Bone marrow	Inathecal and Intravenous injections.	15 in MS treatment group, 19 in ALS treatment group	Improved functional performance in MS patients Slower disease progression rate in ALS patients Enhanced anti-inflammatory response
MS	[97]	Autologous, Bone marrow	Intrathecal injection	7 patients in the treatment group	Clinical stability
MS	[99]	Autologous, Bone marrow	Intravenous injection	10 patients in the treatment group	Enhanced peripheral blood FoxP3 expression Improved visual acuity
MSA	[36]	Autologous, Bone marrow	Intra-arterial and Intravenous injections	11 in treatment group, 16 in placebo group	Increased optic nerve area Smaller increase in clinical severity.
Parkinson's	[106]	Autologous, Bone marrow	Stereotactic injection into the lateral ventricles	7 patients in the treatment group	Reduced size of glucose hypo-metabolism areas. Steady improvement in clinical score in 3/7 patients
Stroke	[3]	Autologous, Bone marrow	Intravenous injection	5 in treatment group, 25 in control group	Decreased brain atrophy Improved functional performance
Stroke	[61]	Autologous, Bone marrow	Intravenous injection	16 in treatment group, 36 in control group	Reduced mortality Improved functional performance
Stroke	[105]	Autologous, Bone marrow	Intravenous injection	12 patients in the treatment group	Improved neurological scores Reduced infarct size
ALS	[101]	Autologous, Bone marrow	Intrathecal injection	8 patients in the treatment group	Slower disease progression rate Elevated anti-inflammatory markers
ALS	[100]	Autologous, Bone marrow	Intrathecal injection	7 patients in the treatment group	Slower disease progression rate
ALS	[103]	Autologous, Bone marrow, ex vivo differentiated	Intramuscular and intrathecal injections.	26 patients in the treatment group	Slower disease progression rate
ALS	Brainstorm Cell therapeutics	Autologous, Bone marrow, ex vivo differentiated	Intramuscular and intrathecal injections.	36 in treatment group, 12 in control group	Decreased inflammatory markers Slower disease progression rate

reported a significant slowing down in the force vital capacity (FVC) scale in four of the patients [100]. Similar improvement in clinical score was also reported by Oh et al. following autologous hBM-MSCs transplantation [101]. A parallel work by this group has also analyzed the relationship between the expression of factors induced by hBM-MSCs and the patient's responsiveness to therapy [102]. They have shown that enhanced secretion of trophic factors such as VEGF, angiogenin, and transforming growth factor β were associated with responsiveness to treatment, providing evidence to the contribution of these factors.

As mentioned earlier, MSC-NTFs developed in our laboratory have also shown beneficial evidence in ALS patients. In a phase I/II study performed by Brainstorm Cell Therapeutics, 87% of the patients treated intrathecally with MSC-NTFs (NurOwn[®]) showed at least 25% improvement in the slope of disease progression at 6 months after treatment, as indicated by the ALS-FRS and the FVC measurements [103].

Recently, Brainstorm Cell Therapeutics announced findings from a randomized, double blind, placebo-controlled phase II study showing that autologous MSC-NTFs treatment in 36 patients indeed led to clinically meaningful benefit (<http://ir.brainstorm-cell.com/phoenix.zhtml?c=142287&p=irol-newsArticle&ID=2186054>). Patients treated with NurOwn[®] showed enhanced levels of NTFs in the CSF and decreased inflammatory markers 2 weeks after intrathecal transplantation. Importantly, these patients exhibited slower disease progression when compared with the placebo group, as measured by the ALS-FRS scale. As announced by the company, a phase III clinical trial is expected to begin in 2017.

Several clinical trials have also demonstrated the potential of hMSCs for the treatment of patients following ischemic stroke. Patients subjected to autologous hBM-MSCs transplantation 6 weeks following infarction within the middle cerebral artery territory, showed decreased brain atrophy and improved daily performance, as measured by the Barthel scale [3]. A follow-up study by this group has reported that hBM-MSCs treated group had lower mortality rate and tended toward improved clinical outcome 5 years after transplantation [104]. In a different trial, autologous hBM-MSCs transplantation following stroke was reported to reduce infarct size by 20% a week following transplantation [105].

Finally, autologous transplantation of hBM-MSCs was also performed in patients with synucleopathy. Venkataramana et al. reported that three of the seven PD patients treated by stereotactic injection of these cells into the sublateral ventricular zone (VZ) have shown improvement in their Unified Parkinson's Disease Rating Scale score [106]. Lee et al. have shown that intra-arterial and intravenous administration of hBM-MSCs to MSA patients resulted in a lesser increase in the severity of their neurological deficits when compared with placebo group, throughout a period of more than 3 years [64].

Although future research will have to determine the efficacy of these treatments in larger cohorts, these clinical results collectively demonstrate the beneficial potential of hMSCs-based therapy in patients with neurodegeneration.

EFFICACY AND LIMITATIONS OF hMSCS-BASED THERAPY

hMSCs are regarded as efficient cell source for therapy, as they can be safely harvested from and transplanted into

donors or patients, have no major ethical concerns, have low immunogenicity and possess a wide therapeutic potential. The results from both preclinical and clinical trials reviewed here indicate the potential of hMSC-based treatment in meeting several key aspects of neurodegeneration, such as neuroprotection, immunomodulation, and protein aggregate clearance. However, meaningful improvement of neurodegeneration would probably require a highly efficient and specific treatment throughout a long period of time.

As hMSCs can be harvested from various tissues, one measure for enhancing the efficiency of hMSCs-based therapy is by revealing the clinical relevance of hMSCs derived from various tissues. hMSCs derived from bone marrow and adipose show similar differentiation potential and expression of common hMSCs surface markers such as CD34, CD44, CD45, CD105, CD29, and CD90 [107–109]. However, recent comparative analyses have indicated variable protein secretion patterns [110, 111] and identified additional markers that differentially express in hMSCs derived from various origins (reviewed in ref. 112). Notably, hA-MSCs were reported to have a higher proliferative capacity over hBM-MSCs and to express a wider range of chemokine receptors, which are considered important for their homing capacity [107–119, 113]. hA-MSCs were also previously reported to possess a higher immunomodulatory potential [108], while a recent report using hA-MSCs and hBM-MSCs from the same donor has reported no major differences in their aspect [114].

Notably, recent publications comparing the administration of hMSCs derived from bone marrow or adipose tissue in mice models of hind limb ischemia have shown mixed results in terms of which source is superior [109, 115]. In a murine model of Crohn's disease, hA-MSCs were reported superior over hBM-MSCs in promoting an anti-inflammatory response [116]. However, there is lack of direct evidence for superiority of specific tissue-derived hMSCs for the treatment of neurodegenerative diseases. As the variation between hMSCs derived from different tissues may result in heterogeneous clinical outcomes, future studies should be performed to determine the relevant hMSCs sources.

Enhancing clinical efficacy for hMSCs-based treatment might also be achieved by extending hMSCs survival following transplantation. hMSCs survival in the CNS following transplantation was repeatedly reported to be limited up to several months in rodent models [18, 19, 33, 44, 74] with one report indicating survival after 45 weeks [41] and some reporting survival of only several days following transplantation [31, 43]. hMSCs survival following transplantation was shown to be affected by several factors including an immune response against the grafted cells [117], hypoxic/ischemic stress [118], or by an oxidative environment in site of damage [119]. Several methods, such as growth factor preconditioning, or pretreatment exposure to oxidative stress, were suggested for enhancing hMSCs survival in vivo following transplantation into the ischemic myocardium (reviewed in ref. 120). However, the factors associated with hMSCs survival in the CNS were not identified yet.

As described earlier, one promising mean of enhancing the beneficial outcome of hMSCs-based therapy is by enhancing the mechanisms throughout which hMSCs function was exerted. This was demonstrated through ex vivo differentiation or by genetically engineering of hMSCs to enhance NTFs

or immunomodulatory molecules expression. Similarly, hMSCs were suggested as a system to deliver shRNA into lesion area. Olson et al. have shown that hMSCs transduced to express shRNA against the huntingtin gene were able to reduce its expression in coculture neurons [121].

Finally, a potential clinical benefit is expected from the combination of hMSCs-based therapy with other novel therapeutic agents. To date, several compounds have shown a synergistic effect upon combined treatment with hMSCs in models of stroke and dementia [122–125] and combined transplantation of multiple cell-type is clinically examined in patients with stroke [126, 127].

CONCLUSION

Upon transplantation into rodent models as well as patients with neurodegenerative diseases, hMSCs are repeatedly reported to home toward lesion sites and secrete a broad range of molecules that modulate various aspects of diseases. Initially, hMSCs broad differentiation capacity was intended to be used for the derivation of neurons for cell regeneration of damaged tissue. However, limited efficacy for the derivation of neuronal cell types using this approach has motivated the use of hMSCs differentiation capacity for the establishment of supportive cells providing neuroprotection for damaged tissue.

This concept is well demonstrated by our group's reports on the beneficial effect of NTF-secreting hMSCs in various models of neurodegeneration as well as in ALS patients. As different neurodegenerative diseases entail abnormalities in various molecular pathways and different cell types, it is expected that the trophic array of molecules essential to support neuronal and glial function may vary as well. While many favorable factors affecting substantial properties of disease have been identified, the potential of this approach is only beginning to unravel, as clinical trials using these cells meet safety criteria. We envisage that the use of hMSCs as vehicles for delivery of therapeutics into lesion area will provide an efficient platform for targeted therapy in various neurodegenerative diseases. Therefore, it seems promising that hMSCs will serve as efficient tools for neurodegenerative disease therapy in the future.

AUTHOR CONTRIBUTIONS

R.V.: manuscript writing; D.O.: manuscript writing, final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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