Title
Evidence for high translational potential of mesenchymal stromal cell therapy to improve recovery from ischemic stroke

Permalink
https://escholarship.org/uc/item/18h6t8cd

Journal
Journal of Cerebral Blood Flow & Metabolism, 33(9)

ISSN
1559-7016

Authors
Eckert, Mark A
Vu, Quynh
Xie, Kate
et al.

Publication Date
2013-06-12

Peer reviewed

REVIEW ARTICLE

Evidence for high translational potential of mesenchymal stromal cell therapy to improve recovery from ischemic stroke

Mark A Eckert[1], Quynh Vu[2], Kate Xie[2], Jingxia Yu[1], Wenbin Liao[3], Steven C Cramer[4] and Weian Zhao[1]

Although ischemic stroke is a major cause of morbidity and mortality, current therapies benefit only a small proportion of patients. Transplantation of mesenchymal stromal cells (MSC, also known as mesenchymal stem cells or multipotent stromal cells) has attracted attention as a regenerative therapy for numerous diseases, including stroke. Mesenchymal stromal cells may aid in reducing the long-term impact of stroke via multiple mechanisms that include induction of angiogenesis, promotion of neurogenesis, prevention of apoptosis, and immunomodulation. In this review, we discuss the clinical rationale of MSC for stroke therapy in the context of their emerging utility in other diseases, and their recent clinical approval for treatment of graft-versus-host disease. An analysis of preclinical studies examining the effects of MSC therapy after ischemic stroke indicates near-universal agreement that MSC have significant favorable effect on stroke recovery, across a range of doses and treatment time windows. These results are interpreted in the context of completed and ongoing human clinical trials, which provide support for MSC as a safe and potentially efficacious therapy for stroke recovery in humans. Finally, we consider principles of brain repair and manufacturing considerations that will be useful for effective translation of MSC from the bench to the bedside for stroke recovery.

INTRODUCTION

Stroke is the second most common cause of death[1] and a major cause of adult-acquired disability[2–4] worldwide. The estimated direct and indirect costs of stroke are over $50 billion a year in the United States alone.[5] Currently, the only drug approved for the treatment of acute ischemic stroke is the thrombolytic drug recombinant tissue plasminogen activator (tPA).[6,7] Unfortunately, the time window for therapeutic intervention with tPA is only 4.5 hours, and as a result only 1 in 20 patients with acute ischemic stroke currently receives this therapy in the United States.[8] Moreover, approximately half of the patients who do receive tPA for acute ischemic stroke and survive to day 90 have a significant long-term disability. As a result, there has been considerable interest in developing new treatment modalities to reduce disability from stroke. One area of increased focus has been restorative therapeutics, particularly the use of stem cells.

Mesenchymal stromal cells (MSC; also termed as mesenchymal stem cells or multipotent stromal cells) are adult non-hematopoietic pluripotent cells that have received considerable study as a cellular therapy due to their multiple modes of action that collectively respond to many of the fundamental pathologic events underlying stroke injury in the central nervous system (CNS).[9] The International Society for Cellular Therapy has defined human MSC based on three criteria:[1] adherence to plastic in standard culture conditions;[2] expression of surface antigens that

include CD105, CD90, and CD73 ($\geqslant$ 95% positive), but not CD34, CD45, HLA-DR, and other hematopoietic markers ($\leqslant$ 2% positive); and[3] the ability to differentiate *in vitro* to osteoblasts, adipocytes, and chondroblasts.[10] Mesenchymal stromal cells are easily derived from multiple sources, including perhaps most prominently the bone marrow, but also from tissues that include adipose, umbilical cord blood, Wharton's jelly, placental tissue, tooth buds, and hepatic tissue.[11,12] Mesenchymal stromal cells can differentiate into several mesodermal lineages, and under certain conditions also have the potential to differentiate into cells that have phenotypic characteristics of neurons, glia, and endothelia.[13–18] Abundant evidence suggests that MSC promote stroke recovery, and do so via multiple different mechanisms of action.[19,20] Importantly, MSC have been established as safe in multiple clinical trials of human populations with wide-ranging diagnoses; indeed, MSC are the basis for the first clinically approved human stem cell therapy in North America beyond bone marrow transplantation.[21] At present, over 240 MSC-based clinical trials registered at clinicaltrials.gov are completed or in progress to investigate both the safety and efficacy of MSC, in multiple disease states. Of these, nine active clinical trials are explicitly investigating the therapeutic benefits of MSC transplantation in ischemic stroke.

This review will consider the extensive experience regarding MSC-based therapies, including their excellent safety profile in preclinical and human studies, with the focus being treatment of

[1]Departments of Pharmaceutical Sciences and Biomedical Engineering, Sue and Bill Gross Stem Cell Research Center, Chao Family Comprehensive Cancer Center, University of California, Irvine, California, USA; [2]Department of Neurology, Sue and Bill Gross Stem Cell Research Center, University of California, Irvine, California, USA; [3]Department of Pathology, State University of New York at Stony Brook, Stony Brook, New York, USA and [4]Departments of Neurology and Anatomy and Neurobiology, Sue and Bill Gross Stem Cell Research Center, University of California, Irvine, California, USA. Correspondence: Dr SC Cramer, Departments of Neurology and Anatomy and Neurobiology, Sue and Bill Gross Stem Cell Research Center, University of California, 845 Health Sciences Road, Suite 3027, Irvine, CA 92697, USA or Dr W Zhao, Departments of Pharmaceutical Sciences and Biomedical Engineering, Sue and Bill Gross Stem Cell Research Center, Chao Family Comprehensive Cancer Center, University of California, 845 Health Sciences Road, Suite 3027, Irvine, CA 92697, USA.
E-mail: scramer@uci.edu or weianz@uci.edu

ischemic stroke, particularly in relation to stroke recovery. A key feature of MSC is that these cells have multiple mechanisms of action. This is somewhat of a paradigm shift in that most therapies are evaluated in relation to a single main mechanism of action. Stem cell therapies have multiple mechanisms of action and have nonetheless been considered to have great potential as stroke therapies.[20,22–25] The current review extends previous reviews that examined the use of MSC to treat stroke by performing a systematic evaluation of preclinical MSC studies in stroke for the first time. These preclinical results are considered in relation to emerging human clinical trial results, as well as the underlying basic biology of MSC and ischemic stroke. The evidence that MSC have a favorable effect on functional outcomes in animal models, across a range of doses and therapeutic time windows, is compelling. In addition, topics important to successful translation of MSC from bench to bedside are considered, including points related to MSC manufacturing, patient stratification, and the time window for MSC therapy in human stroke.

THERAPEUTIC APPLICATIONS OF MESENCHYMAL STROMAL CELLS

Transplantations of MSC in human patients began in 1995, with most early trials focusing on potential benefits of autologous MSC in promoting the engraftment of hematopoietic stem cells in the setting of hematological malignancy.[26,27] Early trials established a positive role for MSC in promoting hematopoietic stem cells engraftment and survival, which was supported by subsequent larger trials.[28] Bolstered by the lack of side effects in these early investigations, additional clinical trials investigated the utility of MSC in patients with Hurler syndrome, metachromatic leukodystrophy, and osteogenesis imperfecta.[28] These early trials were instrumental in both showing the safety of MSC transplantation in humans and establishing baseline criteria for design of MSC-based trials.

In the subsequent years, MSC have been investigated in the context of numerous diseases and disorders, in trials initiated around the world. Current ongoing trials are examining potential therapeutic roles of MSC in diseases ranging from amyotrophic lateral sclerosis to myocardial infarction to hepatic cirrhosis.[26] Due to the ability of MSC to differentiate into cardiomyocytes in vitro and their robust secretion of bioactive molecules, there has been significant interest in their use to treat myocardial infarction, at various stages. Many clinical trials of MSC have shown significantly improved ventricular function after intracoronary injection of MSC in the acute myocardial infarction.[28] To what extent these results are due to direct integration of MSC in cardiac tissue versus paracrine effects of MSC secretions remains an open question. Ongoing clinical trials are expanding on these initial results to establish both the safety and efficacy of MSC in these diseases, among others.

Perhaps, the most encouraging and dramatic clinical application of MSC to date has occurred with graft-versus-host disease (GVHD). In up to 40% of children who receive allogenic bone marrow transplants, immune cells within the transplanted material may attack host's cells. This leads to often life-threatening complications that are most often treated with immunosuppressive regimens, typically corticosteroids.[29] The immunomodulatory effects of MSC have generated significant interest in their potential to change the graft immune response in patients refractory to steroid-based treatments. In 2012, the MSC-based therapy Prochymal (Osiris Therapeutics) was approved for use in children with GVHD in Canada and New Zealand—the first approved stem cell treatment for systemic disease (outside of bone marrow transplants). In clinical trials, Prochymal showed clinically meaningful responses in 64% of patients.[21] This is particularly remarkable as the mortality rate of steroid-refractory GVHD can be as high as 85%.[29] The approval of MSC for treatment

of GVHD emphasizes not only the safety of MSC, but also their potential to treat diseases that are refractory to current therapies. This long-term track record of safety combined with extensive clinical interest suggests that the therapeutic potential of MSC may soon be realized for multiple diseases.

MECHANISMS OF MESENCHYMAL STROMAL CELLS ACTION IN STROKE THERAPY

The experimental rationale for the use of MSC in stroke therapy includes a number of divergent mechanisms of action such as differentiation into cell types relevant to repair, modulation of the immune system, promotion of angiogenesis and neurogenesis, and secretion of neuroprotective and neurotrophic factors (Figure 1). Common to MSC effects in CNS and non-neural tissue is their ability to selectively target regions of injury. Numerous techniques have been used to gain insight into these mechanisms (Table 1). Understanding the relative contribution of each potential mechanism may be useful to optimize future clinical trial design.

Cell Replacement

Initial in vitro studies indicated that MSC are capable of differentiating into cells of multiple germ lineages, including neuronal and glial cells, leading to hopes that transdifferentiation of transplanted MSC might have a role in improving outcome after stroke.[18,30,31] In vitro differentiation of MSC into endothelia has also been described.[32] Multiple groups have described expression of neuronal and glial markers by MSC in the CNS after transplantation for stroke in rodent models.[33–38] Expression of the synaptic receptor protein gamma-aminobutyric acid receptor-associated protein[39,40] has also been reported. However, evidence that MSC become functional replacements for neurons or glia is lacking. For example, MSC do not express the voltage-gated ion channels necessary for generation of action potentials.[41] A further argument against cell replacement being an important mechanism of action is that the improvement in behavioral outcome found when MSC are administered $\geqslant 24$ hours after stroke onset is not accompanied by a reduction in infarct volume;[20,42–44] small infarct volumes might be expected if the MSC were thought to be replacing infarcted brain cells. Note that this pattern is typical of restorative therapies initiated after the stroke injury is fixed: the final behavioral state is improved on the basis of neural plasticity without a concomitant decrease in final infarct volume.[45,46] Overall, the question as to whether MSC directly differentiate into cells that replace injured CNS tissue after stroke remains incompletely addressed, but at this time definitive evidence is lacking.[31,47,48]

Mesenchymal stromal cells may have a more indirect role in modulating changes in cell numbers after stroke, however, the effect of which is to promote recovery. Introduction of MSC after stroke is associated with increased cell proliferation in the subventricular and subgranular zones, suggesting mobilization of endogenous neuronal precursors.[34,49,50] The MSC paracrine secretions (below) promote survival of some cells including neurons, and promote proliferation of other cells such as glia.[51,52] The MSC can also limit secondary neurodegeneration after stroke.[53] Interestingly, MSC can both promote plasticity among damaged neurons and activate astroglial cells to induce secretion of neuroprotective molecules such as brain-derived neurotrophic factor and glial cell derived neurotrophic factor.[54,55] The MSC robustly and specifically upregulate glial cell derived neurotrophic factor in astrocytes in vitro culture,[55] a phenomenon that has also been described after transplantation of MSC. Strikingly, MSC that have infiltrated the ischemic brain upregulate genes involved in neuroregeneration, angiogenesis, and nerve guidance survival.[56] In addition, extracellular matrix
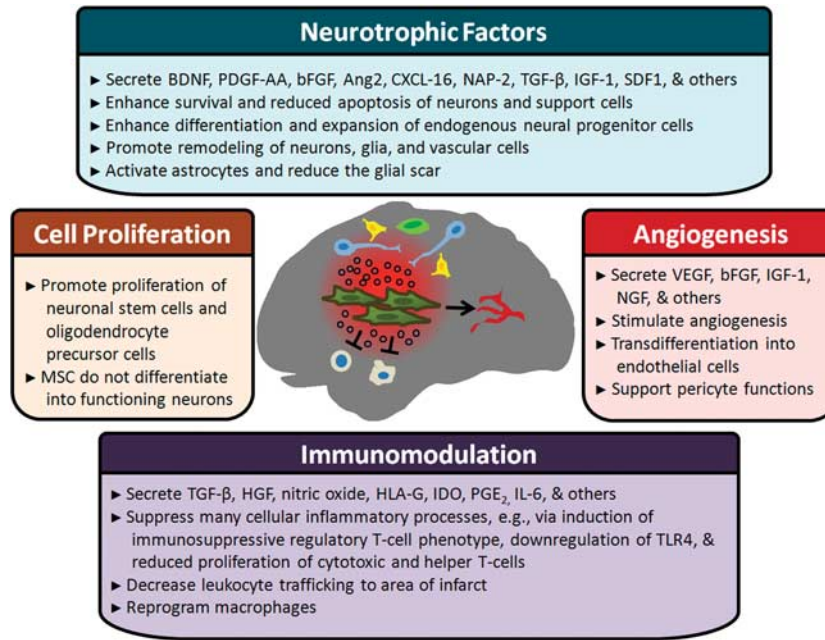
Figure 1. Overview of proposed mechanisms of mesenchymal stromal cells (MSC)-based stroke therapies. Important potential mechanisms mediating MSC action in stroke include secretion of neurotrophic factors,[49,103–105,107,181,182] promotion of angiogenesis,[114,183,184] and modulation of immune responses.[60–63] Note that some of the factors secreted by MSC may have multifactorial roles, i.e., affect multiple pathways. Cell replacement is no longer thought to play a major role in the therapeutic effects of MSC in stroke; although transplanted MSC may express markers of neuronal lineage, there is no evidence that they are functional. In the figure, MSC are olive colored, neurons are blue, glial cells are yellow, neural progenitor cells are green, and immune cells are tan. BDNF, brain-derived neurotrophic factor; PDGF-AA, platelet-derived growth factor AA; bFGF, basic fibroblast growth factor; Ang2, angiopoietin 2; C-X-C motif, chemokine; CXCL-16, ligand 16; NAP-2, neutrophil activating protein 2; TGF-β, transforming growth factor beta; IGF-1, insulin-like growth factor 1; SDF-1, stromal cell-derived factor 1; NGF, nerve growth factor; HGF, hepatocyte growth factor; HLA-G, histocompatibility antigen class I G; IDO, indoleamine 2,3-dioxygenase; PGE$_2$, prostaglandin E$_2$; TLR4, toll-like receptor 4; VEGF, vascular endothelial growth factor; IL-6, interleukin 6.

Table 1. Techniques and markers that provide insight into MSC mechanisms of action after stroke in animal models

Name	Explanation and utility	References
Adhesive removal test	Sensorimotor test of neurologic impairment	105,111,127
BDNF, PDGF-AA, bFGF, Ang2, CXCL-16, NAP-2, TGF-β, IGF-1, SDF-1	Neurotrophic factors secreted by MSC that may have protective and/or regenerative properties in the context of stroke	49,103–105,107,181,182
BrdU, Ki67	Proliferation	36,40,55,104,185
Cleaved caspase	Increase associated with apoptosis	186–188
Connexin-43	Astrocytic gap junctions	185
CXCR4	Promigratory chemokine receptor expressed by MSC	139,182,187
Dcx	Neurogenesis	106
Infarct volume	Extent of stroke; may be histologic or imaging based	34,99,110,111,189,190
mNSS	Extent of neurologic deficit; composite of motor, sensory, reflex, and balance tests	101,106,182
NeuN AND GFAP	Markers of neuronal and glial cells, respectively	36,16,111,183
Rotarod	Sensorimotor test of neurologic impairment	49,107,111,141
SDF-1α	Ligand for CXCR4; secreted by microglia after stroke	154,186,187
Small vessel density analysis	Staining for blood vessels in brain tissue Measures extent of angiogenesis and vascularization	93,187
Synaptophysin	Synapse quantification	185
TTC test	Extent of hypoxia	107,111,187
tPA	Component of fibrinolytic pathway that also has neurogenic properties	99,108
TLR4	Component of innate immune response induced during stroke	187
TUNEL assay	Extent of apoptosis	34,142,187
VEGF, bFGF, IGF-1, NGF	Proangiogenic molecules secreted by MSC	34,114,183,184
vWF	Plasma levels correlate with vascular injury	186,187

Abbreviations: MSC, mesenchymal stromal cells; BDNF, brain-derived neurotrophic factor; PDGF-AA, platelet-derived growth factor AA; bFGF, basic fibroblast growth factor; Ang2, angiopoietin 2; CXCL-16, ligand 16; NAP-2, neutrophil activating protein 2; TGF-β, transforming growth factor beta; IGF-1, insulin-like growth factor 1; SDF-1, stromal cell-derived factor 1; Dcx, doublecortin; TTC, triphenyltetrazolium chloride; BrdU, bromodeoxyuridine; mNSS, modified neurologic severity score; ROS, reactive oxygen species; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; vWF, von Willebrand factor; IL-18, interleukin 18; GFAP, glial fibrillary acidic protein; tPA, tissue plasminogen activator; TLR4, toll-like receptor 4; NGF, nerve growth factor; VEGF, vascular endothelial growth factor.
Some of the more commonly used assays used in the MSC stroke therapy literature are described above.

components such as fibronectin secreted by MSC may promote survival and synaptogenesis of neurons, suggesting other functional roles for the presence of MSC in the CNS other than cell replacement.[57,58] Together, these findings support a role for MSC in the regulation of cell numbers after stroke, regardless as to whether this includes direct cell replacement.

Immunomodulation

Ischemic stroke induces a strong inflammatory response that leads to subsequent recruitment of leukocytes to the infarct zone.[59] Mesenchymal stromal cells are also immunomodulatory, in particular having been found capable of attenuating both innate and adaptive immune responses. They have well-characterized roles in regulating T cell-mediated processes by inhibiting T-cell proliferation, promoting a T-regulatory cell phenotype, and by exerting a non-specific suppressive effect on CD4 + and CD8 + T cells.[60–63] In addition, exposure of MSC to cytokines such as interferon-γ promotes the immunoregulatory phenotype of MSC, leading to further suppression of T cells or natural killer cells.[64] In vitro experiments have shown clear reductions in leukocyte proliferation and alterations in differentiation when cocultured with MSC.[65–67] The MSC can also modulate the function of B cells and antigen-presenting cells, the nature of which varies with environmental signals.[52,68–70]

Importantly, many of the effects that MSC have on the immune system are directly linked to the processes underlying neural repair after stroke. For example, MSC promote resolution of the poststroke inflammatory milieu that can impede repair, and can shift macrophage profiles to those that are more conducive to repair.[68,71] A key feature of MSC transfusion is the remote effect these cells can have on immunologic function. For example, systemically administered MSC may be entrapped in the lung but have immunomodulatory effects on distant organs including the brain.[72–74] Interestingly, the spleen is instrumental in mediating the systemic immune response to ischemic stroke; splenectomy leads to significantly reduced neurodegeneration and reduced immune response after middle cerebral artery occlusion (MCAO) in rats.[75] The dramatic accumulation of MSC in the spleen after intravenous administration hints at possible roles for systemic immunomodulation here,[76,77] although this hypothesis has not yet been tested.

Mesenchymal stromal cells express HLA molecules at very low levels.[78,79] As a result, MSC are immunoprivileged—their activity does not vary in relation to extent of MHC match between donor and recipient. Many of their immunomodulatory effects are therefore seen in both the autologous and allogenic setting, a feature that suggests broad therapeutic application. However, reports of immune responses stimulated by MSC suggest that this may be a relative rather than a absolute property,[68,80] although MSC show no indication of immunologic sensitization in the brain when given after stroke.[72]

Relatively few studies have directly investigated the effects of MSC on the immune response during stroke. Reductions in macrophage recruitment and activation of the innate immune response have been observed after stroke.[22,81,82] The need for such studies is highlighted by the fact that MSC effects on the immune system can vary across levels of specific chemokine, disease settings, and species.[83–86] This need is further underscored by the fact that, at least for MSC given in the initial days after stroke onset, any effects of MSC on the immune system must be interpreted in the context of the immunosuppressed state that arises after stroke.[87,88]

Angiogenesis

Angiogenesis within the infarct zone and the surrounding penumbra is thought to play a key role in mediating survival and regeneration of neurons after stroke. Mesenchymal stromal cells secrete a diverse array of cytokines, many of which are proangiogenic.[86,89] Although the exact molecules may differ depending on cell source, secretion of vascular endothelial growth factor (VEGF), basic fibroblast growth factor, and placental growth factor have been repeatedly described.[89–91] Interestingly, many angiogenic factors have additional neuroprotective roles. For example, VEGF not only induces angiogenesis, but is also anti-inflammatory and promotes the recruitment and differentiation of endogenous neuronal precursors.[92–94] The broad effects of angiogenic molecules may therefore help explain the diverse therapeutic benefits of MSC in stroke. In addition, MSC possess many similarities to pericytes that may allow transplanted MSC to have additional roles relevant to neural repair poststroke such as maintenance of the vascular supply[95] and support of normal pericyte interactions with astrocytes and endothelia to maintain blood–brain barrier integrity.[96]

Neurotrophic Factors

Mesenchymal stromal cells given after an experimental stroke have been found to secrete a wide number of neuroprotective and neurotrophic factors that promote repair and recovery through numerous pathways. Consistent with this, introduction of MSC-conditioned media reproduces many of the effects of direct MSC transplantation.[97,98] The MSC thus serve as a delivery platform for a wide variety of secreted molecules,[97] acting synergistically and in parallel on multiple biologic pathways.

A number of factors secreted by MSC influence cell survival and proliferation after stroke. Stroke leads to necrosis and apoptosis of neurons, endothelial cells, and CNS glia. Studies have consistently found that MSC given after stroke reduce apoptosis and increase proliferation of several cell types including endogenous neural stem cells and glia.[49,82,99,100] This likely occurs via both secretion of prosurvival and antiapoptotic factors and via indirect stimulation of CNS parenchymal cells to secrete neurotrophic, neuroprotective, and prooligodendrogenic factors,[34,91,101,102] e.g., insulin-like growth factor 1, brain-derived neurotrophic factor, epidermal growth factor, and stem cell factor.[49,54,103–107] A particularly important indirect effect of MSC in stroke may be induction of tPA in astrocytes, which both promotes neurite outgrowth and neuroprotection.[99,108] MSC secretion of angiogenic factors such as VEGF may have additional roles in support of repair.[92] The result of these MSC activities includes increased neurogenesis, improved white-matter integrity, and synaptogenesis.[20]

For other neurotrophic factors secreted by MSC, the main effect on stroke recovery is related to amplification of the molecular and cellular events[25] that underlie spontaneous recovery from stroke. Paracrine secretion of neurotrophic factors by MSC after stroke favorably influences numerous restorative brain events.[109] This was well summarized by Li and Chopp,[72] who described transplanted MSC as working like "small molecular factories' by secreting neurotrophins, growth factors and other supportive substances after brain attack, which may continually produce therapeutic benefits in the damaged brain. The poly-pharmacies secreted from MSCs are potentially more important than any single factor to stimulate CNS repair".

EFFECTS OF MESENCHYMAL STROMAL CELLS SOURCE AND DETAILS OF MANUFACTURE

Source of Mesenchymal Stromal Cells

The majority of animal studies of MSC transplantation in stroke have used bone marrow-derived MSC (BM-MSC), with a smaller proportion studying MSC derived from adipose tissue or the umbilical cord.[110,111] Unfortunately, there have been relatively few studies directly comparing the relative efficacy of MSC derived from multiple sources in the setting of stroke. Ikegame et al.[112] injected mice that had undergone an MCAO with MSC derived

from either adipose tissue or bone marrow and found that those from adipose tissue not only promoted reduction in brain infarct volume and neurologic recovery, but were also more refractory to the effects of advanced donor age. Although it is likely that multiple sources of MSC will be used clinically in the future, it is imperative that the differentiation ability, cytokine secretion profile, and immunomodulatory abilities of MSC derived from multiple tissues be fully characterized and directly compared with optimize outcomes.

Mesenchymal stromal cells transplantation may be either autologous, in which the cells to be transplanted are derived from the eventual recipient, or allogenic, in which the source of cells is a genetically similar but not identical donor who is of the same species. Autologous transplantation has little risk of immunoreaction, but requires weeks to culture and expand cells for transplant,[113] which severely limits their use in the acute disease setting.[114] Furthermore, MSC derived from patients with chronic disease or advanced age, features common among patients with stroke, consistently exhibit reduced growth rates in culture.[115,116] The fact that MSC are immunoprivileged suggests safety for an allogenic approach to MSC administration after stroke.[117] Clinical trials in human subjects with many different disease states have established that transplantation of allogenic MSC is safe.[118] Rodent models provide clear evidence for the functional benefits of allogenic MSC after stroke, with no significant difference in functional recovery between autologous and allogenic MSC in a head-to-head comparison when administered 3 hours after tMCAO.[119] In addition, a study in human subjects with chronic ischemic cardiomyopathy treated via transendocardial injection reported no major differences in safety or regenerative activity between autologous and allogenic MSC.[120] An important caveat is that one study found that MSC derived from rats that had undergone MCAO promoted significantly larger improvements in functional outcome and angiogenesis compared with rats treated with MSC derived from control rats.[121] This suggests that stroke may influence the expression of neurotrophic or angiogenic molecules in endogenous MSC *in vivo*.

Details of Mesenchymal Stromal Cells Manufacture

From a broad perspective, issues related to MSC manufacturing could be of critical importance to successful translation of MSC to human trials. Studies in which MSC are given to human subjects must produce these MSC in accordance with Good Manufacturing Practice (GMP),[122] a standard that makes specific and rigorous demands on the materials, processing, documentation, quality assurance testing, release specification, process control, specimen storage, specimen transport, and other features of providing a therapeutic product appropriate for introduction into a human patient. The MSC produced outside of GMP conditions in a preclinical study might not have the same biologic activity, biologic purity, or biologic stability as compared with MSC produced in a GMP-compliant manner for a clinical trial. Differences in the details of manufacture could contribute to differences between preclinical and clinical studies in safety or in behavioral end points. To maximize the success of successful translation, it is critical for preclinical studies not only to report the tissue source and culture conditions, but also to provide detailed information regarding points of compliance with, and points of deviation from, GMP production.

A number of specific issues of MSC manufacture may also be important. Examples include the source of the tissue used to grow the MSC, the expansion rate, choice of culture media, culture conditions, storage conditions, and any transport conditions.[123,124] The number of cell divisions and the number of times cells are replated (passage number) dramatically impact MSC potency and viability.[125,126] For example, MSC that have undergone two passages improve stroke outcome more than MSC that have undergone six passages do,[127] and MSC that have undergone three passages have superior cardioprotective activity compared with MSC that have undergone five passages.[128] Mesenchymal stromal cells are most commonly grown in basal media supplemented with fetal bovine or calf serum. The precise makeup of these supplements can vary, and use of animal products introduces risk, e.g., of infection or allergic reaction, that while small has nevertheless prompted some investigators to develop xeno-free MSC culture methods. Some authors have studied human-derived sera, as well as media defined in relation to specific bioactive molecules such as transforming growth factor beta, platelet-derived growth factor, and basic fibroblast growth factor 2.[129] The utility of these alternative approaches is a topic of current study.[130,131] A number of preclinical studies have modified MSC, e.g., by gene insertion, use of bioscaffolding, or addition of growth factors to culture medium, additional manipulations that could potentially improve MSC restorative effects as well as their safety profile.

PRECLINICAL STUDIES

Fate of Transfused Mesenchymal Stromal Cells

After stroke, MSC can be introduced locally, i.e., by direct injection into an affected brain region (intracerebral), or MSC can be transfused systemically, through the venous (IV) or arterial (IA) routes. The exact pattern of MSC distribution, however, varies with route of delivery.

Mesenchymal stromal cells are selectively attracted to sites of injury and inflammation.[132,133] Tissues release numerous inflammatory cytokines after injury,[134,135] and MSC express a variety of chemokine receptors.[136] Indeed, simply exposing MSC to hypoxia *in vitro* can induce expression of genes involved in migration.[137,138] An example in the setting of stroke is the release of potent chemoattractants such as stromal cell-derived factor 1 alpha (SDF-1α) by microglia and astrocytes in the infarct zone and penumbra, which recruits MSC through receptors such as chemokine (C-X-C motif) receptor 4 (CXCR4).[139,140] The integrity of the cerebral vasculature is compromised after stroke, which can lead to MSC accumulation in the brain via passive entrapment.[73] To what extent and how MSC cross the blood–brain barrier remains to be determined. In addition, the extent to which homing and engraftment within the region of injury are needed for MSC to provide therapeutic efficacy is also incompletely understood. Mesenchymal stromal cells can have remote immunomodulatory effects,[74] e.g., MSC entrapped in the lung affect immune system function systemically,[72,73] prompting some authors to suggest that MSC effects after stroke can be realized in the absence of significant MSC recruitment to the infarct region.[141,142]

Mesenchymal stromal cells are relatively large cells, with diameter 10 to 30 μm.[143] Not surprising, therefore, most MSC are passively entrapped in the lung vasculature after intravenous transfusion.[77] Histologic studies in healthy rodents given intravenous MSC found the MSC to be mainly distributed in lungs and liver at 3 hours; in lungs, liver, and spleen at 24 hours, in bone marrow, spleen, and lung over the next several days; and in bone marrow, spleen, lung, bone, cardiac and skeletal muscle, ear cartilage, and liver several months thereafter.[144,145] After stroke, MSC also home to regions of brain injury;[72] in the brain, the majority are found in the ischemic core and its peri-infarct boundary zone. Mesenchymal stromal cells are predominantly eliminated by the kidneys.[77] One year after stroke, i.e., as far out poststroke as has been examined preclinically, most surviving MSC are found within the ischemic brain, with very few present in other organs.[146]

These findings have been confirmed by whole body imaging of radiolabeled MSC given intravenously to rats with and without

stroke due to MCAO. During the first 2 hours after stroke, MSC are transiently trapped in the lungs, and can be seen to increase within the region of brain ischemia.[77] Interestingly, the clearance and biodistribution of intravenous MSC resembles that of inert microparticles: an initial accumulation in the lungs followed by gradual clearance over a period of days to a week.[147]

The exact distribution of MSC differs according to route of administration.[76,148] Introduction of MSC via the intraarterial or the intracerebral route reduces avoids first-pass pulmonary effects and so results in fewer MSC in the lung, but these approaches are more invasive and so introduce specific risks.[149,150] It remains to be determined whether differences in MSC distribution in relation to route of administration are associated with significant differences in safety or in efficacy of treating stroke recovery.

PRECLINICAL STUDIES OF MESENCHYMAL STROMAL CELLS SAFETY AND EFFICACY FOR PROMOTING STROKE RECOVERY

A systematic search of PubMed and Web of Science identified preclinical studies of MSC in the setting of experimental ischemic stroke. Studies of MSC that have been modified, e.g., genetically or via a bioscaffold were excluded. A total of 46 studies reporting 61 different MSC treatment arms were identified. In 39 of these, behavioral functional outcomes were reported; all but 1 of these 39 found significantly better functional outcomes with MSC as compared with control (see Supplementary Table and References). There has been a dramatic increase in the number of preclinical MSC studies in recent years. The use of MSC to promote stroke recovery has been found safe.

These favorable effects of MSC on functional recovery were robust across rodent and primate species, route of delivery (intravenous, intraarterial, and intracerebral), type of MSC (allogenic versus autologous), time of administration in relation to stroke (from 5 weeks before to 1 month after stroke), and MSC dosage (from 1×10^4 to 3.25×10^7 cells, i.e., 36.3 cells/g to 1.3×10^5 cells/g). Effects were also consistent across species of MSC origin, with studies showing favorable behavioral effects with MSC derived from rat in 24 studies, human in 16, and mouse in 6 studies.

These findings indicate that in animal models of stroke, MSC exert therapeutic effects over a wide dose range, may be administered as late as 1 month after cerebral ischemia, and may be beneficial regardless of cell source or route of delivery. Importantly, the retained efficacy of MSC when given to animals 30 days after stroke[146] mitigates concerns that the time needed to expand MSC in culture for autologous MSC therapy (e.g., 2 to 3 weeks) could be an impediment to this approach after stroke, should this issue be of interest.

Several clinical aspects of stroke could have an important influence on efficacy of MSC in stroke recovery in humans. Few preclinical studies have examined these issues. Several studies have examined the functional effects of MSC in aged rodents (retired Wistar breeder rats) and found evidence of significant improvement in behavioral outcomes as well as in increased proliferation and neurogenesis.[105,106] Similarly, a study found that in spontaneously hypertensive rats, MSC effectively homed to the ischemic lesion, although behavioral outcomes were not examined.[33] Conversely, intravenous administration of MSC 24 hours after MCAO in streptozotocin-induced diabetic rats led to significantly worse functional outcome, including mortality, compared with non-diabetic rats.[151] The authors observed elevated levels of angiogenin and increased blood–brain barrier leakage in the brain of diabetic mice that received MSC, which they speculate led to the adverse effects of MSC. Future preclinical studies can expand on this experience, for example, by examining how MSC effects interact with various forms of rehabilitation or commonly used pharmacological therapies after stroke, or by comparing MSC effects across different forms of stroke as captured by divergent stroke models. The observation of adverse effects of MSC in streptozotocin-induced diabetic rats emphasizes that, as with any new potential therapy, the impact of comorbidities must always be considered, although it must be appreciated that 2 weeks of poststreptozotocin hyperglycemia[151] only models a small fraction of the components of years of diabetes mellitus.[152] Understanding the influence that these and other variables can have on the therapeutic effects of MSC after stroke will be important to identify characteristics of those patients most likely to benefit from MSC, and to understand the fundamental limitations of MSC in the treatment of stroke therapy.

CLINICAL TRIALS IN ISCHEMIC STROKE

Based on the encouraging results of preclinical studies of MSC after stroke, the excellent safety profile of MSC in non-stroke human studies, and the potential advantages of MSC in relation to other cellular therapies (e.g., can be derived from multiple sources, immunoprivileged), significant interest in the application of MSC to stroke treatment is emerging. An overview of completed and ongoing clinical trials of MSC in stroke appears in Tables 2 and 3. Importantly, all completed trials have investigated the utility of MSC at relatively late time points, i.e., at least 23 days after stroke. This underscores the fact that trials enrolling patients >23 days after stroke are focusing on restorative targets, an important distinction as compared with stroke trials that enroll patients in the early hours after stroke and use interventions that focus on reducing acute injury via reperfusion or neuroprotective targets. This issue is considered in greater detail in the following section.

The first report of MSC transplantation in stroke patients was performed by Bang et al.[153] In this study, ischemic stroke patients were randomized into control and experimental groups, with the experimental groups receiving intravenous infusion of 10^8 autologous BM-MSC 4 to 9 weeks after onset of symptoms. The authors observed significantly improved modified Rankin scores and Barthel Indexes up to 6 months after MSC transplantation compared with control groups. Due to concerns regarding the safety of fetal calf serum and fetal bovine serum incorporated into the treatment, a 5-year follow-up study was performed in 2010.[154] The authors observed sustained improved functional outcome in patients treated with MSC with no significant side effects and no changes in mortality rates as compared with control patients in the follow-up, including no malignancy, consistent with the in vitro finding that there is little evidence of neoplastic transformation of MSC even after prolonged culture.[155] An exciting observation was that functional gains in patients receiving MSC correlated positively with serum levels of SDF-1α at the time of MSC delivery, and negatively with the presence of ischemic damage to the subventricular zone.

Honmou et al[114] observed that MSC passaged in autologous human serum expanded more rapidly than in fetal calf serum, reducing cell preparation time and risk of transmissible disorders. They went on to deliver intravenous autologous MSC 36 to 133 days after stroke. Although this study did not include a control group, the authors were able to report that MSC administration was not associated with CNS tumors, abnormal cell growth, or neurologic deterioration. No evidence was found for adverse events such as venous thromboembolism, systemic malignancy, or systemic infection after MSC infusion. A recent report from Bhasin et al[156] reported that intravenous transplantation of autologous MSC in six patients with chronic (3 to 12 months after) stroke was safe as compared with six controls, and that MSC treatment was not associated with any significant change in neurologic function. A second study from this group[157] gave patients with chronic stroke an intravenous transfusion of either mononuclear cells (n = 14) or MSC (n = 6) using an unblinded non-randomized case control study design and found that one of the end points favored the combined cell therapy group at one of the time points examined: patients receiving cell therapy showed a

Table 2. Active clinical trials of MSC in human subjects with ischemic stroke

Location/clinicaltrials.gov ID #	Diagnosis	N	Source	Route	Time poststroke	Doses	Dosage	Inclusion/exclusion criteria	Measures of outcome
University Hospital Grenoble (FR) NCT00875654	Ischemic stroke	30	Autologous hBM-MSC	IV	<6 weeks	1	?	Y	*Primary*: Safety study of feasibility and tolerance *Secondary*: Clinical and functional effects; dosage; criteria for future phase III trial; define target population
National University of Malaysia NCT01461720	Middle cerebral artery infarct	50	Autologous hBM-MSC	IV	1 week – 2 months	1	?	Y	*Primary*: NIH Stroke Scale, Barthel Index, Rankin Scale, size of infarct (MRI) *Secondary:* Stroke-Specific Quality of Life Scale and Stroke Impact Scale
Southern Medical University,China NCT01468064	Middle cerebral artery infarct	90	Autologous hBM-MSC or hEPCs	IV	<1 week	2: 1 & 2 wks	2.5×10^6	Y	*Primary*: Number of adverse events *Secondary*: modified Rankin Scale, Barthel's Index
Stemedica Cell Technologies, Inc NCT01297413	Chronic ischemic stroke	33	Allogenic hBM-MSC; hypoxic growth conditions	IV	>6 months	1	0.5–1.5×10^6	Y	*Primary*: Safety *Secondary*: NIH Stroke Scale, Mini-Mental Status Exam, Barthel Index, Geriatric Depression Scale
Wenzhou Medical College, China NCT01714167	Chronic ischemic stroke	30	Autologous hBM-MSC	IC	3–60 months	1	2–4×10^6	Y	*Primary*: NIH Stroke Scale *Secondary*: Size of infarct (MRI)
Samsung Medical Center NCT01716481	Ischemic stroke	60	Autologous hBM-MSC	IV	<90 days	1	?	Y	*Primary*: modified Rankin Scale *Secondary*: NIH Stroke Scale, Barthel Index, quality of life, mobility and motor function, biomarker panel
Athersys, Inc. NCT01436487	Cortical ischemic stroke	140	MultiStem® proprietary adult stem cells	IV	1–2 days	1	'Low' and 'High'	Y	*Primary*: Dose limiting adverse events and modified Rankin Scale (mRS) *Secondary*: Functional outcome (mRS, NIH Stroke Scale, Barthel Index), adverse events, secondary infections
SanBio, Inc. NCT01287936	Chronic ischemic stroke	18	SB623 notch expressing MSC	IV	6–36 months	1	2.5, 5, or 10×10^6	Y	*Primary*: Safety *Secondary*: Functional outcome (European Stroke Scale, NIH Stroke Scale, mRS, Fugl-Meyer, FDG-PET)
Celgene Corporation NCT01310114	Acute stroke, middle cerebral artery stroke, posterior cerebral artery stroke	44	Human placenta-derived cells (PDA001)	IV	?	1 or 2	2×10^8–8×10^8	Y	*Primary*: Safety and efficacy (mRS) *Secondary*: NIH Stroke Scale & Barthel Index

Abbreviations: FDG-PET, [18F]-fluorodeoxyglucose positron emission tomography; hBM, human bone marrow; hEPCs, human endothelial progenitor cells; IC, intracerebral; IV, intravenous; MRI, magnetic resonance imaging; MSC, mesenchymal stromal cells; NIH, National Institutes of Health.
Currently nine clinical trials registered with clinicaltrials.gov are investigating application of MSC or related cells in the treatment of ischemic stroke in human subjects.

Table 3. Completed clinical trials of MSC therapy in human subjects with ischemic stroke

Study	Study goals	Study design	N	Source	Route	Dose (total number)	Time poststroke	Observations
Bang et al[153]	Safety and efficacy	Unblinded, randomized	5	Autologous BM-MSC	IV	1×10^8	23–37 days	Significant improvement in Rankin Scores and modified Barthel's Index; No adverse events; Established safety of MSC therapy for stroke in humans
Lee et al[154]	Long-term safety and efficacy	Blinded, randomized	16	Autologous BM-MSC	IV	5×10^7	23–37 days	No significant increase in adverse events; Significant improvement in Rankin Scores; Increased serum levels of SDF-1α and decrease in SVZ damage correlated with efficacy
Honmou et al[114]	Feasibility and safety	Unblinded, non-randomized	12	Autologous BM-MSC	IV	$0.5–5 \times 10^8$	<6 months	Significant reduction in lesion volume as measured with magnetic resonance imaging (MRI) (>20%); No significant increase in adverse events; Established feasibility and safety of large doses of MSC for stroke therapy
Bhasin et al[156]	Safety, feasibility, and efficacy	Unblinded, non-randomized	6	Autologous BM-MSC	IV	$5–6 \times 10^7$	3–12 months	No mortality or adverse events; Functional imaging improved
Bhasin et al[157]	Safety, feasibility, and efficacy	Unblinded, non-randomized	6	Autologous BM-MSC	IV	$5–6 \times 10^7$	3–24 months	Intravenous autologous MSC is safe and feasible

Abbreviations: BM-MSC, bone marrow-derived mesenchymal stromal cells; IV, intravenous; SDF-1α, stromal cell-derived factor 1 alpha; SVZ, subventricular zone. All current completed clinical trials to date have investigated autologous transplantation of MSC in relatively small numbers of patients. Although results of the trials are mixed, all studies to date have confirmed the absence of any concerns related to morbidity or mortality in association with MSC therapy.

greater increase in Barthel index score from baseline to 24 weeks (46 to 75) as compared with the control group (47 to 68, $n = 20$, $P = 0.05$). A key principle of brain repair after stroke is the need to pair a restorative therapy with concomitant experience[45,46] (see below), and so a favorable feature of study design in this trial was subjects in all treatment groups received 8 weeks of physical therapy.

Taken together, these data provide preliminary support for the idea that intravenous transfusion of MSC in patients with chronic stroke is safe and might improve quality of life. It is also important to note that the paucity of preclinical data examining the effects of MSC introduced >30 days after stroke onset. A major disparity thus exists in MSC study design between the body of published preclinical studies and completed human trials, in relation to the number of days poststroke when MSC were introduced; this may be reasonable for initial safety studies but will become increasingly important in later phase studies.

A number of guidelines have been published that could maximize the effectiveness with which MSC are translated to human clinical trials. The Stem Cell Therapy as an Emerging Paradigm for Stroke (STEPS) committee[158,159] provided a series of recommendations regarding both preclinical and clinical research into stem cell-based therapies for ischemic stroke, including the necessity of validated animal experiments, robust safety studies in humans, and the importance of selecting appropriate time points and patient populations for clinical trials. In particular, they suggested three areas of research that would advance the field: (1) development of cell labeling techniques that are safe and reliable for clinical testing, (2) identification and validation of surrogate markers of stroke recovery, and (3) formation of a stroke recovery imaging consortium to develop imaging end points for stroke clinical trials. Research into all of these areas would clearly also have enormous benefits for understanding the role of MSC in ischemic stroke therapy. Other guidance comes from the Stroke Treatment Academic Industry Roundtable (STAIR) publications, which in addition to providing recommendations regarding the

design of preclinical and clinical restorative stroke studies, recommended a focus on treatments with multiple mechanisms of action as well as treatments that targeted systems outside the brain, such as the immune system.[160,161] The diverse mechanisms of action of MSC coupled with their potent immunomodulatory functions suggest that they will be promising candidates for future ischemic stroke therapies.

Other clinical trials investigating the safety and efficacy of both allogenic and autologous MSC transplant are ongoing, at multiple study sites (Table 2), some introducing MSC at earlier time points poststroke than currently published studies. Importantly, most of the trials are using stroke-specific measurements of neurologic recovery and are also investigating the role of dosage in therapeutic efficacy. In addition, one of the trials is investigating the safety and efficacy of MSC grown under hypoxic conditions in ameliorating stroke symptoms, with the rationale being that hypoxia better preconditions these MSC to the microenvironment of the infarcted brain. Several of the trials are using proprietary stem cell formulations, including Athersys' Multistem® product and SanBio's MSC modified to express Notch. Celgene is also examining the safety and efficacy of PDA001, a placenta-derived stem cell product that may be similar to MSC, for safety and efficacy in a wide variety of stroke settings. These trials in particular will be instrumental in understanding the safety and utility of modifying MSC for stroke therapy. Results from these initial trials will provide needed data on the potential role of MSC-based therapy for cerebrovascular disease in humans.

MESENCHYMAL STROMAL CELLS FOR STROKE RECOVERY AND PRINCIPLES OF BRAIN REPAIR

Treatment of cerebrovascular disease encompasses a number of different arenas, each with its own strategies. These include stroke prevention, acute reperfusion, and the focus of the current review, restorative therapy. A number of guiding principles for designing poststroke restorative therapeutics have emerged from preclinical

research and human studies.[46,162] Consideration of these principles stands to strengthen emerging trials examining MSC as a restorative stroke therapy.

One key principle to consider is the time sensitivity of stroke therapeutic windows. This is a topic common to acute and restorative stroke therapies: in the setting of acute stroke, neuroprotective drugs and reperfusion therapies are known to have specific therapeutic time windows. The same is true in the setting of brain repair after stroke, where biologic targets and so response to therapy change with time.[163,164] Preclinical data for MSC suggest that efficacy may change in relation to the time of treatment after stroke.[72] There are limited preclinical data for MSC initiated >30 days after stroke onset, and it cannot be assumed that the biologic targets present in the first week poststroke remain months or years later. Other lessons from the experience of translating acute stroke therapies[165–167] inform the design of MSC clinical trials; for example, having human trials match preclinical data as closely as possible, such as for treatment time window and route of administration. Maximum communication and iterative interactions between preclinical scientists and persons designing translational trials are important.[45,168]

A second principle is the need to stratify patients with stroke to maximize study power and the likelihood of detecting a treatment effect in a clinical trial.[169] This is also a topic that is relevant to both acute and restorative stroke therapies. Stroke is an extremely heterogeneous clinical diagnosis, patients differ in numerous important ways, and these differences can influence the likelihood that treatment will improve outcome. In the acute stroke setting, much focus has been on identifying ischemic penumbra with methods such as diffusion-perfusion magnetic resonance imaging mismatch. Clinical trials of MSC as a restorative therapy after stroke will have maximum likelihood of identifying treatment effects if patients are selected, or stratified, such that enrollees have sufficient biologic target to derive a benefit. This is an area of active research, and the optimal choices such as entry criteria will likely vary according to particulars of each trial, but two general guiding principles can be considered. First, enrolling patients whose clinical features are aligned with those reported in the positive preclinical studies increases the likelihood that the trial will identify a treatment effect. This point was illustrated in the analysis of a recent restorative stroke trial of cortical stimulation, where preclinical studies found that stimulation improved motor deficits after stroke but a phase III randomized controlled clinical trial in humans failed to detect a difference in motor outcome between study groups. Each of the preclinical studies in rodents and primates required preserved physiologic integrity of the motor system, but the clinical trial did not. A *post hoc* review[170] of patients randomized to active therapy (stimulation) in the trial found that the treatment effect was significantly greater in those patients who had preserved physiologic integrity of the motor system. This example highlights the need to design clinical trials of MSC in stroke with preclinical studies in mind; for example, when selecting entry criteria, outcome measures, timing of assessments, choice of biomarkers, and more. Second, patient selection can be guided on the basis of known predictors of outcome after stroke. Here again the specific details will vary with the nature of each MSC trial, however, predictors of stroke outcome have been characterized, e.g., infarct volume, extent of corticospinal tract injury, baseline behavioral status, mood, and age, and such measures are likely to retain their predictive power in a clinical trial setting.[171–175] A related perspective in this regard is that entry criteria predict final outcomes;[176] features of study design such as entry criteria inform predictions of outcome values. For example, a study that enrolls a high fraction of patients with lacunar infarcts will have a higher rate of good outcomes in the placebo group,[176] which can obscure any treatment effects, while a study that enrolls patients with very severe deficits might

have a harder time showing a significant difference between treatment arms.[169,177]

A third principle is that brain repair is experience dependent. This is a point where acute stroke and restorative stroke therapies differ sharply. Therapy given in the setting of acute stroke, such as intravenous tPA, does not require any specific behavioral experience to achieve efficacy. In the restorative stroke setting, however, evidence suggests that treatment efficacy varies with behavioral reinforcement. An early demonstration of this point was by Feeney et al,[178] who showed that a stimulant promoted improved motor outcome in rats only when its exposure was paired with training. Introducing a therapy that primes the brain for plasticity sets the stage for improved outcome, but treatment effects are maximized when appropriate training and experience are provided in parallel. Behavioral experience and training are key to shaping brain plasticity during recovery, similar to the principles of brain plasticity during normal development.[179] As such, efficacy of MSC therapy after stroke will likely best be maximized if studies measure, if not control, the behavioral experience that patients receive during the time of brain reformatting. This experience includes standard forms of rehabilitation therapy, such as occupational therapy, physical therapy, and speech therapy, and could also include specific training for patients aiming to maximize recovery.

A fourth principle is the potential value of using modality-specific measures.[180] This point is likely of greater importance to restorative stroke trials than acute trials. Of course, achieving gains in global measures of clinical status is important to both acute and restorative stroke therapies. However, restorative therapies such as MSC achieve their effect by promoting plasticity, and trials are more likely to show treatment effects in those brain networks that have sustained subtotal injury—a behavior whose underlying brain networks are destroyed by stroke is less likely to show treatment-related gains than a behavior for which the underlying networks are accessible to treatment. A neurologic modality for which the neural underpinnings are partially spared, such as gait or arm motor function or language, might show significant gains from treatment while global outcome measures, which reflect treatment effects across many brain networks, might show only modest effects.

CONCLUSIONS AND PERSPECTIVES

Mesenchymal stromal cells are remarkable cells with high potential for treating a wide variety of human disease targets, including stroke recovery. Several features make MSC particularly attractive as a therapeutic candidate, including inherent multipotency; ability to promote repair through multiple avenues in parallel such as immunoregulation, angiogenesis, and neurogenesis; their availability from multiple potential sources; and their immunoprivileged status. A large number of diverse preclinical studies have consistently showed that MSC safely improve behavioral outcome after stroke, and initial human trials are encouraging. Additional basic and preclinical research, including efforts to understand the mechanisms of action most important to MSC effects, will provide a fuller understanding as to how to best apply MSC to improve outcome after stroke. Studies in human subjects are needed, as well, for example, because the human experience of stroke is influenced by factors not easily examined in animals, such as psychosocial factors, and human neuroanatomy and vascular anatomy differ from animal models in ways important to MSC biology. Such studies will greatly inform key issues such as selecting the patient population most likely to derive benefit, or defining the optimal time, dose, and route for introducing MSC after stroke. The current preclinical and clinical experience supports the value of continued translation of MSC as a restorative therapy for stroke.

DISCLOSURE/CONFLICT OF INTEREST

Steven Cramer has served as a consultant to GlaxoSmithKline, Pfizer/Cogstate, and Microtransponder.

REFERENCES

1 Donnan GA, Fisher M, Macleod M, Davis SM. Stroke. Lancet 2008; 371: 1612–1623.
2 NCHS Dataline. Public Health Reports. 126: 433.
3 Johnston SC, Mendis S, Mathers CD. Global variation in stroke burden and mortality: estimates from monitoring, surveillance, and modelling. Lancet Neurol 2009; 8: 345–354.
4 Teasell RW. Long-term sequelae of stroke: how should you handle stroke complications? Can Fam Physician 1992; 38: 381–388.
5 Mauldin PD, Simpson KN, Palesch YY, Spilker JS, Hill MD, Khatri P et al. Design of the economic evaluation for the Interventional Management of Stroke (III) trial. Int J Stroke 2008; 3: 138–144.
6 Ansari S, Rahman M, Waters MF, Hoh BL, Mocco J. Recanalization therapy for acute ischemic stroke, part 1: surgical embolectomy and chemical thrombolysis. Neurosurg Rev 2011; 34: 1–9.
7 Ansari S, Rahman M, McConnell DJ, Waters MF, Hoh BL, Mocco J. Recanalization therapy for acute ischemic stroke, part 2: mechanical intra-arterial technologies. Neurosurg Rev 2011; 34: 11–20.
8 Adeoye O, Hornung R, Khatri P, Kleindorfer D. Recombinant tissue-type plasminogen activator use for ischemic stroke in the United States: a doubling of treatment rates over the course of 5 years. Stroke 2011; 42: 1952–1955.
9 Trounson A. New perspectives in human stem cell therapeutic research. BMC Med 2009; 7: 29.
10 Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006; 8: 315–317.
11 Mohal JS, Tailor HD, Khan WS. Sources of adult mesenchymal stem cells and their applicability for musculoskeletal applications. Curr Stem Cell Res Ther 2012; 7: 103–109.
12 Witkowska-Zimny M, Wrobel E. Perinatal sources of mesenchymal stem cells: Wharton's jelly, amnion and chorion. Cell Mol Biol Lett 2011; 16: 493–514.
13 Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immunol 2008; 8: 726–736.
14 Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci USA 1999; 96: 10711–10716.
15 Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR. Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. Science 2000; 290: 1779–1782.
16 Sanchez-Ramos J, Song S, Cardozo-Pelaez F, Hazzi C, Stedeford T, Willing A et al. Adult bone marrow stromal cells differentiate into neural cells in vitro. Exp Neurol 2000; 164: 247–256.
17 Deng W, Obrocka M, Fischer I, Prockop DJ. In vitro differentiation of human marrow stromal cells into early progenitors of neural cells by conditions that increase intracellular cyclic AMP. Biochem Biophys Res Commun 2001; 282: 148–152.
18 Phinney DG, Prockop DJ. Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair--current views. Stem Cells 2007; 25: 2896–2902.
19 Brooke G, Cook M, Blair C, Han R, Heazlewood C, Jones B et al. Therapeutic applications of mesenchymal stromal cells. Semin Cell Dev Biol 2007; 18: 846–858.
20 Dharmasaroja P. Bone marrow-derived mesenchymal stem cells for the treatment of ischemic stroke. J Clin Neurosci 2009; 16: 12–20.
21 Cyranoski D. Canada approves stem cell product. Nat Biotechnol 2012; 30: 571.
22 Honmou O, Onodera R, Sasaki M, Waxman SG, Kocsis JD. Mesenchymal stem cells: therapeutic outlook for stroke. Trends Mol Med 2012; 18: 292–297.
23 Gögel S, Gubernator M, Minger SL. Progress and prospects: stem cells and neurological diseases. Gene Ther 2011; 18: 1–6.
24 Lindvall O, Kokaia Z. Stem cell research in stroke: how far from the clinic? Stroke 2011; 42: 2369–2375.
25 Hermann DM, Chopp M. Promoting brain remodelling and plasticity for stroke recovery: therapeutic promise and potential pitfalls of clinical translation. Lancet Neurol 2012; 11: 369–380.
26 Sensebé L, Krampera M, Schrezenmeier H, Bourin P, Giordano R. Mesenchymal stem cells for clinical application. Vox Sang 2010; 98: 93–107.
27 Lazarus HM, Haynesworth SE, Gerson SL, Rosenthal NS, Caplan AI. 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: 557–564.
28 Wang S, Qu X, Zhao RC. Clinical applications of mesenchymal stem cells. J Hematol Oncol 2012; 5: 19.
29 Vaes B, Van't Hof W, Deans R, Pinxteren J. Application of MultiStem(®) allogeneic cells for immunomodulatory therapy: clinical progress and pre-clinical challenges in prophylaxis for graft versus host disease. Front Immunol 2012; 3: 345.
30 Woodbury D, Schwarz EJ, Prockop DJ, Black IB. Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res 2000; 61: 364–370.
31 Krabbe C, Zimmer J, Meyer M. Neural transdifferentiation of mesenchymal stem cells--a critical review. APMIS 2005; 113: 831–844.
32 Oswald J, Boxberger S, Jørgensen B, Feldmann S, Ehninger G, Bornhäuser M et al. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells 2004; 22: 377–384.
33 Eglitis MA, Dawson D, Park KW, Mouradian MM. Targeting of marrow-derived astrocytes to the ischemic brain. Neuroreport 1999; 10: 1289–1292.
34 Bao X, Feng M, Wei J, Han Q, Zhao H, Li G et al. Transplantation of Flk-1 + human bone marrow-derived mesenchymal stem cells promotes angiogenesis and neurogenesis after cerebral ischemia in rats. Eur J Neurosci 2011; 34: 87–98.
35 Chen J, Li Y, Wang L, Lu M, Zhang X, Chopp M. Therapeutic benefit of intra-cerebral transplantation of bone marrow stromal cells after cerebral ischemia in rats. J Neurol Sci 2001; 189: 49–57.
36 Li Y, Chopp M, Chen J, Wang L, Gautam SC, Xu YX et al. Intrastriatal transplantation of bone marrow nonhematopoietic cells improves functional recovery after stroke in adult mice. J Cereb Blood Flow Metab 2000; 20: 1311–1319.
37 Skvortsova VI, Gubskiy LV, Tairova RT, Povarova OV, Cheglakov IB, Holodenko RV et al. Use of bone marrow mesenchymal (stromal) cells in experimental ischemic stroke in rats. Bull Exp Biol Med 2008; 145: 122–128.
38 Zhao L-R, Duan W-M, Reyes M, Keene CD, Verfaillie CM, Low WC. Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats. Exp Neurol 2002; 174: 11–20.
39 Shichinohe H, Kuroda S, Yano S, Ohnishi T, Tamagami H, Hida K et al. Improved expression of gamma-aminobutyric acid receptor in mice with cerebral infarct and transplanted bone marrow stromal cells: an autoradiographic and histologic analysis. J Nucl Med 2006; 47: 486–491.
40 Chen J, Li Y, Wang L, Zhang Z, Lu D, Lu M et al. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. Stroke 2001; 32: 1005–1011.
41 Hofstetter CP, Schwarz EJ, Hess D, Widenfalk J, El Manira A, Prockop DJ et al. Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. Proc Natl Acad Sci USA 2002; 99: 2199–2204.
42 Ikeda N, Nonoguchi N, Zhao MZ, Watanabe T, Kajimoto Y, Furutama D et al. Bone marrow stromal cells that enhanced fibroblast growth factor-2 secretion by herpes simplex virus vector improve neurological outcome after transient focal cerebral ischemia in rats. Stroke 2005; 36: 2725–2730.
43 Kurozumi K, Nakamura K, Tamiya T, Kawano Y, Kobune M, Hirai S et al. BDNF gene-modified mesenchymal stem cells promote functional recovery and reduce infarct size in the rat middle cerebral artery occlusion model. Mol Ther 2004; 9: 189–197.
44 Zhao M-Z, Nonoguchi N, Ikeda N, Watanabe T, Furutama D, Miyazawa D et al. Novel therapeutic strategy for stroke in rats by bone marrow stromal cells and ex vivo HGF gene transfer with HSV-1 vector. J Cereb Blood Flow Metab 2006; 26: 1176–1188.
45 Cramer SC, Sur M, Dobkin BH, O'Brien C, Sanger TD, Trojanowski JQ et al. Harnessing neuroplasticity for clinical applications. Brain 2011; 134: 1591–1609.
46 Cramer SC. Repairing the human brain after stroke. II. Restorative therapies. Ann Neurol 2008; 63: 549–560.
47 Hilfiker A, Kasper C, Hass R, Haverich A. Mesenchymal stem cells and progenitor cells in connective tissue engineering and regenerative medicine: is there a future for transplantation? Langenbecks Arch Surg 2011; 396: 489–497.
48 Miller RH, Bai L, Lennon DP, Caplan AI. The potential of mesenchymal stem cells for neural repair. Discov Med 2010; 9: 236–242.
49 Chen J, Li Y, Katakowski M, Chen X, Wang L, Lu D et al. Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat. J Neurosci Res 2003; 73: 778–786.
50 Steffenhagen C, Dechant F-X, Oberbauer E, Furtner T, Weidner N, Küry P et al. Mesenchymal stem cells prime proliferating adult neural progenitors toward an oligodendrocyte fate. Stem Cells Dev 2012; 21: 1838–1851.
51 Mauri M, Lentini D, Gravati M, Foudah D, Biella G, Costa B et al. Mesenchymal stem cells enhance GABAergic transmission in co-cultured hippocampal neurons. Mol Cell Neurosci 2012; 49: 395–405.
52 Ribeiro CA, Salgado AJ, Fraga JS, Silva NA, Reis RL, Sousa N. The secretome of bone marrow mesenchymal stem cells-conditioned media varies with time

and drives a distinct effect on mature neurons and glial cells (primary cultures). *J Tissue Eng Regen Med* 2011; **5**: 668–672.

53 Zhang J, Zhang Y, Xing S, Liang Z, Zeng J. Secondary neurodegeneration in remote regions after focal cerebral infarction: a new target for stroke management? *Stroke* 2012; **43**: 1700–1705.

54 Li Y, Chen J, Chen XG, Wang L, Gautam SC, Xu YX *et al*. Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. *Neurology* 2002; **59**: 514–523.

55 Shen LH, Li Y, Chopp M. Astrocytic endogenous glial cell derived neurotrophic factor production is enhanced by bone marrow stromal cell transplantation in the ischemic boundary zone after stroke in adult rats. *Glia* 2010; **58**: 1074–1081.

56 Yilmaz G, Alexander JS, Erkuran Yilmaz C, Granger DN. Induction of neuro-protective/regenerative genes in stem cells infiltrating post-ischemic brain tissue. *Exp Transl Stroke Med* 2010; **2**: 11.

57 Maltman DJ, Hardy SA, Przyborski SA. Role of mesenchymal stem cells in neurogenesis and nervous system repair. *Neurochem Int* 2011; **59**: 347–356.

58 Sakai T, Johnson KJ, Murozono M, Sakai K, Magnuson MA, Wieloch T *et al*. Plasma fibronectin supports neuronal survival and reduces brain injury following transient focal cerebral ischemia but is not essential for skin-wound healing and hemostasis. *Nat Med* 2001; **7**: 324–330.

59 Iadecola C, Anrather J. The immunology of stroke: From mechanisms to translation. *Nat Med* 2011; **17**: 796–808.

60 Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P *et al*. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood* 2002; **99**: 3838–3843.

61 Nasef A, Mathieu N, Chapel A, Frick J, François S, Mazurier C *et al*. Immuno-suppressive effects of mesenchymal stem cells: Involvement of HLA-G. *Transplantation* 2007; **84**: 231–237.

62 Meisel R, Zibert A, Laryea M, Göbel U, Däubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. *Blood* 2004; **103**: 4619–4621.

63 Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 2005; **105**: 1815–1822.

64 Krampera M, Cosmi L, Angeli R, Pasini A, Liotta F, Andreini A *et al*. Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. *Stem Cells* 2006; **24**: 386–398.

65 Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S *et al*. Mesenchymal stem cells suppress lymphocyte proliferation *in vitro* and prolong skin graft survival *in vivo*. *Exp Hematol* 2002; **30**: 42–48.

66 Bacigalupo A, Valle M, Podestà M, Pitto A, Zocchi E, De Flora A *et al*. T-cell suppression mediated by mesenchymal stem cells is deficient in patients with severe aplastic anemia. *Exp Hematol* 2005; **33**: 819–827.

67 Sato K, Ozaki K, Oh I, Meguro A, Hatanaka K, Nagai T *et al*. Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. *Blood* 2007; **109**: 228–234.

68 Marigo I, Dazzi F. The immunomodulatory properties of mesenchymal stem cells. *Semin Immunopathol* 2011; **33**: 593–602.

69 Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F *et al*. Human mesenchymal stem cells modulate B-cell functions. *Blood* 2006; **107**: 367–372.

70 Beyth S, Borovsky Z, Mevorach D, Liebergall M, Gazit Z, Aslan H *et al*. Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness. *Blood* 2005; **105**: 2214–2219.

71 Maggini J, Mirkin G, Bognanni I, Holmberg J, Piazzón IM, Nepomnaschy I *et al*. Mouse bone marrow-derived mesenchymal stromal cells turn activated macrophages into a regulatory-like profile. *PLoS ONE* 2010; **5**: e9252.

72 Li Y, Chopp M. Marrow stromal cell transplantation in stroke and traumatic brain injury. *Neurosci Lett* 2009; **456**: 120–123.

73 Ankrum J, Karp JM. Mesenchymal stem cell therapy: Two steps forward, one step back. *Trends Mol Med* 2010; **16**: 203–209.

74 Oh JY, Roddy GW, Choi H, Lee RH, Ylöstalo JH, Rosa RH *et al*. Anti-inflammatory protein TSG-6 reduces inflammatory damage to the cornea following chemical and mechanical injury. *Proc Natl Acad Sci USA* 2010; **107**: 16875–16880.

75 Ajmo CT, Vernon DOL, Collier L, Hall AA, Garbuzova-Davis S, Willing A *et al*. The spleen contributes to stroke-induced neurodegeneration. *J Neurosci Res* 2008; **86**: 2227–2234.

76 Gao J, Dennis JE, Muzic RF, Lundberg M, Caplan AI. The dynamic *in vivo* distribution of bone marrow-derived mesenchymal stem cells after infusion. *Cells Tissues Organs* 2001; **169**: 12–20.

77 Detante O, Moisan A, Dimastromatteo J, Richard M-J, Riou L, Grillon E *et al*. Intravenous administration of 99mTc-HMPAO-labeled human mesenchymal stem cells after stroke: *in vivo* imaging and biodistribution. *Cell Transplant* 2009; **18**: 1369–1379.

78 Isa A, Nehlin JO, Sabir HJ, Andersen TE, Gaster M, Kassem M *et al*. Impaired cell surface expression of HLA-B antigens on mesenchymal stem cells and muscle cell progenitors. *PLoS ONE* 2010; **5**: e10900.

79 Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringdén O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol* 2003; **31**: 890–896.

80 Nauta AJ, Westerhuis G, Kruisselbrink AB, Lurvink EGA, Willemze R, Fibbe WE. Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a nonmyeloablative setting. *Blood* 2006; **108**: 2114–2120.

81 Vendrame M, Gemma C, De Mesquita D, Collier L, Bickford PC, Sanberg CD *et al*. Anti-inflammatory effects of human cord blood cells in a rat model of stroke. *Stem Cells Dev* 2005; **14**: 595–604.

82 Mora-Lee S, Sirerol-Piquer MS, Gutiérrez-Pérez M, Gomez-Pinedo U, Roobrouck VD, López T *et al*. Therapeutic effects of hMAPC and hMSC transplantation after stroke in mice. *PLoS ONE* 2012; **7**: e43683.

83 Shi Y, Hu G, Su J, Li W, Chen Q, Shou P *et al*. Mesenchymal stem cells: a new strategy for immunosuppression and tissue repair. *Cell Res* 2010; **20**: 510–518.

84 Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI *et al*. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* 2008; **2**: 141–150.

85 Ren G, Su J, Zhang L, Zhao X, Ling W, L'huillie A *et al*. Species variation in the mechanisms of mesenchymal stem cell-mediated immunosuppression. *Stem Cells* 2009; **27**: 1954–1962.

86 Hwang JH, Shim SS, Seok OS, Lee HY, Woo SK, Kim BH *et al*. Comparison of cytokine expression in mesenchymal stem cells from human placenta, cord blood, and bone marrow. *J Korean Med Sci* 2009; **24**: 547–554.

87 Scheibe F, Ladhoff J, Huck J, Grohmann M, Blazej K, Oersal A *et al*. Immune effects of mesenchymal stromal cells in experimental stroke. *J Cereb Blood Flow Metab* 2012; **32**: 1578–1588.

88 Vogelgesang A, Dressel A. Immunological consequences of ischemic stroke: immunosuppression and autoimmunity. *J Neuroimmunol* 2011; **231**: 105–110.

89 Schinköthe T, Bloch W, Schmidt A. *In vitro* secreting profile of human mesenchymal stem cells. *Stem Cells Dev* 2008; **17**: 199–206.

90 Kagiwada H, Yashiki T, Ohshima A, Tadokoro M, Nagaya N, Ohgushi H. Human mesenchymal stem cells as a stable source of VEGF-producing cells. *J Tissue Eng Regen Med* 2008; **2**: 184–189.

91 Kinnaird T, Stabile E, Burnett MS, Shou M, Lee CW, Barr S *et al*. Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. *Circulation* 2004; **109**: 1543–1549.

92 Ma Y, Zechariah A, Qu Y, Hermann DM. Effects of vascular endothelial growth factor in ischemic stroke. *J Neurosci Res* 2012; **90**: 1873–1882.

93 Horie N, Pereira MP, Niizuma K, Sun G, Keren-Gill H, Encarnacion A *et al*. Transplanted stem cell-secreted vascular endothelial growth factor effects poststroke recovery, inflammation, and vascular repair. *Stem Cells* 2011; **29**: 274–285.

94 Sun Y, Jin K, Xie L, Childs J, Mao XO, Logvinova A *et al*. VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. *J Clin Invest* 2003; **111**: 1843–1851.

95 Toma C, Wagner WR, Bowry S, Schwartz A, Villanueva F. Fate of culture-expanded mesenchymal stem cells in the microvasculature: *in vivo* observations of cell kinetics. *Circ Res* 2009; **104**: 398–402.

96 Fisher M. Pericyte signaling in the neurovascular unit. *Stroke* 2009; **40**: S13–S15.

97 Parekkadan B, Milwid JM. Mesenchymal stem cells as therapeutics. *Annu Rev Biomed Eng* 2010; **12**: 87–117.

98 Cho YJ, Song HS, Bhang S, Lee S, Kang BG, Lee JC *et al*. Therapeutic effects of human adipose stem cell-conditioned medium on stroke. *J Neurosci Res* 2012; **90**: 1794–1802.

99 Xin H, Li Y, Shen LH, Liu X, Wang X, Zhang J *et al*. Increasing tPA activity in astrocytes induced by multipotent mesenchymal stromal cells facilitate neurite outgrowth after stroke in the mouse. *PLoS ONE* 2010; **5**: e9027.

100 Scheibe F, Klein O, Klose J, Priller J. Mesenchymal stromal cells rescue cortical neurons from apoptotic cell death in an *in vitro* model of cerebral ischemia. *Cell Mol Neurobiol* 2012; **32**: 567–576.

101 Liu N, Zhang Y, Fan L, Yuan M, Du H, Cheng R *et al*. Effects of transplantation with bone marrow-derived mesenchymal stem cells modified by Survivin on experimental stroke in rats. *J Transl Med* 2011; **9**: 105.

102 Zhang L, Li Y, Zhang C, Chopp M, Gosiewska A, Hong K. Delayed administration of human umbilical tissue-derived cells improved neurological functional recovery in a rodent model of focal ischemia. *Stroke* 2011; **42**: 1437–1444.

103 Borlongan CV, Lind JG, Dillon-Carter O, Yu G, Hadman M, Cheng C *et al*. Bone marrow grafts restore cerebral blood flow and blood brain barrier in stroke rats. *Brain Res* 2004; **1010**: 108–116.

104 Li Y, Chen J, Zhang CL, Wang L, Lu D, Katakowski M *et al*. Gliosis and brain remodeling after treatment of stroke in rats with marrow stromal cells. *Glia* 2005; **49**: 407–417.

105 Shen LH, Li Y, Chen J, Zacharek A, Gao Q, Kapke A et al. Therapeutic benefit of bone marrow stromal cells administered 1 month after stroke. J Cereb Blood Flow Metab 2007; 27: 6–13.

106 Zhang J, Li Y, Chen J, Yang M, Katakowski M, Lu M et al. Expression of insulin-like growth factor 1 and receptor in ischemic rats treated with human marrow stromal cells. Brain Res 2004; 1030: 19–27.

107 Lin Y-C, Ko T-L, Shih Y-H, Lin M-YA, Fu T-W, Hsiao H-S et al. Human umbilical mesenchymal stem cells promote recovery after ischemic stroke. Stroke 2011; 42: 2045–2053.

108 Xin H, Li Y, Shen LH, Liu X, Hozeska-Solgot A, Zhang RL et al. Multipotent mesenchymal stromal cells increase tPA expression and concomitantly decrease PAI-1 expression in astrocytes through the sonic hedgehog signaling pathway after stroke (in vitro study). J Cereb Blood Flow Metab 2011; 31: 2181–2188.

109 Ross JJ, Verfaillie CM. Evaluation of neural plasticity in adult stem cells. Philos Trans R Soc Lond B Biol Sci 2008; 363: 199–205.

110 Koh S-H, Kim KS, Choi MR, Jung KH, Park KS, Chai YG et al. Implantation of human umbilical cord-derived mesenchymal stem cells as a neuroprotective therapy for ischemic stroke in rats. Brain Res 2008; 1229: 233–248.

111 Lim JY, Jeong CH, Jun JA, Kim SM, Ryu CH, Hou Y et al. Therapeutic effects of human umbilical cord blood-derived mesenchymal stem cells after intrathecal administration by lumbar puncture in a rat model of cerebral ischemia. Stem Cell Res Ther 2011; 2: 38.

112 Ikegame Y, Yamashita K, Hayashi S-I, Mizuno H, Tawada M, You F et al. Comparison of mesenchymal stem cells from adipose tissue and bone marrow for ischemic stroke therapy. Cytotherapy 2011; 13: 675–685.

113 Koç ON, Gerson SL, Cooper BW, Dyhouse SM, Haynesworth SE, Caplan AI 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: 307–316.

114 Honmou O, Houkin K, Matsunaga T, Niitsu Y, Ishiai S, Onodera R et al. Intravenous administration of auto serum-expanded autologous mesenchymal stem cells in stroke. Brain 2011; 134: 1790–1807.

115 Fan M, Chen W, Liu W, Du G-Q, Jiang S-L, Tian W-C et al. The effect of age on the efficacy of human mesenchymal stem cell transplantation after a myocardial infarction. Rejuvenation Res 2010; 13: 429–438.

116 Wilson A, Shehadeh LA, Yu H, Webster KA. Age-related molecular genetic changes of murine bone marrow mesenchymal stem cells. BMC Genomics 2010; 11: 229.

117 Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC. Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. Transplantation 2003; 75: 389–397.

118 Figueroa FE, Carrión F, Villanueva S, Khoury M. Mesenchymal stem cell treatment for autoimmune diseases: A critical review. Biol Res 2012; 45: 269–277.

119 Li Y, McIntosh K, Chen J, Zhang C, Gao Q, Borneman J et al. Allogeneic bone marrow stromal cells promote glial-axonal remodeling without immunologic sensitization after stroke in rats. Exp Neurol 2006; 198: 313–325.

120 Hare JM, Fishman JE, Gerstenblith G, Difede Velazquez DL, Zambrano JP, Suncion VY et al. Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. JAMA 2012; 308: 1–11.

121 Zacharek A, Shehadah A, Chen J, Cui X, Roberts C, Lu M et al. Comparison of bone marrow stromal cells derived from stroke and normal rats for stroke treatment. Stroke 2010; 41: 524–530.

122 Ilic N, Brooke G, Murray P, Barlow S, Rossetti T, Pelekanos R et al. Manufacture of clinical grade human placenta-derived multipotent mesenchymal stromal cells. Methods Mol Biol 2011; 698: 89–106.

123 Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 2006; 24: 1294–1301.

124 Lane TA, Garls D, Mackintosh E, Kohli S, Cramer SC. Liquid storage of marrow stromal cells. Transfusion 2009; 49: 1471–1481.

125 Colter DC, Sekiya I, Prockop DJ. Identification of a subpopulation of rapidly self-renewing and multipotential adult stem cells in colonies of human marrow stromal cells. Proc Natl Acad Sci USA 2001; 98: 7841–7845.

126 Muraglia A, Cancedda R, Quarto R. Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model. J Cell Sci 2000; 113(Pt 7): 1161–1166.

127 Li WY, Choi YJ, Lee PH, Huh K, Kang YM, Kim HS et al. Mesenchymal stem cells for ischemic stroke: Changes in effects after ex vivo culturing. Cell Transplant 2008; 17: 1045–1059.

128 Crisostomo PR, Wang M, Wairiuko GM, Morrell ED, Terrell AM, Seshadri P et al. High passage number of stem cells adversely affects stem cell activation and myocardial protection. Shock 2006; 26: 575–580.

129 Ng F, Boucher S, Koh S, Sastry KSR, Chase L, Lakshmipathy U et al. PDGF, TGF-beta, and FGF signaling is important for differentiation and growth of mesenchymal stem cells (MSCs): transcriptional profiling can identify markers and signaling pathways important in differentiation of MSCs into adipogenic, chondrogenic, and osteogenic lineages. Blood 2008; 112: 295–307.

130 Chase LG, Yang S, Zachar V, Yang Z, Lakshmipathy U, Bradford J et al. Development and characterization of a clinically compliant xeno-free culture medium in good manufacturing practice for human multipotent mesenchymal stem cells. Stem Cells Transl Med 2012; 1: 750–758.

131 Nold P, Brendel C, Neubauer A, Bein G, Hackstein H. Good manufacturing practice-compliant animal-free expansion of human bone marrow derived mesenchymal stroma cells in a closed hollow-fiber-based bioreactor. Biochem Biophys Res Commun 2012; 12: 02142–02150.

132 Chapel A, Bertho JM, Bensidhoum M, Fouillard L, Young RG, Frick J et al. Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome. J Gene Med 2003; 5: 1028–1038.

133 Ortiz LA, Gambelli F, McBride C, Gaupp D, Baddoo M, Kaminski N 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: 8407–8411.

134 Ahmadian Kia N, Bahrami AR, Ebrahimi M, Matin MM, Neshati Z, Almohaddesin MR et al. Comparative analysis of chemokine receptor's expression in mesenchymal stem cells derived from human bone marrow and adipose tissue. J Mol Neurosci 2011; 44: 178–185.

135 Tuttolomondo A, Di Raimondo D, Di Sciacca R, Pinto A, Licata G. Inflammatory cytokines in acute ischemic stroke. Curr Pharm Des 2008; 14: 3574–3589.

136 Ponte AL, Marais E, Gallay N, Langonné A, Delorme B, Hérault O et al. The in vitro migration capacity of human bone marrow mesenchymal stem cells: comparison of chemokine and growth factor chemotactic activities. Stem Cells 2007; 25: 1737–1745.

137 Raheja LF, Genetos DC, Wong A, Yellowley CE. Hypoxic regulation of mesenchymal stem cell migration: the role of RhoA and HIF-1α. Cell Biol Int 2011; 35: 981–989.

138 Hu X, Wei L, Taylor TM, Wei J, Zhou X, Wang J-A et al. Hypoxic preconditioning enhances bone marrow mesenchymal stem cell migration via Kv2.1 channel and FAK activation. Am J Physiol Cell Physiol 2011; 301: C362–C372.

139 Ohtani Y, Minami M, Kawaguchi N, Nishiyori A, Yamamoto J, Takami S et al. Expression of stromal cell-derived factor-1 and CXCR4 chemokine receptor mRNAs in cultured rat glial and neuronal cells. Neurosci Lett 1998; 249: 163–166.

140 Lu D-Y, Tang C-H, Yeh W-L, Wong K-L, Lin C-P, Chen Y-H et al. SDF-1alpha up-regulates interleukin-6 through CXCR4, PI3K/Akt, ERK, and NF-kappaB-dependent pathway in microglia. Eur J Pharmacol 2009; 613: 146–154.

141 Kawabori M, Kuroda S, Sugiyama T, Ito M, Shichinohe H, Houkin K et al. Intracerebral, but not intravenous, transplantation of bone marrow stromal cells enhances functional recovery in rat cerebral infarct: an optical imaging study. Neuropathology 2012; 32: 217–226.

142 Gutiérrez-Fernández M, Rodríguez-Frutos B, Alvarez-Grech J, Vallejo-Cremades MT, Expósito-Alcaide M, Merino J et al. Functional recovery after hematic administration of allogenic mesenchymal stem cells in acute ischemic stroke in rats. Neuroscience 2011; 175: 394–405.

143 Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL 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.

144 Allers C, Sierralta WD, Neubauer S, Rivera F, Minguell JJ, Conget PA. Dynamic of distribution of human bone marrow-derived mesenchymal stem cells after transplantation into adult unconditioned mice. Transplantation 2004; 78: 503–508.

145 Erices AA, Allers CI, Conget PA, Rojas CV, Minguell JJ. Human cord blood-derived mesenchymal stem cells home and survive in the marrow of immunodeficient mice after systemic infusion. Cell Transplantation 2003; 12: 555–561.

146 Shen LH, Li Y, Chen J, Cui Y, Zhang C, Kapke A et al. One-year follow-up after bone marrow stromal cell treatment in middle-aged female rats with stroke. Stroke 2007; 38: 2150–2156.

147 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: 656–662.

148 Walczak P, Zhang J, Gilad AA, Kedziorek DA, Ruiz-Cabello J, Young RG et al. Dual-modality monitoring of targeted intraarterial delivery of mesenchymal stem cells after transient ischemia. Stroke 2008; 39: 1569–1574.

149 Grieve SM, Bhindi R, Seow J, Doyle A, Turner AJ, Tomka J et al. Microvascular obstruction by intracoronary delivery of mesenchymal stem cells and quantification of resulting myocardial infarction by cardiac magnetic resonance. Circ Heart Fail 2010; 3: e5–e6.

150 Willing AE, Lixian J, Milliken M, Poulos S, Zigova T, Song S et al. Intravenous versus intrastriatal cord blood administration in a rodent model of stroke. *J Neurosci Res* 2003; **73**: 296–307.

151 Chen J, Ye X, Yan T, Zhang C, Yang X-P, Cui X et al. Adverse effects of bone marrow stromal cell treatment of stroke in diabetic rats. *Stroke* 2011; **42**: 3551–3558.

152 King AJF. The use of animal models in diabetes research. *Br J Pharmacol* 2012; **166**: 877–894.

153 Bang OY, Lee JS, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. *Ann Neurol* 2005; **57**: 874–882.

154 Lee JS, Hong JM, Moon GJ, Lee PH, Ahn YH, Bang OY. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. *Stem Cells* 2010; **28**: 1099–1106.

155 Bernardo ME, Zaffaroni N, Novara F, Cometa AM, Avanzini MA, Moretta A et al. Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term *in vitro* culture and do not exhibit telomere maintenance mechanisms. *Cancer Res* 2007; **67**: 9142–9149.

156 Bhasin A, Srivastava MVP, Kumaran SS, Mohanty S, Bhatia R, Bose S et al. Autologous mesenchymal stem cells in chronic stroke. *Cerebrovasc Dis Extra* 2011; **1**: 93–104.

157 Bhasin A, Padma Srivastava MV, Mohanty S, Bhatia R, Kumaran SS, Bose S. Stem cell therapy: A clinical trial of stroke. *Clin Neurol Neurosurg* 2012 pii: S0303-8467(12)00533-1-1.

158 The STEPS Participants. Stem Cell Therapies as an Emerging Paradigm in Stroke (STEPS): bridging basic and clinical science for cellular and neurogenic factor therapy in treating stroke. *Stroke* 2009; **40**: 510–515.

159 Savitz SI, Chopp M, Deans R, Carmichael ST, Phinney D, Wechsler L. Stem Cell Therapy as an Emerging Paradigm for Stroke (STEPS) II. *Stroke* 2011; **42**: 825–829.

160 Stroke Therapy Industry Roundtable. Recommendations for standards regarding preclinical neuroprotective and restorative drug development. *Stroke* 1999; **30**: 2752–2758.

161 Albers GW, Goldstein LB, Hess DC, Wechsler LR, Furie KL, Gorelick PB et al. Stroke Treatment Academic Industry Roundtable (STAIR) recommendations for maximizing the use of intravenous thrombolytics and expanding treatment options with intra-arterial and neuroprotective therapies. *Stroke* 2011; **42**: 2645–2650.

162 Kleim JA, Jones TA. Principles of experience-dependent neural plasticity: implications for rehabilitation after brain damage. *J Speech Lang Hear Res* 2008; **51**: S225–S239.

163 Biernaskie J, Chernenko G, Corbett D. Efficacy of rehabilitative experience declines with time after focal ischemic brain injury. *J Neurosci* 2004; **24**: 1245–1254.

164 Ren J, Kaplan PL, Charette MF, Speller H, Finklestein SP. Time window of intra-cisternal osteogenic protein-1 in enhancing functional recovery after stroke. *Neuropharmacology* 2000; **39**: 860–865.

165 Grotta J, Bratina P. Subjective experiences of 24 patients dramatically recovering from stroke. *Stroke* 1995; **26**: 1285–1288.

166 Fisher M, Ratan R. New perspectives on developing acute stroke therapy. *Ann Neurol* 2003; **53**: 10–20.

167 Gladstone DJ, Black SE, Hakim AM. Toward wisdom from failure: lessons from neuroprotective stroke trials and new therapeutic directions. *Stroke* 2002; **33**: 2123–2136.

168 Hachinski V, Donnan GA, Gorelick PB, Hacke W, Cramer SC, Kaste M et al. Stroke: working toward a prioritized world agenda. *Stroke* 2010; **41**: 1084–1099.

169 Cramer SC. Stratifying patients with stroke in trials that target brain repair. *Stroke* 2010; **41**: S114–S116.

170 Nouri S, Cramer SC. Anatomy and physiology predict response to motor cortex stimulation after stroke. *Neurology* 2011; **77**: 1076–1083.

171 Stinear CM, Barber PA, Smale PR, Coxon JP, Fleming MK, Byblow WD. Functional potential in chronic stroke patients depends on corticospinal tract integrity. *Brain* 2007; **130**: 170–180.

172 Riley JD, Le V, Der-Yeghiaian L, See J, Newton JM, Ward NS et al. Anatomy of stroke injury predicts gains from therapy. *Stroke* 2011; **42**: 421–426.

173 Lai S-M, Duncan PW, Keighley J, Johnson D. Depressive symptoms and independence in BADL and IADL. *J Rehabil Res Dev* 2002; **39**: 589–596.

174 Gillen R, Tennen H, McKee TE, Gernert-Dott P, Affleck G. Depressive symptoms and history of depression predict rehabilitation efficiency in stroke patients. *Arch Phys Med Rehabil* 2001; **82**: 1645–1649.

175 Cramer SC, Parrish TB, Levy RM, Stebbins GT, Ruland SD, Lowry DW et al. Predicting functional gains in a stroke trial. *Stroke* 2007; **38**: 2108–2114.

176 Uchino K, Billheimer D, Cramer SC. Entry criteria and baseline characteristics predict outcome in acute stroke trials. *Stroke* 2001; **32**: 909–916.

177 Cramer SC. Brain repair after stroke. *N Engl J Med* 2010; **362**: 1827–1829.

178 Feeney DM, Gonzalez A, Law WA. Amphetamine, haloperidol, and experience interact to affect rate of recovery after motor cortex injury. *Science* 1982; **217**: 855–857.

179 Wiesel TN, Hubel DH. Ordered arrangement of orientation columns in monkeys lacking visual experience. *J Comp Neurol* 1974; **158**: 307–318.

180 Cramer SC, Koroshetz WJ, Finklestein SP. The case for modality-specific outcome measures in clinical trials of stroke recovery-promoting agents. *Stroke* 2007; **38**: 1393–1395.

181 Wilkins A, Kemp K, Ginty M, Hares K, Mallam E, Scolding N. Human bone marrow-derived mesenchymal stem cells secrete brain-derived neurotrophic factor which promotes neuronal survival *in vitro*. *Stem Cell Res* 2009; **3**: 63–70.

182 Cui X, Chen J, Zacharek A, Li Y, Roberts C, Kapke A et al. Nitric oxide donor upregulation of stromal cell-derived factor-1/chemokine (CXC motif) receptor 4 enhances bone marrow stromal cell migration into ischemic brain after stroke. *Stem Cells* 2007; **25**: 2777–2785.

183 Liu H, Honmou O, Harada K, Nakamura K, Houkin K, Hamada H et al. Neuroprotection by PlGF gene-modified human mesenchymal stem cells after cerebral ischaemia. *Brain* 2006; **129**: 2734–2745.

184 Shen LH, Li Y, Chen J, Zhang J, Vanguri P, Borneman J et al. Intracarotid transplantation of bone marrow stromal cells increases axon-myelin remodeling after stroke. *Neuroscience* 2006; **137**: 393–399.

185 Zhang C, Li Y, Chen J, Gao Q, Zacharek A, Kapke A et al. Bone marrow stromal cells upregulate expression of bone morphogenetic proteins 2 and 4, gap junction protein connexin-43 and synaptophysin after stroke in rats. *Neuroscience* 2006; **141**: 687–695.

186 Maas MB, Furie KL. Molecular biomarkers in stroke diagnosis and prognosis. *Biomark Med* 2009; **3**: 363–383.

187 Leu S, Lin Y-C, Yuen C-M, Yen C-H, Kao Y-H, Sun C-K et al. Adipose-derived mesenchymal stem cells markedly attenuate brain infarct size and improve neurological function in rats. *J Transl Med* 2010; **8**: 63.

188 Chen J, Shehadah A, Pal A, Zacharek A, Cui X, Cui Y et al. Neuroprotective effect of human placenta-derived cell treatment of stroke in rats. *Cell Transplant* 2012; **22**: 871–879.

189 Egashira Y, Sugitani S, Suzuki Y, Mishiro K, Tsuruma K, Shimazawa M et al. The conditioned medium of murine and human adipose-derived stem cells exerts neuroprotective effects against experimental stroke model. *Brain Res* 2012; **1461**: 87–95.

190 Yang Y-C, Liu B-S, Shen C-C, Lin C-H, Chiao M-T, Cheng H-C. Transplantation of adipose tissue-derived stem cells for treatment of focal cerebral ischemia. *Curr Neurovasc Res* 2011; **8**: 1–13.