Published in final edited form as:

Annu Rev Biomed Eng. 2010 August 15; 12: 87–117. doi:10.1146/annurev-bioeng-070909-105309.

Mesenchymal Stem Cells as Therapeutics

Biju Parekkadan^{1,*} and Jack M. Milwid^{1,2,*}

Biju Parekkadan: biju_parekkadan@hms.harvard.edu

¹Center for Engineering in Medicine and Surgical Services, Massachusetts General Hospital, Shriners Hospital for Children, and Harvard Medical School, Boston, Massachusetts 02114

²Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Abstract

Mesenchymal stem cells (MSCs) are multipotent cells that are being clinically explored as a new therapeutic for treating a variety of immune-mediated diseases. First heralded as a regenerative therapy for skeletal tissue repair, MSCs have recently been shown to modulate endogenous tissue and immune cells. Preclinical studies of the mechanism of action suggest that the therapeutic effects afforded by MSC transplantation are short-lived and related to dynamic, paracrine interactions between MSCs and host cells. Therefore, representations of MSCs as drug-loaded particles may allow for pharmacokinetic models to predict the therapeutic activity of MSC transplants as a function of drug delivery mode. By integrating principles of MSC biology, therapy, and engineering, the field is armed to usher in the next generation of stem cell therapeutics.

Keywords

mesenchymal stem cell; immunotherapy; clinical trials; paracrine; extracorporeal bioreactor; conditioned medium

1. INTRODUCTION

The discovery of adult stem cells substantiated theories about the presence of regenerative populations of cells in developed organisms and has led to growing interest in the use of these cells as therapeutics. In particular, researchers are now exploring the use of bone marrow–derived mesenchymal stem cells (MSCs) in preclinical and clinical studies to resolve injury by enhancing endogenous repair programs, which represents a powerful new paradigm for treating human disease. From systemic administration of MSCs as an intravenous treatment to the delivery of their molecular secretions by extracorporeal devices, groups around the globe are focusing their attentions on this cell, seeking to harness its full therapeutic potential.

MSCs are an excellent candidate for cell therapy because (a) human MSCs are easily accessible; (b) the isolation of MSCs is straightforward and the cells can expand to clinical scales in a relatively short period of time (1, 2); (c) MSCs can be biopreserved with minimal

DISCLOSURE STATEMENT

The authors are founders of a company developing mesenchymal stem cell (MSC)-based therapeutics.

loss of potency and stored for point-of-care delivery (3, 4); and (*d*) human trials of MSCs thus far have shown no adverse reactions to allogeneic versus autologous MSC transplants, enabling creation of an inventory of third-party donor MSCs to widen the number of patients treated by a single isolation (5–7). MSC transplantation is considered safe and has been widely tested in clinical trials of cardiovascular (8, 9), neurological (10, 11), and immunological disease (12, 13) with encouraging results. Unfortunately, within the past year, several of the pivotal lead trials either have undergone early termination or have failed to meet primary endpoints.

These results suggest an incomplete understanding of the underlying mechanism(s) of action of MSC therapy and point to the importance of further preclinical development. A more refined understanding of the natural functions of MSCs in the bone marrow may provide the basis for insight into their primary mode(s) of action. MSCs were first considered to be stromal progenitor cells in the bone marrow and were originally hypothesized to serve one primary role in their undifferentiated state: replenishment of stromal tissue in the bone marrow. However, MSCs and their stromal progeny also perform a number of alternative functions in the bone marrow, including the secretion of soluble mediators, which support hematopoiesis. These alternative functions are now being characterized in the context of MSC transplantation, whereby paracrine interactions between MSCs and host cells have been shown to relate directly to the therapeutic activity of MSCs.

Although it has not been definitively proven whether engraftment and differentiation of MSCs is necessary to convey this paracrine support, recent studies have suggested that less than 1% of systemically administered MSCs persist for longer than a week following injection (14, 15), and the observed benefits of MSC therapy may result from the relinquishment of their molecular contents upon administration. If this is true, engineering approaches may improve the optimization of MSC dosing and provide for alternative uses of MSCs to deliver drugs as active, dynamic delivery vehicles.

The aim of this review is to present an overview of the field of MSC therapy, with a particular focus on the various hypotheses concerning the mechanism(s) of action of MSCs. The majority of works cited focus on systemic administration of bone marrow–derived MSCs because this therapeutic modality has been explored more extensively than other means of administering MSC-based therapy. For alternative sources and modes of administration of MSCs, we refer the reader to other reviews (16–18). We begin with a brief history of MSCs, from their initial discovery to current clinical programs using cell transplantation. We then revisit the biological origins and natural functions of MSCs and present the initial observations that laid the groundwork for therapeutic testing. Next, we describe retrospective studies that support the theory that MSCs can be conceptualized as drug-releasing particles that deliver their payloads in the course of hours to days. Finally, we propose the development of new pharmacokinetic analysis techniques based on this molecular-particle theory to motivate new clinical trial designs for therapeutic delivery of MSCs. Ultimately, such engineering analyses may better predict the biological activity of MSCs and leverage the therapeutic potential of these cells in a more rational way.

2. ORIGINS AND DEVELOPMENTAL BIOLOGY OF MESENCHYMAL STEM CELLS

In this section, we begin with historical studies that first suggested the existence of MSCs (see Figure 1). We then focus on clinical studies that have used MSCs as an intravenous therapeutic. Finally, we describe the current methods used to characterize MSC populations and highlight the known differentiated functions of MSCs in their native, *in vivo* microenvironments.

2.1. History of Mesenchymal Stem Cells: From Discovery to Clinical Therapy

The existence of a stromal precursor giving rise to mesodermal cells in the bone marrow (19) was originally theorized in the nineteenth century. Cohnheim hypothesized a bone marrow origin of fibroblasts implicated in distal wound healing (20). In the early twentieth century, Maximow described the essential relationship between newly forming blood components and the mesoderm during embryogenesis. He initially postulated the importance of the marrow stromal tissue in supporting the development and maintenance of blood and hematopoietic organs (21). These observations made by Cohnheim and Maximow offered the first indication of a reservoir of stromal cells in the bone marrow that were involved in the natural healing response and hematopoiesis.

In vivo transplantation of bone marrow elements in the 1960s demonstrated that stromal precursors are directly involved in the formation of skeletal tissue cells. Friedenstein et al. first demonstrated that stromal cells could be isolated from whole bone marrow aspirates based on differential adhesion to tissue culture plastic—a method still widely practiced to isolate MSCs. These stromal cells were originally described as adherent, clonogenic, nonphagocytic, and fibroblastic in nature, with the ability to give rise to colony forming units-fibroblastic (CFU-F) (22). Transplantation of these marrow stromal cells under the kidney capsule or in subcutaneous space led remarkably to the formation of ectopic marrow. Decomposition of the origins of ectopic marrow cells revealed donor-derived bony trabeculae, myelosupportive stroma, and adipocytes and host-derived hematopoietic cells that colonized and matured within the space (23–25). Experiments performed with dermal fibroblasts or differentiated connective tissue cells failed to recapitulate the same histological image, demonstrating that this was a phenomenon specific to the marrow stroma. These seminal experiments illustrated an “organizing” function of MSCs similar to other lymphoid stromal cells and suggested that MSCs were a precursor to bone marrow connective-tissue cells.

In the late 1980s, Maureen Owen and Arnold Caplan elaborated on Friedenstein’s initial work and proposed the existence of an adult stem cell that was responsible for mesengensis (25, 26). Owen et al. further characterized the marrow stroma and illustrated the heterogeneity of the population (25, 27–30). At the same time, Caplan and colleagues hypothesized that a subpopulation of the marrow stroma was developmentally linked to the mesenchymal tissues he had been studying during chick embryogenesis (26, 31). In addition, they identified the first set of MSC-expressed antigens that react with SH2 (CD105) and SH3 (CD73) monoclonal antibodies. He coined the term mesenchymal stem cell to describe this subtype of marrow stromal cells involved in the process of mesengensis (32–34).

Shortly following the discovery of methods for isolating and culturing MSCs, the field began to grow rapidly, and many groups began to explore their therapeutic uses. Only a few years after MSCs were identified, human trials were commenced to evaluate the safety and efficacy of MSC therapy. Initially, autologous MSCs were explored to aid in the engraftment and recovery of hematopoiesis after ablation and bone marrow transplantation for the treatment of cancer (35, 36). Concurrently, researchers conducted a number of groundbreaking studies that leveraged the therapeutic potential of allogeneic MSC transplants to treat children with osteogenesis imperfecta, a genetic disorder of skeletal dysplasia (37, 38). Shortly thereafter, more studies were performed to investigate the utility of allogeneic MSCs to treat patients with Hurler syndrome and metachromatic leukodystrophy (39). The focus of these early studies was predicated upon the fact that MSCs functioned as stromal stem cells and therefore might be best suited to treat diseases and conditions afflicting connective and hematopoietic tissue. These first studies were important because they provided preliminary evidence of the safety of MSC therapy as well as the basis for good manufacturing processes to generate MSCs on a clinical scale.

More recently, groups around the world have investigated MSC transplantation for the treatment of myriad diseases based on a newfound appreciation for MSCs' pleiotropic functions that enhance endogenous repair and attenuate immunological dysfunction. Examples of clinical trial designs are provided in Table 1. Currently, there are 79 registered clinical trial sites for evaluating MSC therapy throughout the world (<http://clinicaltrials.gov/>). The United States has the highest concentration of registered trial sites at 28, but the rest of the world accounts for more than half of the total number (19 in Europe, 16 in China, 5 in the Middle East, 4 in India, 3 in Canada, 2 each in Africa and Japan, and 1 in Australia), indicating strong international interest in MSCs as a potential therapy. The majority of trials are sponsored by academic medical centers exploring novel applications of MSCs to treat conditions as diverse as critical-limb ischemia (NCT00883870), spinal cord injury (NCT00816803), and liver cirrhosis (NCT00420134).

Osiris Therapeutics, Inc., founded in 1991, has played a pivotal role in the past decade in shaping the direction of research and development of MSC-based therapies. Concurrent with emerging theories regarding the immunological activity of MSCs, which we discuss in greater depth below, Osiris has developed a clinical trial program to explore the therapeutic utility of MSCs in humans. It has pioneered studies to investigate systemic administration of MSCs as a therapy for steroid-refractive graft-versus-host disease (GvHD), Crohn's disease, type I diabetes mellitus (IDDM), myocardial infarction (MI), and chronic obstructive pulmonary disease (COPD). Because early trials established a good record of safety for direct MSC injection, many of these studies are currently in Phase II or Phase III trials, and MSCs already have been granted expanded access for use in pediatric steroid-refractive GvHD by the United States Food and Drug Administration (FDA). However, in the past year, Osiris reported that several of its Phase II and Phase III studies were either prematurely terminated or failed to meet primary endpoints (<http://www.osiristx.com>). In March 2009, initial results for the Crohn's disease trial found a greater-than-expected placebo response, which led the company to cease recruitment after enrolling 210 patients in a Phase III study. In June 2009, six-month interim data of the Phase II COPD trial were announced: They indicated a statistically significant decrease in systemic inflammation as measured by C-reactive protein in MSC-treated patients but no statistical improvement of lung function. In September 2009, Osiris reported the results of two Phase III double-blind, placebo-controlled trials for adult GvHD in which MSC transplants were tested either as a first-line therapy or in patients that were refractory to standard medical treatment. Both trials showed an insignificant improvement in mortality compared with placebo at a 28-day endpoint (first-line therapy: $N = 192$; 45% response rate with MSCs versus 46% response rate with placebo; refractory therapy: $N = 260$; 35% with MSCs versus 30% with placebo). Stratification of the cohorts based on subclasses of GvHD have yet to be reported but may show benefit in specific patient populations. Nevertheless, the results of these trials may curtail efforts to develop and validate MSC therapy in humans.

These results may indicate an incomplete understanding of the mechanisms of action of MSCs, and therefore an inefficient administration of the cells that does not best convey therapeutic benefit. Many clinical trials entail the administration of MSCs systemically and assume that MSCs engraft and provide long-term support by either directly replenishing damaged tissue or interacting with neighboring cells to promote endogenous repair. Currently, it is widely debated whether MSC engraftment, proliferation, and/or differentiation is necessary for therapeutic benefit. Many new studies are now implicating paracrine signaling as the primary mechanism of action, and a few studies have even demonstrated that direct injection of the molecules secreted by MSCs can provide an improved benefit above and beyond what is conveyed by transplanted whole cells (41–43). In later sections, we discuss this issue further and present a new theory as to how MSCs may

be conveying therapeutic benefit without engraftment and differentiation—i.e., how they instead may be acting as dynamic drug delivery vehicles.

2.2. Phenotype and Multipotency Analysis for the Identification of Mesenchymal Stem Cells

One of the most elusive problems in MSC biology has been the identification of a single marker that distinguishes a purified population of MSCs with a uniquely defined set of functional properties. Without such an identifier, definitively comparing putative MSCs from different tissues has been challenging. Exhaustive phenotypic analysis has therefore been necessary to distinguish MSCs from other cells that exhibit similar fibroblastic, adherent characteristics in culture. Table 2 summarizes the current phenotypic tests used to identify MSCs.

As described in Table 2, the phenotype of MSCs is defined in part by the multipotency of these cells in culture and in vivo. Determining in vivo multipotency is a powerful tool for assessing MSC phenotype. As far back as Friedenstein, ectopic transplantation of MSCs has been used to determine whether MSC-like cells are capable of inducing bone and marrow formation (49, 51, 52). Perhaps the most rigorous method for determining the “stemness” of an MSC population involves the injection of MSCs into the blastocyst of a mouse and studying the developmental progeny from the injected cell populations (50). This method is not commonly used, however; although it was shown to be useful for a subset of MSCs, it does not necessarily reflect the phenotype of MSCs in general.

2.3. Embryonic and Adult Sources of Mesenchymal Stem Cells

The developmental precursor of MSCs has been difficult to identify because MSCs have no distinguishing features to track in vivo. A number of studies support the concept that the typical sites of developmental hematopoiesis, including the placenta, aorta-gonad-mesonephros, and fetal liver, are also populated by embryonic MSCs (53–55). These cells are originally independent of interactions with hematopoietic stem cells and can be found in the embryonic circulation at early stages of ontogeny (56). Counterintuitively, a novel embryological source of MSCs has been identified in the cranial neural crest. Through in situ methods with fluorescent reporting proteins, one group demonstrated a transient proliferation of Sox-1⁺ cells originally from the neuroepithelium that display multipotency and that transitioned through a neural crest stage to give rise to adult MSCs (57). Cells with multilineage differentiation potential and cytoskeletal elements reminiscent of adult MSCs can be isolated from the first branchial arch, ectomesenchymal cells that give rise to the orofacial connective tissue (58–60). These results are consistent with the promiscuous expression of neural proteins in MSCs in their basal state (61, 62). To date, no studies have determined the genetic events that guide the lineage specification of embryonic precursors into MSCs. Such genomic profiling can ultimately lead to new ways to derive these cells from embryonic stem cells or other ontogeny-related cell types.

Although the bone marrow has been established as the primary source of MSCs, because of the invasive nature of bone marrow aspiration, efforts are underway to identify other abundant and reliable sources of MSCs for clinical purposes. The isolation of MSCs from peripheral sources such as umbilical cord blood (UCB) (63, 64), placental tissue (53–55), and adipose tissue (47) has been reported with cells displaying similar immunophenotypes and multipotency, although other contradictory studies report the absence of MSCs in these peripheral locations (65, 66). Whether there is a definitive relationship between these cells from various sources is unclear because rigorous studies of in vivo multipotency have yet to be done. Furthermore, it is important to be wary of interpretations of CFU-F analysis of MSCs from sites other than the bone marrow, given that many adherent and clonogenic

fibroblastoid cells exist in nonhematopoietic tissues. Although phenotypically similar, or even identical in some cases, MSCs derived from different tissues have been shown to exhibit variable function and activity (67). It is thought that the MSC niche, unique to each tissue of origin, is at the root of these variations.

2.4. Mesenchymal Stem Cell Localization and Mobilization In Vivo

The physical location, or niche, of a stem cell provides invaluable information about their role and interactions within the tissue (see Figure 2). Bone marrow MSCs have been explored for therapeutic use more extensively than any other subtype, and the native functions of these cells in the bone marrow have been studied in hopes of revealing clues about their therapeutic activity (68, 69). The MSC niche has been difficult to locate and even harder to observe dynamically because no unique MSC marker has been identified and because the marrow cavity is difficult to probe in vivo. That stated, based on correlations between immunophenotype and ex vivo CFU-F assays, evidence supports the idea that MSCs exist in perivascular locations (70, 71). This theory is consistent with the observations that (a) MSCs are presumably found in many tissue types including synovium, periosteum, adipose, UCB, and placenta (41, 72, 73); (b) the number of MSCs in a given tissue scales with the density of microvasculature; (c) MSCs secrete factors that promote vasculogenesis and endothelial stabilization (74); and (d) they may exhibit different functional characteristics depending on the derivative tissue type (67). Their stromal counterparts may differentiate and migrate from this space to reside on the abluminal side of marrow sinusoids and form a three-dimensional network that invests the capillary bed.

Adventitial reticular cells, or pericytes, that have fibroblastic extensions projecting into the lumen of sinusoids, are likely the in vivo surrogate of CFU-F, although single-cell analytical studies have not been performed (69, 75, 76). These pericytes share a similar surface and intracellular protein expression pattern with MSCs, which implies that the cells are ontologically related (77). This location has been reproduced in artificial systems as well. Ectopic stromal cells displaying platelet derived growth factor receptor (PDGF-R), NG2, and high expression of CD146 are typically localized in perisinusoidal regions (78). In addition, tissue-engineered constructs juxtaposing MSCs and endothelial cells form long-lasting vascular structures, with MSCs naturally displaying pericytic phenotype and function (79). Such localization suggests that MSCs may be intimately involved in angiogenesis, wound healing, and interactions with blood-borne entities.

Given their purported perivascular location, the question of whether MSCs mobilize into the bloodstream during health and disease is an important one that has not been answered. It is unlikely that bona fide MSCs circulate peripherally because of their limited numbers (~0.01% of mononuclear bone marrow cells); it is more likely that they produce lineage-restricted cell types that home to tissues as a mechanism of nonparenchymal cell replenishment during injury. Fibrocytes are circulating bone marrow-derived cells (~0.1–0.5% of nonerythrocytic cells in peripheral blood) that phenotypically resemble a hybrid of monocytes and fibroblasts expressing type I collagen and the surface markers CD11b, CD13, CD34, and CD45RO (80). In sex-mismatched bone marrow chimeras, these cells were found to be the progeny of a radioresistant precursor from the bone marrow (81). During injury, fibrocytes were rapidly and specifically found in the areas of inflammation (81), fibrosis (82), and cancer (83–86), where they are thought to mature into tissue-resident myofibroblasts (87). They express chemotactic receptors such as CCR3, CCR5, CCR7, and CXCR4 and are absent of CCR4, CCR6, and CXCR3 (80). Interestingly, fibrocytes express surface molecules such as major histocompatibility complex class II, CD80, and CD86 and were shown to present pulsed antigens to naive T cells in an efficient manner when compared with monocytes and dendritic cells, although this was not verified in vivo (88). These cells have functional and phenotypic resemblance to bone marrow resident MSCs;

therefore, identifying distinguishable characteristics between the two cell types may lead to a greater understanding of the MSC-fibrocyte axis during health and disease.

2.5. The Interaction Between the Bone Marrow Stroma and Hematopoiesis

MSCs exist within the bone marrow as a precursor to connective-tissue components that act primarily as supportive elements to hematopoiesis. The essential functions of MSCs and their precursors within the marrow can provide insight into mechanisms involved when these cells are used in a therapeutic context. The initial appreciation for the important interaction between stromal cells and hematopoietic cells was obtained from the analysis of two different spontaneous mutations in mouse colonies that led to the same anemic phenotype. Analysis of these mutant mice revealed that a stromal cell ligand known as stem cell factor (SCF) and its associated receptor, c-kit, found on hematopoietic stem cells (HSCs) was essential for the maintenance of HSCs. Other cell-cell interactions between MSC progeny, such as osteoblasts and HSCs, has also proved essential for HSC self-renewal (89, 90). Moreover, stromal elements secrete a number of insoluble and soluble species within the marrow space that promote the growth and differentiation of hematopoietic cell lineages (91–94).

Experimentally, MSCs can act as a surrogate feeder layer and promote the self-renewal and differentiation of HSCs in long-term colony-initiating culture and CFU assays (95). Two types of culture techniques utilizing stromal cell layers and defined chemical supplements allow for the establishment of lymphoid and myeloid cells in vitro. The Whitlock-Witte method cultures bone marrow cells on a confluent layer of irradiated stromal cells with a low-serum-containing medium without corticosteroids (96, 97). It is a lymphoid culture system, which supports the growth of B lymphocytes and which, with some modifications in culture parameters, can also allow selective proliferation and differentiation of all developmental stages of pre-B cells and B lymphocytes. A myelopoietic culture system, known as Dexter cultures, maintains myeloid progenitor cells and differs from Whitlock-Witte cultures by using high concentrations of serum and hydrocortisone and lower incubation temperatures (98, 99). Collectively, the marrow stroma can direct the differentiation of lymphoid and myeloid cells in vitro, and it is likely that the mechanisms underlying this directed differentiation will be relevant to the immune response to MSCs in vivo.

3. PRECLINICAL AND CLINICAL APPLICATIONS OF MESENCHYMAL STEM CELL THERAPY

An extra level of biological understanding is often gained during testing of a new therapeutic. The evolution of MSC therapy over the years reflects a transformation in how investigators view these cells and their best-suited clinical applications. Initially heralded as stem cells, MSCs were first evaluated for regenerative applications. MSCs have since been shown to directly influence the innate and adaptive arms of the immune system (100), enhance proliferation of epithelial cells (101), and promote neovascularization of ischemic tissues (74). These observations have prompted a new age of MSC transplantation as a treatment for immune-mediated and tissue-sparing diseases. In this section, we capture this paradigm shift and focus on important studies that contextualize MSCs as a therapeutic for regenerative medicine and inflammatory diseases. Furthermore, we discuss the potential side effects that should be considered when MSCs are used.

3.1. Mesenchymal Stem Cells for Regenerative Medicine and Inflammatory Diseases

MSCs are currently being explored for use in humans because of their potent ability to treat many devastating diseases in animals (see Table 3). Although the primary mechanisms of

action have not been fully elucidated, studies indicate that MSCs can act on several levels of endogenous repair to bring about resolution of disease. MSCs have been shown to protect cells from injury and directly promote tissue repair (102, 103). When administered to treat animals undergoing acute renal failure, MSCs prevent apoptosis and elicit proliferation of renal-tubule epithelial cells in a differentiation-independent manner (104, 105). When injected into the myocardium after infarction, MSCs can reduce the incidence of scar formation (106–108). When administered to prevent the onset of IDDM, MSCs protect β -islets from autoimmune attack; when administered after onset of the disease, they promote temporary restoration of glucose regulation, suggesting protection and repair of damaged islet tissues (109).

In addition to promoting tissue repair directly, MSCs have also been shown to modulate the immune system and attenuate tissue damage caused by excessive inflammation. Initial indications regarding the immunomodulatory aspects of MSCs were first observed in the context of MSC transplantation studies in animals and humans. Unexpectedly, MSCs seemed to exhibit an unusual ability to evade the immune system. Initial clinical trials showed that autologous and allogeneic MSCs could be transplanted without immune rejection (35, 37). Further preclinical studies presented similar findings: Human MSCs can engraft and persist in many tissues in prenatal and adult sheep with no apparent rejection (125); MSC injection in baboons can prolong the life of a transplanted skin graft and suppress T cell proliferation in a dose-dependent manner (126); and injected MSCs can suppress the immune response in mice, allowing for the expansion of tumor cells (127). The immunosuppressive ability was first exploited clinically in the treatment of an 8-year-old boy with severe, acute GvHD, who was refractory to steroid immunosuppression (128). The patient was successfully treated by MSC transplantation. In recent years, this immunosuppression has been found to be an active process, and the mechanisms underlying MSC immunomodulation operate at different levels of the innate and adaptive immune system.

3.2. Decomposing the Interaction Between Mesenchymal Stem Cells and the Innate and Adaptive Immune System

Many studies suggest that MSCs can promote the conversion from a T_H1 (cell-mediated) immune response to a T_H2 (humoral) immune response (100). In vitro coculture experiments have been used to exemplify the effects of MSCs on individual populations of immune cells that favor this conversion at the cellular and molecular levels. With respect to adaptive immunity, the majority of in vitro studies have shown that MSCs can directly inhibit $CD3^+ CD4^+$ T cell proliferation and secretion of T_H1 lymphokines, such as IL-2 and IFN- γ . These studies have induced T cell activation by various methods including mixed lymphocyte reactions (MLRs), mitogens, and T cell receptor (TCR) or costimulatory receptor engagement. T cells in the presence of MSCs appear to be anergized by the lack of a second danger signal by MSCs, which do not express the costimulatory molecules CD80, CD86, and CD40 (129); however, this has yet to be definitively proven. Several investigations have also shown a direct suppressive effect of MSCs on cytotoxic $CD8^+$ T cells. MSCs prevented cytotoxicity of target cells by alloantigen-specific $CD8^+$ T cells when present during the priming of cytotoxic cells (130). Some investigators attribute the inhibition of cytotoxicity by MSCs to an intrinsic “veto” function or to the generation of suppressor $CD8^+$ cells after coculture (131), although conflicting data exist. Other reports have also observed generation of $CD4^+ CD25^+$ T cells, a cell-surface-marker expression pattern of both newly activated $CD4^+$ lymphocytes and regulatory T cells (100, 132). Although it is unclear whether MSCs directly influence B cells in vivo, some in vitro evidence suggests that MSCs can suppress B cell proliferation (133, 134). In contrast, other reports have shown that MSCs can stimulate antibody secretion and induce polyclonal

differentiation and expansion of healthy human B cells (135, 136), consistent with the supportive role of stromal cells in B lymphopoiesis. In addition, these same supportive mechanisms may advance the progression of B cell-mediated disease such as multiple myeloma and systemic lupus erythematosus (135, 137). However, it is possible that suppression of T cells by MSCs may contribute to decreased B cell activity in vivo (138).

The switch from a cell-mediated to humoral immune response triggered by MSC transplantation may involve the differentiation of innate immune cells to an anti-inflammatory phenotype. Within an inflamed tissue environment, MSCs are capable of influencing many aspects of the cytotoxic responses to injury and disease (139). MSCs can attenuate natural cytotoxic responses of neutrophils by dampening respiratory burst and inhibiting spontaneous apoptosis in vitro via secretion of IL-6 (140). MSCs also possess the ability to suppress proliferation of natural killer (NK) cells (141–143) and attenuate their cytotoxic activity by downregulating the expression of NKp30 and NKG2D, surface receptors involved in NK cell activation (144). This is accomplished even while cytokine-activated NK cells are capable of killing MSCs in vitro, suggesting a possible mechanism for MSC rejection in vivo (141). In vivo studies have yet to be performed to demonstrate this mechanism. MSCs can also revert macrophages to adopt an anti-inflammatory phenotype in the context of sepsis by secreting prostaglandin E2 and conveying a contact-dependent signal to promote IL-10 secretion (145).

Dendritic cells (DCs) serve as the major link between innate and adaptive immunity because of their ability to present antigens to lymphocytes with high efficiency. In coculture with MSCs, monocytes failed to differentiate into DCs when cultured in lineage-specifying growth conditions (146, 147). In addition, MSCs inhibited the maturation of DCs to present appropriate antigens and costimulation to T cells through CD1a, CD40, CD80, CD86, and HLA-DR (146, 148). After coculture with MSCs, DCs were ineffective in their ability to activate lymphocytes by suppressing TNF- α and IFN- γ expression and upregulating IL-10 in DC-CD4⁺ MLRs (147). This interaction was found to be γ -secretase dependent, indicating the role of the Notch pathway in MSC-DC interactions (149). Ultimately, MSCs may drive, or “license,” DCs to a suppressor phenotype that can further attenuate T cell-mediated immunity.

3.3. Potential Side Effects of Mesenchymal Stem Cell Therapy

MSC transplantation has been designated safe by the FDA. Clinical trials of MSC transplantation have shown no adverse events that affected the safety profile of these cells over the past 10 years of testing. Nevertheless, recent preclinical studies have highlighted potential long-term risks associated with MSC therapy that may not be observable in the short time period following administration (see Figure 3). These risks include potential maldifferentiation, immunosuppression, and instigation of malignant tumor growth.

MSCs are multipotent cells and may ectopically differentiate after therapeutic transplantation. Recent animal studies have confirmed that this is possible. MSCs, when administered in the context of acute glomerulonephritis in rats, can engraft in the renal tubules and maldifferentiate into adipocytes that hinder normal function of the kidney and lead to chronic kidney disease (150). When administered in mice, MSCs can also create microemboli and subsequently form osteosarcoma-like pulmonary lesions (151). However, this phenomenon has been observed in immunocompromised mice and therefore may not reflect a considerable risk to immunocompetent hosts. A similar observation was made when MSCs were administered to nonobese diabetic (NOD) mice in the context of IDDM (109). The MSCs formed soft tissue and visceral tumors throughout the mice upon administration. These studies may indicate risks of MSC transplantation that may be of particular importance to immunocompromised patients.

Other potential complications of MSC transplantation are related to the immunosuppressive properties of these cells and the loss of immunosurveillance to foreign and host pathogens. After MSC infusions were used to treat nine patients suffering from GvHD, three developed viral infections (152). Although these patients were at increased risk for developing opportunistic infections through the nature of their disease, concerns were raised that immunosuppression by the MSCs had caused a reduction of immunosurveillance to viruses.

These findings are supported by in vitro observations that lymphocyte proliferation by herpes viruses is suppressed by MSCs (152). Cancer is another potential serious side effect. Theoretically, MSCs could be tumorigenic through direct transformation, metabolism of chemotherapeutic agents, and/or suppression of the antitumor immune response. All these phenomena have been reported previously. MSCs have the potential to transform into sarcomas when Wnt signaling is suppressed (153). Mesenchymal cells have been shown to regulate the response of acute lymphoblastic leukemia to asparaginase chemotherapy by metabolizing the drug via their high expression of asparaginase synthetase (154, 155). Bone marrow stromal cells, highly enriched for MSCs, promote survival of B- and plasma-cell malignancies by inducing hedgehog signaling (156). Moreover, MSCs used for the treatment of GvHD limited the graft-versus-leukemia effect of allogeneic bone marrow transplantation, leading to a higher rate of relapse compared with control groups (157, 158). Patients with increased risk of malignancy or opportunistic infections may not be suitable candidates for MSC therapy; researchers should take this into account when assessing trials until more conclusive experimental data become available.

MSCs also associate with tumors and promote tumor growth when administered systemically into animals with existing malignancy (159, 160). Animal studies by Djouad and colleagues have revealed enhanced tumor growth after MSC transplantation when tumor cells were implanted (127, 161). It is unclear whether the MSCs enhanced tumor growth by immunomodulatory, trophic, or other effects. Karnoub et al. demonstrated that MSCs within tumor stroma promote breast cancer metastasis via cancer cell-induced de novo secretion of the chemokine CCL5 in MSCs (160). Recent work has shown that MSCs can differentiate into tumor-associated fibroblasts (TAFs) that provide stromal support to growing tumors (162). When mixed with tumor cells and transplanted in vivo, MSCs fulfill the following four criteria that are required for them to be considered TAFs: (a) expression of fibroblast markers FAP and FSP; (b) secretion of neovascularization promoters VEGF, desmin, and α -smooth muscle actin; (c) secretion of tissue remodeling and invasion proteins TSP-1, Tn-C, and SL-1; and (d) secretion of tumor-promoting factors hepatocyte growth factor (HGF), epidermal growth factor (EGF), and IL-6 (162). It should be noted, however, that the number of MSCs mixed with the cancer cells exceeded a typical systemic dose, and therefore they may represent a risk only if excessive numbers of MSCs are administered. Although MSCs were not specifically demonstrated to arise from the bone marrow in these studies, evidence that MSCs possess the ability to differentiate into TAFs is consistent with other studies showing that bone marrow stromal cells are recruited to the sites of indolent tumors and promote growth (163). Osteopontin is implicated as one of the key hormonal mediators of this effect, and it has been shown to be secreted by MSCs that have begun to differentiate down the osteoblast lineage (46). Future studies will be required to demonstrate direct causality.

Interestingly, some investigators are using the “tumor-homing” properties of MSCs for therapeutic use by genetically engineering the cells with cytolytic drugs to kill tumorigenic tissue selectively (164). MSCs that are genetically engineered to express TRAIL, a ligand for death receptors on the surface of tumor cells, can suppress tumor growth among subcutaneous tumors, pulmonary metastatic tumors, and highly malignant glioblastoma tumors in mice (165, 166). [TRAIL stands for tumor necrosis factor (TNF)-related apoptosis

inducing ligand.] Also, MSCs engineered to deliver IFN- β are capable of migrating into the brain and providing survival benefit to mice with gliomas (167). Using the homing properties of MSCs as such may present a new opportunity for use of MSCs as drug delivery vehicles in the context of cancer.

4. MOLECULAR PARTICLE THEORY OF MESENCHYMAL STEM CELL THERAPY

Therapeutic studies in different injury models accompanied by cell-tracking studies have revealed two peculiar observations: First, infusion of undifferentiated MSCs leads to therapeutic effects in different injury models without MSC differentiation. Second, the majority of MSCs cannot be located by sensitive, whole-body imaging techniques days after transplantation. In this section, we discuss studies that suggest that MSCs may impart therapeutic benefit by secreting soluble factors, and then we paint a molecular portrait of MSCs. Next, we highlight particular biodistribution studies that describe the rapid kinetics of MSC clearance after transplantation similar to what might be found when inert particles are injected. Finally, we close with a new proposed framework for pharmacokinetic analysis of MSC therapy.

4.1. Molecular View of Mesenchymal Stem Cells

Recent work has shown that the therapeutic benefits observed when MSCs are transplanted can be completely recapitulated, and in some cases improved upon, by administration of MSC secreted factors alone. This is not surprising because suppression of effector functions in most MSC-immune cell coculture studies was reproduced in the absence of cell-cell contact and in a dose-dependent manner, indicating the role of soluble factors. Furthermore, these inhibitory molecules can exert their effects across species barriers as evidenced by suppression of MLRs in xenogeneic cultures (127, 168). MSC-conditioned supernatants have no antiproliferative effect on T cells, yet they are capable of suppressing the stimulation of B cells (133). This suggests that MSCs can dynamically react to their immunological environment in the context of T cells while also secreting immunomodulatory molecules in their quiescent, undifferentiated state in the context of B cell development. Also, the numbers of MSCs needed to suppress T cell activity compared with B cell activity differ by approximately one to two orders of magnitude (134, 169). These studies hint at the interesting dynamics and dosing of MSC-derived molecules that researchers should consider when evaluating MSC therapeutic applications.

It remains highly debated as to which soluble mediators are involved in MSC therapy (reviewed in Reference 170), although a clear distinction can be made that some molecules are considered naturally secreted by MSCs and others are inducible. Many candidates such as HGF, transforming growth factor β_1 (TGF- β_1), or the metabolic byproduct of indoleamine 2,3-dioxygenase (IDO) (100, 171–175) are basally secreted by these cells. However, stimulation of MSCs by Toll-like receptor ligands or inflammatory cytokines causes an alteration of the MSC secretome and a different set of chemical species (176, 177). For example, lipopolysaccharide (LPS) found in serum leads to the rapid upregulation of prostaglandin E_2 (PGE $_2$), likely through an immediate early gene response related to NF- κ B. Recently, researchers demonstrated a direct correlation between the upregulation of an anti-inflammatory protein, TSG-6, upon engraftment of MSCs in the lungs and the recovery of myocardial function after infarction (14).

In our laboratory, we have sought to leverage this collection of bioactive molecules to treat animals undergoing inflammatory organ injury. We have developed methods for intravenously administering MSC-derived molecules in the form of concentrated

conditioned medium, as well as in a dynamic and continuous manner by using an MSC extracorporeal bioreactor. Initially, it was our intent to treat rats undergoing D-galactosamine-mediated organ injury by transplanting human MSCs. However, upon transplantation of the cells, we observed no benefit to the animals as measured by 7-day survival (42). In contrast, by systemically administering the equivalent mass of lysed cells, we observed a survival trend, suggesting that the molecules relinquished upon lysis provide the primary benefit. We confirmed this hypothesis by collecting the molecules secreted in MSC-conditioned medium (MSC-CM) and administering a concentrated form of MSC-CM intravenously in a single bolus dose to animals after the induction of disease. We found that MSC-CM provides a statistically significant survival benefit, causing an increase in survival from 14% in our control groups to 50% in our treatment group (43). We then engineered a delivery platform for the continuous and dynamic administration of these molecules into the bloodstream (42) and found that this treatment method provided an improved survival benefit of 71% compared with 14% in controls.

These studies collectively suggest that the secreted factors of MSCs account for the majority of beneficial effects in response to MSC transplantation. Considering the putative natural functions of MSCs, it is reasonable to postulate that the same secretory mechanisms by which MSCs maintain hematopoiesis are therapeutic in the context of disease. In fact, many of the MSC factors that have been implicated in hematopoiesis have also been shown to provide therapeutic benefit in certain disease models. Many insist that upon transplantation of the cells, engraftment, proliferation, differentiation, and homing to the site of injury are equally important. However, new evidence is suggesting that MSC engraftment rarely occurs, and observed therapeutic benefits of MSC transplantation may arise from other alternative explanations.

4.2. Particle View of Mesenchymal Stem Cells

Early qualitative studies using MSCs stably transfected with a fluorescent reporter gene for bone regeneration revealed interesting dynamics of the MSC grafts in vivo. First, engraftment of osteoprogenitor cells was found to be saturated, suggesting that higher doses of cells would be an ineffective strategy to improve engraftment (178). Second, temporal tracking of enhanced green fluorescent protein (eGFP)-expressing MSCs showed that transplanted cells exhibited limited proliferation and self-renewal capacity after engraftment, yet they could be serially passaged and repopulate another host (179). Similar studies have evaluated different reporter strategies, but most techniques used are not amenable to whole-subject imaging by nature and thereby may be confounded by selective tissue sampling.

Quantitative examination of the biodistribution of MSCs within the body after transplantation suggests that the dynamics of an MSC graft are similar to that of inert micrometer-scale particles injected into the bloodstream of animals (180). Recent studies using sensitive cell-tracking methods have revealed these counterintuitive results (Figure 4). Most homing and engraftment studies have demonstrated little, if any, long-term engraftment (>1 week) of MSCs upon systemic administration. Studies have shown that the majority of administered MSCs (>80%) accumulate immediately in the lungs and are cleared with a half-life of 24 h (14, 181, 182). Tissue-specific homing has been demonstrated, indicating a response of the administered MSCs to injured tissue (45, 183). It was shown in a mouse model of myocardial infarction that MSCs are capable of engrafting in the site of injury and differentiating into cardiomyocyte-like cells that were shown via immunohistochemistry to express typical cardiomyocyte markers (184). Another study showed that in mice undergoing cisplatin-mediated acute renal failure, systemic injection of MSCs resulted in accumulation of MSCs in the kidney and differentiation into tubular epithelial cells that exhibited the characteristic brush border of the proximal tubule (185). Nevertheless, the majority of studies have shown that only a small percentage of the original

systemically administered cell mass is capable of engrafting even under the best conditions, and of those that do engraft, only a small percentage have been shown to differentiate into functional replacement tissue.

These homing, engraftment, and differentiation studies illustrate one of the most persistent paradoxes in MSC therapy: Systemically administered MSCs appear to convey potent therapeutic responses in a variety of diseases, and yet they have not been shown to exhibit long-term engraftment. Adding further complexity, recent studies have suggested that allogeneic MSCs are not immunoprivileged, that they enjoy only a minimally prolonged residence time compared with allogeneic fibroblasts (15), and that they may elicit a memory response leading to a rapid clearance of subsequent doses by the immune system. It is not unreasonable to suggest, therefore, that instead of engrafting and differentiating, MSCs convey therapeutic benefit by relinquishing their molecular contents. In this way, MSCs may be better viewed as drug delivery particles that, when administered, are subject to distribution and clearance similar to other intravenous therapeutics. Therefore, if the cells are to be delivered optimally, quantitative analysis regarding their pharmacokinetics will better inform dosing regimens to account for the limits and advantages of particle drug delivery as such.

4.3. The Pharmacokinetics of a Mesenchymal Stem Cell Graft

A reductionist view of MSCs as particles loaded with a molecular drug may enable efforts to predict the efficacy of this cellular therapeutic within the confines of known physical models of drug delivery systems. By extracting quantitative parameters from retrospective studies, we plan to take a mathematical approach to propose designs of new formulations and dosing regimens composed of MSC therapy. The objective of this exercise is to maximize the therapeutic activity of MSCs while minimizing potential side effects associated with MSC transplantation such as maldifferentiation and tumor growth.

As with most physical models, we make a few basic assumptions and approximations to simplify the problem in order to display analytical solutions. It would perhaps be more fitting to use computational approaches to solve the complex set of partial differential equations that form the basis of this theory, but doing so would exceed the scope of this initial model. We begin by modeling MSCs as inert, spherical ($d = 20\ \mu\text{m}$) particles that have no interactions with host structures. Furthermore, we assume that a single molecule of fixed concentration is encapsulated within each MSC and contains 100% of the bioactivity. The transport of this single molecule from the cell directly into the bloodstream is not rate limiting. Finally, we assume that a defined therapeutic index exists with respect to the single molecule that directly correlates to serum concentration profile.

With these assumptions in place, we first consider the cell mass used in clinical studies and employ order-of-magnitude approximations to determine if the current clinical dosing of MSCs is justified. If cells secrete $\sim 100\ \text{pg}$ per 10^6 cells per day of a therapeutic mediator, which corresponds to $\sim 0.1\ \text{fg}$ per cell (assuming the same intracellular levels of the mediator), then we can estimate that when a clinical-scale mass of MSCs ($\sim 100 \times 10^6$ cells) is infused intravenously, this equates to $\sim 10\ \text{ng}$ of a therapeutic molecule. In comparison with other biologics, which are administered in the microgram to milligram range, we can immediately see that the clinically administered dose of molecules relinquished from MSCs is at least two to three orders of magnitude lower than that of other single-molecule therapeutics. This simple analysis would suggest that a greater cell mass should be administered, but it does, of course, grossly underestimate the biological complexity of the molecular mixture within MSCs, which is multifactorial in nature and may have synergistic effects.

With respect to the temporal dosing of an MSC graft, we have taken known kinetics data concerning MSC transplantation from selected studies (14, 15) and have represented these data in a new form to illustrate this concept graphically (Figure 5). In the extreme case, and perhaps consistent with early notions, effective MSC transplantation assumed that nearly 100% of the cells remain viable and active after infusion into a subject. Therefore, the units of activity of the transplant would approach steady state in the timescale of days to weeks after administration. However, if we plot the normalized cell concentration found in tissues within 1–120 h based on previous studies, we see that the kinetics of an MSC graft is much more transient than expected with a half-life of approximately 24 h. Using a two-compartment pharmacokinetic model, we can extract parameters that explain the discrepancy between theoretical and apparent bioavailability of MSCs. Assuming an intravenous bolus where the infusion rate (R_i) is eliminated and the dimensionless plasma concentration $C_p(t=0)$ is equal to 1, we find that the rate of tissue intravasation (K_2) and the rate of clearance (R_c) are significantly greater than the theorized tissue extravasation (K_1), resulting in a much shorter half-life of the cellular therapeutic. Furthermore, we see that the apparent activity of the treatment, represented as the time to reach maximal secretion of a molecular mediator, is extremely sensitive to the cellular viability and reinforces the concept of a short therapeutic window associated with MSC therapy. If we arbitrarily choose a minimum effective concentration of MSC therapy and transpose this timescale to a biological response, we see that transplanted MSCs are only therapeutically active for a short period of time (in this case, less than 24 h). This timescale corresponds precisely with measured serum cytokine levels that are directly associated with MSC therapy (145) and that potentially can be considered surrogate biomarkers for effective therapy. Ultimately, successive doses of MSCs within a shorter treatment period may allow for the maintenance of MSC therapy within a therapeutic window that can ultimately sustain a long-term biological response.

5. CONCLUSIONS

Developing new therapies that affect multiple disease pathways is of growing importance for patient care. MSC transplantation represents an exciting approach that could potentially treat complex diseases by providing combinatorial therapy. Furthermore, the continued use of MSC therapy can be recast to improve our understanding of the natural role of these cells during health and disease in vivo. The collected efforts of scientists, engineers, physicians, and industry will be necessary to realize the promise of MSC therapy.

Optimization of MSC therapy may not be achievable until the primary mechanism(s) of action afforded by intravenous administration of MSCs is determined. Current optimization approaches are based on the number of MSCs used, but this parameter alone may not capture the true activity of this complex therapy. The therapeutic activity of an MSC graft was once thought to be a function of engraftment and differentiation. However, mounting evidence is indicating that MSCs can impart activity independent of these functions. Trafficking studies have reported that systemically administered MSCs fail to engraft in most tissues and that engrafted cells may eventually be rejected, resulting in immunological memory to subsequent treatments (14, 15). This new evidence suggests that the activities of MSC formulations may have half-lives on the order of an inert particle. This timescale should be considered when new dosing regimens for MSC therapy are being defined.

To simplify the explanation of cellular biodistribution, we have considered MSCs as inert particles. However, in reality they are by no means inert. Bone marrow MSCs actively participate in the maintenance of hematopoiesis and therefore influence the development of cells in the immune system. MSCs can differentiate into stromal lineages that provide the cellular and structural elements required to support hematopoiesis. Beyond cellular

differentiation, MSCs fortify the chemical milieu of the bone marrow by secreting many immunoregulatory and trophic factors that contribute to the successful development of the blood cells. In essence, MSCs are intimately associated with homeostatic mechanisms that tightly regulate activity in the bone marrow microenvironment. As a consequence, MSCs may be naturally equipped to interact directly with the immune system and influence it by mechanisms that are concordant with the origins of MSCs. Indeed, the natural target tissue of MSC therapy may very well be the hematopoietic system.

By using previous examples of molecular therapeutics as an initial framework, we have attempted to describe the theoretical behavior of MSCs upon administration in order to identify some options to consider when developing dosing regimens. Using our simplified model, we can glean that cell therapy may need to be administered at a greater magnitude and/or frequency to sustain a long-term biological response. Moreover, methods that improve the half-life of the graft *in vivo* by increasing cell survival and engraftment or by decreasing cell clearance may also be viable options to enhance therapeutic activity. Furthermore, this model underscores the need to identify the mechanisms that govern cell fate *in vivo* as well as practical and relevant biomarkers that can be used to monitor the activity of MSCs after administration.

MSCs have the potential to treat many unmet medical conditions that afflict patients every day in a manner that is consistent with the human body's natural capacity to heal itself. Development of quantitative means for harnessing the source of MSC therapeutic activity will therefore ensure we make the most of everything these cells can accomplish.

Acknowledgments

We thank Drs. Zaki Megeed, Arno Tilles, and Martin Yarmush for thoughtful discussions and refinements to this review. The authors' work in this area is supported by grants from the National Institutes of Health (1K01DK087770-01), Broad Medical Research Foundation (BMRP498382), and the Shriners Hospitals for Children.

Abbreviation

MSC	mesenchymal stem cell
CFU-F	colony forming units-fibroblastic
GvHD	graft-versus-host disease
IDDM	type I diabetes mellitus
MI	myocardial infarction
COPD	chronic obstructive pulmonary disease
FDA	United States Food and Drug Administration
UCB	umbilical cord blood
PDGF-R	platelet derived growth factor receptor
SCF	stem cell factor
HSC	hematopoietic stem cell
MLR	mixed lymphocyte reaction
TCR	T cell receptor
NK	natural killer

DC	dendritic cell
NOD	nonobese diabetic
HGF	hepatocyte growth factor
EGF	epidermal growth factor
TGF-β_1	transforming growth factor β_1
IDO	indoleamine 2,3-dioxygenase
LPS	lipopolysaccharide
MSC-CM	mesenchymal stem cell-conditioned medium
eGFP	enhanced green fluorescent protein

LITERATURE CITED

- Colter DC, Class R, DiGirolamo CM, Prockop DJ. Rapid expansion of recycling stem cells in cultures of plastic-adherent cells from human bone marrow. *Proc Natl Acad Sci USA*. 2000; 97:3213–18. [PubMed: 10725391]
- Sekiya I, Larson BL, Smith JR, Pochampally R, Cui JG, Prockop DJ. Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality. *Stem Cells*. 2002; 20:530–41. [PubMed: 12456961]
- Lee MW, Yang MS, Park JS, Kim HC, Kim YJ, Choi J. Isolation of mesenchymal stem cells from cryopreserved human umbilical cord blood. *Int J Hematol*. 2005; 81:126–30. [PubMed: 15765780]
- Kotobuki N, Hirose M, Takakura Y, Ohgushi H. Cultured autologous human cells for hard tissue regeneration: preparation and characterization of mesenchymal stem cells from bone marrow. *Artif Organs*. 2004; 28:33–39. [PubMed: 14720286]
- Fouillard L, Chapel A, Bories D, Bouchet S, Costa JM, et al. Infusion of allogeneic-related HLA mismatched mesenchymal stem cells for the treatment of incomplete engraftment following autologous hematopoietic stem cell transplantation. *Leukemia*. 2007; 21:568–70. [PubMed: 17252011]
- Marmont AM, Gualandi F, Piaggio G, Podesta M, Teresa van Lint M, et al. Allogeneic bone marrow transplantation (BMT) for refractory Behcet's disease with severe CNS involvement. *Bone Marrow Transplant*. 2006; 37:1061–63. [PubMed: 16633360]
- Le Blanc K, Ringden O. Immunobiology of human mesenchymal stem cells and future use in hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2005; 11:321–34. [PubMed: 15846285]
- Ripa RS, Haack-Sorensen M, Wang Y, Jorgensen E, Mortensen S, et al. Bone marrow derived mesenchymal cell mobilization by granulocyte-colony stimulating factor after acute myocardial infarction: results from the Stem Cells in Myocardial Infarction (STEMMI) trial. *Circulation*. 2007; 116:I24–30. [PubMed: 17846310]
- Chen SL, Fang WW, Ye F, Liu YH, Qian J, et al. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am J Cardiol*. 2004; 94:92–95. [PubMed: 15219514]
- Lee PH, Kim JW, Bang OY, Ahn YH, Joo IS, Huh K. Autologous mesenchymal stem cell therapy delays the progression of neurological deficits in patients with multiple system atrophy. *Clin Pharmacol Ther*. 2008; 83(5):723–30. [PubMed: 17898702]
- Bang OY, Lee JS, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. *Ann Neurol*. 2005; 57:874–82. [PubMed: 15929052]
- Lazarus HM, Koc ON, Devine SM, Curtin P, Maziarz RT, et al. Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. *Biol Blood Marrow Transplant*. 2005; 11:389–98. [PubMed: 15846293]

13. Ringden O, Uzunel M, Rasmusson I, Remberger M, Sundberg B, et al. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation*. 2006; 81:1390–97. [PubMed: 16732175]
14. Lee R, Pulin A, Seo M, Kota D, Ylostalo J, et al. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. *Cell Stem Cell*. 2009; 5:54–63. [PubMed: 19570514]
15. Zangi L, Margalit R, Reich-Zeliger S, Bachar-Lustig E, Beilhack A, et al. Direct imaging of immune rejection and memory induction by allogeneic mesenchymal stromal cells. *Stem Cells*. 2009; 27(11):2865–74. [PubMed: 19750539]
16. Bajada S, Mazakova I, Richardson J, Ashammakhi N. Updates on stem cells and their applications in regenerative medicine. *J Tissue Eng Regen Med*. 2008; 2(4):169–83. [PubMed: 18493906]
17. Bianco P, Robey P. Stem cells in tissue engineering. *Nature*. 2001; 414:118–21. [PubMed: 11689957]
18. Minguell J, Erices A, Conget P. Mesenchymal stem cells. *Exp Biol Med*. 2001; 226:507–20.
19. Prockop D. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science*. 1997; 276:71–74. [PubMed: 9082988]
20. Cohnheim J. Über Entzündung und Eiterung. *J Arch Path Anat Physiol Klin Med*. 1867; 40:1–79.
21. Maximow A. Relation of blood cells to connective tissues and endothelium. *Physiol Rev*. 1924; 4:533–63.
22. Friedenstein A, Gorskaja J, Kulagina N. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. *Exp Hematol*. 1976; 4:267–74. [PubMed: 976387]
23. Friedenstein A, Piatetzky-Shapiro I, Petrakova K. Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol*. 1966; 16:381–90. [PubMed: 5336210]
24. Friedenstein A, Chailakhyan R, Latsinik N, Panasyuk A, Keiliss-Borok I. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues: cloning in vitro and retransplantation in vivo. *Transplantation*. 1974; 17:331–40. [PubMed: 4150881]
25. Owen M. Marrow stromal stem cells. *J Cell Sci Suppl*. 1988; 10:63–76. [PubMed: 3077943]
26. Caplan A. Mesenchymal stem cells. *J Orthop Res*. 1991; 9:641–50. [PubMed: 1870029]
27. Owen M. Lineage of osteogenic cells and their relationship to the stromal system. *J Bone Miner Res*. 1985; 3:1–25.
28. Owen, M. The marrow stromal cell system. In: Beresford, JN.; Owen, ME., editors. *Marrow Stromal Cell Culture*. New York: Cambridge Univ. Press; 1988. p. 1-9.
29. Owen M, Friedenstein A. Stromal stem cells: marrow-derived osteogenic precursors. *Ciba Found Symp*. 1988; 136:42–60. [PubMed: 3068016]
30. Ashurst D, Ashton B, Owen M. Bone marrow stromal cells raised in diffusion chambers produce typical bone and cartilage matrices. *Calcif Tissue Int*. 1988; 42(Suppl):2.
31. Goshima J, Goldberg V, Caplan A. The osteogenic potential of culture-expanded rat marrow mesenchymal cells assayed in vivo in calcium phosphate ceramic blocks. *Clin Orthop Relat Res*. 1991; 262:298–311. [PubMed: 1984928]
32. Haynesworth S, Baber M, Caplan A. Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies. *Bone*. 1992; 13:69–80. [PubMed: 1316137]
33. Horwitz E, Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, et al. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy*. 2005; 7:393–95. [PubMed: 16236628]
34. Haynesworth S, Goshima J, Goldberg V, Caplan A. Characterization of cells with osteogenic potential from human marrow. *Bone*. 1992; 13:81–88. [PubMed: 1581112]
35. Lazarus H, Haynesworth S, Gerson S, Rosenthal N, Caplan A. Ex vivo expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): implications for therapeutic use. *Bone Marrow Transplant*. 1995; 16:557–64. [PubMed: 8528172]
36. Koc O, Gerson S, Cooper B, Dyhouse S, Haynesworth S, et al. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells

- in advanced breast cancer patients receiving high-dose chemotherapy. *J Clin Oncol*. 2000; 18:307–16. [PubMed: 10637244]
37. Horwitz E, Prockop D, Fitzpatrick L, Koo W, Gordon P, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med*. 1999; 5:309–13. [PubMed: 10086387]
 38. Horwitz E, Gordon P, Koo W, Marx J, Neel M, et al. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone. *Proc Natl Acad Sci USA*. 2002; 99:8932–37. [PubMed: 12084934]
 39. Koc O, Day J, Nieder M, Gerson S, Lazarus H, Krivit W. Allogeneic mesenchymal stem cell infusion for treatment of metachromatic leukodystrophy (MLD) and Hurler syndrome (MPS-IH). *Bone Marrow Transplant*. 2002; 30:215–222. [PubMed: 12203137]
 40. Mazzini L, Fagioli F, Boccaletti R, Mareschi K, Oliveri G, et al. Stem cell therapy in amyotrophic lateral sclerosis: a methodological approach in humans. *Amyotroph Lateral Scler*. 2003; 4:158–61.
 41. Phinney D, Prockop D. Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair—current views. *Stem Cells*. 2007; 25:2896–902. [PubMed: 17901396]
 42. Parekkadan B, van Poll D, Suganuma K, Carter E, Berthiaume F, et al. Mesenchymal stem cell-derived molecules reverse fulminant hepatic failure. *PLoS One*. 2007; 2:e941. [PubMed: 17895982]
 43. van Poll D, Parekkadan B, Cho C, Berthiaume F, Nahmias Y, et al. Mesenchymal stem cell-derived molecules directly modulate hepatocellular death and regeneration in vitro and in vivo. *Hepatology*. 2008; 47:1634–43. [PubMed: 18395843]
 44. Owen M, Cave J, Joyner C. Clonal analysis in vitro of osteogenic differentiation of marrow CFU-F. *J Cell Sci*. 1987; 87(5):731–38. [PubMed: 3499442]
 45. Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. *Stem Cells*. 2007; 25:2739–49. [PubMed: 17656645]
 46. Pittenger M, Mackay A, Beck S, Jaiswal R, Douglas R, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999; 284:143–47. [PubMed: 10102814]
 47. Zuk P, Zhu M, Ashjian P, De Ugarte D, Huang J, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell*. 2002; 13:4279–95. [PubMed: 12475952]
 48. Tavassoli M, Crosby W. Transplantation of marrow to extramedullary sites. *Science*. 1968; 161:54–56. [PubMed: 4871792]
 49. Bianco P, Riminucci M, Gronthos S, Robey P. Bone marrow stromal stem cells: nature, biology, and potential applications. *Stem Cells*. 2001; 19(3):180–92. [PubMed: 11359943]
 50. Jiang Y, Jahagirdar B, Reinhardt R, Schwartz R, Keene C, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*. 2002; 418:41–49. [PubMed: 12077603]
 51. Friedenstein A, Petrakova K, Kurolesova A, Frolova G. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. *Transplantation*. 1968; 6:230–47. [PubMed: 5654088]
 52. Friedenstein A, Deriglasova U, Kulagina N, Panasuk A, Rudakowa S, et al. Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay method. *Exp Hematol*. 1974; 2:83–92. [PubMed: 4455512]
 53. Battula VL, Treml S, Abele H, Buhning HJ. Prospective isolation and characterization of mesenchymal stem cells from human placenta using a frizzled-9-specific monoclonal antibody. *Differentiation*. 2007; 76(4):326–36. [PubMed: 17924962]
 54. Battula VL, Bareiss PM, Treml S, Conrad S, Albert I, et al. Human placenta and bone marrow derived MSC cultured in serum-free, b-FGF-containing medium express cell surface frizzled-9 and SSEA-4 and give rise to multilineage differentiation. *Differentiation*. 2007; 75:279–91. [PubMed: 17288545]
 55. Chang CJ, Yen ML, Chen YC, Chien CC, Huang HI, et al. Placenta-derived multipotent cells exhibit immunosuppressive properties that are enhanced in the presence of interferon-gamma. *Stem Cells*. 2006; 24:2466–77. [PubMed: 17071860]

56. Mendes SC, Robin C, Dzierzak E. Mesenchymal progenitor cells localize within hematopoietic sites throughout ontogeny. *Development*. 2005; 132:1127–36. [PubMed: 15689383]
57. Takashima Y, Era T, Nakao K, Kondo S, Kasuga M, et al. Neuroepithelial cells supply an initial transient wave of MSC differentiation. *Cell*. 2007; 129:1377–88. [PubMed: 17604725]
58. Deng MJ, Jin Y, Shi JN, Lu HB, Liu Y, et al. Multilineage differentiation of ectomesenchymal cells isolated from the first branchial arch. *Tissue Eng*. 2004; 10:1597–606. [PubMed: 15588419]
59. Bhattacharjee V, Mukhopadhyay P, Singh S, Johnson C, Philipose JT, et al. Neural crest and mesoderm lineage-dependent gene expression in orofacial development. *Differentiation*. 2007; 75:463–77. [PubMed: 17286603]
60. Yan Z, Lin Y, Jiao X, Li Z, Wu L, et al. Characterization of ectomesenchymal cells isolated from the first branchial arch during multilineage differentiation. *Cells Tissues Organs*. 2006; 183:123–32. [PubMed: 17108683]
61. Deng J, Petersen BE, Steindler DA, Jorgensen ML, Laywell ED. Mesenchymal stem cells spontaneously express neural proteins in culture and are neurogenic after transplantation. *Stem Cells*. 2006; 24:1054–64. [PubMed: 16322639]
62. Blondheim NR, Levy YS, Ben-Zur T, Burshtein A, Cherlow T, et al. Human mesenchymal stem cells express neural genes, suggesting a neural predisposition. *Stem Cells Dev*. 2006; 15:141–64. [PubMed: 16646662]
63. Gang EJ, Jeong JA, Hong SH, Hwang SH, Kim SW, et al. Skeletal myogenic differentiation of mesenchymal stem cells isolated from human umbilical cord blood. *Stem Cells*. 2004; 22:617–24. [PubMed: 15277707]
64. Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. *Br J Haematol*. 2000; 109:235–42. [PubMed: 10848804]
65. Wexler SA, Donaldson C, Denning-Kendall P, Rice C, Bradley B, Hows JM. Adult bone marrow is a rich source of human mesenchymal “stem” cells but umbilical cord and mobilized adult blood are not. *Br J Haematol*. 2003; 121:368–74. [PubMed: 12694261]
66. Romanov YA, Svintsitskaya VA, Smirnov VN. Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. *Stem Cells*. 2003; 21:105–10. [PubMed: 12529557]
67. Gardner JM, Devoss JJ, Friedman RS, Wong DJ, Tan YX, et al. Deletional tolerance mediated by extrathymic Aire-expressing cells. *Science*. 2008; 321:843–47. [PubMed: 18687966]
68. Muguruma Y, Yahata T, Miyatake H, Sato T, Uno T, et al. Reconstitution of the functional human hematopoietic microenvironment derived from human mesenchymal stem cells in the murine bone marrow compartment. *Blood*. 2006; 107:1878–87. [PubMed: 16282345]
69. Jones E, McGonagle D. Human bone marrow mesenchymal stem cells in vivo. *Rheumatology*. 2008; 47:126–31. [PubMed: 17986482]
70. Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. *J Bone Miner Res*. 2003; 18:696–704. [PubMed: 12674330]
71. Zannettino AC, Paton S, Arthur A, Khor F, Itescu S, et al. Multipotential human adipose-derived stromal stem cells exhibit a perivascular phenotype in vitro and in vivo. *J Cell Physiol*. 2008; 214:413–21. [PubMed: 17654479]
72. Yoshimura H, Muneta T, Nimura A, Yokoyama A, Koga H, Sekiya I. Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle. *Cell Tissue Res*. 2007; 327:449–62. [PubMed: 17053900]
73. da Silva Meirelles L, Chagastelles P, Nardi N. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J Cell Sci*. 2006; 119:2204–13. [PubMed: 16684817]
74. Tang Y, Zhao Q, Zhang Y, Cheng L, Liu M, et al. Autologous mesenchymal stem cell transplantation induce VEGF and neovascularization in ischemic myocardium. *Regul Pept*. 2004; 117:3–10. [PubMed: 14687695]
75. Bianco P, Riminucci M, Kuznetsov S, Robey PG. Multipotential cells in the bone marrow stroma: regulation in the context of organ physiology. *Crit Rev Eukaryot Gene Expr*. 1999; 9:159–73. [PubMed: 10445154]
76. Bianco P, Costantini M, Dearden LC, Bonucci E. Alkaline phosphatase positive precursors of adipocytes in the human bone marrow. *Br J Haematol*. 1988; 68:401–3. [PubMed: 3377984]

77. Traktuev DO, Merfeld-Clauss S, Li J, Kolonin M, Arap W, et al. A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. *Circ Res.* 2008; 102:77–85. [PubMed: 17967785]
78. Sacchetti B, Funari A, Michienzi S, Di Cesare S, Piersanti S, et al. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell.* 2007; 131:324–36. [PubMed: 17956733]
79. Au P, Tam J, Fukumura D, Jain RK. Bone marrow-derived mesenchymal stem cells facilitate engineering of long-lasting functional vasculature. *Blood.* 2008; 111(9):4551–58. [PubMed: 18256324]
80. Abe R, Donnelly SC, Peng T, Bucala R, Metz CN. Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. *J Immunol.* 2001; 166:7556–62. [PubMed: 11390511]
81. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. *Mol Med.* 1994; 1:71–81. [PubMed: 8790603]
82. Quan TE, Cowper SE, Bucala R. The role of circulating fibrocytes in fibrosis. *Curr Rheumatol Rep.* 2006; 8:145–50. [PubMed: 16569374]
83. Barth PJ, Ebrahimsade S, Ramaswamy A, Moll R. CD34+ fibrocytes in invasive ductal carcinoma, ductal carcinoma in situ, and benign breast lesions. *Virchows Arch.* 2002; 440:298–303. [PubMed: 11889601]
84. Barth PJ, Ebrahimsade S, Hellinger A, Moll R, Ramaswamy A. CD34+ fibrocytes in neoplastic and inflammatory pancreatic lesions. *Virchows Arch.* 2002; 440:128–33. [PubMed: 11964041]
85. Barth PJ, Schenck zu Schweinsberg T, Ramaswamy A, Moll R. CD34+ fibrocytes, α -smooth muscle antigen-positive myofibroblasts, and CD117 expression in the stroma of invasive squamous cell carcinomas of the oral cavity, pharynx, and larynx. *Virchows Arch.* 2004; 444:231–34. [PubMed: 14758552]
86. Nimphius W, Moll R, Olbert P, Ramaswamy A, Barth PJ. CD34+ fibrocytes in chronic cystitis and noninvasive and invasive urothelial carcinomas of the urinary bladder. *Virchows Arch.* 2007; 450:179–85. [PubMed: 17149610]
87. Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB. Myofibroblasts. I. Paracrine cells important in health and disease. *Am J Physiol.* 1999; 277:C1–9. [PubMed: 10409103]
88. Chesney J, Bacher M, Bender A, Bucala R. The peripheral blood fibrocyte is a potent antigen-presenting cell capable of priming naive T cells in situ. *Proc Natl Acad Sci USA.* 1997; 94:6307–12. [PubMed: 9177213]
89. Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, et al. Osteoblastic cells regulate the hematopoietic stem cell niche. *Nature.* 2003; 425:841–46. [PubMed: 14574413]
90. Zhang J, Niu C, Ye L, Huang H, He X, et al. Identification of the hematopoietic stem cell niche and control of the niche size. *Nature.* 2003; 425:836–41. [PubMed: 14574412]
91. Zhang CC, Lodish HF. Insulin-like growth factor 2 expressed in a novel fetal liver cell population is a growth factor for hematopoietic stem cells. *Blood.* 2004; 103:2513–21. [PubMed: 14592820]
92. Stier S, Ko Y, Forkert R, Lutz C, Neuhaus T, et al. Osteopontin is a hematopoietic stem cell niche component that negatively regulates stem cell pool size. *J Exp Med.* 2005; 201:1781–91. [PubMed: 15928197]
93. Hattori K, Heissig B, Wu Y, Dias S, Tejada R, et al. Placental growth factor reconstitutes hematopoiesis by recruiting VEGFR1+ stem cells from bone-marrow microenvironment. *Nat Med.* 2002; 8:841–49. [PubMed: 12091880]
94. Dexter TM, Heyworth CM, Spooner E, Ponting IL. The role of growth factors in self-renewal and differentiation of hemopoietic stem cells. *Philos Trans R Soc Lond Ser B.* 1990; 327:85–98. [PubMed: 1969664]
95. Majumdar MK, Thiede MA, Mosca JD, Moorman M, Gerson SL. Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (MSCs) and stromal cells. *J Cell Physiol.* 1998; 176:57–66. [PubMed: 9618145]
96. Whitlock CA, Witte ON. Long-term culture of murine bone marrow precursors of B lymphocytes. *Methods Enzymol.* 1987; 150:275–86. [PubMed: 3501518]

97. Whitlock CA, Witte ON. Long-term culture of B lymphocytes and their precursors from murine bone marrow. *Proc Natl Acad Sci USA*. 1982; 79:3608–12. [PubMed: 6980414]
98. Dexter TM, Moore MA, Sheridan AP. Maintenance of hemopoietic stem cells and production of differentiated progeny in allogeneic and semiallogeneic bone marrow chimeras in vitro. *J Exp Med*. 1977; 145:1612–16. [PubMed: 325171]
99. Dexter TM, Allen TD, Lajtha LG. Conditions controlling the proliferation of hemopoietic stem cells in vitro. *J Cell Physiol*. 1977; 91:335–44. [PubMed: 301143]
100. Aggarwal S, Pittenger M. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood*. 2005; 105:1815–22. [PubMed: 15494428]
101. Herrera M, Bussolati B, Bruno S, Fonsato V, Romanazzi G, Camussi G. Mesenchymal stem cells contribute to the renal repair of acute tubular epithelial injury. *Int J Mol Med*. 2004; 14:1035–41. [PubMed: 15547670]
102. Ortiz L, Gambelli F, McBride C, Gaupp D, Baddoo M, et al. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc Natl Acad Sci USA*. 2003; 100:8407–11. [PubMed: 12815096]
103. Rojas M, Xu J, Woods C, Mora A, Spears W, et al. Bone marrow-derived mesenchymal stem cells in repair of the injured lung. *Am J Respir Cell Mol Biol*. 2005; 33:145–52. [PubMed: 15891110]
104. Tögel F, Hu Z, Weiss K, Isaac J, Lange C, Westenfelder C. Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms. *Am J Physiol Renal Physiol*. 2005; 289:F31–42. [PubMed: 15713913]
105. Tögel F, Weiss K, Yang Y, Hu Z, Zhang P, Westenfelder C. Vasculotropic, paracrine actions of infused mesenchymal stem cells are important to the recovery from acute kidney injury. *Am J Physiol Renal Physiol*. 2007; 292:F1626–35. [PubMed: 17213465]
106. Shake J, Gruber P, Baumgartner W, Senechal G, Meyers J, et al. Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. *Ann Thorac Surg*. 2002; 73:1919–26. [PubMed: 12078791]
107. Amado L, Saliaris A, Schuleri K, St John M, Xie J, et al. Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. *Proc Natl Acad Sci USA*. 2005; 102:11474–79. [PubMed: 16061805]
108. Miyahara Y, Nagaya N, Kataoka M, Yanagawa B, Tanaka K, et al. Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. *Nat Med*. 2006; 12:459–65. [PubMed: 16582917]
109. Fiorina P, Jurewicz M, Augello A, Vergani A, Dada S, et al. Immunomodulatory function of bone marrow-derived mesenchymal stem cells in experimental autoimmune type 1 diabetes. *J Immunol*. 2009; 183:993–1004. [PubMed: 19561093]
110. Kale S, Karihaloo A, Clark P, Kashgarian M, Krause D, Cantley L. Bone marrow stem cells contribute to repair of the ischemically injured renal tubule. *J Clin Invest*. 2003; 112:42–49. [PubMed: 12824456]
111. Lange C, Tögel F, Ittrich H, Clayton F, Nolte-Ernsting C, et al. Administered mesenchymal stem cells enhance recovery from ischemia/reperfusion-induced acute renal failure in rats. *Kidney Int*. 2005; 68:1613–17. [PubMed: 16164638]
112. Morigi M, Introna M, Imberti B, Corna D, Abbate M, et al. Human bone marrow mesenchymal stem cells accelerate recovery of acute renal injury and prolong survival in mice. *Stem Cells*. 2008; 26:2075–82. [PubMed: 18499895]
113. Nagaya N, Fujii T, Iwase T, Ohgushi H, Itoh T, et al. Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis. *Am J Physiol Heart Circ Physiol*. 2004; 287:H2670–76. [PubMed: 15284059]
114. Quevedo H, Hatzistergos K, Oskouei B, Feigenbaum G, Rodriguez J, et al. Allogeneic mesenchymal stem cells restore cardiac function in chronic ischemic cardiomyopathy via trilineage differentiating capacity. *Proc Natl Acad Sci USA*. 2009; 106:14022–27. [PubMed: 19666564]

115. Abdi R, Fiorina P, Adra C, Atkinson M, Sayegh M. Immunomodulation by mesenchymal stem cells: a potential therapeutic strategy for type 1 diabetes. *Diabetes*. 2008; 57:1759–67. [PubMed: 18586907]
116. Lin P, Chen L, Yang N, Sun Y, Xu Y. Evaluation of stem cell differentiation in diabetic rats transplanted with bone marrow mesenchymal stem cells. *Transplant Proc*. 2009; 41(5):1891–93. [PubMed: 19545751]
117. Vija L, Farge D, Gautier J, Vexiau P, Dumitrache C, et al. Mesenchymal stem cells: stem cell therapy perspectives for type 1 diabetes. *Diabetes Metab*. 2009; 35:85–93. [PubMed: 19230736]
118. Madec A, Mallone R, Afonso G, Abou Mrad E, Mesnier A, et al. Mesenchymal stem cells protect NOD mice from diabetes by inducing regulatory T cells. *Diabetologia*. 2009; 52:1391–99. [PubMed: 19421731]
119. Chung N, Jeong D, Park S, Choi B, Cho B, et al. Cotransplantation of marrow stromal cells may prevent lethal graft-versus-host disease in major histocompatibility complex mismatched murine hematopoietic stem cell transplantation. *Int J Hematol*. 2004; 80:370–76. [PubMed: 15615264]
120. Maitra B, Szekeley E, Gjini K, Laughlin M, Dennis J, et al. Human mesenchymal stem cells support unrelated donor hematopoietic stem cells and suppress T-cell activation. *Bone Marrow Transplant*. 2004; 33:597–604. [PubMed: 14716336]
121. Sun L, Akiyama K, Zhang H, Yamaza T, Hou Y, et al. Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans. *Stem Cells*. 2009; 27(6):1421–32. [PubMed: 19489103]
122. Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, et al. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. *Blood*. 2005; 106:1755–61. [PubMed: 15905186]
123. Parr A, Tator C, Keating A. Bone marrow-derived mesenchymal stromal cells for the repair of central nervous system injury. *Bone Marrow Transplant*. 2007; 40:609–19. [PubMed: 17603514]
124. Rafei M, Campeau P, Aguilar-Mahecha A, Buchanan M, Williams P, et al. Mesenchymal stromal cells ameliorate experimental autoimmune encephalomyelitis by inhibiting CD4 Th17 T cells in a CC chemokine ligand 2-dependent manner. *J Immunol*. 2009; 182:5994–6002. [PubMed: 19414750]
125. Liechty K, MacKenzie T, Shaaban A, Radu A, Moseley A, et al. Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep. *Nat Med*. 2000; 6:1282–86. [PubMed: 11062543]
126. Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, et al. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol*. 2002; 30:42–48. [PubMed: 11823036]
127. Djouad F, Plence P, Bony C, Tropel P, Apparailly F, et al. Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. *Blood*. 2003; 102:3837–44. [PubMed: 12881305]
128. Le Blanc K, Rasmusson I, Sundberg B, Gotherstrom C, Hassan M, et al. Treatment of severe acute graft-versus-host disease with third-party haploidentical mesenchymal stem cells. *Lancet*. 2004; 363:1439–41. [PubMed: 15121408]
129. Klyushnenkova E, Mosca JD, Zernetkina V, Majumdar MK, Beggs KJ, et al. T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression. *J Biomed Sci*. 2005; 12:47–57. [PubMed: 15864738]
130. Rasmusson I, Uhlin M, Le Blanc K, Levitsky V. Mesenchymal stem cells fail to trigger effector functions of cytotoxic T lymphocytes. *J Leukoc Biol*. 2007; 82:887–93. [PubMed: 17609339]
131. Potian JA, Aviv H, Ponzio NM, Harrison JS, Rameshwar P. Veto-like activity of mesenchymal stem cells: functional discrimination between cellular responses to alloantigens and recall antigens. *J Immunol*. 2003; 171:3426–34. [PubMed: 14500637]
132. Prevosto C, Zancolli M, Canevali P, Zocchi MR, Poggi A. Generation of CD4+ or CD8+ regulatory T cells upon mesenchymal stem cell-lymphocyte interaction. *Haematologica*. 2007; 92:881–88. [PubMed: 17606437]

133. Augello A, Tasso R, Negrini S, Amateis A, Indiveri F, et al. Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. *Eur J Immunol.* 2005; 35(5):1482–90. [PubMed: 15827960]
134. Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, et al. Human mesenchymal stem cells modulate B-cell functions. *Blood.* 2006; 107:367–72. [PubMed: 16141348]
135. Traggiai E, Volpi S, Schena F, Gattorno M, Ferlito F, et al. Bone marrow-derived mesenchymal stem cells induce both polyclonal expansion and differentiation of B cells isolated from healthy donors and systemic lupus erythematosus patients. *Stem Cells.* 2008; 26:562–69. [PubMed: 18024418]
136. Rasmusson I, Le Blanc K, Sundberg B, Ringden O. Mesenchymal stem cells stimulate antibody secretion in human B cells. *Scand J Immunol.* 2007; 65:336–43. [PubMed: 17386024]
137. Arnulf B, Lecourt S, Soulier J, Ternaux B, Lacassagne MN, et al. Phenotypic and functional characterization of bone marrow mesenchymal stem cells derived from patients with multiple myeloma. *Leukemia.* 2007; 21:158–63. [PubMed: 17096013]
138. Gerdoni E, Gallo B, Casazza S, Musio S, Bonanni I, et al. Mesenchymal stem cells effectively modulate pathogenic immune response in experimental autoimmune encephalomyelitis. *Ann Neurol.* 2007; 61:219–27. [PubMed: 17387730]
139. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol.* 2008; 8:726–36. [PubMed: 19172693]
140. Raffaghello L, Bianchi G, Bertolotto M, Montecucco F. Human mesenchymal stem cells inhibit neutrophil apoptosis: a model for neutrophil preservation in the bone marrow niche. *Stem Cells.* 2008; 26:151–62. [PubMed: 17932421]
141. Spaggiari G, Capobianco A, Abdelrazik H, Becchetti F, Mingari M, Moretta L. Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. *Blood.* 2008; 111:1327–33. [PubMed: 17951526]
142. Sotiropoulou P, Perez S, Gritzapis A, Baxevas C, Papamichail M. Interactions between human mesenchymal stem cells and natural killer cells. *Stem Cells.* 2006; 24:74–85. [PubMed: 16099998]
143. Poggi A, Prevosto C, Massaro A, Negrini S, Urbani S, et al. Interaction between human NK cells and bone marrow stromal cells induces NK cell triggering: role of NKp30 and NKG2D receptors. *J Immunol.* 2005; 175:6352–60. [PubMed: 16272287]
144. Spaggiari G, Capobianco A, Becchetti S, Mingari M, Moretta L. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. *Blood.* 2006; 107:1484–90. [PubMed: 16239427]
145. Németh K, Leelahavanichkul A, Yuen P, Mayer B, Parmelee A, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E2–dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat Med.* 2008; 15:42–49. [PubMed: 19098906]
146. Beyth S, Borovsky Z, Mevorach D, Liebergall M, Gazit Z, et al. Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness. *Blood.* 2005; 105:2214–19. [PubMed: 15514012]
147. Jiang XX, Zhang Y, Liu B, Zhang SX, Wu Y, et al. Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. *Blood.* 2005; 105:4120–26. [PubMed: 15692068]
148. Zhang W, Ge W, Li C, You S, Liao L, et al. Effects of mesenchymal stem cells on differentiation, maturation, and function of human monocyte-derived dendritic cells. *Stem Cells Dev.* 2004; 13:263–71. [PubMed: 15186722]
149. Li YP, Paczesny S, Lauret E, Poirault S, Bordigoni P, et al. Human mesenchymal stem cells license adult CD34+ hemopoietic progenitor cells to differentiate into regulatory dendritic cells through activation of the Notch pathway. *J Immunol.* 2008; 180:1598–608. [PubMed: 18209056]
150. Kunter U, Rong S, Boor P, Eitner F, Muller-Newen G, et al. Mesenchymal stem cells prevent progressive experimental renal failure but maldifferentiate into glomerular adipocytes. *J Am Soc Nephrol.* 2007; 18:1754–64. [PubMed: 17460140]

151. Aguilar S, Nye E, Chan J, Loebinger M, Spencer-Dene B, et al. Murine but not human mesenchymal stem cells generate osteosarcoma-like lesions in the lung. *Stem Cells*. 2007; 25:1586–94. [PubMed: 17363552]
152. Sundin M, Örvell C, Rasmusson I, Sundberg B, Ringden O, Le Blanc K. Mesenchymal stem cells are susceptible to human herpesviruses, but viral DNA cannot be detected in the healthy seropositive individual. *Bone Marrow Transplant*. 2006; 37:1051–59. [PubMed: 16604097]
153. Matushansky I, Hernando E, Socci ND, Mills JE, Matos TA, et al. Derivation of sarcomas from mesenchymal stem cells via inactivation of the Wnt pathway. *J Clin Invest*. 2007; 117:3248–57. [PubMed: 17948129]
154. Iwamoto S, Mihara K, Downing JR, Pui CH, Campana D. Mesenchymal cells regulate the response of acute lymphoblastic leukemia cells to asparaginase. *J Clin Invest*. 2007; 117:1049–57. [PubMed: 17380207]
155. Williams DA. A new mechanism of leukemia drug resistance? *N Engl J Med*. 2007; 357:77–78. [PubMed: 17611211]
156. Dierks C, Grbic J, Zirikli K, Beigi R, Englund NP, et al. Essential role of stromally induced hedgehog signaling in B-cell malignancies. *Nat Med*. 2007; 13:944–51. [PubMed: 17632527]
157. Ning H, Yang F, Jiang M, Hu L, Feng K, et al. The correlation between cotransplantation of mesenchymal stem cells and higher recurrence rate in hematologic malignancy patients: outcome of a pilot clinical study. *Leukemia*. 2008; 22:593–99. [PubMed: 18185520]
158. Vianello F, Dazzi F. Mesenchymal stem cells for graft-versus-host disease: a double-edged sword? *Leukemia*. 2008; 22:463–65. [PubMed: 18335000]
159. Klopp A, Spaeth E, Dembinski J, Woodward W, Munshi A, et al. Tumor irradiation increases the recruitment of circulating mesenchymal stem cells into the tumor microenvironment. *Cancer Res*. 2007; 67:11687–95. [PubMed: 18089798]
160. Karnoub A, Dash A, Vo A, Sullivan A, Brooks M, et al. Mesenchymal stem cells within tumor stroma promote breast cancer metastasis. *Nature*. 2007; 449:557–63. [PubMed: 17914389]
161. Djouad F, Bony C, Apparailly F, Louis-Plence P, Jorgensen C, Noel D. Earlier onset of syngeneic tumors in the presence of mesenchymal stem cells. *Transplantation*. 2006; 82:1060–66. [PubMed: 17060855]
162. Spaeth E, Dembinski J, Sasser A, Watson K, Klopp A, et al. Mesenchymal stem cell transition to tumor-associated fibroblasts contributes to fibrovascular network expansion and tumor progression. *PLoS One*. 2009; 4:e4992. [PubMed: 19352430]
163. McAllister S, Gifford A, Greiner A, Kelleher S, Saelzler M, et al. Systemic endocrine instigation of indolent tumor growth requires osteopontin. *Cell*. 2008; 133:994–1005. [PubMed: 18555776]
164. Kidd S, Spaeth E, Dembinski J, Dietrich M, Watson K, et al. Direct evidence of mesenchymal stem cell tropism for tumor and wounding microenvironments using in vivo bioluminescence imaging. *Stem Cells*. 2009; 27:2614–23. [PubMed: 19650040]
165. Loebinger M, Eddaoudi A, Davies D, Janes S. Mesenchymal stem cell delivery of TRAIL can eliminate metastatic cancer. *Cancer Res*. 2009; 69:4134–42. [PubMed: 19435900]
166. Sasportas L, Kasmieh R, Wakimoto H, Hingtgen S, van de Water J, et al. Assessment of therapeutic efficacy and fate of engineered human mesenchymal stem cells for cancer therapy. *Proc Natl Acad Sci USA*. 2009; 106:4822–27. [PubMed: 19264968]
167. Nakamizo A, Marini F, Amano T, Khan A, Studeny M, et al. Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. *Cancer Res*. 2005; 65:3307–18. [PubMed: 15833864]
168. Liu J, Lu XF, Wan L, Li YP, Li SF, et al. Suppression of human peripheral blood lymphocyte proliferation by immortalized mesenchymal stem cells derived from bone marrow of Banna Minipig inbred-line. *Transplant Proc*. 2004; 36:3272–75. [PubMed: 15686744]
169. Le Blanc K. Immunomodulatory effects of fetal and adult mesenchymal stem cells. *Cytotherapy*. 2003; 5:485–89. [PubMed: 14660044]
170. van Poll D, Parekkadan B, Borel Rinkes I, Tilles A, Yarmush M. Mesenchymal stem cell therapy for protection and repair of injured vital organs. *Cell Mol Bioeng*. 2008; 1:42–50.

171. Di Nicola M, Carlo-Stella C, Magni M, Milanese M, Longoni P, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood*. 2002; 99:3838–43. [PubMed: 11986244]
172. Le Blanc K, Tammik L, Sundberg B, Haynesworth S, Ringden O. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. *Scand J Immunol*. 2003; 57:11–20. [PubMed: 12542793]
173. Klyushnenkova E, Mosca J, McIntosh K, Thiede M. Human mesenchymal stem cells suppress allogeneic T cell responses in vitro: implications for allogeneic transplantation. *Blood*. 1998; 92(Suppl 1, Pt. 1):642a.
174. Tse W, Beyer W, Pendleton J, D'Andrea A, Guinan E. Bone marrow derived mesenchymal stem cells suppress T cell activation without inducing allogeneic anergy. *Blood*. 2000; 96:241a.
175. Meisel R, Zibert A, Laryea M, Gobel U, Daubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. *J Am Soc Nephrol*. 2004; 103:4619–21.
176. Yagi H, Parekkadan B, Sukanuma K, Soto-Gutierrez A, Tompkins R, et al. Long-term superior performance of a stem cell/hepatocyte device for the treatment of acute liver failure. *Tissue Eng Pt A*. 2009; 15(11):3377–88.
177. Block G, Ohkouchi S, Fung F, Frenkel J, Gregory C, et al. Multipotent stromal cells are activated to reduce apoptosis in part by upregulation and secretion of stanniocalcin-1. *Stem Cells*. 2009; 27:670–81. [PubMed: 19267325]
178. Marino R, Martinez C, Boyd K, Dominici M, Hofmann TJ, Horwitz EM. Transplantable marrow osteoprogenitors engraft in discrete saturable sites in the marrow microenvironment. *Exp Hematol*. 2008; 36:360–68. [PubMed: 18179857]
179. Dominici M, Marino R, Rasini V, Spano C, Paolucci P, et al. Donor cell-derived osteopoiesis originates from a self-renewing stem cell with a limited regenerative contribution after transplantation. *Blood*. 2008; 111(8):4386–91. [PubMed: 18182575]
180. Sjöholm I, Edman P. Acrylic microspheres in vivo. I. Distribution and elimination of polyacrylamide microparticles after intravenous and intraperitoneal injection in mouse and rat. *J Pharmacol Exp Ther*. 1979; 211:656–62. [PubMed: 512927]
181. Gao J, Dennis J, Muzic R, Lundberg M, Caplan A. The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. *Cells Tissues Organs*. 2001; 169:12–20. [PubMed: 11340257]
182. Schrepfer S, Deuse T, Reichenspurner H, Fischbein M, Robbins R, Pelletier M. Stem cell transplantation: the lung barrier. *Transplant Proc*. 2007; 39:573–76. [PubMed: 17362785]
183. Francois S, Bensidhoum M, Mouiseddine M, Mazurier C, Allenet B, et al. Local irradiation not only induces homing of human mesenchymal stem cells at exposed sites but promotes their widespread engraftment to multiple organs: a study of their quantitative distribution after irradiation damage. *Stem Cells*. 2006; 24:1020–29. [PubMed: 16339642]
184. Toma C, Pittenger M, Cahill K, Byrne B, Kessler P. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circ Res*. 2002; 105:93–98.
185. Morigi M, Imberti B, Zoja C, Corna D, Tomasoni S, et al. Mesenchymal stem cells are renotropic, helping to repair the kidney and improve function in acute renal failure. *J Am Soc Nephrol*. 2004; 15:1794–804. [PubMed: 15213267]

Figure 1.
A brief history of mesenchymal stem cells (MSCs). Abbreviation: Tx, transplantation.

Figure 2.

Natural functions of mesenchymal stem cells (MSCs) in the bone marrow. (a) MSCs can differentiate into skeletal tissue cells within the marrow cavity. (b) MSCs secrete a number of soluble factors that are involved in hematopoietic development. (c) Given their purported perivascular localization, MSCs may serve cellular functions similar to pericytes that surround bone marrow sinusoids. (d) MSCs maintain the mechanical microenvironment of the marrow by secreting and remodeling ECM. Abbreviation: ECM, extracellular matrix.

Figure 3.

Potential risks associated with MSC transplantation. (a) MSCs have been shown to maldifferentiate into glomerular adipocytes and osteosarcomas when administered systemically. (b) Systemic administration of MSCs may impair immune surveillance, making the recipient more susceptible to opportunistic infections. (c) When transplanted with cancer cells, MSCs can adapt a tumor-associated fibroblast phenotype and support the growth of the cancer by directly promoting tumor growth, metastasis, and angiogenesis. Abbreviations: MSC, mesenchymal stem cell; TAF, tumor-associated fibroblast.

Figure 4. Representative studies describing the in vivo distribution of MSCs upon systemic administration. Tracking studies generally consist of intravenous injection of the cells and then tracking of the cells using a variety of known methods. The representative studies featured here used two sensitive methods available for whole-organism analysis: polymerase chain reaction of a human gene to quantify human MSC engraftment in a number of mouse tissues, and MSCs labeled with luciferase to qualitatively trace their engraftment. Abbreviation: iv, intravenous.

Figure 5.

Pharmacokinetic analysis of MSC therapy. (a) Schematic of two-compartment pharmacokinetic model of MSC drug delivery incorporating the following parameters: R_i , injection rate; R_c , clearance rate; K_1 , rate of extravasation; K_2 , rate of intravasation. (b) Theoretical engraftment of MSCs with a hypothetical retention of nearly 100% of MSCs over the course of 120 h. The apparent activity is the product of the unit activity per cell and the number of cells remaining after injection. Assuming a minimum effective activity level well below the apparent activity, the biological response would be expected to rise as soon as the minimum effective activity level is reached and to be sustained thereafter. (c) Apparent engraftment of MSCs with a decaying retention of MSCs. Assuming an exponential decay with a 24-h half-life, the apparent activity peaks above the minimum effective activity level only for a brief period of time. This results in a brief and temporary biological response that does not persist beyond 24 h. These data are consistent with the cytokine response associated with MSC transplantation or MSC-derived molecules when the latter were administered to animals undergoing systemic inflammation.

Table 1
Examples of clinical trials of mesenchymal stem cell therapy reported in the academic literature

Indication	Enrollment (N_{total} ; N_{control} , N_{cells})	Trial design	Cell mass MOA	Measured parameters and follow-up time after CTx	Study conclusions	Reference
BMTx	(23; 15, 8)	Local advanced or metastatic breast cancer with high-dose chemotherapy and PBPC Tx given CTx at 1 or 24 h later	$1.5\text{--}3.9 \times 10^6$ CD34+ cells kg^{-1} and 2.2×10^6 MSCs kg^{-1}	Neutrophil and platelet counts (daily) CFU (42 days) Mortality (100 days)	<ol style="list-style-type: none"> 1 Prompt hematopoietic engraftment of 8 days 2 70% CFU recovery 3 One patient death after 100 days 	35
OI	(7; 2, 5)	Diagnosed severe type III OI with similar growth rates	$5.7\text{--}7.5 \times 10^6$ bone marrow cells kg^{-1} iv	Growth analysis (6 months)	Increased growth rates that slowed over time	38
MLD	(5; 1, 4)	Patients with successfully matched bone marrow transplants	$2\text{--}10 \times 10^6$ MSCs kg^{-1} iv	Nerve conduction velocity Mental/physical exams	<ol style="list-style-type: none"> 1 No toxicity 2 Improved nerve conduction velocity 3 No clinical change in overall mental or physical status 	39
ALS	(7; 5, 2)	Diagnosed ALS with severe lower-limb and mild upper-limb impairment	MSCs suspended in CSF infused into T7–T9 exposed spinal cord	MRI (3, 6 months) Neuromuscular exam (3 months)	<ol style="list-style-type: none"> 1 No adverse reactions 2 No structural MRI changes 3 Mild increase in muscle strength of a lower-limb muscle group 	40
MI	(69; 35, 34)	CTx 10 days after PCI	$48\text{--}60 \times 10^9$ MSCs into coronary artery lesion	Echocardiography (monthly) PET (3, 6 months) EKG (3 months)	<ol style="list-style-type: none"> 1 Decreased akinetic, dyskinetic, hypokinetic segments 2 Increased wall movement at infarct 3 LVEF higher 	9
GvHD	(56; 10, 46)	Open-label, multicenter trial of hematological cancer patients treated with CTx 4 days prior to haploidentical BMTx	1, 5, or 10×10^6 MSCs kg^{-1} iv	Hematopoietic recovery (daily) Acute GvHD	<ol style="list-style-type: none"> 1 Prompt hematopoietic engraftment in most patients 2 23 of 46 patients did not undergo acute GvHD, with 11 showing longer relapse time 	12

Abbreviations: ALS, amyotrophic lateral sclerosis; BMTx, bone marrow transplant; CFU, colony forming unit; CSF, cerebrospinal fluid; CTx, cell transplant; EKG, electrocardiogram; GvHD, graft-versus-host disease; iv, intravenous; LVEF, left ventricular ejection fraction; MI, myocardial infarction; MLD, metachromatic leukodystrophy; MOA, method of administration; MRI, magnetic resonance imaging; MSC, mesenchymal stem cell; OI, osteogenesis imperfecta; PBPC, peripheral blood progenitor cell; PCI, percutaneous coronary intervention; PET, positron emission tomography; Tx, transplantation.

Table 2

Phenotype tests for mesenchymal stem cells and known MSC phenotypic characteristics

Phenotype test	Known MSC phenotype	Method(s) used	Reference(s)
Colony formation	Will form fibroblastic colonies after isolation	CFU-F assay	22, 44
Immunophenotype	CD11 ⁺ , CD14 ⁺ , CD18 ⁺ , CD31 ⁺ , CD34 ⁺ , CD40 ⁺ , CD45 ⁺ , CD56 ⁺ , CD80 ⁺ , CD86 ⁺ , MHCII ⁺ , CD29 ⁺ , CD44 ⁺ , CD71 ⁺ , CD73 ⁺ , CD90 ⁺ , CD105 ⁺ , CD106 ⁺ , CD120a ⁺ , CD124, CD166 ⁺ , Stro-1 ⁺ , ICAM-1 ⁺ , MHCII ⁺	FACS	45, 46
In vitro multipotency	Will differentiate down multiple pathways: Osteogenic Chondrogenic Adipogenic	Induction via specialized media	47
Ectopic marrow formation	Will form ectopic bone marrow in the presence of bone minerals	Subcutaneous transplantation, kidney capsule transplantation, diffusion chamber transplantation	48, 49
In vivo multipotency	Subset of MSCs, termed MAPCs, are capable of contributing to all somatic cell types in mice	Blastocyst transplantation	50

Abbreviations: CFU-F, colony forming units-fibroblastic; FACS, fluorescent activated cell sorting; MAPC, multipotent adult progenitor cell.

Table 3

Animal studies of diseases shown to respond to administration of mesenchymal stem cells

Disease	Animal model(s)	Method of administration	Evidence of MSC efficacy	References
Acute renal failure	Rodent Cisplatin Ischemia/reperfusion	Intravenous infusion	Decreased serum creatinine Decreased apoptosis Increased epithelial proliferation Suppression of proinflammatory cytokine gene expression	104, 105, 110, 111, 112
Myocardial infarction	Rodent LAD ligation Pig Temporary LAD occlusion	Intravenous infusion Intramyocardial transplantation	Reduction in scar formation Improvement in cardiac function Differentiation of MSCs into functioning myocardium	106, 107, 108, 113, 114
Type I diabetes mellitus	Rodent NOD mice Streptozotocin	Intravenous infusion	Partial restoration of glucose management Reduction in anti-insulin T cells Prevention of FOXP3 ⁺ cell apoptosis	109, 115–118
Graft-versus-host disease	Rodent HLA-mismatched bone marrow transplantation	Intravenous infusion Intravenous coinfusion with bone marrow transplant	Increased survival	119, 120
Systemic lupus erythematosus	Rodent MRL/lpr mouse	Intravenous infusion	Recapitulation of bone marrow osteoblastic niche Reduction in autoantibody levels Improvement in kidney function Reduction in ANA Increase in FOXP3 ⁺ cells	121
Acute disseminated encephalomyelitis (multiple sclerosis)	Rodent Experimental autoimmune encephalomyelitis	Intravenous infusion	Improved clinical score Reduced demyelination Reduced immune-cell infiltration into CNS	122–124
Pulmonary fibrosis	Rodent Bleomycin-induced lung injury	Intravenous infusion	Reduced inflammation Reduced collagen deposition Reduced MMP activation	102, 103

Abbreviations: ANA, anti-nuclear antigen; CNS, central nervous system; LAD, left anterior descending; NOD, nonobese diabetic; HLA, human leukocyte antigen; MMP, matrix metalloproteinase.