Mesenchymal Stem Cells: New Approaches for the Treatment of Neurological Diseases

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Abstract: Cellular therapies represent a new frontier in the treatment of neurological disease. Mesenchymal stem cells (MSCs), which can be harvested from bone marrow, adipose tissue, and umbilical cord blood, among many other sources, possess several qualities which may be used to treat diseases of the central nervous system. MSCs migrate to sites of malignancy, a property which may be used for the treatment of brain cancer. MSCs possess immunosuppressive properties, which may be used for the treatment of neurological disorders with an inflammatory etiology. Finally, MSCs restore injured neural tissue, a property which may be used for the treatment of neural injury. Approximately 23 clinical trials have been completed to date, with many more ongoing, and all have been listed in this review. The long-term safety of MSC-based therapies is not well established, and continues to be one major limitation to clinical translation. More broadly, only a small minority of clinical trials have employed rigorous designs that include prospective randomization, patients from multiple centers, clinically-relevant and reproducible endpoints, and adequate long-term follow-up. These limitations must be addressed before MSCs can enter widespread clinical use. Nevertheless, MSCs represent a promising new approach to treating diseases of the central nervous system that are traditionally associated with morbid outcomes. With additional pre-clinical and clinical studies that focus on their potential benefits as well as dangers, MSCs may one day find translation to clinical use in the setting of neurological disease.

Keywords: Mesenchymal stem cells, brain neoplasms, nervous system diseases, Parkinson disease, Alzheimer disease, stroke, clinical trial, cell transplantation.

INTRODUCTION

Stem cell-based therapies hold tremendous promise for the treatment of human disease. Each of the 5 types of human stem cells—embryonic, epithelial, hematopoietic, neural, and mesenchymal—have received considerable attention from the scientific community for their potentially therapeutic properties. This review focuses on the possible clinical applications of one subset of stem cells for the treatment of diseases of the central nervous system (CNS): the mesenchymal stem cell.

The term *mesenchymal stem cell* (MSC) classically refers to a cultured cell line that has the capability for self-renewal and differentiation into mesenchymal lineages—osteocytic, chondrocytic, and adipogenic. These cells possess three properties which can be used for therapeutic purposes: (1) migration toward cancer, (2) suppression of the immune system, and (3) restoration of injured tissue. This review describes each of these three properties in detail, and then addresses how they may be used for the treatment of neurological disease, including brain cancer, immune-related disease, and neurodegenerative disease and injury. A full review of published and ongoing clinical trials is then presented to establish the feasibility and current state of clinical translation.

DEFINITION OF A MESENCHYMAL STEM CELL

The *in vivo* correlate of cultured MSCs is poorly characterized in terms of phenotypic markers and origin; therefore, the definition of MSCs is based purely on the *in vitro* characteristics of these cells [1]. The following are the minimal criteria that define a MSC, as set forth in 2006 by the International Society of Cellular Therapy (ISCT):

1) Plastic-adherence in culture;
2) Possession of the mesenchyme markers CD105, CD73 and CD90, and no markers of endothelial, hematopoietic, or immunological cells (CD45, CD34, CD14 or CD11b, CD79α, or CD19 and HLA-DR surface markers); and
3) The capacity to differentiate into osteocytes, adipocytes and chondrocytes [2, 3], as shown in Fig. (**1**).

The ISCT definition specifies *combinations* of immunophenotypic markers because there is not one single marker expressed *in vitro* that reliably predicts behavior that is characteristic of MSCs. In the laboratory setting, cells that strictly match the ISCT criteria can be directly isolated from cultures by prospective, multiparametric immunoselection of adherent cells for the characteristic combinations of markers, followed by confirmation that these cell populations are capable of differentiating into osteocytes, adipocytes, and chondrocytes [2, 3]. Despite their name, MSCs do not fulfill the true definition of a stem cell, as they are incapable of maintaining a whole tissue population [1, 4, 5], and they display replicative senescence [6].

Although the ISCT criteria have greatly consolidated the defining characteristics of MSCs, there remain several definitional problems regarding the characteristics of these cells. First, the protocols for their isolation from various types of tissue must still be standardized. Because there is no standard isolation protocol, observations from cells isolated at one laboratory still may not be reproducible in another laboratory which employs a different protocol, and at worst, may influence the results of clinical trials [7]. Second, because the ISCT criteria have been adopted only recently, most of the existing research has been performed on cells that only partially match what is now agreed upon as a true MSC. Finally, the ISCT criteria do not account for those characteristics of MSCs that are relevant for therapeutic purposes, such as the ability to differentiate into, and perhaps partially reconstitute, neural tissue [8, 9]. Therefore, as with any field that is still in its infancy, definitional problems still exist, despite a standardized definition.

The original terms for what are now called mesenchymal stem cells were *colony-forming unit fibroblasts* (CFU-F) and *marrow stromal cells* [10]. These descriptive terms are closely tied to the early experiments which first isolated these cells solely from bone marrow aspirates [10]. Using simple density gradient separation, bone marrow aspirates can be dissociated into a single suspension containing hematopoietic stem cells (HSCs) and marrow stromal

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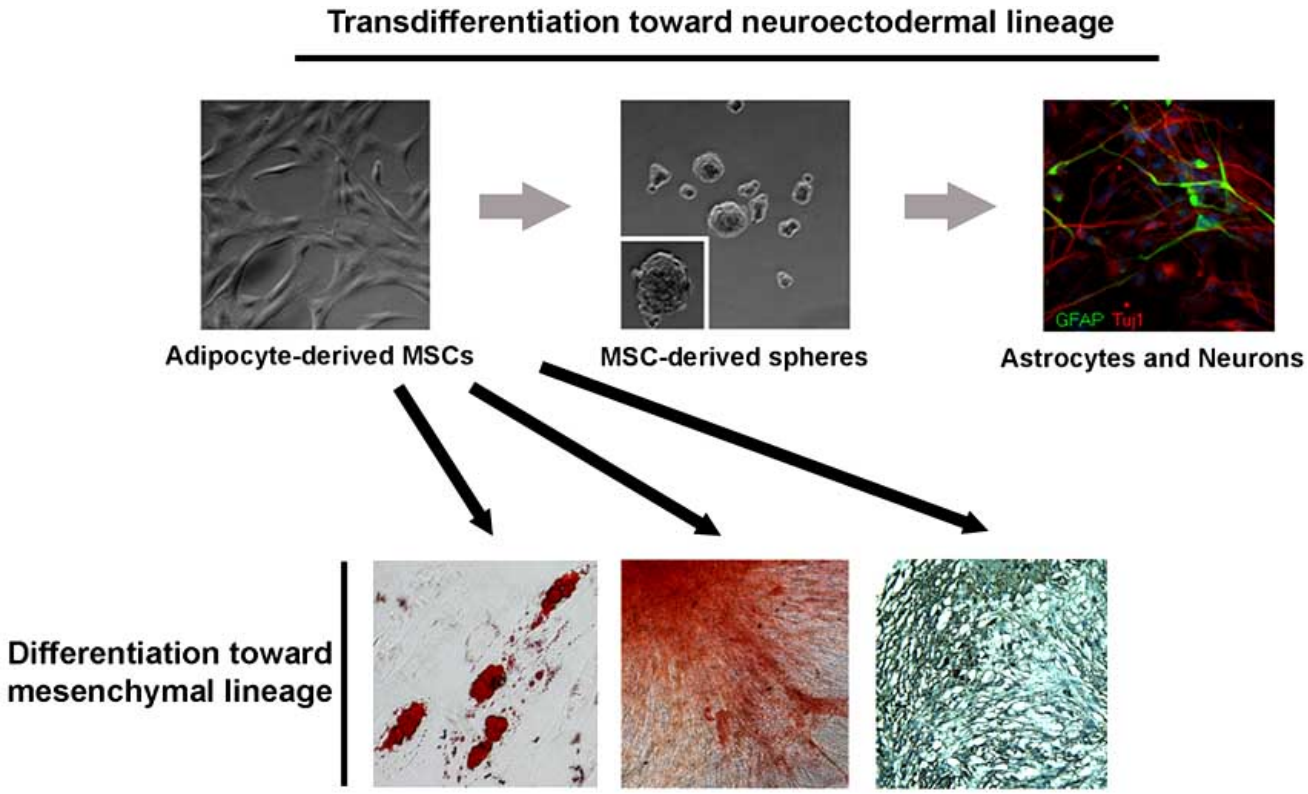

Fig. (1). Differentiation of mesenchymal stem cells toward adipocytes (oil red-o stain), osteocytes (alizarin red stain) and chondrocytes (immunostained for type II collagen). MSCs also posses the ability to transdifferentiate toward the neuroectodermal lineage as GFAP+ astrocytes and Tuj1+ neurons.

cells. The term *marrow stromal* refers to the role of these cells in the structural matrix of bone marrow and their support of hematopoiesis. When cells from this suspension are cultured, only the marrow stromal cells will adhere to the surface of the flask, and can therefore be separated from HSCs by repeatedly changing the media. The marrow stromal cells are rapidly adherent, and proliferate extensively *in vitro*, forming spindle shaped, fibroblast-like colonies within about 7-14 days, at a rate of about 1-10 colonies per 10^5 cells plated [11]. These cells were therefore also called *colony-forming unit fibroblasts*. CFU-F clonal outgrowth is heterogenous, composed of multiple cell types including osteoblasts, fibroblasts, adipocytes, macrophages, and endothelial cells. Over several passages, the progeny of CFU-F colonies begin to exhibit signs of senescence and loses the capacity for proliferation and differentiation [11]. This explains why, despite historical importance, CFU-F outgrowth has not been widely used for isolating MSCs.

WHERE ARE MESENCHYMAL STEM CELLS LOCATED IN THE HUMAN BODY?

It may accurately be stated that a mesenchymal *system* is present in the adult body [12]. Viable lines of MSCs have been isolated from nearly every organ, and many tissues [13]. This includes brain [13], liver [13], kidney [13], lung [13], bone marrow [13], muscle [13], thymus [13], pancreas [13], skin [13], aorta [13], vena cava [13], glomeruli [13], salivary glands [14], adipose tissue [15], subcutaneous scalp tissue [16], palatine tonsil [17], periodontal ligament [18], fetal tissues [19-21], lymph node [22], spleen [23], thymus [24], umbilical cord [25], Wharton's jelly [26], and placenta [27]. Even some cancers harbor MSC-like cells [28]. The presence of MSCs in circulating blood and umbilical cord blood, however, is controversial [23, 29-31]. The diasporic distribution of MSCs in the body suggests that there are many possible sources from which to derive cells for clinical applications. However, from a therapeutic standpoint, the problem is that of finding a source of cells that will be most practical, effective, and generalizable for patients in the clinical setting.

HARVESTING MESENCHYMAL STEM CELLS FOR CLINICAL USE

For MSCs to be used for therapeutic purposes, they must be harvested using techniques that are amenable to clinical translation. Therapeutic translation of a technique requires that it possess one or more characteristics that make it practical for use in the clinical setting. First, the technique should require no more than a minimally invasive procedure that poses minimal inconvenience to the patient. Second, the technique should yield abundant quantities of cells. Finally, it should reliably yield cells which satisfy the ISCT criteria that define a MSC [10, 32]. These criteria explain in part why bone marrow, which was the original source of MSCs in experimental studies, is not the most practical source available for the clinical context. Harvesting bone marrow requires an invasive procedure which yields a small number of cells, and the number, differentiation potential, and lifespan of bone marrow-derived MSCs decline with patient age [33-36]. Therefore alternate sources of MSCs for clinical use are required. Two alternate sources for harvesting MSCs that have received considerable attention in the literature are adipose tissue and umbilical cord blood.

Mesenchymal Stem Cells from Adipose Tissue

Subcutaneous adipose tissue represents the most plentiful potential source for the harvest of MSCs. Approximately 220,000 liposuction procedures are performed in the United States every year [32, 37]. Each liposuction procedure can yield up to several liters of lipoaspirate [38], which is otherwise discarded. However, using simple digestion techniques, this tissue can serve as an abundant source of MSCs for clinical use. Liposuction has a relatively low complication rate [37-39] to the extent that the hazards of this procedure are minor with respect to the morbidity and mortality of

brain cancer and stroke, which may be treated with the harvested cells. The wide availability of adipose tissue from liposuction procedures also means that adipose-derived MSCs are more practical to study in the laboratory setting. In most academic medical centers, a steady stream of adipose tissue is usually available from various plastic surgical and some neurosurgical procedures, whereas bone marrow and other tissues are much more scarce. The plentiful volume of adipose tissue therefore makes this source of MSCs highly amenable for clinical and research use.

Adipose-derived MSCs are also amenable for clinical translation because they can be isolated reliably using simple techniques. Most protocols call for digestion of the lipoaspirate in collagenase, followed by centrifugation, which separates mature adipocytes from the stromal vascular fraction that forms the pellet and contains MSCs [15, 32, 36, 40-41]. The stromal vascular fraction is then cultured in stromal media, which is repeatedly changed to remove non-adherent cells. Using this approach, our laboratory is able to process and plate adipose cells within 90 minutes after receiving a tissue specimen. In about 20 days, a viable cell line can be established (unpublished data). This technique reliably yields cells with an immunophenotype consistent with the ISCT definition for MSCs, and capable of differentiating into the 3 mesenchymal lineages [36]. Furthermore, the MSCs derived from adipose tissue are nearly identical to those isolated from bone marrow in terms of morphology, the success rate of isolating MSCs, expansion potential, differentiation capacity, and immunophenotype [36]. Therefore the protocols for the isolation of MSCs from adipose tissue are thought to be highly amenable for widespread clinical use.

Mesenchymal Stem Cells from Umbilical Cord Blood

Umbilical cord blood, which is a well-established source of HSCs [42-43], may also provide a clinically feasible source of MSCs [44]. After removal of the placenta, about 100 to 150 ml of blood can be collected by allowing it to drain from the cord into a mixture of culture media and heparin. This procedure, which makes use of a biological specimen that is normally discarded, poses no risk, discomfort, or inconvenience to the mother or newborn because the umbilical cord is detached at the time of blood collection. From the blood, mononuclear cells can be separated and cultured, and a heterogenous adherent layer will form. Initial studies indicated that 24% of cells in this adherent layer have a fibroblastiod morphology, and express CD13, CD29, CD49e, CD54, CD90, but not CD14, CD31, CD34, CD45, CD49d, nor CD106, among others [45]. The cells possess an expansion capacity that exceeds that of bone marrow and adipose-derived MSCs [36, 46], which may be due in part to higher telomerase activity [47]. These cells have also been shown to be capable of differentiating reliably into osteocytes [45, 48-50] and chondrocytes [36, 48-49, 51], consistent with the properties of MSCs. However, their adipogenic differentiation is controversial [1].

The use of umbilical cord blood clinically may be limited by unreliable and often low isolation efficiency. The isolation success rate of MSCs from cord blood has been reported as anywhere from none to 60%, and is probably highly dependent on the time between harvest and processing [29, 45-46, 52-56]. Furthermore, the therapeutic use of umbilical cord blood as a source of MSCs would usually require allogeneic transfer, which raises the possibility that banking and typing of various lines of MSCs may be necessary, or at least that the safety of allogeneic transfer in humans must become more well-established through additional clinical studies [57]. By contrast, allogeneic transfer is not necessary for adipose-derived MSCs, in which case an autograft can easily be harvested from any patient. Therefore, the use of MSCs from umbilical cord blood may be less translatable to the clinic than MSCs derived from adipose tissue.

An overall therapeutic approach to the use of MSCs, which follows the cells from their initial isolation by the techniques described above, to their possible clinical use in brain cancer, neurodegenerative disease, and neural injury, is shown in Fig. (**2**). The subsequent portions of this review describe, in turn, each of these 3 therapeutic applications.

MESENCHYMAL STEM CELLS FOR THE TREATMENT OF BRAIN CANCER

MSCs possess migratory properties which are highly relevant for clinical application in the treatment of brain cancer [58]. The first studies of MSC migration took place in non-cancer animal models, and gradually led to the study of MSC migration in the context of animals engrafted with tumors. In 1998, Pereira *et al.* demonstrated that murine MSCs migrate primarily to bone marrow, cartilage, and lung, but also to spleen, brain, and skin, in an osteogenesis imperfecta mouse model combined with irradiation [59]. A subsequent study showed that MSCs administered to undiseased, wildtype rats localized to the lungs, liver, and bone marrow; and that the administration of sodium nitroprusside increased levels in liver and bone marrow [60]. Two subsequent studies in 2004 demonstrated that MSCs may possess selective migratory tropism for sites of inflammation. Houghton *et al*. demonstrated that endogenous MSCs repopulate the stomach in mice with chronic gastrointestinal inflammation from *Helicobacter pylori* infection, and undergo progression from metaplasia to dysplasia, and cancer [61]. A case report by Le Blanc *et al.* published that same year described a patient with acute graft-versus-host disease who was infused with human bone marrow-derived MSCs, which localized to the colon and lymph node [62]. Taken together, these initial studies in non-cancer contexts indicated that exogenously administered MSCs distribute somewhat widely in the body, but that they may migrate more specifically to sites of injury and possibly inflammation [63].

These initial results led to numerous studies exploring how the migratory properties of MSCs could be used for the treatment of various medical conditions, one of which was cancer. Initial indications that MSCs could have anti-neoplastic properties came indirectly from the demonstration that in murine Lewis lung carcinoma and B16 melanoma lung metastasis models, tumor seeding and metastasis numbers were greatly decreased when the carcinoma cells were co-administered with MSCs [64]. A separate study on a different type of stem cell, the neural stem cell (NSC), was published one year later by Aboody *et al.*, and demonstrated that NSCs home to the site of brain cancer. By modifying NSCs to secrete the therapeutically relevant molecule cytosine deaminase, selective drug delivery to the site of the tumor was achieved, resulting in increased survival compared to untreated groups. These two studies, together with growing interest in MSC migration patterns, motivated the hypothesis that—as with NSCs—MSCs might migrate selectively to sites of cancer, and could be modified to serve as gene-delivery vehicles.

Under this experimental approach, MSCs are genetically modified to secrete an anti-neoplastic compound, and then used to treat animals possessing tumor grafts. The usual hypothesis of such studies is that MSCs will localize to the site of the tumor and selectively deliver the anti-neoplastic gene product, which will result in a therapeutic effect. The first set of studies to employ this experimental paradigm evaluated the effects of interferon β-secreting human bone marrow MSCs administered in various murine tumor models. These included models of human melanoma lung metastasis [65], breast cancer and pulmonary metastasis [66], and several glioma models [67]. In these studies, the MSCs localized to the site of malignancy, and in most models resulted in extended survival [58]. Many other studies employing the same experimental approach with different gene products have since been published [68-78], and are reviewed separately [58, 79]. These subsequent studies almost universally demonstrate that MSCs home to the site of cancer, but they also reinforce the initial findings that MSCs localize in small numbers to other organs, even in animals with tumors [75, 78].

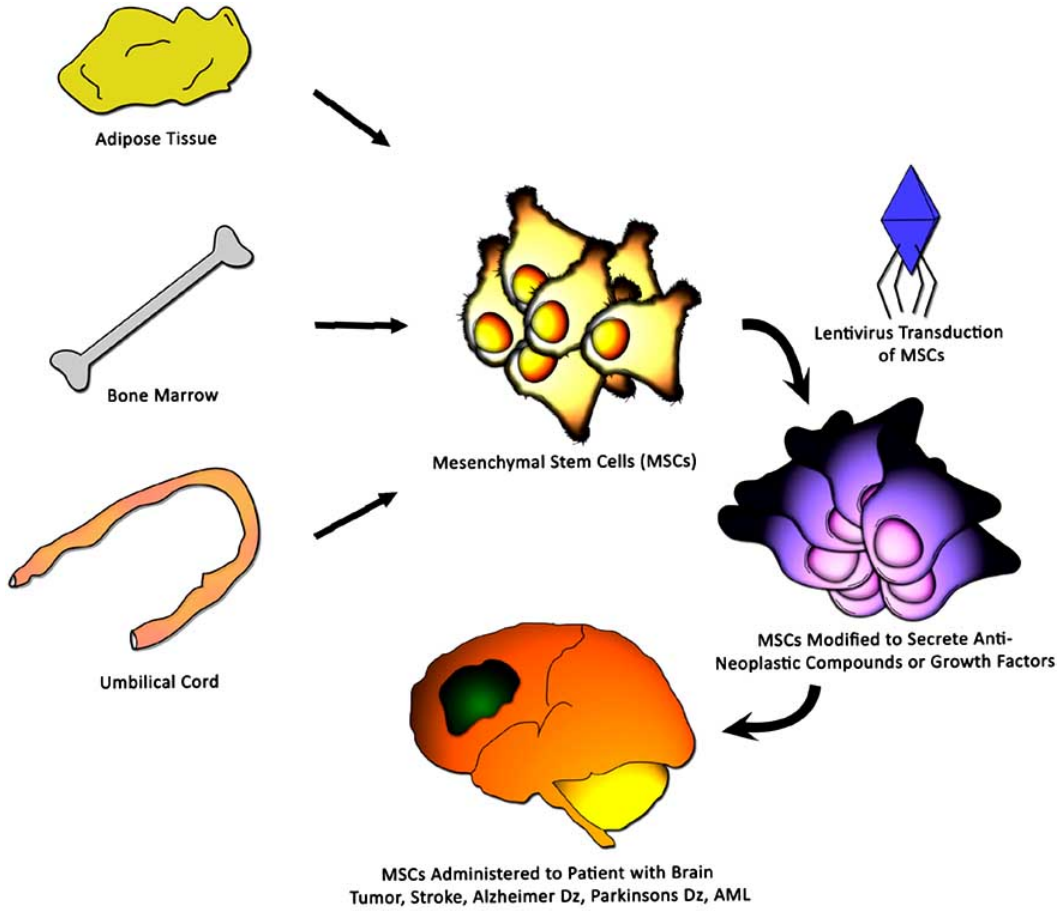

Fig. (2). Therapeutic paradigm for the use of mesenchymal stem cells in the treatment of neurological disease. MSCs are harvested from adipose tissue, bone marrow, or umbilical cord blood and subsequently culture-expanded. Lentiviral transduction can be used to modify the MSCs to secrete a therapeutic compound of interest. These cells can then be administered to patients with neurological disease, such as brain cancer or stroke, with potential therapeutic effect.

These include the lung, liver, spleen, and kidney [75, 78], although cells were shown to clear from the lungs and liver by 20 days after administration [72]. The actions of MSCs at these other organs have not been well-characterized. Animal studies on the migration of MSCs toward cancer have comprehensively been reviewed elsewhere [80].

Do Unmodified Mesenchymal Stem Cells Enhance Tumor Growth?

Although MSCs, when modified to secrete anti-neoplastic compounds, have successfully been used to extend survival in animal cancer models, the effects of *unmodified* MSCs on cancer remain controversial. Despite initial results which suggested that MSCs may possess anti-neoplastic properties [64, 81], some studies that followed demonstrated tumor-enhancing effects [82]. In animals with subcutaneous implantation of Renca adenocarcinoma, the co-implantation or intravenous infusion of MSCs resulted in earlier tumor occurrence, although tumor growth characteristics are not modified [83]. In mice implanted subcutaneously with human colon cancer cell lines, the administration of MSCs resulted in higher tumor incidence, with elevated proliferation, angiogenesis, and metastatic ability of the cancer cells [84]. MSCs have also been shown to increase the metastatic potential of breast cancer cell lines [85]. Finally, some animal studies have demonstrated diminished survival when unmodified MSCs are administered to animals bearing tumors [78], whereas others demonstrated enhanced survival [74]. At least mechanistically, there is reason to believe that immunosuppressive properties of MSCs, which are described in detail below, may aid brain cancer cells is escaping immune surveillance, and thereby enhance tumor growth. Whether this explains the diminished survival observed in animals with both tumor and MSC implantation is unclear, but should motivate future research on the immunosuppressive properties of MSCs that may enhance tumor growth. Immunosuppression by MSCs, and its clinical relevance, is addressed next.

MESENCHYMAL STEM CELLS ARE IMMUNOSUPPRESSIVE

MSCs are both immunosuppressive and non-immunogenic. The immunosuppressive properties of MSCs underlie the potential therapeutic benefit of using MSCs to treat neurological disorders with an underlying inflammatory component [86]. The non-immunogenic properties of MSCs raise the possibility of transplantation from allogeneic donors, which represents the most convenient stream of cells for clinical use, for both therapeutic and regenerative applications. In animals, including primate, canine, and goat, allogeneic MSCs do not elicit a lymphoproliferative response [87-90]. The immunoregulatory properties of MSCs arise from the interactions of MSCs with most cells of the adaptive and innate immune systems. These interactions are discussed below.

Mesenchymal Stem Cells and T Lymphocytes

MSCs suppress the proliferation of activated T cells through several mechanisms. MSCs secrete the soluble isoform of the human leukocyte antigen class I molecule HLA-G5, an HLA molecule which is a well-established component of maternal tolerance to the

fetus [91-94]. The secretion of HLA-G5 by MSCs requires contact with activated T cells and is IL-10 dependent [93-94]. HLA-G5 is important as it has been shown to suppress T cell proliferation and increase the production of T-regulatory cells [94-97], suggesting that this is one critical pathway by which MSCs suppress the immune system. A second mechanism of T cell suppression involves two pathways mediated by interferon gamma (IFN γ). First, IFN γ causes MSCs to produce indoleamine 2,3-dioxygenase (IDO) [98-100]. IDO is important because it catalyzes the breakdown of the essential amino acid tryptophan, which is necessary for lymphocyte proliferation [101-102]. Therefore, lymphocyte proliferation is inhibited by high IDO levels from MSCs. Second, the immunosuppressive function of MSCs on T cells is elicited by the presence of IFN γ in combination with any of the 3 inflammatory cytokines TNF α, IL-1α, or IL-1β [98]. In response to these cytokine combinations, MSCs initiate an immunosuppressive response by secreting two sets of compounds: inducible NOS and the chemokines CXCL9 and CXCL10 [98]. Inducible NOS results in high local levels of nitric oxide (NO). NO is involved in suppression of Stat5 phosphorylation and suppression of subsequent T cell proliferation [103], and the chemokines secreted by MSCs are responsible for attracting T cells into this suppressive, NO-rich environment [98]. Finally, indirect suppression of T cells also occurs *via* the primary actions of MSCs on dendritic cells (DCs), which are described in detail in subsequent sections of this review.

MSCs selectively support the survival and proliferation of T cells that are in a quiescent, anti-inflammatory state [104]. MSCs induce a non-responsive state in T-cells which resembles division arrest anergy. In the presence of MSCs, T cells become arrested in the G1 phase with concomitant inhibition of cyclin D2 expression and upregulation of p27(kip1) [104]. MSCs also inhibit proliferation of activated T cells and promote the survival of T cells in a quiescent state by interfering with the Fas receptor-mediated program of cell death [105]. Following suppression by MSCs, T cells convert to an anti-inflammatory state, characterized by decreased IFN γ production by TH1 cells [96], decreased IL-4 production by TH2 cells [96], and diminished production of TNF α [106]. Thus, MSCs suppress the immune system in part by supporting a quiescent, anergic state in T cells.

Mesenchymal Stem Cells and B Lymphocytes

The major mechanism for suppression of B cells is likely through the suppression of T-cells. Direct inhibition of B cells by MSCs probably occurs to a limited extent, as suggested by the finding that co-culture of bone marrow-derived MSCs with B cells results in arrest at the G0/G1 phase of the cell cycle and impaired chemotactic potential of B cells [107]. Suppression of B cells may occur in part due to soluble factors secreted by MSCs; however, cell surface interactions between MSCs and B cells appear to have an important role as well [107, 108]. In particular, the interaction between the membrane proteins programmed death 1 (PD-1) and its ligands PD-L1 and PD-L2 contributes to B cell suppression [108]. The presence of IFN γ may be necessary for this immunosuppressive effect to occur [99]. Despite these results suggesting an immunosuppressive effect on B cells, MSCs have also been shown to induce polyclonal expansion and differentiation of B cells [109] and to stimulate antibody secretion [110]. These results suggest a possibly dualistic effect of MSCs on B cells, one which future research may help to elucidate.

Mesenchymal Stem Cells and Dendritic Cells

Immature dendritic cells (DC), which are derived from HSCs in the bone marrow, sample their surrounding environment for pathogenic material. Following recognition of a pathogen-associated molecular pattern, these cells undergo a process of maturation and subsequent presentation of the antigenic material to other cells in the immune system. These two critical processes of DCs—maturation and antigen presentation—are both inhibited by MSCs.

MSCs reversibly inhibit the generation of CD34+ -derived and monocyte-derived DCs from precursor cells in a dose-dependent fashion [111, 112]. The mechanism behind this inhibition of differentiation appears to involve activation of the Notch pathway and cell cycle arrest of precursor cells [112]. In the presence of MSCs, cyclin D2 is downregulated, and monocyte precursors fail to enter the G1 phase of the cell cycle, resulting in accumulation of cells in the G0 phase [113]. This effect is similar to that observed when MSCs are co-cultured with T cells, but with slightly different gene expression patterns [113]. The mechanism likely involves soluble factors secreted by MSCs [111], although direct co-culture of MSCs with DCs results in stronger immunosuppresion than simple culture in MSC-conditioned media [112]. In addition to inhibition of DC maturation, MSCs cause matured DCs to exhibit characteristics of an immature phenotype, including reduced expression of CD83, fewer antigen presentation molecules, and decreased IL-12 secretion [114]. The inhibition of DC maturation by MSCs is coupled with diminished expression of cell surface molecules, which include MHC II, CD40, and CD86 co-stimulatory molecules [115]. Thus MSCs have an inhibitory effect on both the maturation and antigen presentation capabilities of DCs.

In addition to antigen presentation, DCs signal other cells in the immune system by releasing cytokines. This process is also altered by MSCs. MSCs diminish TNF-α secretion from mature type 1 DCs, and increase IL-10 secretion from mature type 2 DCs [96]. Thus MSCs also inhibit cytokine secretion patterns of DCs.

Mesenchymal Stem Cells, Natural Killer Cells, and Neutrophils

Natural killer (NK) cell-mediated cytolysis and IFN γ secretion are inhibited by the HLA-G5 mechanism described above [93-94]. The IDO mechanism described above also mediates inhibition of NK cells [116]. In addition, a unique mechanism involving reciprocal inhibition of MSCs and NK cells takes place. MSCs express surface ligands and adhesion molecules (ULBP, PVR, Nectin-2, and ICAM-1) which interact with NK surface receptors and antigens (NKp30, NKG2D, DNAM-1, and LFA-1), and initiate the efficient lysis of MSCs by NK cells [117, 118]. Upon binding with MSCs, NK cells release IFN γ and TNF α, which appear to mediate this cytotoxic effect [117]. However, MSCs exert opposing inhibitory effects on NK cells by downregulating NKp30, NKp44, and NKG2D, on the surface of NK cells, thus limiting cytotoxicity [116]. In addition to these "defensive" mechanisms, MSCs also alter NK cell phenotype, suppress their proliferation, cytokine secretion, and cytotoxicity, through soluble factors, including transforming growth factor β1 and prostaglandin E2, and mechanisms which require cell contact [119].

The interactions between MSCs and neutrophils are not well-characterized, but likely play an important role in the neutrophil storage pool in bone marrow. Low numbers of MSCs are capable of inhibiting apoptosis of neutrophils and dampening the respiratory burst, through the secretion of IL-6 and subsequent activation of the STAT-3 transcription factor [120]. However phagocytosis and chemotaxis of neutrophils are unaffected [120].

In summary, MSCs affect many components of both innate and adaptive immunity, and these effects can be understood at the cellular level for T cells, B cells, DCs, NK cells, and neutrophils. The next section describes how the immunosuppressive effects of MSCs can be useful from the perspective of treating diseases of the CNS which have an inflammation-related pathogenesis.

MESENCHYMAL STEM CELLS FOR THE TREATMENT OF NEURODEGENERATIVE DISORDERS

The progressive degeneration of specific cell populations within the nervous system is a distinguishing feature of neurodegenerative disorders [121]. Recent developments in the understanding of the pathogenesis of these diseases have identified as a possible thera-

peutic target a process of neuroinflammation. These pathophysiological features, taken together with the lack of effective treatment modalities, may make some neurodegenerative diseases and many auto-immune neurological disorders appropriate candidates for MSC-based therapies. The rationale behind this MSC-based strategy lies in this stem cell's unique capacity to both transdifferentiate to neural cells and modulate the immune system, while possibly circumventing the problem of immune rejection. This subsection focuses on three neurological diseases which are thought to have an immune-related component; specifically Parkinson's disease (PD), Alzheimer's disease (AD) and Amyotrophic Lateral Sclerosis (ALS), where cell loss is a distinguishing pathological feature and evidence of an inflammatory immune component has been implicated [122]. MSCs provide one possible therapeutic approach for patients with such disorders.

Parkinson's Disease

PD is characterized by the selective loss of dompamingeric neurons in the substantia nigra. Although efforts to restore striatal dopaminergic transmission by engrafting embryonic dopaminergic neurons failed, the prospect of using cellular therapies remains feasible [123, 124]. MSCs have been shown to reproducibly differentiate into dopaminergic neurons with the full range of enzymes to synthesize dopamine in a potassium-dependent manner [125]. Furthermore MSCs have been engineered to synthesize L-DOPA which led to improved function in a rat model of PD [126, 127]. Remarkably, MSCs have been modified or have been shown directly to express nerve grow factor (NGF), brain derived growth factor (BDNF), and glial derived growth factor (GDNF); which are implicated in neuronal survival, neurite formation and dopminergic differentiation [128]. The ability to release such trophic factors and their propensity to home to sites of injury makes MSCs an attractive cellular therapy in the treatment of PD. Their immunomodualting capacity has even strengthened this argument. Several studies have reported activation of a microglial and astroglial inflammatory component in the pathogenesis of PD [129]. Two of the proinflammatory molecules implicated in this mechanism include tumor necrosis factor-α (TNF-α) and interleukin (IL-1) which, as previously discussed, are negatively modulated by MSCs *via* the release of IFN-γ [98].

Alzheimer's Disease

AD is characterized by degeneration and progressive loss of neurons throughout the brain with particular loss of cholinergic neurons in the basal forbrain [130]. While there is limited evidence that MSCs can differentiate into cholinergic cells there have been some reports of the expression of the acteycholine-synthesizing enzyme, choline acetyltransferase (ChAT), upon differentiation of MSCs [121]. The therapeutic mechanism of MSCs within the context of AD would most likely not be attributable to cellular regenerative effects, but rather to the secretion of cytokines and growth factors. There is increasing evidence that microglia and astrocyte activation contribute to the pathology seen in AD where the expression of the cytokines S100β and IL-10 play a major role in the relationship between stressed neurons and β-amyloid plaques [121, 122]. As in PD, IL-1 appears to be a major upstream regulator of this inflammatory response making immunomodulation of IL-1 with MSC therapy a more suitable target [122]. Perhaps the most compelling proposal for the use of MSCs in the treatment of AD lies in their ability to be engineered to produce growth factors. The use of NGF has now emerged as a primary candidate for the treatment of Alzheimer's disease as several studies have shown protection of neurons and improvement of memory in animal models of aging, excitioxocity, and amyloid formation [131, 132]. Recently, a phase I clinical trial using NGF-engineered autologous fibroblasts transplanted into the forebrain of AD patients demonstrated a slowing of the rate of cognitive decline [133]. MSCs may offer better homing capabilities and added immunomodulating capabilities than fibroblasts with comparable safety profiles to prevent degeneration of basal forebrain cholinergic neurons.

Amyotrophic Lateral Sclerosis

ALS is characterized by the selective loss of upper and lower motor neurons in the cerebral cortex, brainstem and spinal cord with eventual death within 5 years after symptom onset [134]. Cell replacement and regeneration are not the most feasible therapeutic strategies because newly administered cells would have to traverse extremely long distances to establish new synapses. However, replacement of neurotrophic factors may be a more realistic approach [135]. MSCs have been engineered to release GDNF a potent neurtrophic factor that has been shown to protect motor neuron loss in a rat model of facial motor nucleus injury [136]. In addition, the use of intrathecal VEGF injection with its neurotrophic and neuroprotective effects has recently been shown to delay disease onset and extend the survival of animals in an ALS model [137]. VEGF is an attractive growth factor to consider in MSC therapy of neurological diseases because it has already been implicated as a key trophic factor in MSC-mediated cardiac repair. Infiltrating leukocytes and activated microglia and astrocytes have been found in tissues of ALS patients, underscoring the importance of an inflammatory process in the progression of the disease [121]. As described previously, MSCs provide an anti-inflammatory environment, and even have anti-proliferative effects on microglial cells and astrocytes [138]. These findings have led to a phase I clinical trial that demonstrated the safety of autologous transplantation of MSCs in 10 ALS patients [139].

MESENCHYMAL STEM CELLS RESTORE INJURED NEURAL TISSUE

Perhaps one of the most exciting discoveries in MSC research is their ability to differentiate into tissues other than those of the mesodermal lineage. Transdifferentiation is a property congruent with the origins of mesenchymal tissue which is now appreciated to include mesoderm and to a lesser extent cranial neural crest [140]. Although most studies rely on the use of neuronal and glial markers as evidence for transdifferentiation of MSCs, a few studies have validated this observation by being able to differentiate MSCs *in vitro* into excitable neuron-like cells that respond to a spectrum of excitatory and inhibitory neurotransmitters [141]. This compelling evidence behind the neuroectodermal differentiation potential of these cells has made them attractive therapeutic agents for the treatment of neurological disease. Since MSCs can easily be derived through the previously described methods, their use to exert neurorestorative effects in the treatment of stroke has been the subject of vigorous laboratory investigation and has already led to one clinical trial in stroke patients [142].

The mechanism by which MSCs exert their neurorestorative effects remains unknown. Although it has been hypothesized that the functional improvement seen in stroke animals after MSC therapy is a result of transdifferentiation, as evidenced by the expression of neuronal-specific markers including NeuN, microtubule associated protein 2, and neuron specific gamma-aminobutyric acid receptors from differentiated MSCs [143-145], this hypothesis remains controversial. Two high profile studies using Cre-lox recombination have demonstrated that bone marrow derived stem cells fuse spontaneously with neural progenitors *in vitro* and *in vivo* with no evidence of transdifferntiation events without fusion [146, 147]. In contrast to these findings, several groups agree that there is a low frequency of transdifferentiated cells that are seen *in vivo* [148-151]. However the paucity of these events has led many investigators to believe that the therapeutic efficacy of MSCs lies in their ability to release growth factors and immune modulating cytokines (reviewed above) to modulate brain injury, promote angiogenesis, prevent cell death, and recruit local progenitors through paracrine

signaling, rather than direct engraftment and differentiation into functionally distinct neuronal cell types.

MESENCHYMAL STEM CELLS FOR THE TREATMENT OF ISCHEMIC NEURAL INJURY

Biological Function of MSCs in the Brain and Access Past the Blood Brain Barrier

One of the most challenging aspects of delivering therapeutic molecules to the CNS is the traversal of the blood brain barrier. Several mesodermally-derived cell types including T-cells, B-cells, and macrophages, have been shown to migrate throughout the CNS [152-155]. These observations have been further validated with studies showing bone marrow progenitors differentiating into microglia and astrocytes when injected into the tails of irradiated mice [156]. An intravascular approach such as this may even work in humans. Examination of postmortem brain samples from females who received bone marrow transplants from male donors showed neurons and astrocytes with Y chromosomes in the brain parenchyma [157]. These observations have collectively led many researchers to believe that MSCs can access the CNS. Some studies even suggest that bone marrow progenitor cells may play a critical role in the physiological renewal of brain cells [158].

Mesenchymal Stem Cells for the Treatment of Stroke in Animal Models

MSCs have been evaluated most frequently in animal models of *ischemic* stroke, with very few data on efficacy for *hemorrhagic* stroke. A recent review on the therapeutic use of non-human and human MSCs to treat ischemic stroke in rodent models cited more than 20 studies which reported neurological improvement [159]. This effect was observed when MSCs were administered by intracerebral transplantation, intra-arterial infusion, or intravenous infusion. Although there has been no controlled comparison of efficacy between the various routes of MSC administration, one study did report improved functional recovery in a rodent middle cerebral artery occlusion model, when umbilical cord blood was delivered intravenously, as compared with intracranial injection [160]. In another study which employed a rodent intracerebral hemorrhage model, the administration of MSCs either through the ipsilateral carotid artery or the lateral ventricle resulted in significant improvements in neurological function, as compared to MSC injection into the cervical vein [160]. While the proposed mechanisms behind the efficacy of each route is a subject of intense debate, there is some consensus that MSCs display a strong affinity to regions of brain injury in much the same way as inflammatory cells have a tropism towards injured tissue [159]. MSCs are often found targeting the ischemic penumbra with preferential migration to the ischemic hemisphere rather than the non-ischemic hemisphere [161]. Genetically-modified MSCs expressing the growth factors BDNF [162], fibroblast growth factor-2 (FGF-2) [163], and hepatocyte growth factor [164], have been shown to further reduce the size of the infract, and FGF or placental growth factor-engineered MSCs significantly improved functional recovery after stroke, as compared with MSC transplantation alone [163, 165]. Furthermore, human MSCs increased the local expression of BDNF and NGF in ischemic tissue resulting in reduced apoptosis in the ischemic penumbra and higher proliferation of cells from the subventricular zone [166]. In a hindlimb ischemia model, hypoxic preconditioning of MSCs resulted in increased motility and improved therapeutic potential, suggesting that the natural hypoxic niche found in bone marrow may be a critical signaling mechanism to maintain the tissue regenerative potential of MSCs. Collectively these data from animal studies suggest that MSCs improve stroke outcome in animal models and that the efficacy of these cells is dependent on several variables including growth factor support, cell number, and type of stroke.

Mesenchymal Stem Cells for the Treatment of Stroke in Patients

The only clinical use of MSCs for the treatment of stroke patients was reported in 2005 [142]. In this study, 5 ischemic stroke patients received intravenous injections of *Ex vivo*-cultured autologous MSCs, compared with 25 untreated stroke patients in a control group. The neurological outcomes in MSC-treated patients, as determined by the Barthel index and modified Rankin score, improved consistently during follow-up at 3, 6, and 12 months. Whether these results reflect true efficacy, or simply the timecourse of normal recovery, is impossible to know, given several limitations of the study. These include a high attrition rate (10 patients from the control group were lost) and an unblinded study design [167]. Nevertheless, such preliminary studies as this will be helpful and necessary to establish the safety and efficacy of MSCs within the context of stroke, and could lay the groundwork for larger and more rigorously-designed clinical studies.

ARE MESENCHYMAL STEM CELLS SAFE TO ADMINISTER TO PATIENTS?

The possibility that MSCs possess the capability for malignant transformation is the most pressing, unresolved question. Not only is there an overall paucity of research that addresses the topic, but few authors have studied the malignant transformation of MSCs specifically within the context of brain cancer. MSCs are susceptible to malignant transformation at one of two stages: *in vitro* during preparation and expansion of the cells, and *in vivo*, after the cells have been administered. These possibilities are addressed in turn below.

The *in vitro* malignant transformation of MSCs is controversial due to conflicting published results. In a study by Bernardo *et al*., MSCs were cultured to senescence or 25 passages, and subsequently analyzed for signs of malignant potential. No chromosomal anomalies were found, and telomerase activity and telomere shortening were both normal [168]. Further results demonstrated that MSCs do not undergo malignant conversion after only 6 to 8 weeks in culture [169]. However, after 4 to 5 months in culture, MSCs transform to a malignant phenotype *via* the upregulation of *c-myc* and downregulation of p16, and these cell lines were capable of forming tumor nodules in multiple body organs of immunodeficient mice [169]. These results are supported by a subsequent study which demonstrated that, following isolation of bone marrow MSCs under standard protocols, an adherent population of cells forms *in vitro* with a spherical, cuboidal morphology [170]. This population of cells demonstrated high levels of telomerase activity, chromosomal aneuploidy, and translocations, and was capable of forming tumors in multiple organs in NOD/SCID mice [170]. From a clinical standpoint, these initial indications of possible *in vitro* malignant transformation may point to a prohibitively low or unpredictable biosafety profile of MSCs. Additional studies on MSCs that have been rigorously isolated by standardized flow cytometric methods, as well as careful time course studies of MSC *in vitro* cultures, will be required before MSCs expanded *in vitro* can be deemed safe enough for clinical use.

In vivo, MSCs may undergo malignant conversion either by direct transformation, or by interactions with cells of an existing tumor (one which they were perhaps administered to treat). There is little evidence to support the *direct* malignant transformation of MSCs *in vivo*. Although several animal studies have documented the distribution of exogenously administered MSCs, we have not come across a single report of tumor seeding or formation by MSCs in tumor-naïve animals. Admittedly, few animal studies employ methods that are sufficiently rigorous to detect developing tumors at any location in the body, however.

Because one potential clinical application of MSCs is the treatment of cancer, various authors have studied whether *interactions*

Table 1. Published Clinical Trials of MSCs. To obtain this list of clinical trials, Pubmed was searched on April 24, 2010, for "Mesenchymal Stem Cells [Mesh]" with a "clinical trial" limit, and studies that were not clinical trials were omitted. Abbreviations: *BM:* Bone Marrow. *CABG:* Coronary Artery Bypass Grafting. *CT:* Computed Tomography. *G1PLIL:* Encodes Nonexpressing β-galactosidase and Neo^r Sequences with ATG→CTG Mutations. *HRC/GVHDP:* Hematopoietic Reconstitution / Graft Versus Host Disease Prophylaxis. *LNc8*: A Clone of the Vector Encoding the Neomycin Phosphotransferase Gene (Neo^r). *LVEF:* Left Ventricular Ejection Fraction. *MLD:* Metachromic Leukodystrophy. *MPC:* Mesenchymal Progenitor Cell. *MSC:* Mesenchymal Stem Cell. *NYHA:* New York Heart Association Score. *PCI:* Percutaneous Coronary Intervention Procedure

Citation	Location of Trial	Disease	Enrolled	Follow-Up Length	MSC source	MSC Administration Technique	Major Outcomes
Kebriaei *et al.* 2009 [178]	USA	HRC/GVHDP	32	2 years by CT scans	BM-MSC from unmatched donor	Intravenous infusion + corticosteroids	No evidence of ectopic tissue formation. 94% response rate to MSC therapy, but no untreated control group.
MacMillan *et al.* 2009 [179]	USA	HRC/GVHDP	15	2 years by skeletal survey, 6.8 years survival	BM-MSC from haploidentical parent donor	Intravenous infusion + umbilical cord blood hematopoietic stem cells	No adverse events or evidence of ectopic tissue formation. Trend toward higher survival in MSC-treated groups. Platelet and neutrophil engraftment observed.
Gan *et al.* 2008 [180]	China	Adjuvant to posterior spinal fusion surgery	41	35 months	Autologous BM-MSC	Part of a surgically-implanted bone graft	No reports of malignancy or visible masses on CT scan at 12 months post-operation. 95.1% spinal fusion success rate with graft.
Centeno *et al.* 2008 [181]	USA	Degenerative joint disease	1	6 months	Autologous BM-MSC	Percutaneous injection into knee synovium	Elevated cartilage and meniscus growth. Better range of motion and less knee pain. No evidence of malignancy.
Meijer *et al.* 2008 [182]	Netherlands	Tissue engineering for jaw defect	6	15 months	Autologous BM-MSC which were differentiated to osteocytes on a porous matrix	Surgical implantation	One patient had bone formation attributable to the implant.
Müller *et al.* 2008 [183]	Germany	HRC/GVHDP	7	Maximum 29 months	BM-MSC from HSC donor	Intravenous transfusion	Three deaths most likely from GVHD complications. Others survived and/or improved.
Le Blanc *et al.* 2008 [184]	Sweden, Netherlands, Italy, Australia	HRC/GHVDP	55	Median 16 months	BM-MSC from either HLA-identical sibling donor, haploidentical donor, or third-party HLA-mismatched donor	Intravenous infusion	No immediate adverse effects. One patient developed *de novo* AML of recipient origin. About 50% of patients had complete response.
Mazzini *et al.* 2008 [185]	Italy	Amyotrophic Lateral Sclerosis	9	4 years	Autologous BM-MSC	Intraspinal injection at the thoracic level	No adverse events reported. No signs of abnormal cell proliferation on MRI scans. Four patients had diminished rate of decline of forced vital capacity and the ALS-FRS score.
Mohyeddin Bonab *et al.* 2007 [186]	Iran	Multiple sclerosis	10	19 months	Autologous BM-MSC	Intrathecal infusion	On disability scale: 5 patients improved; others no change. On functional scale: 6 improved, 1 no change, 3 deteriorated. On MRI: 2 had new plaque, 7 no change, 1 decreased plaque number.

Table 1. Contd….

Citation	Location of Trial	Disease	Enrolled	Follow-Up Length	MSC source	MSC Administration Technique	Major Outcomes
Mohyeddin-Bonab *et al.* 2007 [187]	Iran	Myocardial infarction	8	12 months or longer	Autologous BM-MSC	Intramyocardial during CABG (5 patients) or intracoronary during PCI (3 patients)	Reduced infarction size, and higher NYHA scores, in treatment groups. LVEF not significantly different.
Mohamadnejad *et al.* 2007 [188] P1	Iran	Decompensated liver cirrhosis	4	12 months	Autologous BM-MSC	Intravenous infusion	No side effects, no abnormalities noted on abdominal CT. Quality of life, SF-36 physical component, and mental components increased.
Ball *et al*. 2007 [189]	Netherlands and Italy	HRC/GVHDP	14	Maximum 28 months	BM-MSC from HSC donor	Intravenous infusion	No rejection of graft in patients who received MSCs, as compared to rejection rate of 7 in 47 patients who did not receive MSCs.
Liu *et al.* 2006 [190]	China	Healthy volunteers	12	1 month	Autologous BM-MSC	Intravenous infusion	No adverse events reported. No changes in ECG, chest x-ray, liver and kidney function tests, immune cell populations, or bone marrow biopsy composition following injection.
Moviglia *et al.* 2006 [191]	Argentina	Chronic spinal cord injury	2	6 months and 3 months	Human BM-MSC which were transdifferentiated to NSCs	Intraarterial infusion to artery feeding lesion site	No adverse events reported. Improved motor and sensory spinal cord levels.
Chen *et al.* 2006 [192]	China	Ischemic Cardiomyopathy	48	12 months	Allogeneic BM-MSC	Intracoronary arterial infusion	3 patients experienced pulmonary edema during injection of MSCs. Improved exercise tolerance, LVEF, NYHA score, and time to death in treatment group.
Ringdén *et al.* 2006 [193]	Sweden	HRC/GVHDP	8	Variable	BM-MSC from haploidentical and HLA-mismatched donors	Intravenous infusion	Complete remission of GVHD in 6 patients. Deaths in 2 patients with no obvious response.
Assmus *et al.* 2006 [194]	Germany	Post-Myocardial infarction with stable ischemic heart disease	75	3 months	Autologous bone marrow cells isolated by Ficoll density-gradient centrifugation.	Intraarterial infusion to artery feeding lesion site	Significant increase in left ventricular ejection fraction in treated groups. Significant increase in left ventricular function when controls crossed over to treatment regimen.
Bang *et al.* 2005 [142]	South Korea	Ischemic stroke	30	12 months	Autologous BM-MSC	Intravenous infusion	Improved Barthel index and modified Rankin score in the treatment groups.
Lazarus *et al.* 2005 [195]	USA, Italy	HRC/GVHDP	56	24 months	BM-MSC from HLA-identical sibling donors	Intravenous infusion	No adverse events. 53% disease- or progression-free survival. Neutrophil and platelet engraftment at 14 and 20 days respectively.
Chen *et al.* 2004 [196, 197]	China	Myocardial Infarction	69	6 months	BM-MSC	Intracoronary arterial infusion	No deaths. No arrythmias at 3 months follow up. Improved perfusion defect, and LVEF in treatment group.

Table 1. Contd.…

Citation	Location of Trial	Disease	Enrolled	Follow-Up Length	MSC source	MSC Administration Technique	Major Outcomes
Cahill *et al.* 2004 [198]	USA	Bone marrow transplant	3	Unspecified	Human BM-MSC	Intravenous infusion	High engraftment in bone marrow.
Horwitz *et al.* 2002 [199]	USA	Osteogenesis imperfecta	6	6 months	Allogeneic BM-MSC transfected with LNc8 and G1PLII genes	Intravenous infusion	Controllable urticaria in one patient after infusion. Engraftment in bone, skin, marrow stroma. Likely immune recognition of LNc8 cells, but not G1PLII cells.
Koç *et al.* 2002 [200]	USA	MLD and Hurler syndrome	MLD: 5 HS: 6	24 months	Allogeneic BM-MSC	Intravenous infusion	Improvements in nerve conduction. Improved bone mineral density.
Koç *et al.* 2000 [201]	USA	Hematopoietic recovery following chemotherapy in breast cancer patients	28	9 months	PBSC + BM-MSC	Intravenous infusion	No immediate toxicity. Hematopoietic recovery within about 8 days. At 9 months, 3 patients died from breast cancer progression. One patient died within 100 days from an unknown cause.
Lazarus *et al.* 1995 [202]	USA	Phase I trial, patients with hematologic malignancy in full remssion	23	28 to 49 days	Autologous MPCs	Intravenous infusion	No adverse reactions.

Table 2. Active, Recruiting, or Completed but Unpublished Clinical Trials of MSCs. To obtain this list of clinical trials, Clinicaltrials.gov was searched on August 8, 2009, for "Mesenchymal Stem Cells," and studies that did not use MSCs were omitted. Abbreviations: Same as in Table 1, with the Following Additions: *UC:* Umbilical Cord. *BMSC:* Bone Marrow Stem Cell

NCT ID	Title	Conditions	Interventions	Phase	Enrollment	Anticipated Completion Date
NCT00294112	Prochymal™ Adult Human Mesenchymal Stem Cells for Treatment of Moderate-to-Severe Crohn's Disease	Crohn's Disease	Prochymal™ MSC	II	10	Complete
NCT00136903	Safety and Efficacy Study of Adult Human Mesenchymal Stem Cells to Treat Acute GVHD	HRC/GVHDP	Prochymal™ MSC	II	33	Complete
NCT00366145	Efficacy and Safety of Adult Human Mesenchymal Stem Cells to Treat Patients Who Have Failed to Respond to Steroid Treatment for Acute Graft Versus Host Disease (GVHD)	HRC/GVHDP	MSC	III	240	Complete
NCT00284986	Safety and Efficacy of Prochymal for the Salvage of Treatment-Refractory Acute GVHD Patients	HRC/GVHDP	Prochymal™ MSC	II	30	Complete
NCT00187018	Marrow Mesenchymal Cell Therapy for Osteogenesis Imperfecta: A Pilot Study	Osteogenesis Imperfecta	Marrow-implanted MSC		14	Complete
NCT00186914	Stromal Therapy of Osteodysplasia After Allogeneic Bone Marrow Transplantation	Osteodysplasia	MSC infusion	I	8	Complete
NCT00260338	Stem Cell Therapy for Vasculogenesis in Patients With Severe Myocardial Ischemia	Myocardial Ischemia	Stem cell	I/II	40	Complete
NCT00135850	The Effect of Mobilized Stem Cell by G-CSF and VEGF Gene Therapy in Patients With Stable Severe Angina Pectoris	Ischemic Heart Disease	VEGF-A165 plasmid	I/II	48	Complete

Table 2. Contd….

NCT ID	Title	Conditions	Interventions	Phase	Enrollment	Anticipated Completion Date
NCT00953485	Allogeneic Mesenchymal Stem Cells Transplantation for Primary Sjögren's Syndrome (pSS)	Sjogren's Syndrome	Allogeneic MSC	I/II	20	Dec 2011
NCT00395200	Mesenchymal Stem Cells in Multiple Sclerosis (MSCIMS)	Multiple Sclerosis	MSC	I/II	10	Oct 2010
NCT00504803	Mesenchymal Stem Cell Infusion as Prevention for Graft Rejection and Graft-Versus-Host Disease	Hematological Malignancies	MSC	II	30	May 2011
NCT00823316	Safety and Efficacy Study of Umbilical Cord Blood-Drived Mesenchymal Stem Cells to Promote Engraftment of Unrelated Hematopoietic Stem Cell Transplantation	Acute Leukemia	UC-MSC	I/II	10	Dec 2009
NCT00891501	The Use of Autologous Bone Marrow Mesenchymal Stem Cells in the Treatment of Articular Cartilage Defects	Degenerative Arthritis, Chondral Defects, Osteochondral Defects	BM-MSC	II/III	25	Dec 2014
NCT00883870	Mesenchymal Stem Cells in Critical Limb Ischemia	Critical Limb Ischemia	MSC	I/II	20	Dec 2011
NCT00420134	Use of Autograft Mesenchymal Stem Cells Differentiated Into Progenitor of Hepatocytes for Treatment of Patients With End-Stage Liver Disease	Liver Failure, Cirrhosis	Hepatocyte progenitor from MSC	I/II	30	
NCT00314483	Evaluation of the Role of Mesenchymal Stem Cells in the Treatment of Graft Versus Host Disease	HRC/GVHDP	MSC	I/II	25	June 2008
NCT00646724	Cotransplantation of Islet and Mesenchymal Stem Cell in Type 1 Diabetic Patients	Type 1 Diabetes	MSC + Islet Cell	I/II	30	Jan 2011
NCT00850187	Autologous Transplantation of Mesenchymal Stem Cells (MSCs) and Scaffold in Full-Thickness Articular Cartilage	Knee Cartilage Defects/Osteoarthritis	BM-MSC	I	6	May 2010
NCT00603330	Mesenchymal Stem Cell Infusion as Treatment for Steroid-Resistant Acute GVHD or Poor Graft Function	HRC/GVHDP	MSC	II	100	Oct 2012
NCT00447460	Treatment of Refractory (Acute or Chronic) Graft-Versus-Host Disease by the Infusion of Expanded in-Vitro Allogenic Mesenchymal Stem Cell	HRC/GVHDP	MSC	I/II	15	Aug 2009
NCT00476060	Mesenchymal Stem Cell Transplantation in Decompensated Cirrhosis	Cirrhosis	Autologous MSC	II	50	Feb 2010
NCT00885729	Mesenchymal Stem Cells in a Clinical Trial to Heal Articular Cartilage Defects	Cartilage Defects	MSC	I	50	Apr 2018
NCT00911365	Trial of Autologous Mesenchymal Stem Cells in Patients With Multiple System Atrophy	Multiple System Atrophy	MSC	II		
NCT00250302	Autologous Implantation of Mesenchymal Stem Cells for the Treatment of Distal Tibial Fractures	Tibial Fracture	Autologous MSC	I/II	24	
NCT00498316	Cord Blood Expansion on Mesenchymal Stem Cells	Myelodysplastic Syndrome/Leukemia	Cord Blood Infusion	I	100	
NCT00752479	Mesenchymal Stem Cells Under Basiliximab/Low Dose RATG to Induce Renal Transplant Tolerance	Kidney Transplant	MSC infusion, Basiliximab, RATG	I/II	6	Mar 2010
NCT00543374	Extended Evaluation of Prochymal™ Adult Human Stem Cells for Treatment-Resistant Moderate-to-Severe Crohn's Disease	Crohn's Disease	Prochymal™ MSC	III	200	Dec 2009

Table 2. Contd.…

NCT ID	Title	Conditions	Interventions	Phase	Enrollment	Anticipated Completion Date
NCT00587990	Prospective Randomized Study of Mesenchymal Stem Cell Therapy in Patients Undergoing Cardiac Surgery (PROMETHEUS)	Stem Cell Transplantation/Ventricular Dysfunction, Left	MSC	I/II	45	June 2011
NCT00698191	Mesenchymal Stem Cells Transplantation for Refractory Systemic Lupus Erythematosus	Refractory SLE	Allogeneic MSC	I/II	20	Dec 2012
NCT00476762	Follow-up Study to Evaluate the Safety of Prochymal for the Treatment of GVHD Patients	HRC/GVHDP	Prochymal™ MSC	II	50	
NCT00683722	Prochymal™ (Human Adult Stem Cells) for the Treatment of Moderate to Severe Chronic Obstructive Pulmonary Disease (COPD)	COPD	Prochymal™ MSC	II	60	
NCT00690066	Prochymal™ (Human Adult Stem Cells) for the Treatment of Recently Diagnosed Type 1 Diabetes Mellitus (T1DM)	Type 1 Diabetes	Prochymal™ MSC	II	60	
NCT00826046	Expanded Access of Prochymal® (Ex-Vivo Cultured Adult Human Mesenchymal Stem Cells) Infusion for the Treatment of Patients Who Have Failed to Respond to Steroid Treatment for Acute GVHD	Graft-Versus-Host Disease	Prochymal™ MSC			
NCT00515307	Bone Marrow Stem Cells as a Source of Allogenic Hepatocyte Transplantation in Homozygous Familial Hypercholesterolemia	Hypercholesterolemia	BM-MSC	I	1	June 2008
NCT00114452	Safety Study of Adult Mesenchymal Stem Cells (MSC) to Treat Acute Myocardial Infarction	Myocardial Infarction	Prochymal™ MSC	I	48	Sep 2006
NCT00361049	Donor Mesenchymal Stem Cell Infusion in Treating Patients With Acute or Chronic Graft-Versus-Host Disease After Undergoing a Donor Stem Cell Transplant	Cancer	Allogeneic MSC	I	24	
NCT00767260	Autologous Mesenchymal Stem Cell and Bone Marrow Stem Cell Infusion Combined With Hyperbaric Oxygen Therapy in Type 2 Diabetes Mellitus	Type 2 Diabetes Mellitus	MSC, BMSC	I/II	100	Mar 2010
NCT00482092	Evaluation of Prochymal™ Adult Human Stem Cells for Treatment-Resistant Moderate-to-Severe Crohn's Disease	Crohn’s Disease	MSC	III	270	Dec 2011
NCT00658073	Mesenchymal Stem Cell Transplantation in Recipients of Living Kidney Allografts	Kidney Transplant	MSC		60	Mar 2009
NCT00883727	*Ex vivo* Cultured Bone Marrow Derived Allogenic MSCs in AMI	Myocardial Infarction	MSC	I/II	20	Dec 2011
NCT00609661	Adult Stem Cell Response to Burn Injury	Burn Injury			50	June 2010
NCT00877903	Prochymal™ (Human Adult Stem Cells) Intravenous Infusion Following Acute Myocardial Infarction (AMI)	Myocardial Infarction	Prochymal™ MSC	II	220	Mar 2012
NCT00827398	Treatment of Steroid Resistant GVHD by Infusion MSC	HRC/GVHDP	MSC	I/II	10	
NCT00759018	Expanded Access of Prochymal Infusion for the Treatment of Pediatric Patients Who Have Failed to Respond to Steroid Treatment for Acute GVHD	HRC/GVHDP	Prochymal™ MSC			
NCT00609232	Prochymal™ to Treat Crohn’s Disease	Crohn’s Disease	Prochymal™ MSC	III	15	Dec 2010
NCT00418418	Combined CABG and Stem-Cell Transplantation for Heart Failure	Heart Failure	Intramyocardial MSC	II	60	Dec 2010

Table 2. Contd….

NCT ID	Title	Conditions	Interventions	Phase	Enrollment	Anticipated Completion Date
NCT00768066	The Transendocardial Autologous Cells (hMSC or hBMC) in Ischemic Heart Failure Trial (TAC-HFT)	Heart Failure	Autologous MSC, autologous human BM cells	I/II	60	
NCT00781872	Mesenchymal Stem Cells for the Treatment of MS	Multiple Sclerosis	Autologous MSC	I/II	20	
NCT00710411	Inflammatory Response After Muscle and Skeleton Trauma	Multiple Trauma			90	Dec 2011
NCT00913289	Liver Regeneration Therapy Using Autologous Adipose Tissue Derived Stromal Cells	Liver Cirrhosis	Adipose MSC		9	Mar 2011
NCT00790413	Haploidentical Stem Cell Transplantation in Neuroblastoma	Neuroblastoma	Allogeneic MSC + allogeneic lymphocyte infusion		15	
NCT00081055	OTI-010 for Graft-Versus-Host Disease Prophylaxis in Treating Patients Who Are Undergoing Donor Peripheral Stem Cell Transplantation for Hematologic Malignancies	GVHD, Leukemia, Myelodysplastic Syndromes	Autologous MSC, OTI-010, peripheral blood stem cell transplantation	II		
NCT00629096	Intracoronary Infusion of Autologous Bone Marrow Cells for Treatment of Idiopathic Dilated Cardiomyopathy	Dilated Cardiomyopathy	Intracoronary autologous BM stem cells	II	30	Aug 2010
NCT00555828	Safety Study of Allogeneic Mesenchymal Precursor Cells in Subjects With Recent Acute Myocardial Infarction	Myocardial Infarction	Allogeneic MPC	I/II	25	Dec 2013
NCT00721045	A Phase II Dose-Escalation Study to Assess the Feasibility and Safety of Transendocardial Delivery of Three Different Doses of Allogeneic Mesenchymal Precursor Cells (MPCs) in Subjects With Heart Failure	Heart Failure	MPC	II	60	July 2011

with tumor cells can induce malignant transformation in MSCs. The potential carcinogenic transformation of MSCs in the context of cancer has been proposed based on accumulating evidence that the stroma of the tumor—comprised of those cells which surround cancer cells—is important in supporting the growth of the tumor [171]. Tumor-associated stromal cells scavenge the lactate produced by hypoxic tumor cells and convert it to pyruvate, which can be used for oxidative phosphorylation [172]. One important component of this tumor stroma in some cancers is the carcinoma associated fibroblast (CAF). CAFs are bone marrow-derived cells which have been shown to promote growth of breast cancer cells and angiogenesis through the tumor [173-175]. Notably, cells that are similar to CAFs can be derived from human bone marrow-derived MSCs, when exposed to tumor-conditioned medium [176]. These cells derived from MSCs are similar to CAFs in terms of phenotype, expression of SDF-1, and the ability to enhance tumor growth both *in vivo* and *in vitro* [176]. This observation raises the possibility that a similar malignant transformation could occur *in vivo* if MSCs are exposed to tumor stroma. Also, human MSCs have been shown to increase the metastatic potential of breast cancer [85]. These observations have led some authors to believe that MSCs, when exposed to the microenvironment of a tumor, could be a source of CAFs, and that administration of MSCs could promote tumor growth [171]. Whether such a carcinogenic stromal environment exists in brain tumors is unknown. However, it has been demonstrated that human adipose derived MSCs promote the growth of a tumor when co-injected intracranially with either H460 (human lung cancer) or U87 (human glioblastoma) cancer cells lines into nude mice [177]. An increase in tumor cell viability and lower levels of apoptotic cell death were also observed in this study [177]. Therefore the stromal environment in brain tumors may potentially cause MSCs to differentiate into a cancer-supporting phenotype.

Further uncertainties arise due to conflicting results from other cancer models. Whereas subcutaneous injections of melanoma cells into allogeneic recipients led to tumor formation only in the presence of MSCs [82], MSCs do not enhance tumor growth in a xenograft model of ovarian cancer [69], and human MSCs do not modify colon cancer cell growth *in vitro* [72]. Such conflicting results, taken together with the paucity of studies on the interactions of MSCs with glioma cells, underlie the need for further research before it will be possible to have full confidence in the safety of MSCs.

Evidence from Clinical Trials

In all the clinical trials of MSCs, which are described and cited in Table **1**, there have been no acute nor long-term adverse events reported. These clinical trials assessed the safety and therapeutic potential of MSCs in the treatment of mostly non-neurological diseases, such as myocardial infarction and graft-versus-host disease, and they may give an early indication of the safety of MSC-based therapies for the treatment of neurological disease. Notably, there were no reports of carcinogenesis, and there were no reported adverse events from the use of allogeneic transplants. Although clinical trials provide the best available evidence of MSC characteristics in patients, assessing the safety of MSCs from these early-stage

clinical trials is nevertheless very difficult. First, a reliable assessment of carcinogenic potential is often only possible many years after the exposure which is in question. Follow-up intervals in trials of MSCs tend to be short in comparison with the drawn-out pathogenesis of many cancers, and long-term carcinogenic potential therefore remains unknown. Second, systemically-administered MSCs have tremendous migratory capacity, making it difficult to predict possible sites for seeding, and therefore to select the optimal imaging studies to detect the presence of growing cancer. Few studies have employed comprehensive imaging modalities, such as whole body PET, that are capable of capturing developing cancers from a whole body perspective. Third, clinical trials of novel therapies, such as MSCs, tend to be reserved for very ill patients with a poor prognosis. The poor outcomes of these patient populations may obscure possible adverse effects. Fourth, few clinical trials to date have evaluated MSCs that have been genetically modified, although the introduction of foreign genes may increase the carcinogenic potential of a cell line through insertional mutagenesis. Finally, few clinical trials using MSCs have employed sufficiently rigorous designs which include prospective randomization and clinically-relevant, standardized end-points. Although clinical trials provide the best existing evidence regarding the safety of MSCs, these five limitations in their designs—limited follow-up intervals, lack of adequate follow-up diagnostic testing, morbid subject baseline characteristics, the use of only unmodified stem cells, and the lack of prospective randomization—suggest that further studies on the potential hazards of MSC-based therapies are still required. The results of ongoing and future clinical trials (Table **2**), and continued follow up from existing patient cohorts, will undoubtedly shed light on any possible long term hazards of MSC-based therapies.

CONCLUSION

MSCs hold tremendous promise for therapeutic application in many different disease categories. Fig. (**2**) summarizes a therapeutic paradigm in which MSCs are harvested from human tissue, culture expanded, and modified to secrete a therapeutic compound of interest. We believe that clinical translation of MSCs for the treatment of neurological disease will likely follow this paradigm or a similar approach. This review presented an overview of all the MSC clinical trials which have been conducted to date and those which are ongoing. Within the context of the CNS, MSCs have been evaluated for the treatment of autoimmune diseases, brain cancers, and neural injury, and are at or near clinical trials within each category. The poor prognosis of these CNS disorders motivates the need for radical new therapeutic approaches such as the use of MSCs. The major limitation to clinical translation remains their poorly characterized oncogenic potential, and in particular, the oncogenic potential that arises from genetic modification. A better understanding of these possible hazards may be obtained by conducting new clinical trials; however, a more practical approach would be the institution of more rigorous follow-up protocols in existing patient cohorts, over longer periods of time. More generally, research on MSCs would be greatly aided by improved clinical trial designs that incorporate multiple centers, prospective randomization, and clinically-relevant, reproducible endpoints. Other areas of potential investigation include elucidation of the mechanisms of action that underlie the clinically-important effects of MSCs, and the study of which effects are dependent on the administered dose of MSCs. Exploring these subjects is a colossal task, but one well worth undertaking for a therapeutic approach which may hold tremendous potential for treating morbid illnesses of the CNS.

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