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Genetically Engineered Mesenchymal Stem Cells as a Proposed Therapeutic for Huntington's Disease

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Abstract There is much interest in the use of mesenchymal stem cells/marrow stromal cells (MSC) to treat neurodegenerative disorders, in particular those that are fatal and difficult to treat, such as Huntington's disease. MSC present a promising tool for cell therapy and are currently being tested in FDA-approved phase I–III clinical trials for many disorders. In preclinical studies of neurodegenerative disorders, MSC have demonstrated efficacy, when used as delivery vehicles for neural growth factors. A number of investigators have examined the potential benefits of innate MSC-secreted trophic support and augmented growth factors to support injured neurons. These include over-expression of brain-derived neurotrophic factor and glial-derived neurotrophic factor, using genetically engineered MSC as a vehicle to deliver the cytokines directly into the microenvironment. Proposed regenerative approaches to

neurological diseases using MSC include cell therapies in which cells are delivered via intracerebral or intrathecal injection. Upon transplantation, MSC in the brain promote endogenous neuronal growth, encourage synaptic connection from damaged neurons, decrease apoptosis, reduce levels of free radicals, and regulate inflammation. These abilities are primarily modulated through paracrine actions. Clinical trials for MSC injection into the central nervous system to treat amyotrophic lateral sclerosis, traumatic brain injury, and stroke are currently ongoing. The current data in support of applying MSC-based cellular therapies to the treatment of Huntington's disease is discussed.

Keywords Human mesenchymal stem cells · Neurite outgrowth · Neurodegenerative diseases · Hypoxia · Tissue repair · Huntington's disease · Clinical trials · BDNF

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Abbreviations

ALS	Amyotrophic lateral sclerosis
BDNF	Brain-derived neurotrophic factor
CSF	Cerebrospinal fluid
FGF-2	Fibroblast growth factor-2
GDNF	Glial-derived neurotrophic factor
GMP	Good Manufacturing Practice
GLP	Good laboratory practice
HD	Huntington's disease
HGF	Hepatocyte growth factor
hESC	Human embryonic stem cells
HSCT	Hematopoietic stem cell transplantation
HTT	Huntingtin gene, mRNA, or protein
IGF-1	Insulin-like growth factor-I
MSC	Mesenchymal stem cells
NSC	Neural stem cells
OEC	Olfactory ensheathing cells

siRNA	Small interfering ribonucleic acid
SOP	Standard operating procedure
SVZ	Subventricular zone
TBI	Traumatic brain Injury
QA	Quinolinic acid
QA/QC	Quality assurance/quality control
VEGF	Vascular endothelial growth factor

Introduction

Human mesenchymal stem cells are known to secrete a variety of cytokines and growth factors that have both paracrine and autocrine activities for damaged tissues, including the brain (reviewed in [1]). The mechanism of action of adult MSC obtained from bone marrow or adipose tissue is based on the innate functions of these stem cells: the injected cells home to the injured area, in particular to hypoxic, apoptotic, or inflamed areas, and release trophic factors that hasten endogenous repair. These secreted bioactive products can suppress local inflammation, enhance angiogenesis, reduce levels of free radicals, inhibit fibrosis and apoptosis, and stimulate recruitment, retention, proliferation, and differentiation of tissue-residing stem cells (reviewed in [2]). These paracrine effects are distinct from the classical model of direct differentiation of stem cells into the tissue to be regenerated.

MSC are ideally suited for cellular therapy due to their ease of isolation, manipulation, and safety. They can be expanded from normal qualified human donors in large quantities and can be infused without tissue matching, since they shield themselves from the immune system [3]. The ability to be transplanted without tissue matching has allowed large multicenter trials to be conducted with direct comparison of the same batches of MSC across hundreds of patients, without adverse events or rejection reactions [4, 5]. Due to the promise of MSC in cellular therapies, a variety of studies have focused initially not only on their characterization but also on their utility in treatment of several diseases in animal models. MSC contributed significantly to the recovery of tissues in models of myocardial infarction [6], stroke [7, 8], meniscus injury [9], and limb ischemia [10]. However, the number of engrafted MSC was consistently low in the damaged tissue, suggesting that their efficacy relies upon actions other than direct differentiation. Kinnaird et al. [11] demonstrated that MSC-conditioned media stimulated endothelial cell proliferation and migration *in vitro*, and the injection of MSC-conditioned media into mice that had undergone hind limb ischemia was sufficient to mediate regeneration of the blood flow in the injured limb. Similar results have been shown with a cardiac infarction model [12], and the secretion of multiple angiogenic cytokines from MSC has been demonstrated.

Hepatocyte growth factor (HGF), fibroblast growth factor-2 (FGF-2), insulin-like growth factor-1 (IGF-1), and vascular endothelial growth factor (VEGF) have all been detected in MSC-conditioned medium. The increasing body of evidence points toward the theory that a complex set of trophic factors secreted by MSC significantly contributes to injury repair *in vivo*, through stimulating angiogenesis, reducing oxidative stress, and decreasing apoptosis.

MSC have been found to produce improvements in disease models even though a limited number of the cells could be demonstrated to be stably engrafted. A mystery that remains in the MSC field is that, while MSC persist long-term in the tissues of non-injured or chronically damaged mice, in cases of acute injury or inflammation, MSC respond to the injury robustly, but only transiently and do not become an enduring part of the repaired tissue or vasculature to any significant degree. We have studied this disparity in immune deficient mice that cannot reject the human cells, so the disappearance of MSC is independent of an immune rejection. One month post-infusion MSC are often virtually undetectable at the area of acute tissue damage [13–15]. The same phenomenon has been observed in large animal models. In contrast, when labeled MSC, cultured in the same way, are infused intravenously into immune deficient mice that have low-level systemic damage from irradiation, a chronic disease, or no damage at all, the cells migrate through all tissues and persist in a relatively evenly dispersed and long-lasting manner [16, 17]. We have recovered human MSC from numerous organs of the mice at time points from 1 to 18 months post-transplantation in those cases [16–20], whereas in the acute injury setting, they are only transiently recovered.

In our previous studies, hypoxic preconditioning was found to enhance MSC efficacy in a severely hypoxic tissue ischemia model, perhaps by priming MSC to respond to the low oxygen environment [14]. We are currently using the hypoxic preconditioning method to prepare MSC for injection into the striatum in murine models of HD. Further studies must be done to better understand the duration of the residence of infused MSC in tissues, including the brain, and the mechanisms that recruit and retain them in chronic vs. acute tissue damage environments. This knowledge will allow the most effective use of MSC-based cellular therapies.

MSC in Neural Repair—Overview

There is currently much interest in the use of MSC to treat neurodegenerative diseases. In particular degenerative diseases like Huntington's disease that are fatal and difficult to treat, MSC may prove therapeutic by providing neuro-

trophic factors to encourage repair and potentially new growth of neurons from endogenous neural progenitor cells. Proposed regenerative approaches include delivery via intracerebral or intrathecal injection or even infusion via an intranasal route [21]. Therapies will capitalize upon the innate trophic support from MSC or on additional augmented growth factor support. Genetically engineered MSC can serve as the vehicle to deliver brain-derived neurotrophic factor (BDNF) or glial-derived neurotrophic factor (GDNF) into the brain to support injured neurons, as reviewed in this report.

Upon transplantation, MSC in the brain promote endogenous neuronal growth, decrease apoptosis, and regulate inflammation through the use of secreted factors. Rossignol et al. [22] demonstrated that after xenotransplantation of human MSC into rat brain, there were higher levels of messenger RNAs (mRNAs) for the anti-inflammatory molecules IL-6 and TGF- β_1 than for the pro-inflammatory cytokines IL-8 and IL-12. MSC can mediate modification of the damaged tissue microenvironment to enhance endogenous neural regeneration and protection. MSC transplanted at sites of nerve injury have been shown to promote functional recovery by producing trophic factors that induce survival and regeneration of host neurons [23]. Transplantation of human bone marrow stem cells into the brains of immunodeficient mice markedly increased the proliferation of endogenous neural stem cells [24]. In an experimental allergic encephalomyelitis model of multiple sclerosis, rodents that received an intraventricular infusion of MSC were found to have almost twice the number of axons as control animals [25]. Busch et al. [26] demonstrated that human multipotent adult progenitor cells, a precursor of MSC, prevented axonal dieback and significantly enhanced neurite growth into the lesion core after a spinal cord injury. Although candidate molecules are under investigation, further detailed studies are needed to carefully define the factors responsible for the MSC-mediated induction of proliferation and enhancement of axonal extension and synaptic connection in resident neural stem cells, in order to best capitalize upon this type of therapy for the repair of neurodegenerative diseases.

Several groups have shown that intraparenchymal delivery of human MSC is safe and can delay loss of motor neurons in rodents. Vercelli et al. [27] transplanted human MSC directly into the lumbar spinal cords of SOD1 ALS mice. The MSC migrated throughout the spinal cord and delayed loss of motor neurons, prolonging motor performance. Another study compared the efficacy of transplanting olfactory ensheathing cells (OEC) and rat MSC intrathecally through the fourth ventricle in the spinal cord. The OEC distributed widely, but significant changes in clinical outcomes were observed only with addition of MSC transplantation when female ALS mice

showed statistically longer lifespans than males and control mice [28].

Numerous clinical trials have demonstrated the low risk of systemic infusion of allogeneic MSC into patients with various diseases. Biosafety monitoring of MSC infusion has been performed throughout these trials, with no serious adverse events reported. For direct infusion of MSC into the brain or spinal cord, the biosafety aspects should be monitored extremely carefully. Possible risks include the development of cytogenetic abnormalities in cultured cells or ectopic differentiation to other tissue lineages. The development of cytogenetic abnormalities when MSC were cultured past the crisis point has been reported in rodent MSC [29]. In our lab, we have found that, in general, rodent MSC cultures are frequently contaminated with hematopoietic elements and do not well reflect human MSC biology. There has been only one report of a karyotypic abnormality happening in human MSC that were cultured in conditions that would never be allowed in a good manufacturing practice (GMP) or good laboratory practice (GLP) setting [30]. It was later found that the tumor arose due to cross-contamination of MSC cultures with fibrosarcoma and glioma cells [31]. When human MSC are cultured under GLP/GMP conditions, with appropriate release criteria, adverse events have not been observed throughout the field, both in vivo and in numerous human clinical trials [1, 2, 32–38]. We have published a decade-long biosafety study on the clinical biosafety profile of genetically engineered MSC [18].

A potential risk for infusion of MSC into the spinal cord or brain is that they have potent revascularization capacity and will home to the hypoxic region around a tumor bed [39, 40]. This tropism for the tumor or wound bed could result in enhanced revascularization and survival of a growing tumor. Therefore, in proposed cellular therapy trials, an important exclusion criterion is the lack of prior brain tumors or other cancers in the past 5 years. Magnetic resonance imaging (MRI) or other functional imaging modalities should be done prior to treatment in HD trials to establish the baseline striatal volume [41, 42] and would be also used to rule out existing brain tumors.

Several groups have reported that MSC can be induced to express neural markers in vitro, in particular after cocultivation with human or murine neurons or conditioned medium. However, although mature neurons with functional activity can be generated in culture from neural stem cells (NSC) [43, 44], it has not been shown conclusively that mature neurons with signaling capacity can be generated from MSC [45]. While attractive in theory, MSC do not appear to be a viable alternative to NSC as a neural tissue replacement strategy. It has been reported that undifferentiated MSC often express a number of proteins used as markers of neural differentiation [46].

Additionally, MSC can assimilate proteins and pieces of membrane from other cells, requiring more definitive proof of “trans-differentiation” of MSC to neurons, particularly in co-cultures and *in vivo* where MSC have extensive contact with neural tissue. Recent studies have shown that RNA species and proteins can be passed from mammalian donor cell to target cell, through gap junctions, exosomes, virtosomes, or tunneling nanotubules [47–49]. RNA interference (RNAi) molecules were found to be secreted in microvesicles, which later fused with other cell [50–53]. Ratajczak et al. [54] described horizontal transfer of mRNA from cell to cell through embryonic stem cell-derived microvesicles. Virtosomes were recently described to be complexes of RNA–lipoprotein, which can readily enter other cells where they can modify the biology of the recipient cells [47]. Tunneling nanotubes have also been described for transferring RNA species and organelles between cells [48]. Our group is currently exploring an MSC-based platform for producing and delivering RNAi moieties targeted to the huntingtin mRNA as a potential therapeutic avenue for treating HD [55]. We chose to explore human MSC, which we and others have previously shown to be excellent *in vivo* delivery vehicles for enzymes and proteins, as the cell type to produce and convey RNAi [2, 17]. Transfer of organelles as large as mitochondria from MSC to damaged cells has also been described [56]. Therefore, reports of MSC trans-differentiating to neural phenotypes, particularly after co-cultivation with neurons, must be cautiously interpreted. However, the ability of MSC to migrate to areas of damage in the brain and to secrete neurorestorative factors is significant and could be harnessed to treat neurodegenerative disease.

Expression of Neuroregulatory Factors by MSC

Understanding the innate capacity of MSC to influence neural cell growth, survival, and neurite extension is currently a very important field of study. Detailed determination of which factors MSC express, under which conditions, is key. Crigler et al. [23] demonstrated that specific subpopulations of human MSC expressed BDNF and beta-NGF, but not neurotrophins NT-3 or NT-4. The authors used a co-culture assay to show that BDNF expression levels correlated with the ability of MSC subclones to induce survival and neurite outgrowth in the SH-SY5Y neuroblastoma cell line. The effects were only partially inhibited by a neutralizing anti-BDNF antibody, indicating that other factors secreted by the MSC also had neuroregulatory effects. The authors identified production of other neurite-inducing factors, axon guidance, and neural cell adhesion molecules, which contributed to the capacity

of the MSC to induce neuronal cell survival and nerve regeneration. Wilkins et al. [57] have also demonstrated that MSC secrete BDNF and that addition of anti-BDNF neutralizing antibodies attenuated their neuroprotective effects. These studies help to at least partially determine the mechanisms of MSC-mediated neural survival and also validate the important role of BDNF in neuronal protection.

In addition to neurotrophic factors, the extracellular matrix (ECM) molecules produced by MSC have also been shown to support neural cell attachment, growth, and axonal extension [58]. Neurons on ECM derived from MSC formed more complex neurite networks than those cultured on poly-D-lysine [58]. Croft et al. [59] showed that soluble factors produced by MSC promoted the survival of neurons in culture and promoted axonal growth in neuronal progeny. The authors proposed mechanisms to manipulate MSC *in vitro* prior to transplantation that could potentially further enhance the endogenous neurogenic response to injury. Different extracellular matrix scaffolds have also been examined to enhance survival of stem cells after implantation to damaged areas of the brain, and some ECM molecules can synergize with the neurorestorative effects of MSC [60]. However, a highly important consideration is to not use matrix proteins such as fibronectin that could enhance fibroblastic differentiation from MSC, which could risk scarring and microglial activation. Therefore, such scaffolds for implantation must be carefully tested in animal models of HD before consideration for future clinical application.

MSC provide a very promising platform from which to produce factors for neural regulation. Viral vectors under inducible promoters can be used to customize MSC for the treatment of specific diseases, as described in more detail below. Overexpression of BDNF is a particularly promising modification for HD. A traditional gene therapy approach, where the neurons would be directly infected *in vivo* by live lentiviral or AAV vectors carrying BDNF would suffer from a number of safety concerns. Integrating virus can pool at the injection site, superinfecting neighboring cells and limiting distribution beyond a small area. Viral integrations can be controlled *ex vivo* with MSC and limited to one to two viral integrants per MSC genome, as suggested by the FDA for stem cell gene therapy trials. Using MSC as the delivery vehicle, a “suicide gene” such as thymidine kinase can also be used to eliminate a graft if anything went wrong. This would not be possible with vector-mediated delivery since the “suicide approach” would destroy the neuron into which the gene had integrated. The natural reparative characteristics of MSC can act synergistically with the growth factors that they produce. MSC could potentially migrate to injured cells to deliver BDNF locally.

For any planned delivery method, it will be important to provide a precise and quantitative evaluation of BDNF protein level in the human CNS, perhaps by conducting a spinal tap after the therapy. Methods for precise quantitation of BDNF have been described by Zuccato et al. [61, 62]. BDNF is a complex protein that could have unwanted side effects if expressed at very high levels. It will also be important to regulate BDNF levels using an inducible promoter system, since transgenic mouse models have shown that high levels of BDNF overexpression in the brain can interfere with normal brain function by causing learning impairments and increased excitability [63].

Discussion of potential therapeutic approaches in the context of MSC-based treatments and growth factor delivery for HD is presented in this review.

Huntington's Disease

HD is an incurable inherited genetic disorder where the condition of the patients inexorably worsens and their prognosis is eventual death, following decline in control of emotion, movement, and cognition. The pathology of HD is caused by a variable sized polyglutamine expansion of the protein product of the huntingtin (HTT) gene. Multiple disease mechanisms have been described for HD neurodegeneration and are currently under investigation in the search for therapeutics. HD is a challenging disease to treat. Not only do the affected, dying neurons need to be rescued or replaced, but the levels of the toxic mutant protein must also be diminished to prevent further neural damage and to halt progression of the movement disorders and physical and mental decline associated with HD.

The best hope for halting HD progression is to reduce or eliminate the mutant Htt protein in the affected cells [64, 65]. Small interfering RNAs (siRNA) have been shown to be effective at reducing Htt levels and ameliorating disease symptoms in animal models [66, 67]. Exciting new data shows that the mutant HTT mRNA can be specifically silenced by targeting conserved single nucleotide polymorphisms in the mutant allele, while sparing the transcript produced by the normal allele [68–70]. The challenge for this technology is to deliver the siRNA into the human brain in a sustained, safe, and effective manner. Our group is currently funded by the California Institute for Regenerative Medicine to develop an siRNA delivery system using human MSC as the “production factories” in the brain. Safety and efficacy studies are ongoing [55].

Many studies have demonstrated the safety and efficacy of MSC injection into the brain and central nervous system. Lescaudron and colleagues [71] transplanted autologous bone marrow stem cells into the damaged striatum of the rat HD model and found that the transplant significantly

reduced working memory deficits. The transplanted MSC remained undifferentiated, but exerted trophic effects on the injured tissue. Bantubungi et al. [72] found that the lesioned environment of the striatum favored the proliferation and intralésional distribution of MSC. Several groups have shown that striatal atrophy in models of HD is accompanied by the subsequent enlargement of lateral ventricles [73, 74]. The reduction in volume of the lateral ventricle can thus be a good indicator of repair in HD correction studies. Amin et al. [73] demonstrated that after MSC implantation into the striata of rats with a unilateral damage model of HD, striatal atrophies were significantly reduced. Consequently, the volume of the lateral ventricle returned to a significantly smaller size, approaching the normal contralateral ventricle. Their results demonstrated the potential for MSC in the treatment of microanatomical defects in the motor disorders of HD.

Groups who have evaluated the impact of intrastriatal transplantation of MSC in rodent models of HD are summarized in Table 1 [73, 75–82]. Some groups had hoped to observe MSC becoming neurons after transplantation. However, as discussed above, although some neuronal markers can be observed on transplanted MSCs due to their propensity for “sharing” membrane and cytoplasmic markers and natural expression of a number of traditionally neural markers like nestin [46], bona fide transdifferentiation into functional neurons has not been convincingly demonstrated. In contrast to “transdifferentiation” to neural cells, through the ability of MSC to secrete neurotrophic factors, reduce inflammation, reduce apoptosis, enhance axonal extension, and reduce reactive oxygen species, significant positive effects were seen in alleviating HD progression in the studies listed.

Data suggest that MSC implantation into the striatum can potentially delay the unrelenting loss of medium spiny neurons in HD. An important factor to consider is that the MSC implanted at adjacent sites have the capacity to migrate into the most damaged areas in response to stress or death of medium spiny neurons, which are lost in HD. MSC are known to migrate robustly in the brain, in response to injury. Delcroix et al. [83] demonstrated that iron nanoparticle-labeled MSC migrated from an implantation site in the subventricular zone microenvironment toward the olfactory bulb through the rostral migratory stream only when a mechanical lesion of the olfactory bulb was performed. They confirmed the known potential of MSC to migrate toward areas of damage, even over a great distance in the brain. The migratory capacity of MSC was further examined by transplantation of the cells a distance from a quinolinic acid (QA)-induced striatal lesion, a rat model for Huntington's disease [82, 84]. Migration was monitored using MRI scans followed by histology. The engrafted MSC were shown to have migrated a great

Table 1 Mesenchymal stem-cell-based treatment of HD in preclinical rodent models

References	Animal model	Transplanted cells	Histology	Lesion volume	Functional outcome
Amin et al., [73]	Rat, QA	Rat BM-MSCs	Reduced striatal atrophy	Improved striatal volume	ND
Lee et al., [79]	Rat, QA	Human adipose MSCs	Reduced striatal atrophy Reduced apoptosis	Decreased lesion volume	Reduced Apo-M induced rotations
Lin et al., [80]	Mice, QA	Human BM-MSCs	Increased cell proliferation in striatum	Decreased lesion volume	Improved rotarod performance Extended survival time
Lin et al., [80]	R6/2-J2 mice	Human BM-MSCs	Improved cell differentiation	ND	Improved survival
Jiang et al., [78]	Rat, QA	Human BM-MSCs	Reduced striatal atrophy	Decreased lesion volume	Reduced motor dysfunction
Lee et al., [79]	R6/2 mouse	Human adipose MSCs	Reduced HTT aggregates Attenuated loss of striatal neuron	Improved striatal volume	Improved rotarod Reduced claspings Improved survival
Rosignol et al., [81]	Rat, 3NP	Rat BM-MSCs	ND	Prevented 3NP-mediated ventricle enlargement	Improved rotarod Improved paw placement
Sadan et al., [82]	Rat, QA	Rat BM-MSCs	Improved MSC migration to lesion	Decreased lesion volume	Regenerated striatal network Reduced Apo-M induced rotations
Im et al., [77]	YAC 128 mice	Human adipose MSCs	Reduced striatal atrophy	Improved striatal volume	Improved rotarod performance/motor function
Edalatmanesh et al., [76]	Rat, QA	Rat BM-MSCs	ND	ND	Reduced Apo-M induced rotations Improved beam walk Improved hang wire time
Dey et al., [93]	YAC 128 mice	BM-MSCs engineered to produce BDNF	Reduced striatal atrophy	Improved striatal volume	Improved rotarod performance Reduced hindlimb claspings

distance along the internal capsule toward the QA-induced lesion in the striatum. They were shown to have regenerated the damaged striatal dopaminergic nerve terminal network in this animal model for HD [82, 84].

A human cellular therapy trial has already demonstrated progress in the treatment of HD by intracerebral implantation. Bachoud-Levi and colleagues [85, 86] transplanted human fetal neurons into the brains of five patients with HD. Three out of five patients with HD produced motor and cognitive improvements 2 years after the fetal neural graft. The authors obtained convincing evidence that these human fetal striatal grafts were capable of reconnection and activation of the frontal lobes. Clinical improvement plateaued after 2 years and then faded off variably 4–6 years after the implantation [85, 86]. These and other studies demonstrate that fetal striatal neurons can be safely implanted into the brain without adverse events. The group is currently conducting phase II clinical trials.

Since fetal striatal tissue could be a limiting factor, an alternative might be derivation of striatal neurons from human embryonic stem cells. However, a major challenge yet to be robustly achieved from embryonic stem cell-derived neurons is to coerce the new neurons make bona fide synaptic connections, as the Bachoud-Levi group had seen from the fetal neurons. Co-transplantation of fetal or embryonic stem cell-derived neural grafts with MSC to

enhance neuritogenesis and to guide axonal pathfinding through areas of damage could be considered [26].

A confounding factor for the treatment of HD by cellular therapy is the continued presence of the mutant HTT mRNA and protein in neighboring cells causing chronic damage, even if new, healthy neurons are implanted. Efforts to specifically knock down the expression of this mutant HTT mRNA and protein prior to or simultaneously with the MSC-based therapies must be considered [68–70].

Neural Growth Factor Delivery by MSC

An interesting development candidate that we and others are characterizing is allogeneic human MSC engineered to secrete BDNF (Fig. 1). This candidate therapy could provide a potential strategy to enhance striatal neuron survival and regeneration in HD. BDNF levels are very low in mice and humans with HD. In rodent models, BDNF has been shown to ameliorate symptoms and to extend survival, making BDNF therapy a leading candidate for use in treating HD. Striatal neurons depend on BDNF for function and survival [87, 88]. In the later stages of HD, available BDNF levels plummet since the mutant protein prevents production at the mRNA level [61]. This reduction in BDNF affects the onset and severity of the disease in HD

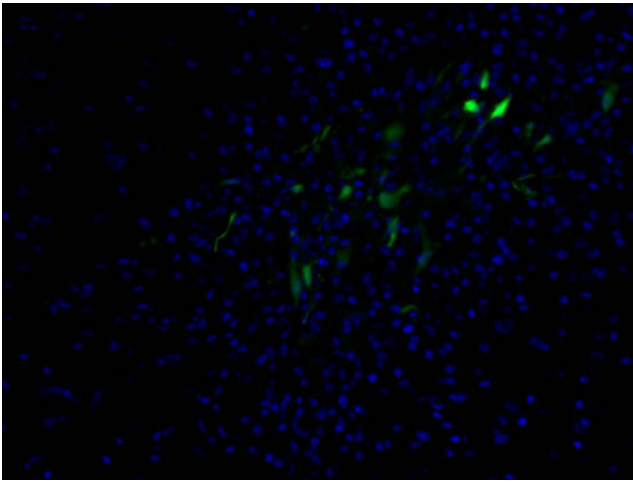


Fig. 1 MSC/BDNF in the brain of an immune deficient mouse. Human mesenchymal stem cells engineered to secrete brain-derived neurotrophic factor (BDNF) and a reporter gene (enhanced green fluorescent protein, or eGFP) were transplanted into the brain of immune-deficient mice using stereotactic injection. Tissues were harvested for assessment of human cell engraftment and biosafety at different timepoints. Shown is engraftment at 6 days post-injection

mice. Up-regulation of BDNF in the brains of transgenic rodent models of HD has shown amelioration of the disease phenotype [89]. Adenoviral astrocyte-specific expression of BDNF in the striata of mice transgenic for HD was shown to delay the onset of the motor phenotype [90]. Due to its pro-survival effects in striatal neuropathology, BDNF is a leading candidate for neuroprotective therapies in HD [62, 91, 92].

In transgenic mouse models of HD, there is a significant reduction in the aberrant behavioral phenotype following administration of MSC/BDNF [93]. The Dunbar laboratory has shown that MSC, especially those engineered to over-express BDNF, have significant ameliorative effects on disease progression in a transgenic mouse model of HD [93]. These studies have demonstrated that MSC engineered to produce BDNF and implanted into the striata significantly increased time to fall in rotorod testing, demonstrating an increase in coordination and reduction in movement disorders in treated mice during the 13 months of the study. The MSC/BDNF therapeutic also significantly reduced limb clasping, a hallmark behavioral defect in HD mice, over the same time period [93].

A major impediment for the clinical use of neurotrophic factors is their inability to cross the blood–brain barrier in therapeutic amounts. In the quest to begin translating promising studies such as those published by Dey et al. [93] to the clinic, we have shown biosafety of intracranial administration of MSC in long-term rodent and non-human primate models. Our group uses human MSC as safe and long-lived delivery factories for cytokines and growth

factors [2, 17, 94]. We are currently evaluating the safety and efficacy of MSC engineered to secrete BDNF in peripheral tissues and in the brain to enhance neuro-restorative capacity in HD (Fig. 1).

The use of MSC to deliver factors, both through their own innate responses and through engineering, has benefits beyond direct protein administration. Transplanted MSC can provide sustained and long-term delivery of factors at supraphysiological levels, as we and others have shown over the past two decades [17, 19, 20, 95–98]. Using immune-deficient mouse models, we have recovered human MSC from numerous organs at timepoints from 1 to 18 months post-transplantation, with continued expression of the gene product [16, 17, 19, 94, 95, 97, 99]. We also performed a decade-long biosafety study to demonstrate that genetically engineered human MSC are safe, with no adverse events ever having been observed *in vivo* [18]. Efforts from our group and others are currently evaluating the effects of BDNF expression from human MSC implanted into the striata of HD mice. Taken together, the data discussed in the current review provide support for the potential for MSC to deliver augmented neurotrophic support.

Clinical Trials of MSC for Neural Repair

The ability of MSC to secrete factors to decrease motor neuron death when implanted into the CNS of ALS patients was assessed in a human clinical trial [100, 101]. Ten patients with ALS who had severe functional impairment of their legs were enrolled in the MSC clinical trial with no adverse events. Autologous MSC were isolated from the patient's own bone marrow and expanded using good manufacturing practice conditions. Expanded MSC were suspended in autologous cerebrospinal fluid (CSF) and transplanted into the recipient's spinal cord at a high thoracic level. No immediate or delayed transplant-related toxicities were observed. Patients were regularly monitored before and after transplantation using clinical, psychological, neuroradiological, and neurophysiological evaluations. Three months after cell implantation, a trend toward slowing of the decline in muscular strength was observed in the legs of four of the first seven patients treated. No significant acute or late side effects were evidenced, and four of the patients showed significant slowing of disease progression [101]. These studies demonstrated that MSC infusion into the cerebrospinal fluid can be tolerated without adverse events in patients with ALS [101]. A phase II clinical trial using MSC is underway in Europe, and the FDA has recently approved a phase I trial of MSC delivery to the CNS for ALS in the United States.

A pilot trial using either intravenous- or intrathecal-injected MSC has been conducted by Karussis and colleagues in 12 patients diagnosed with untreatable neurodegenerative diseases—ALS or multiple sclerosis [102]. Their intent was to demonstrate the safety of the delivery methods and treatment with autologous MSC. They initiated the pilot trial after *in vitro* and pre-clinical investigations had demonstrated immunomodulatory effects of MSC with suppression of self-reactive T lymphocytes. In addition, animal studies in mice with chronic progressive experimental autoimmune encephalomyelitis had mitigation of their clinical course accompanied by histopathological evidence of neuroregeneration following IC and IV injection of MSC [103]. Their study was extended to include 10 ALS and 10 MS patients showing proof of concept that both delivery methods were feasible and safe. They are currently conducting a larger phase I/II trial delivering autologous MSC as treatment in patients with severe refractory MS [104]. Additional case reports and a small pilot study including 10 patients with multiple sclerosis treated with autologous MSC have been conducted with intriguing results (Bonab et al. [105–107]). Currently, there are at least four clinical trials being conducted at centers in UK, Israel, Spain and the USA

evaluating the safety and efficacy of bone-marrow-derived MSC in treatment of MS.

Direct injection of MSC into the injured region of the brain during surgery following traumatic brain injury (TBI) has also been performed without adverse events. Seven TBI patients each received up to 10^9 expanded MSC during the cranial repair operation [108]. Patients were followed for 6 months and demonstrated significant improvements in neurologic function. Placebo-controlled trials for MSC injection into the CNS for TBI and stroke, as well as spinal cord injury and neurodegenerative disorders, are currently ongoing in countries outside of the USA (clinicaltrials.gov). No adverse events have been reported from these studies. As one example, Venkataramana et al. [109] recently reported safety data from an open label phase I clinical trial where autologous bone-marrow-derived MSC were transplanted into the striata of patients with advanced PD. No adverse events occurred and possible clinical improvement was observed.

Clinical trials of an MSC-like multipotent cellular product, Multistem [110, 111], has been approved by the FDA to treat stroke in the USA, by the company Athersys. Celgene is approved for a multicenter phase II clinical trial to use placental-derived MSC for treating stroke, and San-Bio is conducting a phase I trial of gene-modified

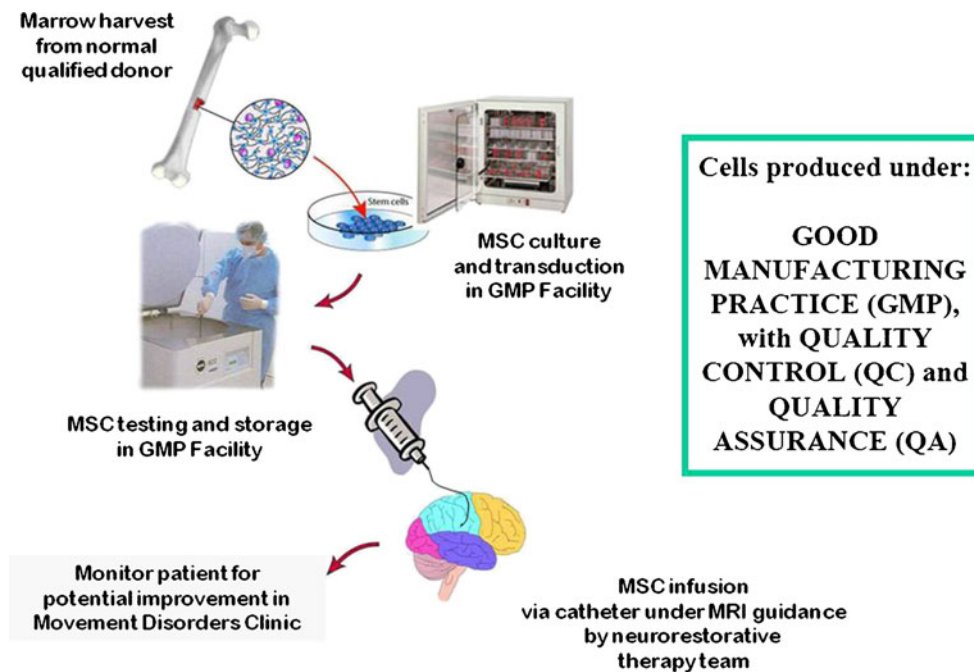


Fig. 2 Schema of proposed human MSC therapy for neurodegenerative disease. Bone marrow is harvested from a normal, qualified donor. MSC are expanded and transduced with viral vectors in the good manufacturing practice facility using qualified reagents and well-established standard operating procedures. The transduced cells are expanded, tested extensively, and banked. Following FDA clearance of the phase I clinical trial, the qualified cells will be implanted near the affected portion of the brain in symptomatic HD patients. MSC will be thawed, tested, and

infused under MRI guidance by an experienced Neurorestorative Therapy Team. Patients will then be followed by experienced clinicians in the movement disorders clinic for evaluation of potential neurorestorative effects: slowing of disease progression as measured by total functional capacity score and delay in volumetric MRI changes known to occur in HD. Potential clinical improvement in severity of movement disorders and cognitive impairment as measured by the Unified HD Rating Scale (UHDRS) and a battery of cognitive tests will be monitored

MSC for stroke. Osiris therapeutics has conducted multiple clinical trials using allogeneic MSC administered through systemic infusion. No stem-cell-related events have occurred and their studies provide extensive safety and provisional efficacy data for allogeneic bone-marrow-derived MSC administration to patients through FDA-approved clinical trials [4, 5, 33].

Patient safety and the potential benefit to risk ratio are always the foremost considerations by the Food and Drug Administration. This review has covered the potential benefits of MSC-based therapies for the treatment of neurodegenerative diseases, and Huntington's disease in particular. We and many others have documented the biosafety of MSC therapies, which are now in phase III trials for some indications, and further safety and efficacy data are being collected by numerous groups. The need for safe and effective cellular therapies to treat HD is great. Current therapies only target symptoms, and there are no drugs or other treatments that effectively delay the relentless loss of striatal volume in affected patients. Extending MSC-based therapies to neurodegenerative diseases, in particular those for which there are currently no effective treatments, such as HD (Fig. 2), could have a high potential benefit to risk ratio.

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