Published in final edited form as:

Prog Neurobiol. 2014 April ; 115: 92–115. doi:10.1016/j.pneurobio.2013.11.007.

Cell based therapies for ischemic stroke: From basic science to bedside

Xinfeng Liu^{a,*}, Ruidong Ye^{a,1}, Tao Yan^{b,c}, Shan Ping Yu^d, Ling Wei^d, Gelin Xu^a, Xinying Fan^a, Yongjun Jiang^a, R. Anne Stetler^e, George Liu^f, and Jieli Chen^{b,**}

^aDepartment of Neurology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China

^bDepartment of Neurology, Henry Ford Hospital, Detroit, MI, USA

^cDepartment of Neurology, Tianjin General Hospital, Tianjin University School of Medicine, Tianjin, China

^dDepartment of Anesthesiology, Emory University School of Medicine, Atlanta, GA, USA

^eCenter of Cerebrovascular Disease Research, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

^fInstitute of Cardiovascular Sciences, Peking University Health Science Center, Beijing, China

Abstract

Cell therapy is emerging as a viable therapy to restore neurological function after stroke. Many types of stem/progenitor cells from different sources have been explored for their feasibility and efficacy for the treatment of stroke. Transplanted cells not only have the potential to replace the lost circuitry, but also produce growth and trophic factors, or stimulate the release of such factors from host brain cells, thereby enhancing endogenous brain repair processes. Although stem/progenitor cells have shown a promising role in ischemic stroke in experimental studies as well as initial clinical pilot studies, cellular therapy is still at an early stage in humans. Many critical issues need to be addressed including the therapeutic time window, cell type selection, delivery route, and *in vivo* monitoring of their migration pattern. This review attempts to provide a comprehensive synopsis of preclinical evidence and clinical experience of various donor cell types, their restorative mechanisms, delivery routes, imaging strategies, future prospects and challenges for translating cell therapies as a neurorestorative regimen in clinical applications.

Keywords

Stem cells; Cell-based therapies; Ischemic stroke; Neurorestoration

© 2013 Elsevier Ltd. All rights reserved.

*Corresponding author at: Department of Neurology, Jinling Hospital, Nanjing University School of Medicine, Nanjing 210002, China. Tel.: +86 25 84801861; fax: +86 25 84664563. xfliu2@vip.163.com. **Corresponding author at: Department of Neurology, Henry Ford Hospital, Detroit, MI 48202, USA. Tel.: +1 313 916 1991; fax: +1 313 916 1318. jieli@neuro.hfh.edu.

¹Both these authors contributed equally to this work.

Conflict of interest

None declared.

1. Introduction

Stroke remains a worldwide health burden, causing high morbidity, mortality, and costs to health care (Feigin et al., 2009; Johnston et al., 2009), and is the primary cause of serious long-term disability in the United States, leading to \$38.6 billion in direct and indirect costs in 2009 (Go et al., 2013). Ischemic stroke accounts for over 80% of the total number of strokes. Currently the only validated therapy for ischemic stroke is thrombolysis, which must be administered within 4.5 h after onset (Del Zoppo et al., 2009). Due to its narrow therapeutic time window and the concern of hemorrhagic complication, thrombolysis is still not used regularly (Liu, 2012). Approximately 5% of stroke patients benefit from reperfusion therapies, and even so, only 10% of the stroke survivors return to independent living. In this context, development of neurorestorative therapies to improve neurological deficits after ischemic stroke is a great challenge for both bench scientists and clinical investigators.

After decades of research focused on acute neuroprotection and the failure of produce much in the way of tangible results (Ginsberg, 2008; Fisher, 2011), the Stroke Progress Review Group has identified neurorestoration as a major priority for stroke research (Grotta et al., 2008). Cell therapy is emerging as a viable neurorestorative therapy for stroke (Zhang and Chopp, 2009). A paucity of studies was reported in previous decades, yet the past 5 years have witnessed a remarkable surge in publications on this topic. Based on these reports, this review attempts to provide a comprehensive synopsis of preclinical evidence and clinical experience using various donor cell types, their restorative mechanisms, delivery methods, imaging strategies, future prospects and challenges for translating cell therapies as neurorestorative therapy for stroke in clinical applications.

2. Restorative mechanisms of cell-based therapies

In this section, we discuss the potential mechanisms of cell-based therapy-induced neurorestorative effects after stroke, which includes cell replacement, enhanced trophic/regenerative support from transplanted cells, immunomodulation, and stimulation of endogenous brain repair processes (such as angiogenesis, arteriogenesis, neurogenesis, synaptogenesis and white matter remodeling).

2.1. Cell replacement

The initial goal of stem cell transplantation was to reconstruct the disrupted cytoarchitecture of stroke-damaged tissue. However, the context of stroke is a complex entity, which would require the survival of grafted cells in an inhospitable environment that includes inflammatory reactions, necrotic cell leakage and glial scar formation (Buhnemann et al., 2006). For stem/progenitor cell therapy, usually several million cells are transplanted into stroke animals. Once locally or systematically injected, stem/progenitor cells exhibit a certain degree of targeted migration toward the damaged regions (*i.e.* pathotropism) (De Feo et al., 2012). Implanted stem/progenitor cells can follow the gradients of chemoattractants, including vascular cell adhesion molecule 1 (VCAM-1), stromal-derived factor 1 (SDF-1), monocyte chemotactic protein-1 (MCP-1), chemokine (C-C motif) ligand 2 (CCL2), and other cytokines that aid in the localization to the damaged central nervous system (CNS)

parenchyma (Guzman et al., 2008c). By quantitative estimation, approximately 1/3 of the locally injected cells migrate to the focal infarct area (Kelly et al., 2004; Darsalia et al., 2007). Contralateral parenchymal grafting yielded similar migration efficiency along the corpus callosum (Modo et al., 2002c; Veizovic et al., 2001). However, upon intravascular delivery, as expected, significantly fewer (1–10%) exogenous cells arrive to the lesion area (Li et al., 2001b, 2002).

Among these migrated cells, one may ask, how many will integrate into the lost circuits? Many groups have reported variable numbers of grafted cells differentiating into mature neurons. The success of attaining a mature neuronal phenotype appears to depend on the source of the stem cells: 34–60% of neural stem cells (NSCs) (Takagi et al., 2005; Darsalia et al., 2007; Ishibashi et al., 2004), 40–66% of induced pluripotent stem cells (iPSCs) (Oki et al., 2012; Jensen et al., 2013), 30% of embryonic stem cells (ESCs) (Buhneemann et al., 2006), and 2–20% of mesenchymal stem cells (MSCs) (Chen et al., 2001a, 2001b) differentiated into neurons expressing mature or immature neuronal markers like NeuN, HuD, and MAP2. A 1-year follow-up study demonstrated that 16.8% of intra-arterially injected bone marrow stromal cells (BMSCs) became neurons (Shen et al., 2007). Specifically, most neuronal phenotypes residing in the damaged area could be regenerated from grafted cells, including GABAergic (GAD67+) neurons, glutamatergic (vGlut+) neurons, dopaminergic (TH+) neurons, interneurons (calbindin+ and parvalbumin+), and medium spiny projection neurons (DARPP-32+) (Darsalia et al., 2007; Takagi et al., 2005; Emborg et al., 2013). Maturation into astrocytes and microglia has also been reported, but to a lesser extent (Chu et al., 2004). The maturation into a neuronal phenotype was further confirmed by the electrophysiological detection of voltage-gated sodium currents (Buhneemann et al., 2006; Oki et al., 2012; Daadi et al., 2009). The presence of these currents allow for the firing of action potentials in mature neurons.

2.2. Enhanced trophic/regenerative support from transplanted cells

Despite the aforementioned histological and electrophysiological evidence, it is difficult to attribute graft-mediated behavioral recovery to the small number of cells replaced. Above all, even in a rodent stroke model, a moderate to severe middle cerebral artery occlusion (MCAO) would cause over 2×10^7 cells die, approximately 75% of which are neurons (Williams and Herrup, 1988). Neural integration may not always be necessary for beneficial outcomes afforded by transplantation-based therapy (Borlongan et al., 2004; Leong et al., 2012).

To this end, a possible novel role for cell-based therapy has been proposed and explored. A considerable portion of grafted cells maintains an undifferentiated phenotype nearby or far away from the lesion of host tissue, where these undifferentiated stem/progenitor cells can directly release growth and trophic factors, or promote the release of such factors from host brain cells (Smith and Gavins, 2012), providing so-called bystander effect. This function may thus trump cell replacement and underpin the recovery seen in experimental stroke with stem cells independent of differentiation (Martino and Pluchino, 2006). The bystander effect was initially described as a feature of NSCs but has also been proposed to explain the

therapeutic effect by other stem/ progenitor cells with lower capacity for neural differentiation (Smith and Gavins, 2012).

A landmark study from Borlongan's group revealed that systemic transplantation-induced benefit effects against stroke could occur without evidence of the grafted cells entering the CNS (Borlongan et al., 2004). Instead, it is most likely due to the transplanted cells-released trophic or growth factors, which traffic across the blood-brain barrier (BBB) and into the injured brain site. This concept was also supported by recent observations that conditioned media from various types of stem/progenitor cells protected the brain from ischemic impairment (Egashira et al., 2012; Cho et al., 2012; Inoue et al., 2013). The trophic factors like glial cell-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), and insulin-like growth factor 1 (IGF-1) have been implicated in grafted cell-induced protection against MCAO or neonatal hypoxia/ischemia (Wei et al., 2009; Borlongan et al., 2004). Trophic and growth factors are survival and/or differentiation factors for murine neuronal progenitor cells (Mehler et al., 1993) and they may play an important role in proliferation or differentiation of host neural tissue.

2.3. Immunomodulation

Recent investigations of various CNS diseases have shed light on the interaction between stem cells and cells belonging to the innate and adaptive branches of the immune system (Kokaia et al., 2012). Immune cells can have double-edged effects on the migration/survival of grafted cells, but stem cells also appear to possess modulatory functions targeted to immune cells in a constitutive, and possibly an evolutionarily conserved way (Martino and Pluchino, 2006), as evidenced by the significant percentage (18%) of immune-related genes (Pluchino et al., 2009; Kokaia et al., 2012).

Furthermore, stem cells have been demonstrated to possess the capacity to inhibit T lymphocyte proliferation and dendritic cell maturation (Pluchino et al., 2009; Kokaia et al., 2012). In experimental autoimmune encephalomyelitis and multiple sclerosis, transplantation of NSCs suppresses proliferation and/or promotes apoptosis of CNS-infiltrating T cells (Pluchino et al., 2005; Einstein et al., 2007; Martino et al., 2010). In the case of stroke, various types of cell transplantation, including NSCs (Bacigaluppi et al., 2009; Lee et al., 2008), MSCs (Sheikh et al., 2011; McGuckin et al., 2013), iPSCs (Chang et al., 2013b), bone marrow mononuclear cells (Franco et al., 2012), and umbilical cord matrix cells (Hirko et al., 2008), have been reported to decrease post-ischemic inflammatory damage. Perilesional activation of astrocytes and microglial cells and CNS recruitment of blood-derived inflammatory cells can be strongly inhibited in the sub-acute phase of stroke by umbilical cord matrix cell or MSC transplantation (Sheikh et al., 2011; Hirko et al., 2008). MSC transplantation also down-regulated a series of pro-inflammatory mediators, involving a non-canonical Janus kinase/Signal Transducer and Activator of Transcription (JAK-STAT) signaling of unphosphorylated signal transducer and activator of transcription 3 (STAT3) (McGuckin et al., 2013).

Interestingly, it is worth noting that immune modulation in the infarct zone by grafted cells may be independent of differentiation into the neuronal phenotype, or even tissue integration into the infarcted region. A large population of systemically administered stem cells (NSCs

or BMSCs) remained in spleen, despite exerting protective effects in ischemic brain (Lee et al., 2008). Furthermore, transplanted NSCs attenuated splenic inflammatory mediators, and splenectomy eliminated the reduction of cerebral inflammation and brain edema afforded by NSCs in a model of hemorrhagic stroke (Lee et al., 2008). Further support stems from the findings that immune modulation is usually accompanied with increased bioavailability of soluble cytokines such as leukemia inhibitory factor (LIF), GDNF, and BDNF, which regulate factors of circulating immune cells (Martino et al., 2011). As discussed above and elsewhere in this review, GDNF and BDNF have been implicated in the neuroprotection afforded by stem cell therapy (Wei et al., 2009; Borlongan et al., 2004), and transplanted NPCs or BMMCs exerted modulatory effects on microglia and suppressed inflammation in the brain (Franco et al., 2012; Bacigaluppi et al., 2009). It is thus suggested that the immunomodulatory effect can be, at least partially, attributed to the release of neurotrophic factors from non-neuronally differentiated grafted cells, especially those undifferentiated cells outside CNS (Kokaia et al., 2012).

2.4. Stimulation of endogenous brain repair processes

2.4.1. Angiogenesis—Angiogenesis is a well-established event occurring in stroke-affected regions. Although excessive angiogenesis may have adverse consequences through worsening edema and hemorrhage in acute stages (Chen et al., 2011b), angiogenesis is essential for ischemic brain repair because it stimulates blood flow and metabolism in the ischemic boundary zone (IBZ) (Zhang and Chopp, 2009). In a small-sample autopsy study, stroke patients with a high density of cerebral blood vessels seemed to survive longer than patients with low vessel density (Krupinski et al., 1994). Proliferating endothelial cells increase as early as 1 day following cerebral ischemia and new vessels continue to grow in the penumbra for at least 21 days (Hayashi et al., 2003). This proangiogenic state may be due to the rapid upregulation of growth factors related to mitogen and endothelial formation (Ergul et al., 2012). For instance, vascular endothelial growth factor (VEGF) exerts remarkable proangiogenic and vascular permeability effects after stroke (Valable et al., 2005; Chen et al., 2003b), and is required for the formation of newly formed vessels by vasculogenesis or angiogenesis. On the other hand, angiopoietin-1 (Ang-1) is involved in maturation, stabilization, and remodeling of blood vessels (Kocsis and Honmou, 2012). Transplanted cells are also able to promote angiogenic cytokines, thereby increasing the proliferation of existing vascular endothelial cells in the first 2 weeks after cerebral ischemia (Zhang et al., 2011b; Liu et al., 2006; Nakano-Doi et al., 2010). Accordingly, increased neovasculature and microvascular perfusion are observed at later times following transplantation (Shyu et al., 2006; Bao et al., 2011). VEGF was increased the conditioned medium derived from MSCs (Tate et al., 2010), demonstrating that these cells are capable of releasing angiogenic factors into the extracellular milieu. Kocsis's and other groups further demonstrated that stem cells genetically modified to overexpress VEGF, Ang-1, or placental growth factor (PIGF) had greater angiogenic and therapeutic effects (Liu et al., 2006; Onda et al., 2008; Toyama et al., 2009; Miki et al., 2007; Lee et al., 2007b; Zhu et al., 2005).

2.4.2. Neurogenesis—Neurons are consistently generated from progenitor cells within the subventricular zone (SVZ) and the hippocampal dentate gyrus in naïve rodents (Zhao et al., 2008). For the most part, neural progenitors in the SVZ of adult rodents travel through

the rostral migratory stream (RMS) to the olfactory bulb, where they differentiate into interneurons (Alvarez-Buylla and Garcia-Verdugo, 2002). Stroke can induce a transient increase of cell proliferation in the ipsilateral SVZ with maximum at 1–2 weeks after cerebral ischemia. Instead of migrating to the olfactory bulb, neuroblasts migrate from RMS to the ischemic boundary regions, mature into resident neurons, and integrate into the parenchymal tissue (Arvidsson et al., 2002; Jin et al., 2001). Neurogenesis is also associated with cognitive function in adult brain, centering around newly maturing granule cells in the dentate gyrus (Aimone et al., 2011). After global cerebral ischemia, ionizing radiation applied to the subgranular zone of the dentate gyrus reduced neurogenesis and impaired working memory recovery (Raber et al., 2004), suggesting stroke sequelae might be worse in the absence of constitutive neurogenesis.

Stem cell transplantation can accelerate the proliferation of progenitors and neuroblasts in the ipsilateral SVZ over an extended timeframe (up to 14 weeks after MCAO) (Jin et al., 2011; Bao et al., 2011; Mine et al., 2013). Implantation of human NSCs into MCAO-affected striatum led to the migration and survival of approximately 3-times more young neurons in the infarcted region (Mine et al., 2013). However, there is no direct evidence whether upregulation of neurogenesis is powerful enough to harness for functional recovery after stroke. These critical issues need to be evidence for further improvement of functional benefits of cell therapy.

2.4.3. Neurovascular niche—Several groups have observed concurrent enhanced angiogenesis and neurogenesis upon cell transplantation after cerebral ischemia (Bao et al., 2011; Wei et al., 2012; Zhang et al., 2011b; Taguchi et al., 2004). Therefore, cell-based therapy may promote neurovascular function thereby promoting functional outcome after stroke. Admittedly, stem cell-augmented angiogenesis and micro-perfusion in the infarct area provide an instructive niche for neurogenic plasticity (Goldman and Chen, 2011). The migration of neuroblasts from the SVZ to the site of injury appears to occur in conjunction with vascular remodeling or angiogenesis, such that blood vessels may function as a scaffold for NPCs migration toward the damaged brain region (Ohab et al., 2006). *In vivo* blockage of angiogenesis in the endostatin, a direct angiogenesis inhibitor, substantially attenuated migration of neuroblasts in the SVZ to the ischemic region with or without stem/progenitor cell therapy (Ohab et al., 2006; Nakano-Doi et al., 2010), indicating a causal link between angiogenesis and neurogenesis. Vascular productions of SDF-1 and Ang-1 play pivotal roles in this coordination by attracting neighboring neuroblasts expressing the receptors C-X-C chemokine receptor type 4 (CXCR4) and Tie2 (Ohab et al., 2006). The angiogenically active microvasculature modulates the local guidance of NSC through endothelial cell-derived chemoattractants. These experiments define a novel brain environment for neuronal regeneration after stroke and identify molecular mechanisms such as vascular and neuronal growth factors (BDNF, VEGF, SDF-1, Tropomyosin receptor kinase B (TrkB), neuropilin-1, etc.) and CXCR4 that are shared between angiogenesis and neurogenesis during functional recovery from brain injury.

2.4.4. White matter remodeling—Stem cell therapeutics also involve white matter remodeling (Chopp et al., 2009). Though only a small portion of grafted cells matures into

an oligodendrocyte phenotype (Hicks et al., 2009; Takagi et al., 2005), endogenous progenitor and mature oligodendrocytes were significantly increased in the ipsilateral ischemic hemisphere after stem cell grafting (Li et al., 2005; Daadi et al., 2009; van Velthoven et al., 2010). The ischemic reduction of the corpus callosum was also significantly prevented with stem cell (MSCs) treatment (Li et al., 2005; van Velthoven et al., 2012b). Further detailed histological assessments revealed that cell transplantation promoted dendritic plasticity, which coincides with functional recovery. Corticocortical, corticostriatal, corticothalamic and corticospinal axonal rewiring from the contralateral side and axonal transport were also restored (Andres et al., 2011b). This process was correlated with the secretion of developmental guidance molecules such as slit and thrombospondin 1 and 2 (Andres et al., 2011b), which are critical for the development of ultrastructurally normal synapses (Christopherson et al., 2005). Stem/progenitor cell treatment may create new circuitry in both ipsilateral and contralateral hemispheres, and even in spinal cord (Liu et al., 2007), through reconstructing of axons and myelin (Shen et al., 2006; Li et al., 2006).

3. Cellular therapies from different cell sources

In contrast to Parkinson's disease or amyotrophic lateral sclerosis, which involves the degeneration of a specific population of neurons, stroke affects a heterogeneous population of brain cell types and vascular cells over large brain regions. Thus the ideal cell therapies for stroke require not only the direct replacement of multiple lost cell types and restoration of functional and appropriate neuronal connections, but also the reconstruction of disrupted vascular systems. Multiple stem/progenitor cells have been tested as potential sources for cell based therapy for stroke. As summarized in Table 1, these cells have demonstrated the ability to survive, mature, migrate to the lesion, and decrease neurological sequelae induced by stroke attack.

3.1. Embryonic stem cells (ESCs)

ESCs are pluripotent stem cells derived from the inner cell mass of an early stage embryo (blastocyst) (Thomson et al., 1998). It has been reported that ESCs can give rise to functional neurons, astrocytes, and oligodendrocytes *in vitro* (Lee et al., 2000; Fraichard et al., 1995; Wichterle et al., 2002), thereby demonstrating the regenerative capacity of ESCs in CNS. Murine ESCs implanted into the contralateral hemisphere following transient cerebral ischemia migrated along the corpus callosum to the ventricular walls, massively populating the border zone of the damaged brain tissue (Hoehn et al., 2002), and correlated with improvements in histological and behavioral outcomes (Nagai et al., 2010; Yanagisawa et al., 2006). Grafted mouse ESCs also form synaptic connection in the recipient brain (Tae-Hoon and Yoon-Seok, 2012).

However, undifferentiated ESCs tend to generate teratomas or even highly malignant teratocarcinomas in intact animals (Reubinooff et al., 2000; Thomson et al., 1998) and in the stroke rodents (Erdo et al., 2003; Nagai et al., 2010). Interestingly, xenotransplantation (*i.e.*, stem cells derived from a different species) appears to suppress tumorigenic formation compared to homologous grafting (Erdo et al., 2003), but raises many issues concerning graft rejection. One possible approach is to use *in vitro* pre-differentiated ESCs that become post-mitotic to minimize their tumorigenic potential. Although one group found teratoma

formation was independent of pre-differentiation (Erdo et al., 2003), several other groups have found efficacy of pre-differentiated grafts without evidence of tumor formation. ESCs-derived neural progenitor cells (Takagi et al., 2005; Fujimoto et al., 2012; Hayashi et al., 2006), vascular progenitor cells (Oyamada et al., 2008), and mesenchymal stem cells (MSCs) (Liu et al., 2009) have been shown to exert salutary effects after stroke without noticeable tumorigenesis.

Neuronal precursors derived from human ESCs reduced infarct volume, increased neurogenesis, and improved behavioral out-come after distal MCAO in both young adult (3-month-old) and aged (24-month-old) rats (Jin et al., 2010, 2011). In such cases, ESCs may provide more homogeneous and vital daughter cells. Currently there are no clinical studies using ESCs for stroke treatment. Several drawbacks of ESCs limit their potential for clinical translation, including ethical concerns, immunological response, limited availability, and heterogeneity of donor cells. These issues need to be resolved to encourage more extensive investigations on ESCs.

3.2. Neural stem/precursor cells

The use of regenerative CNS tissue for stroke can be traced back to the 1980s, when Polezhaev et al. transplanted homologous embryo brain cortex tissue into brain regions of rats subjected to hypoxia (Polezhaev and Alexandrova, 1984). The grafted cells established a close morphological connection with neighboring neurons and improved electrophysiological performance (Polezhaev et al., 1985). These beneficial effects would be assumedly attributed to the proliferative NSCs within the grafted tissues.

3.2.1. Lateral ganglionic eminence (LGE) cells—Fetal cells from porcine primordial striatum, also called lateral ganglionic eminence (LGE), has been shown to survive for at least 3 months when transplanted into striatum of ischemic rats, and induce synaptogenesis within the graft and the host (Savitz et al., 2002). Rodents transplanted with LGE at 14 days after middle cerebral artery occlusion (MCAO) showed significant functional improvement compared with controls when assessed 4 weeks after implantation (Savitz et al., 2002). Unfortunately, the phase I clinical trial assessing LGE cell feasibility for basal ganglia infarcts was terminated after 2 patients developed worsening motor deficits and seizures (Savitz et al., 2005).

3.2.2. Neural stem cells (NSCs)—Later on, highly purified NSCs were isolated from fetal SVZ (Kelly et al., 2004; Ishibashi et al., 2004), fetal temporal lobe (Zhang et al., 2009a) or adult hippocampus (Toda et al., 2001), and were expanded *in vitro*. After intraparenchymal transplantation of these human NSCs into rodent brain, neurospheres displayed robust tropism toward lesions, even when injected 7 days before an ischemic episode (Guzman et al., 2008a). Not surprisingly, embryonic NSCs gave rise to more surviving cells (almost 16 times higher) with less inflammatory response compared to adult NSCs (Takahashi et al., 2008). The transplanted cells that migrated to the ischemic lesion mainly matured into a neuronal phenotype (Darsalia et al., 2007), with a much smaller population converting into astrocytes, microglia (Guzman et al., 2008a; Kelly et al., 2004), or even endothelial cells (Ii et al., 2009). Accordingly, sensorimotor and spatial learning

functions were improved upon NSC supplementation into ischemic rodents (Mine et al., 2013; Andres et al., 2011b; Toda et al., 2001; Zhang et al., 2009b). In addition to the evidence from rodent models, a macaque stroke model demonstrated that intraparenchymal transplantation of human NSCs could survive up to 105 days with partial neuronal differentiation (Roitberg et al., 2006), but neurologic outcomes were not assessed in this study. To avoid parenchymal damage caused by direct injection, intrathecal transplantation of NSCs by lumbar puncture has also been performed. NSCs migrated from cerebrospinal fluid into the subependymal ischemic region, and improved the behavioral deficits (Seyed Jafari et al., 2011).

Intravascular transplantation of NSCs has also been fairly well-studied. Although intravenous infusion results in transplanted cells becoming trapped in filtering organs (Fischer et al., 2009), thus delivering reduced numbers NSCs into the brain than intra-arterial methods (Pendharkar et al., 2010), both intravascular paradigms have been effective in preserving neurologic function following ischemic stroke (Shen et al., 2010a; Bacigaluppi et al., 2009; Rosenblum et al., 2012; Guzman et al., 2008c). Intra-arterial transplantation of NSCs at 3 days after hypoxia–ischemia results in the highest cell engraftment and neuronal expression than delivered at other time points (Rosenblum et al., 2012), but it is not known whether this is associated with improved behavioral outcomes.

3.2.3. MHP36 cell line—An alternative to NSC therapy is to immortalize precursor cells *via* the incorporation of an immortalizing oncogene into the NSC genome to generate clonal neural precursor cells, as exemplified by MHP36 cells (Patkar et al., 2012). MHP36 rodent hippocampal clone cells are conditionally immortalized stem cells derived from the H-2K^b-tsA58 transgenic mouse neuroepithelium (Jat et al., 1991). Modified to express the temperature sensitive tsA58 oncogene, MHP36 cells can be expanded without limit at low temperature (33 °C) *in vitro*. At 37 °C, the oncogene is switched off, thereby halting cell division and inducing differentiation following transplantation into the brain (Wong et al., 2005). A series of reports have certified MHP36 cells as functionally efficacious grafts in promoting recovery after experimental stroke (Veizovic et al., 2001; Modo et al., 2002c; Patkar et al., 2012). Although grafting was associated with an acute inflammatory reaction, immunosuppression might not be required for the survival of MHP36 cells (Modo et al., 2002b; Wong et al., 2005). In a long-term observational study, implanted MHP36 cells migrated into the ischemic regions and survived for at least 1 year, at which time the histological and functional improvement was sustained (Modo et al., 2009). MHP36 grafting following of stroke promoted neuronal differentiation, synaptic plasticity, and axonal projections, as well as improved functional outcomes in mice (Patkar et al., 2012). However, the fate of implanted MHP36 cells and whether their daughter cells fully integrate into the infarcted tissue remains largely unclarified. Other examples of immortalized NSCs include CTX0E03 human NSCs (Pollock et al., 2006; Smith et al., 2012; Stroemer et al., 2009), C17.2 mouse NSCs (Guzman et al., 2008c), and Hb1.F3 human NSCs (Lee et al., 2007a).

3.3. Induced pluripotent stem cells (iPSCs)

iPSCs can be established by transduction of defined transcriptional factors (*Oct3/4*, *Sox2*, *Klf4*, and *c-Myc*) into skin fibroblasts (Takahashi et al., 2007a; Takahashi and Yamanaka,

2006). iPSCs were recently introduced as a potential cell source have been used as an appealing cell source for cell transplantation to repair neuronal network disrupted by various CNS diseases, including ischemic stroke (Ito et al., 2012). iPSCs share similar features compared to ESCs in morphology, proliferative abilities, epigenetic status of pluripotent cell-specific genes, and telomerase activity, but with the benefit that iPSCs can be produced from a patient's own skin fibroblasts, thereby sparing the risk of immune rejection and ethical issues (Abe et al., 2012). In a side-by-side comparison between the therapeutic efficacies of murine iPSCs and ESCs in experimental cerebral ischemia, both stem cell sources demonstrated comparable effects in functional, histological, and metabolic recovery (Wang et al., 2013). Four weeks after transplantation, most of the grafted cells of both types had integrated into the area close to the infarction and expressed astrocyte or mature neuronal markers (Wang et al., 2013). Murine iPSCs generated from mouse embryonic fibroblasts and transplanted prior to MCAO remarkably reduced infarct volume and ameliorated the neurological outcomes (Chen et al., 2010b). Human iPSCs also improved short-term sensorimotor recovery following ischemia (Jiang et al., 2011a).

Like ESCs, tumorigenicity of grafted iPSCs is a critical problem that needs to be addressed. Secondary neurospheres from differently sourced iPSCs formed teratomas in mouse brains in a source-dependent manner (Miura et al., 2009). Abe's group demonstrated that intracerebrally transplanted mice undifferentiated iPSCs expanded and formed much larger tumors in postischemic brain than in naive brain (Kawai et al., 2010), which was consistent with other reports (Chen et al., 2010b). iPSC-derived tumors had increased the expression of matrix metalloproteinase-9 (MMP-9) and phosphorylated vascular endothelial growth factor receptor2 (p-VEGFR2), which might be involved in promoting the teratoma formation (Yamashita et al., 2011).

Alternatively, many researchers have begun to take advantage of *in vitro* neural differentiation methods, such as serum-free culture of embryoid body-like aggregates (SFEB) (Eiraku et al., 2008) to generate neural precursor cell lines from iPSCs. When these iPSCs-derived NPCs were used as donor cells for stroke therapy (Oki et al., 2012; Gomi et al., 2012; Chang et al., 2013b; Polentes et al., 2012), no tumor formation or transplant overgrowth was detected. Despite some remaining undifferentiated cells (Jensen et al., 2013), the majority of the grafted cells differentiated to electrophysiologically functional neurons of a different phenotypes (Oki et al., 2012; Gomi et al., 2012). The neurorestorative effects of iPSC-derived NPC grafts were comparable with those reported using NSCs or ESCs-derived NSCs (Polentes et al., 2012).

More recently, functional dopaminergic or glutaminergic neurons have been generated directly from human skin fibroblasts without reversion to a pluripotent state (Caiazzo et al., 2011; Pang et al., 2011). These induced neuronal cells (iNCs) may be safer with low tumorigenicity compared with iPSCs. However, it remains to be determined whether iNCs exert therapeutic effects in stroke without tumor formation or immune rejections (Abe et al., 2012).

3.4. Mesenchymal stem cells (MSCs)

The terms *mesenchymal stem cells* and *marrow stromal cells* have been used interchangeably. Mesenchyme is embryonic connective tissue that is derived from the mesoderm that differentiates into hematopoietic and connective tissue, whereas marrow stromal cells do not differentiate into hematopoietic cells. MSCs are spindle-shaped plastic-adherent cells consisting of a heterogeneous collection of mesenchymal stem and progenitor cells and were first defined as a population of plastic-adherent fibroblastic cells isolated by Percoll density centrifugation (Glover et al., 2012). MSCs can be derived from almost all tissues of the body including bone marrow, placenta, muscle, skin, dental pulp, adipose tissue, umbilical cord, and Wharton's jelly. Mounting evidence indicated that these MSCs are a favorable cell type for autologous cell transplantations in the scenario of stroke (Honmou et al., 2012). Though MSCs from different sources are not entirely the same (Sanberg et al., 2012), their therapeutic difference for stroke treatment is still unclear. In this review, we will focus on MSCs derived from bone marrow and placenta.

3.4.1. Bone marrow derived MSCs (BMSCs)—In animal models, post-stroke human BMSC transplantation improved sensorimotor function (Huang et al., 2013; Chen and Chopp, 2006; Chen et al., 2001b; Zhao et al., 2002), enhanced synaptogenesis, stimulated nerve regeneration (Tohill et al., 2004), decreased tissue plasminogen activator (tPA)-induced brain damage (Liu et al., 2012a) and mediated immunomodulatory effects (Yoo et al., 2009). We have demonstrated that BMSC therapy can reduce neurological functional deficits when administered intravenously at 1 day or 7 days after stroke (Chen et al., 2001b). Transplanted adult BMSCs migrate to damaged tissue in brain and decrease post-stroke functional deficits (Li et al., 2000, 2001a). Migration may be aided by the disruption of BBB enabling selective entry of BMSCs into ischemic brain compared to normal cerebral tissue.

Interestingly, the mechanisms of action of BMSCs are not in line with the original concept for cellular replacement by stem/progenitor cells transplantation. The totipotent nature of stem and progenitor cells was first used with the intention to replace dead and injured tissue when placed in injured brain (Riess et al., 2002). Although BMSCs also contain a subpopulation of stem-like cells, which are capable of differentiating into brain cells (Sanchez-Ramos et al., 2000), these cells are only a minor subpopulation of the BMSCs. Only a very small percentage of the BMSCs assume parenchymal cell phenotype (Li et al., 2002). Thus it does not appear likely that this subpopulation significantly contributes to functional recovery following stroke. Instead, several lines of evidence point to the concept that transplanted BMSCs promote neurological recovery by stimulating endogenous repair *via* the release of soluble trophic factors and cytokines (Smith and Gavins, 2012). We and others have shown that exogenous cells such as BMSCs do indeed produce trophic factors, as well as stimulate the production of neuroprotective and neurorestorative factors in parenchymal cells (Li et al., 2002; Chen et al., 2003b; Hess and Borlongan, 2008; Takahashi et al., 2008; Yasuhara et al., 2010). In particular, intravenously injected BMSCs enter the brain and stimulate local production of growth factors from endogenous cells like astrocytes and endothelial cells (Chen et al., 2002b, 2003b; Yang et al., 2009; Annabi et al., 2003; Shen et al., 2010b). These growth factors, including VEGF/Flk1 and Ang-1/Tie2 (Zacharek et al., 2007), lead to angiogenesis and vascular stabilization (Chen et al., 2001b).

BMSCs express a wide spectrum of angiogenic/arteriogenic cytokines including VEGF, basic fibroblast growth factor 2 (bFGF/ FGF2), placental growth factor (PIGF), IGF, and Ang-1 (Tang et al., 2005; Kinnaird et al., 2004a, 2004b; Ponte et al., 2007; Wu et al., 2007). Intravenous administration of BMSCs leads to a time-dependent release of neurotrophins and angiogenic growth factors like BDNF, nerve growth factor (NGF), VEGF, IGF-1, hepatocyte growth factor (HGF), and GDNF (Chen et al., 2002a, 2002b, 2003b; Shen et al., 2010b; Zhu et al., 2005). Taken together, the analogy can be made that BMSCs are, in effect, molecular “factories” that induce vascular and parenchymal cells, leading to the production of many cytokines and trophic factors (Eaves et al., 1991; Majumdar et al., 1998). The production of these molecules all contributes to and likely coordinates the improvement in neurological function post stroke. These cytokines and growth factors have both paracrine and autocrine activities (Matsuda-Hashii et al., 2004) and are signaling molecules which regulate cell survival, proliferation and differentiation. For example, GDNF promotes neurogenesis, endogenous cell repairs, neuroblast proliferation and migration from SVZ and decreases apoptosis (Kobayashi et al., 2006). Multipotent BMSCs can modulate tPA activity, in part by stimulating astrocytic secretion of tPA and down-regulating the tPA inhibitor plasminogen activator inhibitor-1 (Xin et al., 2010). Endogenous tPA promoted BMSC-induced neurite outgrowth (Shen et al., 2011), leading to an increased synaptic plasticity that corresponds to an improved functional outcome (Xin et al., 2010).

In cerebral focal ischemic models, BMSC therapy reduces neuronal damage and apoptosis, by preventing the down-regulation of survivin and Bcl-2 (Okazaki et al., 2008). Survivin, a cell cycle regulator and a member of the inhibitor of apoptosis (IAP) family, functions by inhibiting caspase-3 and 7 activity and suppressing default apoptosis in G2/M phase of cell cycle (Li et al., 1998; Shin et al., 2001). Bcl-2 is also an anti-apoptotic and anti-necrotic factor that inhibits caspase-3 activity and upon over expression, Bcl-2 improves survival of neurons from damage (Zhao et al., 2003). In a rat model of stroke, BMSCs transduced with survivin increased survival of transplanted cells, improved neurological recovery, reduced cerebral infarct volume and up-regulated the expression of protective cytokines like VEGF and bFGF in ischemic tissue (Liu et al., 2011). Further evidence of the protective nature of BMSCs against stroke was demonstrated by intravenous administration of male BMSCs into female rats following MCAO. Systemic BMSC grafting in female ischemic rats induced neurogenesis, reduced apoptosis, increased expression of bFGF in IBZ and enhanced endogenous cellular proliferation in SVZ, all of which contributed to an improved functional recovery (Chen et al., 2003a).

In addition to neuroprotective effects, BMSCs effectively recruit elements of and participate in angiogenesis/arteriogenesis (Kinnaird et al., 2004b; Li et al., 2002; Chen et al., 2001a, 2001b, 2003b; Al-Khaldi et al., 2003). Conditioned media collected from BMSC cultures promoted proliferation and integration of endothelial cells and smooth muscle cells (SMCs), and enhanced collateral flow recovery and remodeling when directly injected into mouse ischemic hind limb (Kinnaird et al., 2004b). These findings demonstrated that tissue incorporation of the cellular graft may not be necessary for at least part of the action of BMSCs. In animal models, BMSCs targeted and participated in arteriogenesis and angiogenesis in injured regions (Al-Khaldi et al., 2003), as well as induced a significant

increase in blood flow to the ischemic area by stimulating neovascularization (Cui et al., 2009). The BMSC-stimulated angiogenic and arteriogenic vessels also produced trophic and growth factors that contributed to brain plasticity and recovery of neurological function post stroke (Chen et al., 2003b; Zacharek et al., 2007).

Beneficial neurorestorative effects of BMSCs was achieved *via* a dose-dependent release of trophic factors that can mediate reduction of brain damage and cell death, promote white matter remodeling and enhance angiogenesis, synaptogenesis, and neurogenesis (Tate et al., 2010). Other beneficial effects observed upon BMSC treatment include enhanced structural neuroplasticity and increased axonal outgrowth from healthy brain tissue (Andrews et al., 2008). Intra-carotid BMSC transplantation promoted white matter remodeling by facilitating axonal sprouting and re-myelination in cortical IBZ and corpus callosum (Shen et al., 2006). In a model of chronic stroke, BMSC transplantation improved white matter survival, which may contribute to improved cognitive outcomes (Onda et al., 2008).

Recently, exosome-mediated microRNA (miRNA) shuttling has been suggested to be involved in the BMSCs-induced stroke benefits. Exosomes are nanovesicles that are released from cells as a mechanism of cell-free intercellular communication (Valadi et al., 2007; Vlassov et al., 2012; Koh et al., 2010). BMSCs have been shown to secrete exosomes and thus reduce infarct size in myocardial ischemia/reperfusion injury (Lai et al., 2010; Arslan et al., 2013). Moreover, miRNAs were found to be enriched in the BMSC-derived exosomes (Chen et al., 2010c). Chopp's group has identified miR-133b to be a principle small RNA transferred from BMSCs to the astrocytes and neurons *via* exosomes. miR-133b, targeting and down-regulating Foxl2 expression, can sequentially promote neural plasticity (Xin et al., 2012) and functional recovery after cerebral ischemia (Xin et al., 2013). Other activated miRNAs including miR-210 and miR-107, exerted significant anti-apoptotic effects in BMSCs by targeting caspase 8-associated protein-2 and programmed cell death-10 mRNAs, respectively. Differentiation-related functions of HIF-1 β were partially inhibited by miR-107 in bone marrow-derived EPCs after hypoxic induction (Meng et al., 2012). This novel role of exosomes highlights a new perspective into intercellular mediation of tissue injury and repair, and engenders novel approaches to the development of biologics for tissue repair.

Using BMSCs as a source of cells for cell therapy raises significantly fewer ethical barriers compared to the use of fetal cells, and thus may be the best bet as an attractive alternative to develop for clinical translation at present. However, the longer timeframe required for obtaining cells from autologous sources limits their potential application in patients with acute stroke. In clinical practice, transplantation of BMSCs has been widely tested and appears to be a feasible and safe therapy (Bang et al., 2005; Suarez-Monteagudo et al., 2009; Lee et al., 2010). The first report was from Korea. Bang and colleagues intravenously infused autologous BMSCs into 5 patients with chronic stroke, and compared their neurological improvements with another 25 placebo-treated patients for 1 year. No adverse cell-related effects were observed. In patients with severe cerebral infarcts, the intravenous infusion of autologous BMSCs appears to be a feasible and safe therapy that may improve functional recovery (Bang et al., 2005). Bang's group further evaluated the long-term (5 years) safety and efficacy of BMSC transplantation in 52 patients, among which 16 were

treated with BMSCs. Significant side effects were not observed following BMSC treatment. BMSCs may somewhat contribute to improving functional recovery (Lee et al., 2010). A Phase I/II clinical trial in Spain revealed feasibility, safety and improved neurological outcome in MCA stroke patients transfused intra-arterially at 5 and 9 days after stroke with autologous bone marrow mononuclear cells (Moniche et al., 2012). During the follow up period of 6 months, no adverse effects, deaths, tumor formation or stroke recurrence was reported except for two isolated partial seizures at 3 months. Currently several clinical trials are under way in United States (NCT01297413), Spain (NCT01678534), France (NCT00875654), China (NCT01714167), and Korea (NCT01716481) investigating the feasibility and efficacy of BMSCs for stroke treatment (see Table 3).

3.4.2. Placenta-derived mesenchymal stem cells (PD-MSCs)—MSCs are found as a component of adult human bone marrow, adult peripheral blood and in term cord blood, but are relatively rare, representing 0.01% or less of the total cells. Numbers and function of BMSCs significantly decrease with age (Stenderup et al., 2003; Stephan et al., 1998). However, MSCs can be readily derived from chorion, amnion, and villous stroma of human placenta, independent of pregnancy stage (Park et al., 2011), and are easy to isolate for the generation of large amounts of MSCs in culture.

PD-MSCs can be expanded *in vitro* without the loss of phenotype and without showing signs of karyotypic changes. Allogeneic PD-MSCs should not require histocompatible tissue matching and thus would be considerably more convenient to use than bone marrow-or adipose-derived MSCs (Prather et al., 2009). In addition, placental cells, including PD-MSCs, express MHC class I chain-related (MIC) proteins A and B, human ligands of the activating natural killer cell receptor NKG2D, which play an important role in the placenta as an unique immunosuppressive organ (Hedlund et al., 2009). Thus, in terms of feasibility for development and potential immune compatibility, PD-MSCs are attractive for clinical translation.

The multi lineage differentiation potential of PD-MSCs is similar to BMSCs in terms of morphology, cell-surface antigen expression, and gene expression patterns (Fukuchi et al., 2004; Igura et al., 2004). In addition, PD-MSCs have the ability to differentiate into many different types of cells, including neuronal cells (Portmann-Lanz et al., 2006). For example, dopaminergic differentiation of these progenitors could restore locomotor activity in an animal model of hypoxic–ischemia. PD-MSCs can survive, migrate and orient to IBZ when administered intracerebrally (Schoeberlein et al., 2011) or intravenously (Yarygin et al., 2009) following ischemic stroke. Intravenous administration of PD-MSCs 4 h after MCAO improved functional outcome and reduced lesion volume (Chen et al., 2013b). Another study further detailed the treatment paradigm, observing that intravenous infusion of PD-MSCs at 8 h and 24 h post-MCAO improved functional outcome and decreased infarct volume better than either a single infusion at 24 h (Kranz et al., 2010). The protective effects of PD-MSC were more robust than those observed with fetal-derived MSCs (Kranz et al., 2010).

PD-MSCs also induced a pro-angiogenic effect including increased blood flow, enhanced VEGF expression and formation of new blood vessel in ischemic limbs in mice (Nishishita

et al., 2004). Amniotic membrane derived PD-MSCs are rich in proangiogenic factors and can induce rapid vascularization and renew endothelial cells in hypoxic brain (Warrier et al., 2012).

3.5. Human umbilical cord blood cells (HUCBCs)

Human umbilical cord blood cells (HUCBCs) have great potential as therapeutic agents, since they are easy to isolate without serious ethical and technical problems. HUCBCs can be used for autologous transplantation or allogeneic transplantation, do not require full human leukocyte antigen (HLA) matching, and do not pose grave ethical concerns associated with cells of embryonic origin. HUCBCs are an extremely rich source of both hematopoietic and mesenchymal progenitor cells. The total content of hematopoietic progenitor cells in HUCBCs equals or exceeds that in bone marrow, and the highly proliferative hematopoietic progenitor cells are eightfold higher in HUCBCs than in bone marrow (Almici et al., 1995). Transplant rejection, such as graft-*versus*-host disease (GVHD), is the leading cause of death in stem cell transplant patients. HUCBCs are associated with low risk of GVHD. Numerous studies demonstrated that HUCBC treatment in rodents does not lead to GVHD (Henning et al., 2004; Lu et al., 2002). To date, HUCBCs have been used in more than 8000 transplants for children and adults. Patients who receive HUCBC transplants from a relative are at a significantly lower risk of GVHD and are less prone to rejection than either bone marrow or peripheral blood stem cells (Morgado et al., 2008; Takahashi et al., 2007b). HUCB-derived mononuclear cells have potential to proliferate, differentiate, and to secrete factors possibly beneficial for the host brain tissue *in vivo* (Neuhoff et al., 2007). Umbilical cord blood can also be a rich source of neural stem/progenitor cells (Kozłowska et al., 2007). Transplantation of human umbilical cord blood-derived neural-like stem cells (HUCB-NSCs) in a rat model of cortical infarction, showed substantial survival and migration of transplanted cells into damaged brain (Kozłowska et al., 2007). Although these cells largely differentiated to neurons and, to a lesser extent, astrocytes, their long-term survival (>1 month) was minimal and occurred in the context of an acute host rejection in brain. Intravenous HUCBCs also selectively migrated to the ischemic area in the brain and enhanced functional recovery after stroke (Chen et al., 2001c; Zhang et al., 2011a). Freshly isolated mononuclear cells derived from HUCB are significantly better than neurally directed progenitors and neural-like stem cells in reducing lesion volume and improving functional outcome when transplanted intra arterially (Gornicka-Pawlak et al., 2011). Umbilical cord blood cell derived CD34+ cells promote angiogenesis, neurogenesis (Chen et al., 2013c), and neuronal regeneration resulting in enhanced neovascularization and improve behavioral recovery after systemic administration to MCAO rats and immune compromised mice (Taguchi et al., 2004). It is worth to note that several groups observed neutral effects of HUCBCs on stroke rehabilitation. Makinen et al. (2006) found that a bolus intravenous injection of HUCBCs at 24 h post-stroke failed to improve neurological and cognitive recovery. Another investigation using multiple intravenous infusions at 1, 2, 3 and 7 days after ischemia displayed HUCBCs failed to migrate/survive in the ischemic brain and did not provide significant neurological benefits (Zawadzka et al., 2009). These contradictory results may be due to the different cell implant and the use of immunosuppressive drugs.

The therapeutic benefit derived from HUCBC treatment may derive from stimulation of an endogenous remodeling recovery mechanism. In our study, only a small percentage of HUCBCs expressed neural-like phenotypes when infused following ischemia *via* intravenous delivery, yet functional recovery was found within days after administration of HUCBCs (Chen et al., 2001c). Given the short timeframe, it is highly unlikely that the significant functional recovery would be due to the functional integration of HUCBCs into the cerebral tissue (Vendrame et al., 2005). In addition, only a limited number of cells entered the damaged region, and the tissue replacement would constitute less than a cubic millimeter. Furthermore, the CNS entry of HUCBCs does not correlate positively with reduced cerebral infarction and improved behavioral functions (Borlongan et al., 2004). Thus, it is more likely that HUCBCs migrate toward the injured tissue and act there as localized sources of trophic factor production.

The multicellular nature of HUCBCs presents an interesting scenario. HUCBCs are comprised not only of mesenchymal stem cells which differentiate to neural cells, but also include a large proportion of hematopoietic colony-forming cells (Roura et al., 2012). These cells can release a variety of trophic factors when cultured *in vitro*, including CSF-1 and cytokines (Chen et al., 2010a), which have roles in promoting the proliferation or differentiation of endogenous neural tissue. Indeed, systemic administration of hematopoietic (CD34+) cells derived from HUCBCs following fluid percussion injury or stroke promoted angiogenesis and neurogenesis, and improved behavioral recovery (Chen et al., 2013c; Nystedt et al., 2006; Taguchi et al., 2004). Interestingly, these observations with CD34+ cells were without significant benefit in total infarct volume or neuroprotection (Nystedt et al., 2006), leading to the strong suggestion that HUCBC-derived hematopoietic cells may promote endogenous repair *via* trophic support rather than *via* cell restoration. In addition to trophic support of neural tissue, HUCBCs (including both mesenchymal and hematopoietic cells) could also promote neural recovery indirectly *via* inflammatory modulation. HUCBCs induce strong immunomodulatory properties by the host. Administration of HUCBCs following cerebral ischemia attenuated neuroinflammation (Vendrame et al., 2005; Leonardo et al., 2010). Specifically, HUCBC infusion in an animal model of stroke opposed the pro-inflammatory T helper cell type 1 (Th1) response by promoting a strong anti-inflammatory T-helper 2 (Th2) response (Vendrame et al., 2004; Nikolic et al., 2008).

Inflammatory suppression by HUCBCs has been attributed to decreased presence of pro-inflammatory factors including cytokines, CD45/CD11b- and CD45/B220+ cells, nuclear factor- κ B (NF- κ B) DNA binding activity (Vendrame et al., 2005) and pro-inflammatory isolectin binding cells (Leonardo et al., 2010). HUCBC treatment also suppressed MCAO-associated T-cell proliferation by increasing the production of IL-10 while decreasing interferon- γ (IFN- γ) (Chen et al., 2013a). In the setting of acute myocardial infarction, HUCBCs also limited the expression of pro-inflammatory elements, including tumor necrosis factors- α (TNF- α), MCP-1, macrophage inflammatory protein (MIP), and IFN- γ (Henning et al., 2007). Furthermore, emerging studies have suggested that, in addition to suppression of inflammatory factors, HUCBCs may indirectly protect neural function by inducing oligodendrocyte protection and survival (Rowe et al., 2010, 2012). Together, these

anti-inflammatory and white matter effects of HUCBCs suggest a strong indirect pathway for HUCBC-mediated neural protection.

With functional recovery observed within a few days of therapy and maximum cell migration observed when initiated treatment at 24 h after MCAO, early intravenous treatment with HUCBCs appears to be optimal for clinical treatment of stroke. However, intravenous administration of HUCBCs requires higher doses (up to 4 times) than required for local injection or intra-arterial injection to improve functional outcomes and reduce infarct size (Henning et al., 2007). Intrathecal administration of HUCBCs *via* lumbar puncture in rats is also safe and feasible. Additionally, intrathecal route led to significantly better cell migration, differentiation to neuronal cells and improved functional recovery compared to intravenous administration (Lim et al., 2011).

Limitations of HUCBC transplantation primarily revolve around immunological concerns. A major concern in hematopoietic disorders is post transplantation susceptibility to viral infections mostly within the first 3–4 months (Rubinstein et al., 1998; Parody et al., 2006) caused by delayed immune reconstitution when using unrelated cord blood (Szabolcs and Niedzwiecki, 2007). Other issues are related to delayed engraftment (Rocha and Gluckman, 2006). The limited cell yield which is being overcome by using a combination of two partially HLA matched cord blood units, but then carries the risk of larger incidence of GVHD (Sideri et al., 2011). While cord blood remains an attractive source for cell-based therapy, much work remains to better understand and target its functions.

4. *In vivo* tracking of implanted cells

Longitudinal noninvasive tracking of grafted stem/progenitor cells in the brain will undoubtedly aid our understanding of spatiotemporal dynamics of the graft and how these cells mediate functional recovery. Several imaging methods have been developed to follow the graft in the CNS (Adamczak and Hoehn, 2012). In this section, we discuss the application of optical imaging, magnetic resonance imaging (MRI), and nuclear imaging as potential imaging strategies in stroke injury.

4.1. Optical imaging

Optical imaging refers to two major techniques: bioluminescence and fluorescence imaging. Despite the limitation of low spatial resolution due to high absorption and scatter, optical imaging possesses the highest sensitivity for *in vivo* tracking (Sutton et al., 2008).

Bioluminescence occurs as a result of an enzymatic reaction between a luciferase enzyme with its substrate that converts the chemical energy into light. Bioluminescence imaging relies on the expression of a luminescent protein which is not endogenous to most mammalian cells, and thus requires transduction of the transplant cell population with a transgene encoding a luciferase enzyme. Following transplantation, cells expressing the luciferase enzyme can be visualized by systemic injection of the luciferase substrates, D-luciferin or coelenterazine (Manley and Steinberg, 2012). The simplicity of this approach, combined with its accuracy and ability to quantify signals, makes bioluminescence imaging attractive in terms of feasibility to track the migration (Andres et al., 2011a), biodistribution

(Pendharkar et al., 2010), and survival (Rosenblum et al., 2012) of the grafted cells. Bioluminescence has been used imaging to longitudinally track NPCs after transplantation in a murine model of stroke (Kim et al., 2004). The stem/progenitor cells were transfected to express β -galactosidase as well as firefly luciferase for validation of *in vivo* findings. An excellent correspondence between histological and *in vivo* bioluminescence imaging was found with respect to bulk mass of NPCs (Kim et al., 2004). In another study using human NSCs, the cell dose to bioluminescence relationship existed *in vivo* and remained stable for at least 8 weeks; simultaneous MRI scans further corroborated this, as similar cell numbers could be extrapolated from the cell dose to magnetic resonance signal relationship (Daadi et al., 2009).

Shichinohe et al. introduced whole-body fluorescence imaging (Yang et al., 2000) to follow intrastrially implanted green fluorescent protein (GFP)-expressing BMSCs in living mice for 12 weeks. Due to the relatively short wavelength of fluorescence emitted from GFP (520 nm), the emitted light was inevitably scattered by the surrounding tissue. Thus the skull needed to be removed or thinned for more clear fluorescence images (Shichinohe et al., 2004). Recent advances in nanotechnology have enabled us to apply biocompatible fluorescent semiconductor nanocrystals called quantum dots (Michalet et al., 2005). Near-infrared (NIR)-emitting quantum dots have much longer wavelengths and high resistance to photobleaching (Jaiswal and Simon, 2007). They have been introduced for *in vivo* tracking of cells in the brain. In the context of stroke, Kuroda's group monitored quantum dots-labeled BMSCs in a rat model of permanent MCAO over 8 weeks after transplantation (Sugiyama et al., 2011). NIR fluorescence imaging was well supported by the *ex vivo* brain slice NIR fluorescence imaging and histological analysis (Kawabori et al., 2012; Sugiyama et al., 2011).

4.2. Magnetic resonance imaging (MRI)

As the most often used imaging modality in the CNS, MRI offers ultra-high spatial resolution. Its capacity for detecting even single labeled cell has been reported (Shapiro et al., 2006). For tracking of exogenous stem cells *in vivo*, the graft must be labeled with contrast agents *in vitro* before transplantation to be distinguishable from host tissue. Gadolinium rhodamine dextran (GRID) and superparamagnetic iron oxide (SPIO) are two groups of commonly used contrast agents. GRID appears as hyperintense signals on T1-weighted MR images. Although early reports claimed they do not affect cell differentiation and migration either *in vitro* or *in vivo* (Modo et al., 2002a, 2004), one study indicated GRID particles may have deleterious effects on the long-term histological and functional benefits of NSCs (Modo et al., 2009). As an alternative, SPIO-based contrast agents to label stem/progenitor cells are more sensitive and safe, and induce a strong hypointensity on T2 and especially on T2* images (Hoehn et al., 2002; Jendelova et al., 2003; Walczak et al., 2008). Immunostaining of SPIO-labeled grafts captured with confocal microscopy confirmed the MRI data over 2 months (Daadi et al., 2009; Guzman et al., 2007). Simultaneous and accurate representations of the grafts and the stroke were possible by rendering three dimensional reconstructions from the MR scans (Manley and Steinberg, 2012), allowing for direct comparison between stroke and graft sizes. Unfortunately, iron-rich glial cells and infiltrating macrophages that accumulate in the lesion boundary may also

generate strong T2* contrast signals (Vandeputte et al., 2011), presenting a significant confound. Immunohistochemistry for ED1 and Prussian blue staining indicated that iron-containing macrophages presented in the tissue in accordance with the area of MRI signal augmentation (Hoehn et al., 2007). Recently, new contrast agents such as fluorescent-magnetitenanocluster (Wang et al., 2011) and ^{19}F (Bible et al., 2012) are emerging for MRI cell tracking in stroke. They are believed to have the potential to increase the sensitivity and accuracy of signals.

One may ask, what happen to the incorporated contrast agents if the cells die? While no contrast changes were observed on MR images of animals that received transplantations of dead SVZ cells (Adamczak and Hoehn, 2012), Guzman et al. (2008a) displayed 10–15% of the SPIO-labeled grafted cells were phagocytosed by microglia and could still be detected on MRI. Thus MRI analysis may lead to an overestimation of the graft survival and must be interpreted with caution.

4.3. Nuclear imaging

Radiolabeling is an established method in clinical nuclear medicine for evaluation of stem/progenitor cells, especially for whole-body biodistribution studies because of its picomolar sensitivity and excellent tissue penetration (Welling et al., 2011). Most commonly used radioisotopes include Indium-111 (^{111}In), Technetium-99m ($^{99\text{m}}\text{Tc}$), and ^{18}F -fluorodeoxyglucose (^{18}F -FDG). The first two are single photon emission computed tomography (SPECT) tracers, while the last is for positron emission tomography (PET) imaging. ^{111}In has a half-life of 67 h and thus allows tracking for up to several days after implantation (Blackwood et al., 2009). Inflammation scintigraphy with ^{111}In -oxine-labeled leukocytes is a standard nuclear medicine procedure (Becker and Meller, 2001). It has been introduced to label and monitor cell transplantation for the treatment of stroke (Mitkari et al., 2013; Lappalainen et al., 2008). $^{99\text{m}}\text{Tc}$ has short half-life of 6 h; therefore it is better suited than ^{111}In for short tracking of stem/progenitor cells *in vivo*. Scintillation counting of isolated organ indicated that IV-injected $^{99\text{m}}\text{Tc}$ -labeled bone marrow mononuclear cells accumulated more in the MCAO-attacked hemisphere than contralateral hemisphere (Detante et al., 2009). The clinical feasibility of monitoring cell transplantation using $^{99\text{m}}\text{Tc}$ after ischemic stroke has been reported (Correa et al., 2007; Rosado-de-Castro et al., 2013; Barbosa da Fonseca et al., 2009, 2010), where nuclear tomography was taken within 8 h, and in some cases was extended to 24 h after transplantation (Barbosa da Fonseca et al., 2010). Intra-arterial injection of $^{99\text{m}}\text{Tc}$ -labeled bone marrow mononuclear cell between 9 and 89 days after stroke onset could induce intense accumulation of radioactivity in the infarcted regions.

Collectively, all imaging modalities have their inherent benefits and drawbacks. Some of them are still in their infancy. Continued exploration is required to optimize tracking techniques, so that they can eventually be used for maximal assessments in clinical setting.

5. Cell delivery routes

As aforementioned, positive behavioral improvements have been observed with intracerebral, intracerebroventricular, intravascular and intranasal deliveries of stem/

progenitor cells. Among those, is there an optimal delivery route for specific cell types? Currently, there is no definitive answer. Here we discuss the pros and cons of various delivery paradigms.

5.1. Intracerebral and intracerebroventricular injections

Intracerebral delivery results in more implanted cells in the infarcted region compared with other delivery routes. It may be most suitable for NSC transplantation. However, the procedural risk for stereotaxic injection inevitably raises safety issues. Early clinical trials using intraparenchymal cell implantation have reported severe adverse events involving motor worsening, seizures, syncope, and chronic subdural hematoma (Savitz et al., 2005; Kondziolka et al., 2005). Although no cell-related tumors or ectopic tissue formation have been observed to date, the tumorigenicity concerns remain. In contrast, an intracerebroventricular method is less invasive than direct stereotaxic implantation. However, in a directly comparative study, intracerebroventricular injection of CTX0E03 human NSCs after stroke did not lead to the improvement conferred by intraparenchymal cell implants (Smith et al., 2012). Nonetheless, intrathecal umbilical cord mesenchymal stem cells (UCMSCs) seemed to improve motor function and reduced ischemic damage of stroke in rats (Lim et al., 2011). Only one clinical trial using the intracerebroventricular paradigm to treat stroke has been performed, where a pilot study that showed some stroke patients developed fever and meningeal signs after cell implant *via* intracerebroventricular delivery (Rabinovich et al., 2005). Thus, direct CNS grafting of stem cells for stroke has yet to adequately prove safety for implementation in clinic.

5.2. Intravascular injections

Most of the current clinical trials employ intravascular (intravenous and intra-arterial) paradigms to deliver stem/ progenitor cells in stroke patients (see Tables 2 and 3). It has several advantages, including ease of administration, minimal invasiveness, potential for widespread cell distribution, as well as widespread secretion of neuroprotective, proangiogenic, and immunomodulatory factors (Misra et al., 2012). Systemically grafted cells appear to follow a chemoattractant gradient generated from the injured brain and penetrate through BBB (Guzman et al., 2008b), and it is increasingly recognized that grafted cells do not have to be near the lesion to be effective (Borlongan et al., 2004). Of the two intravascular methods intravenous and intra-arterial, intra-arterial appears to be the most attractive option. Intravenously delivered cells circulate through the systemic and pulmonary vascular systems, which markedly compromises cell homing to the injured brain. Very few cells could integrate into the infarcted area. The majority of cells become trapped in the lung, liver, and spleen upon intravenous administration. Intra-arterial delivery, in contrast, bypasses the peripheral filtering organs, leading to higher cell engraftment to the brain (Zhang et al., 2012b; Li et al., 2010), and greater efficacy (Pendharkar et al., 2010; Kamiya et al., 2008) compared to intravenous infusion.

Despite the advantages, intravascular routes also have safety issues. Cells may stick together and cause microemboli, including lethal pulmonary emboli from intravenous administration or reduction in cerebral blood flow associated with microstrokes due to intra-arterial delivery (Walczak et al., 2008). The use of a microneedle injection technique might preserve

anterograde blood flow throughout the transplantation process and avoid the development of microstrokes (Chua et al., 2011). In a pilot study from our own group, infusion of UCMSCs into M1 portion of the MCA *via* a micro catheter did not affect distal blood flow and perfusion, thereby verifying the safety of intra-MCA administration of stem cells for stroke (Jiang et al., 2012b). However, it should be noted that this is based on the follow-ups of only 4 patients.

5.3. Intranasal delivery

During the recent years, intranasal delivery has emerged as a novel strategy of bypassing the BBB to deliver therapeutic agents to the brain. This non-invasive method facilitates cell homing toward the CNS and reduces the potential side effects associated with intravascular administration. Intranasal administrations of peptides (Alcala-Barraza et al., 2010; Ma et al., 2007), small molecules (Lu et al., 2011; Akpan et al., 2011; Liu et al., 2012b), viruses (Jiang et al., 2012a), plasmid (Han et al., 2007) and bacterial phages (Frenkel and Solomon, 2002) have been demonstrated allow therapeutic molecules entry into the brain. More recently, stem/progenitor cells, exemplified by MSCs and NSCs, were also observed to gain access to the brain *via* the nasal cavity, and render therapeutic benefits in Parkinson's disease (Danielyan et al., 2011), malignant gliomas (Reitz et al., 2012), and stroke (van Velthoven et al., 2013; Wei et al., 2013). Intranasally delivered cells are capable of traveling across the cribriform plate and migrating throughout the forebrain and olfactory bulbs (Jiang et al., 2011b). Although the exact mechanism of intranasal delivery has not been elucidated, accumulating evidence suggests the pathways may involve the olfactory nerve, trigeminal nerve, vascular, and cerebrospinal fluid perfusion (Dhuria et al., 2010; Danielyan et al., 2009). Hypoxic preconditioned BMSCs exhibited enhanced pathotropism after intranasal administration (Wei et al., 2013). Hypoxia-treated BMSCs showed increased levels of CXCR4, MMP-2, and MMP-9. As early as 1.5 h after administration, these cells reached the ischemic cortex and were deposited outside of blood vessels. Accordingly, hypoxic preconditioned BMSCs resulted in reduced lesion volume and improved motor function (Wei et al., 2013). Likewise, van Velthoven's group has tested the neuror-estorative effects of intracranial (van Velthoven et al., 2010, 2012b) and intranasal (Donega et al., 2013; van Velthoven et al., 2013) administration of BMSCs. Though a direct comparison of regenerative efficiency between two delivery routes cannot be made because different numbers of cells were used, functional outcomes of the intranasal and intracranial route were similar in models of neonatal ischemia (van Velthoven et al., 2012a). Currently, transnasal delivery devices like *ViaNase*, *OptiNose*, and *Impel's Precision Olfactory Delivery (POD)*, have been developed, and several clinical trials are ongoing to assess the safety and efficacy of intranasal regimen for the treatment of neurological diseases (Liu, 2011). Whether intranasal delivery of cells can be clinically applicable for stroke patients still awaits future exploration.

Collectively, each cell delivery method has its safety issues, strengths, and weaknesses. Unfortunately, to date there is a paucity of study directly comparing the differentiated efficacy of delivery methods. Thus, the optimum cell delivery route for stroke treatment has not been determined. Stroke subtype, cell delivery timing, cell type and working

mechanisms should be taken into consideration together with the selection of cell delivery route.

6. Manipulation and enhancement of cell based therapy

6.1. Gene modification

With regard to the paracrine-mediated mechanisms of stem/ progenitor cells, enhancement of their trophic activities by overexpression of related genes would be of particular value to magnify the efficacy of cell therapies in stroke treatment (Chen et al., 2013a). A variety of genes was pre-incorporated into the implanted cells and has been reported to induce greater functional recovery. These therapeutic genes include angiogenic factors like VEGF (Zhu et al., 2005), Ang-1 (Onda et al., 2008), HGF (Zhao et al., 2006), PIGF (Liu et al., 2006), neurotrophic factors like BDNF (van Velthoven et al., 2013; Kurozumi et al., 2004; Chang et al., 2013a), ciliary neurotrophic factor (CNTF) (Kurozumi et al., 2005), NGF (Andsberg et al., 1998), GDNF (Kurozumi et al., 2005; Chen et al., 2009; Horita et al., 2006; Ou et al., 2010), neurotrophin-3 (NT-3) (Park et al., 2006), Neurogenin 1 (Kim et al., 2008), and other genes like survivin (Liu et al., 2011), Noggin (Chen et al., 2011a; Ding et al., 2011), copper/zinc-superoxide dismutase (Sakata et al., 2012) and CXCR4 (Yu et al., 2012). Some investigators further tried to transfect multiple genes into cells *ex vivo* and examine the potential therapeutic benefits of the synergistic effects (Ding et al., 2011; Toyama et al., 2009). Kocsis's group transfected human BMSCs with 2 angiogenesis-inducing genes—Ang-1 and VEGF—and found rats intravenously receiving Ang-VEGF-hBMSCs showed the greatest structural–functional recovery as compared to the those rats receiving hBMSCs, Ang-hBMSCs, or VEGF-hBMSCs (Toyama et al., 2009).

Among these studies, MSCs and NSCs are the most frequently used recipient cell types for gene incorporation. They can be easily transduced with common vectors like lentivirus, adenovirus, or adeno-associated virus carrying target genes. According to the aforementioned differential restorative mechanisms of NSCs and MSCs, it is not surprising to find that NSCs are often utilized with neurotrophic factor genes or pro-survival genes, so as to increase the percentage of migrated neurons, whereas MSCs are often utilized to deliver angiogenic factor genes to enhance their paracrine effects. Unfortunately, it remains uncertain whether the same therapeutic gene delivered by different cells could generate differential benefits.

There has been no clinical experience using the gene-modified stem/progenitor cell therapy for stroke treatment so far. It is challenging to prove the safety and efficacy of transferring exogenous genes into patients *via* cells. The first concern is the potential of malignant transformation after gene incorporation. Although exogenous genes are transferred into stem/progenitor cells rather than the host genome, the viral vectors may increase the risk of genotoxicity by insertional mutagenesis and transcriptional activation of adjacent oncogenes. To avoid tumorigenesis in clinical practice, the vector can be rendered self-inactivating and only contains nonviral, physiologic promoter/enhancer elements (Payen and Leboulch, 2012). Second, the therapeutic gene may exert double-edged effects *per se*. For example, VEGF enhances post-stroke angiogenesis and neurogenesis on one hand but increases vascular permeability and may worsen brain edema on the other (Greenberg and

Jin, 2013). Chopp's group has demonstrated that early post-ischemic (1 h) administration of VEGF significantly exacerbated BBB leakage, hemorrhagic transformation, and ischemic lesions (Zhang et al., 2000). Whether VEGF-overexpressing cells could generate a similar deterioration is unknown, but these findings are vital to call attention to the administration paradigm of cell therapy. Third, the delivery of gene-modified cell therapy is difficult to achieve in an early period after stroke. In animal stroke models, most gene-modified cell therapies were administered immediately or soon after cerebral ischemia (Horita et al., 2006; Kurozumi et al., 2004; Liu et al., 2006). However, most completed and ongoing clinical trials employ autologous stem/progenitor cell transplantation, the gene manipulation of which is time consuming and will inevitably delay the cell delivery. Further investigations should be carried out on the effects of delivering gene-modified stem/progenitor cells in a later period after stroke, which allows for transformation and *in vitro* expansion of autologous cells before transplantation (Chen et al., 2013a).

6.2. Preconditioning

In addition to cells modified with exogenous genes, some recent effort has been shifted to mobilization of endogenous mechanisms for optimizing therapeutic potential of cell-based stroke therapy. Being a transcription factor in response to hypoxia, HIF-1 acts as a sensor to low-oxygen in the microenvironment. Increased HIF-1 α and its downstream genes play central roles in hypoxia-induced defense responses (Ogle et al., 2012). Initial evidence from Yu's and Wei's groups provided evidence that, as in many other cell types, a sub-lethal hypoxia exposure significantly increases the tolerance and regenerative properties of stem/progenitor cells *in vitro* and after transplantation (Yu et al., 2013; Wei et al., 2005, 2012, 2013; Theus et al., 2008; Hu et al., 2011; Francis and Wei, 2010). In their reports on BMSCs, ESC-or iPSC-derived neural progenitor cells, hypoxic preconditioning was achieved *via* pre-treatment with sub-lethal hypoxia (e.g. 0.5% O₂, 10–24 h). The hypoxic preconditioned cells survived much better *in vitro* and after transplantation into the ischemic brain (30–50% reduction in cell death) (Theus et al., 2008). The hypoxia exposure up-regulated HIF-1 α and trophic/growth factors such as BDNF, GDNF, VEGF and its receptor FIK-1, erythropoietin (EPO) and its receptor EPOR, stromal derived factor-1 (SDF-1) and its CXC chemokine receptor 4 (CXCR4). Meanwhile, many pro-inflammatory cytokines/chemokines were down-regulated in hypoxia-treated cells (Wei et al., 2012). Compared to normoxic control cells, transplanted hypoxic-preconditioned cells showed enhanced homing ability due to increased expression of CXCR4 (Wei et al., 2012, 2013). Hypoxic preconditioned cells become functional neurons firing repetitive action potential and exhibiting synaptic activities (Song et al., 2012; Francis and Wei, 2010). After transplantation, hypoxic-preconditioned cells showed increased differentiation into neuronal and endothelial cells, and stimulated endogenous angiogenesis and neurogenesis. These cells also showed a greater effect of suppressing microglia activity in the post-ischemic brain (Wei et al., 2012). Stroke animals that received hypoxic-preconditioned cells showed better functional recovery compared to animals transplanted with normoxic cells (Wei et al., 2013; Hu et al., 2011).

Up to now, a number of preconditioning triggers have been tested in stem cells and stem cell-derived progenitor cells for the treatments of ischemic heart and brain disorders. These

triggers are often sublethal insults such as ischemia (Li et al., 2005), hypoxia (Stubbs et al., 2012; Yan et al., 2012; Aly et al., 2011; Peterson et al., 2011; Das et al., 2010), anoxia (Wang et al., 2009; Li et al., 2008; He et al., 2009), hydrogen sulfide (H₂S) (Xie et al., 2012), hydrogen dioxide (H₂O₂) (Zhang et al., 2012a), and carbon monoxide (CO) (Kondo-Nakamura et al., 2010). Alternatively, preconditioning can be achieved using preconditioning mediators such as erythropoietin (EPO) (Theus et al., 2008; Li et al., 2007), SDF-1 (Pasha et al., 2008; Zemani et al., 2008), insulin-like growth factor-1 (IGF-1) (Lu et al., 2012), heat shock proteins (Tilkorn et al., 2012; Jiang et al., 2005), or pharmacological agents such as diazoxide (Niagara et al., 2007; Idris et al., 2012; Afzal et al., 2010), apelin (Li et al., 2012), isoflurane (Kim et al., 2011), lipopolysaccharide (Yao et al., 2009), and cobalt protoporphyrin (Cai et al., 2012). It is expected that the preconditioning strategy will be further explored for their enhanced benefits of cell-based transplantation therapies.

7. Prospects and conclusion

Current evidence shows great promise for cell transplantation as a new therapeutic modality for stroke (Mir and Savitz, 2013). Nonetheless, important lessons should be learned from previous investigations on the development of stroke therapy. The past few decades witnessed over 1000 agents that have been tested as for their neuroprotective effects in stroke (O'Collins et al., 2006), and thus far, none of them has been proved to be of clinical value yet. This sobering reality should not be misconstrued as evidence that neuroprotection or neurorestoration is unattainable in patients (Tymianski, 2010). On the contrary, an abundance of laboratory studies have defined and characterized the pathophysiology of ischemic brain injury and have provided scientifically irrefutable proof-of-principle that stroke restoration, in fact, is feasible with a variety of interventions (Sahota and Savitz, 2011; Ye et al., 2011, 2012). Moreover, the stroke community has accumulated ample experience, positive and negative, from those prior failures (Ye et al., 2013). We recognize that many fundamental questions related to the cell characterization, cell dosage, cell fate, biodistribution, safety indices, outcome measures, patient selection, therapeutic timing, *etc.*, are critical for the successful development of a cell product (Bliss et al., 2010). To address these issues, basic scientists, clinicians, industry partners, and funding bodies collaborated to draw up a guideline called Stem Cell Therapy as an Emerging Paradigm for Stroke (STEPS) in 2009 (The STEPS Participants, 2009), which was further revised in 2011 (Savitz et al., 2011a). The guideline contained recommendations for both experimental and early-stage clinical studies on the cell therapy for stroke. Some of the items involve cell characterization, preclinical safety evaluation, biodistribution and cell persistence, action mechanism exploration, evaluation in heterogeneous stroke types, route of therapy, therapy timing, and immunosuppression (Savitz et al., 2011a). Based on the experience on the stroke therapy academic industry roundtable (STAIR) recommendations (Fisher et al., 2009), we can infer that, although STEPS guidelines may not guarantee a clinical success, these important, logical issues will undoubtedly increase the degree of confidence needed to the certainty of the findings obtained. In summary, cell transplantation for stroke treatment in humans is still in its infancy. There remains a need for further basic and translational studies before it becomes a scientifically proven strategy in clinical setting.

Acknowledgments

This work was supported by National Natural Science Foundation of China 31300900 (RY), 81300993 (TY), Jiangsu Provincial Special Program of Medical Science (BL2013025), National Institute on Aging of USA AG031811 (JC), and National Institute of Health of USA NS057255 (SPY), NS058710 (WL) and R41NS080329 (JC).

Abbreviations

Ang-1	angiopoietin-1
BBB	blood–brain barrier
BDNF	brain-derived neurotrophic factor
bFGF	basic fibroblast growth factor
BMSCs	bone marrow stromal cells
CNS	central nervous system
CXCR4	chemokine (C-X-C motif) receptor 4
ESCs	embryonic stem cells
GDNF	glial cell-derived neurotrophic factor
GFP	green fluorescent protein
GRID	gadolinium rhodaminexdextran
GVHD	graft- <i>versus</i> -host disease
HGF	hepatocyte growth factor
HLA	human leukocyte antigen
hMSCs	human mesenchymal stem cells
HUCBCs	human umbilical cord blood cells
IA	intra-arterial
IBZ	ischemic boundary zone
IC	intracerebral
IGF-1	insulin-like growth factor 1
iPSCs	induced pluripotent stem cells
IV	intravenous
LGE	lateral ganglionic eminence
MCAO	middle cerebral artery occlusion
MCP-1	monocyte chemotactic protein-1
miRNA	microRNA
MRI	magnetic resonance imaging

mRS	modified Rankin Scale
MSCs	mesenchymal stem cells
NGF	nerve growth factor
NIHSS	National Institutes of Health Stroke Scale
NIR	near-infrared
NPCs	neural progenitor cells
NSCs	neural stem cells
PD-MSCs	placenta-derived mesenchymal stem cells
PIGF	placental growth factor
RMS	rostral migratory stream
SDF-1	stromal-derived factor 1
SPIO	superparamagnetic iron oxide
STEPS	Stem Cell Therapy as an Emerging Paradigm for Stroke
SVZ	subventricular zone
tPA	tissue plasminogen activator
UCMSCs	umbilical cord mesenchymal stem cells
VEGF	vascular endothelial growth factor

References

- Abe K, Yamashita T, Takizawa S, Kuroda S, Kinouchi H, Kawahara N. Stem cell therapy for cerebral ischemia: from basic science to clinical applications. *Journal of Cerebral Blood Flow and Metabolism*. 2012; 32:1317–1331. [PubMed: 22252239]
- Adamczak J, Hoehn M. In vivo imaging of cell transplants in experimental ischemia. *Progress in Brain Research*. 2012; 201:55–78. [PubMed: 23186710]
- Afzal MR, Haider H, Idris NM, Jiang S, Ahmed RP, Ashraf M. Preconditioning promotes survival and angiomyogenic potential of mesenchymal stem cells in the infarcted heart via NF-kappaB signaling. *Antioxidants & Redox Signaling*. 2010; 12:693–702. [PubMed: 19751147]
- Aimone JB, Deng W, Gage FH. Resolving new memories: a critical look at the dentate gyrus, adult neurogenesis, and pattern separation. *Neuron*. 2011; 70:589–596. [PubMed: 21609818]
- Akpan N, Serrano-Saiz E, Zacharia BE, Otten ML, Ducruet AF, Snipas SJ, Liu W, Vellozo J, Cohen G, Sosunov SA, Frey WH 2nd, Salvesen GS, Connolly ES Jr, Troy CM. Intranasal delivery of caspase-9 inhibitor reduces caspase-6-dependent axon/neuron loss and improves neurological function after stroke. *Journal of Neuroscience*. 2011; 31:8894–8904. [PubMed: 21677173]
- Al-Khalidi A, Eliopoulos N, Martineau D, Lejeune L, Lachapelle K, Galipeau J. Postnatal bone marrow stromal cells elicit a potent VEGF-dependent neoangiogenic response in vivo. *Gene Therapy*. 2003; 10:621–629. [PubMed: 12692590]
- Alcala-Barraza SR, Lee MS, Hanson LR, McDonald AA, Frey WH 2nd, McLoon LK. Intranasal delivery of neurotrophic factors BDNF, CNTF, EPO, and NT-4 to the CNS. *Journal of Drug Targeting*. 2010; 18:179–190. [PubMed: 19807216]

- Almici C, Carlo-Stella C, Wagner JE, Rizzoli V. Umbilical cord blood as a source of hematopoietic stem cells: from research to clinical application. *Haematologica*. 1995; 80:473–479. [PubMed: 8566892]
- Alvarez-Buylla A, Garcia-Verdugo JM. Neurogenesis in adult subventricular zone. *Journal of Neuroscience*. 2002; 22:629–634. [PubMed: 11826091]
- Aly A, Peterson K, Lerman A, Lerman L, Rodriguez-Porcel M. Role of oxidative stress in hypoxia preconditioning of cells transplanted to the myocardium: a molecular imaging study. *Journal of Cardiovascular Surgery (Torino)*. 2011; 52:579–585.
- Andres RH, Choi R, Pendharkar AV, Gaeta X, Wang N, Nathan JK, Chua JY, Lee SW, Palmer TD, Steinberg GK, Guzman R. The CCR2/CCL2 interaction mediates the transendothelial recruitment of intravascularly delivered neural stem cells to the ischemic brain. *Stroke*. 2011a; 42:2923–2931. [PubMed: 21836091]
- Andres RH, Horie N, Slikker W, Keren-Gill H, Zhan K, Sun G, Manley NC, Pereira MP, Sheikh LA, McMillan EL, Schaar BT, Svendsen CN, Bliss TM, Steinberg GK. Human neural stem cells enhance structural plasticity and axonal transport in the ischaemic brain. *Brain*. 2011b; 134:1777–1789. [PubMed: 21616972]
- Andrews EM, Tsai SY, Johnson SC, Farrer JR, Wagner JP, Kopen GC, Kartje GL. Human adult bone marrow-derived somatic cell therapy results in functional recovery and axonal plasticity following stroke in the rat. *Experimental Neurology*. 2008; 211:588–592. [PubMed: 18440506]
- Andersberg G, Kokaia Z, Bjorklund A, Lindvall O, Martinez-Serrano A. Amelioration of ischaemia-induced neuronal death in the rat striatum by NGF-secreting neural stem cells. *European Journal of Neuroscience*. 1998; 10:2026–2036. [PubMed: 9753090]
- Annabi B, Lee YT, Turcotte S, Naud E, Desrosiers RR, Champagne M, Eliopoulos N, Galipeau J, Beliveau R. Hypoxia promotes murine bonemarrow-derived stromal cell migration and tube formation. *Stem Cells*. 2003; 21:337–347. [PubMed: 12743328]
- Arslan F, Lai RC, Smeets MB, Akeroyd L, Choo A, Aguor EN, Timmers L, van Rijen HV, Doevendans PA, Pasterkamp G, Lim SK, de Kleijn DP. Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. *Stem Cell Research*. 2013; 10:301–312. [PubMed: 23399448]
- Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nature Medicine*. 2002; 8:963–970.
- Bacigaluppi M, Pluchino S, Peruzzotti-Jametti L, Kilic E, Kilic U, Salani G, Brambilla E, West MJ, Comi G, Martino G, Hermann DM. Delayed post-ischaemic neuroprotection following systemic neural stem cell transplantation involves multiple mechanisms. *Brain*. 2009; 132:2239–2251. [PubMed: 19617198]
- Bang OY, Lee JS, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. *Annals of Neurology*. 2005; 57:874–882. [PubMed: 15929052]
- Bao X, Feng M, Wei J, Han Q, Zhao H, Li G, Zhu Z, Xing H, An Y, Qin C, Zhao RC, Wang R. Transplantation of Flk-1+ human bone marrow-derived mesenchymal stem cells promotes angiogenesis and neurogenesis after cerebral ischemia in rats. *European Journal of Neuroscience*. 2011; 34:87–98. [PubMed: 21692879]
- Barbosa da Fonseca LM, Battistella V, de Freitas GR, Gutfilen B, Dos Santos Goldenberg RC, Maiolino A, Wajnberg E, Rosado de Castro PH, Mendez-Otero R, Andre C. Early tissue distribution of bone marrow mononuclear cells after intra-arterial delivery in a patient with chronic stroke. *Circulation*. 2009; 120:539–541. [PubMed: 19667245]
- Barbosa da Fonseca LM, Gutfilen B, Rosado de Castro PH, Battistella V, Goldenberg RC, Kasai-Brunswick T, Chagas CL, Wajnberg E, Maiolino A, Salles Xavier S, Andre C, Mendez-Otero R, de Freitas GR. Migration and homing of bone-marrow mononuclear cells in chronic ischemic stroke after intra-arterial injection. *Experimental Neurology*. 2010; 221:122–128. [PubMed: 19853605]
- Battistella V, de Freitas GR, da Fonseca LM, Mercante D, Gutfilen B, Goldenberg RC, Dias JV, Kasai-Brunswick TH, Wajnberg E, Rosado-de-Castro PH, Alves-Leon SV, Mendez-Otero R, Andre C. Safety of autologous bone marrow mononuclear cell transplantation in patients with nonacute ischemic stroke. *Regenerative Medicine*. 2011; 6:45–52. [PubMed: 21175286]

- Becker W, Meller J. The role of nuclear medicine in infection and inflammation. *Lancet Infectious Diseases*. 2001; 1:326–333. [PubMed: 11871805]
- Bhasin A, Padma Srivastava MV, Mohanty S, Bhatia R, Kumaran SS, Bose S. Stem cell therapy: a clinical trial of stroke. *Clinical Neurology and Neurosurgery*. 2013; 115:1003–1008. [PubMed: 23183251]
- Bhasin A, Srivastava MV, Kumaran SS, Mohanty S, Bhatia R, Bose S, Gaikwad S, Garg A, Airan B. Autologous mesenchymal stem cells in chronic stroke. *Cerebrovascular Diseases Extra*. 2011; 1:93–104. [PubMed: 22566987]
- Bible E, Dell'Acqua F, Solanky B, Balducci A, Crapo PM, Badylak SF, Ahrens ET, Modo M. Non-invasive imaging of transplanted human neural stem cells and ECM scaffold remodeling in the stroke-damaged rat brain by (19)F and diffusion-MRI. *Biomaterials*. 2012; 33:2858–2871. [PubMed: 22244696]
- Blackwood KJ, Lewden B, Wells RG, Sykes J, Stodilka RZ, Wisenberg G, Prato FS. In vivo SPECT quantification of transplanted cell survival after engraftment using (111)In-tropolone in infarcted canine myocardium. *Journal of Nuclear Medicine*. 2009; 50:927–935. [PubMed: 19470852]
- Bliss TM, Andres RH, Steinberg GK. Optimizing the success of cell transplantation therapy for stroke. *Neurobiology of Disease*. 2010; 37:275–283. [PubMed: 19822211]
- Borlongan CV, Hadman M, Sanberg CD, Sanberg PR. Central nervous system entry of peripherally injected umbilical cord blood cells is not required for neuroprotection in stroke. *Stroke*. 2004; 35:2385–2389. [PubMed: 15345799]
- Borlongan CV, Kaneko Y, Maki M, Yu SJ, Ali M, Allickson JG, Sanberg CD, Kuzmin-Nichols N, Sanberg PR. Menstrual blood cells display stem cell-like phenotypic markers and exert neuroprotection following transplantation in experimental stroke. *Stem Cells and Development*. 2010; 19:439–452. [PubMed: 19860544]
- Buhnemann C, Scholz A, Bernreuther C, Malik CY, Braun H, Schachner M, Reymann KG, Dihne M. Neuronal differentiation of transplanted embryonic stem cell-derived precursors in stroke lesions of adult rats. *Brain*. 2006; 129:3238–3248. [PubMed: 17018551]
- Cai C, Teng L, Vu D, He JQ, Guo Y, Li Q, Tang XL, Rokosh G, Bhatnagar A, Bolli R. The heme oxygenase 1 inducer (CoPP) protects human cardiac stem cells against apoptosis through activation of the extracellular signal-regulated kinase (ERK)/NRF2 signaling pathway and cytokine release. *Journal of Biological Chemistry*. 2012; 287:33720–33732. [PubMed: 22879597]
- Caiazzo M, Dell'Anno MT, Dvoretzskova E, Lazarevic D, Taverna S, Leo D, Sotnikova TD, Menegon A, Roncaglia P, Colciago G, Russo G, Carninci P, Pezzoli G, Gainetdinov RR, Gustincich S, Dityatev A, Broccoli V. Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. *Nature*. 2011; 476:224–227. [PubMed: 21725324]
- Chang DJ, Lee N, Choi C, Jeon I, Oh SH, Shin DA, Hwang TS, Lee HJ, Kim SU, Moon H, Hong KS, Kang KS, Song J. Therapeutic effect of BDNF-overexpressing human neural stem cells (HB1.F3.BDNF) in a rodent model of middle cerebral artery occlusion. *Cell Transplantation*. 2013a; 22:1441–1452. [PubMed: 23044072]
- Chang DJ, Lee N, Park IH, Choi C, Jeon I, Kwon J, Oh SH, Shin DA, Do JT, Lee DR, Lee H, Moon H, Hong KS, Daley GQ, Song J. Therapeutic potential of human induced pluripotent stem cells in experimental stroke. *Cell Transplantation*. 2013b; 22:1427–1440. [PubMed: 23044029]
- Chen B, Gao XQ, Yang CX, Tan SK, Sun ZL, Yan NH, Pang YG, Yuan M, Chen GJ, Xu GT, Zhang K, Yuan QL. Neuroprotective effect of grafting GDNF gene-modified neural stem cells on cerebral ischemia in rats. *Brain Research*. 2009; 1284:1–11. [PubMed: 19520066]
- Chen C, Cheng Y, Chen J. Transfection of Noggin in bone marrow stromal cells (BMSCs) enhances BMSC-induced functional outcome after stroke in rats. *Journal of Neuroscience Research*. 2011a; 89:1194–1202. [PubMed: 21544853]
- Chen C, Wang Y, Yang GY. Stem cell-mediated gene delivering for the treatment of cerebral ischemia: progress and perspectives. *Current Drug Targets*. 2013a; 14:81–89. [PubMed: 23170799]
- Chen J, Chopp M. Neurorestorative treatment of stroke: cell and pharmacological approaches. *NeuroRx*. 2006; 3:466–473. [PubMed: 17012060]

- Chen J, Li Y, Katakowski M, Chen X, Wang L, Lu D, Lu M, Gautam SC, Chopp M. Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat. *Journal of Neuroscience Research*. 2003a; 73:778–786. [PubMed: 12949903]
- Chen J, Li Y, Wang L, Lu M, Zhang X, Chopp M. Therapeutic benefit of intracerebral transplantation of bone marrow stromal cells after cerebral ischemia in rats. *Journal of Neuroscience Research*. 2001a; 189:49–57.
- Chen J, Li Y, Wang L, Zhang Z, Lu D, Lu M, Chopp M. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke*. 2001b; 32:1005–1011. [PubMed: 11283404]
- Chen J, Sanberg PR, Li Y, Wang L, Lu M, Willing AE, Sanchez-Ramos J, Chopp M. Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. *Stroke*. 2001c; 32:2682–2688. [PubMed: 11692034]
- Chen J, Shehadah A, Pal A, Zacharek A, Cui X, Cui Y, Roberts C, Lu M, Zeitlin A, Hariri R, Chopp M. Neuroprotective effect of human placental derived cell treatment of stroke in rats. *Cell Transplantation*. 2013b; 22:871–879. [PubMed: 22469567]
- Chen J, Ye X, Yan T, Zhang C, Yang XP, Cui X, Cui Y, Zacharek A, Roberts C, Liu X, Dai X, Lu M, Chopp M. Adverse effects of bone marrow stromal cell treatment of stroke in diabetic rats. *Stroke*. 2011b; 42:3551–3558. [PubMed: 21940967]
- Chen J, Zhang ZG, Li Y, Wang L, Xu YX, Gautam SC, Lu M, Zhu Z, Chopp M. Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. *Circulation Research*. 2003b; 92:692–699. [PubMed: 12609969]
- Chen N, Newcomb J, Garbuzova-Davis S, Davis Sanberg C, Sanberg PR, Willing AE. Human umbilical cord blood cells have trophic effects on young and aging hippocampal neurons in vitro. *Aging and Disease*. 2010a; 1:173–190. [PubMed: 21234285]
- Chen SH, Wang JJ, Chen CH, Chang HK, Lin MT, Chang FM, Chio CC. Umbilical cord blood-derived CD34+ cells improve outcomes of traumatic brain injury in rats by stimulating angiogenesis and neurogenesis. *Cell Transplantation*. 2013c
- Chen SJ, Chang CM, Tsai SK, Chang YL, Chou SJ, Huang SS, Tai LK, Chen YC, Ku HH, Li HY, Chiou SH. Functional improvement of focal cerebral ischemia injury by subdural transplantation of induced pluripotent stem cells with fibrin glue. *Stem Cells and Development*. 2010b; 19:1757–1767. [PubMed: 20192839]
- Chen TS, Lai RC, Lee MM, Choo AB, Lee CN, Lim SK. Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs. *Nucleic Acids Research*. 2010c; 38:215–224. [PubMed: 19850715]
- Chen X, Katakowski M, Li Y, Lu D, Wang L, Zhang L, Chen J, Xu Y, Gautam S, Mahmood A, Chopp M. Human bone marrow stromal cell cultures conditioned by traumatic brain tissue extracts: growth factor production. *Journal of Neuroscience Research*. 2002a; 69:687–691. [PubMed: 12210835]
- Chen X, Li Y, Wang L, Katakowski M, Zhang L, Chen J, Xu Y, Gautam SC, Chopp M. Ischemic rat brain extracts induce human marrow stromal cell growth factor production. *Neuropathology*. 2002b; 22:275–279. [PubMed: 12564767]
- Cho YJ, Song HS, Bhang S, Lee S, Kang BG, Lee JC, An J, Cha CI, Nam DH, Kim BS, Joo KM. Therapeutic effects of human adipose stem cell conditioned medium on stroke. *Journal of Neuroscience Research*. 2012; 90:1794–1802. [PubMed: 22535477]
- Chopp M, Li Y, Zhang ZG. Mechanisms underlying improved recovery of neurological function after stroke in the rodent after treatment with neurorestorative cell-based therapies. *Stroke*. 2009; 40:S143–S145. [PubMed: 19064763]
- Christopherson KS, Ullian EM, Stokes CC, Mallowney CE, Hell JW, Agah A, Lawler J, Mosher DF, Bornstein P, Barres BA. Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell*. 2005; 120:421–433. [PubMed: 15707899]
- Chu K, Kim M, Park KI, Jeong SW, Park HK, Jung KH, Lee ST, Kang L, Lee K, Park DK, Kim SU, Roh JK. Human neural stem cells improve sensorimotor deficits in the adult rat brain with experimental focal ischemia. *Brain Research*. 2004; 1016:145–153. [PubMed: 15246850]

- Chua JY, Pendharkar AV, Wang N, Choi R, Andres RH, Gaeta X, Zhang J, Moseley ME, Guzman R. Intra-arterial injection of neural stem cells using a microneedle technique does not cause microembolic strokes. *Journal of Cerebral Blood Flow and Metabolism*. 2011; 31:1263–1271. [PubMed: 21157474]
- Correa PL, Mesquita CT, Felix RM, Azevedo JC, Barbirato GB, Falcao CH, Gonzalez C, Mendonca ML, Manfrim A, de Freitas G, Oliveira CC, Silva D, Avila D, Borojevic R, Alves S, Oliveira AC Jr, Dohmann HF. Assessment of intra-arterial injected autologous bone marrow mononuclear cell distribution by radioactive labeling in acute ischemic stroke. *Clinical Nuclear Medicine*. 2007; 32:839–841. [PubMed: 18075415]
- Cui X, Chopp M, Zacharek A, Roberts C, Lu M, Savant-Bhonsale S, Chen J. Chemokine, vascular and therapeutic effects of combination simvastatin and BMSC treatment of stroke. *Neurobiology of Disease*. 2009; 36:35–41. [PubMed: 19591934]
- Daadi MM, Li Z, Arac A, Grueter BA, Sofilos M, Malenka RC, Wu JC, Steinberg GK. Molecular and magnetic resonance imaging of human embryonic stem cell-derived neural stem cell grafts in ischemic rat brain. *Molecular Therapy*. 2009; 17:1282–1291. [PubMed: 19436269]
- Danielyan L, Schafer R, von Ameln-Mayerhofer A, Bernhard F, Verleysdonk S, Buadze M, Lourhmati A, Klopfer T, Schaumann F, Schmid B, Koehle C, Proksch B, Weissert R, Reichardt HM, van den Brandt J, Buniatian GH, Schwab M, Gleiter CH, Frey WH 2nd. Therapeutic efficacy of intranasally delivered mesenchymal stem cells in a rat model of Parkinson disease. *Rejuvenation Research*. 2011; 14:3–16. [PubMed: 21291297]
- Danielyan L, Schafer R, von Ameln-Mayerhofer A, Buadze M, Geisler J, Klopfer T, Burkhardt U, Proksch B, Verleysdonk S, Ayturan M, Buniatian GH, Gleiter CH, Frey WH 2nd. Intranasal delivery of cells to the brain. *European Journal of Cell Biology*. 2009; 88:315–324. [PubMed: 19324456]
- Darsalia V, Kallur T, Kokaia Z. Survival, migration and neuronal differentiation of human fetal striatal and cortical neural stem cells grafted in strokedamaged rat striatum. *European Journal of Neuroscience*. 2007; 26:605–614. [PubMed: 17686040]
- Das R, Jahr H, van Osch GJ, Farrell E. The role of hypoxia in bone marrowderived mesenchymal stem cells: considerations for regenerative medicine approaches. *Tissue Engineering Part B: Reviews*. 2010; 16:159–168. [PubMed: 19698058]
- De Feo D, Merlini A, Laterza C, Martino G. Neural stem cell transplantation in central nervous system disorders: from cell replacement to neuroprotection. *Current Opinion in Neurology*. 2012; 25:322–333. [PubMed: 22547103]
- Del Zoppo GJ, Saver JL, Jauch EC, Adams HP Jr. Expansion of the time window for treatment of acute ischemic stroke with intravenous tissue plasminogen activator: a science advisory from the American Heart Association/American Stroke Association. *Stroke*. 2009; 40:2945–2948. [PubMed: 19478221]
- Detante O, Moisan A, Dimastromatteo J, Richard MJ, Riou L, Grillon E, Barbier E, Desruet MD, De Fraipont F, Segebarth C, Jaillard A, Hommel M, Ghezzi C, Remy C. Intravenous administration of ^{99m}Tc-HMPAO-labeled human mesenchymal stem cells after stroke: in vivo imaging and biodistribution. *Cell Transplantation*. 2009; 18:1369–1379. [PubMed: 19849895]
- Dhuria SV, Hanson LR, Frey WH 2nd. Intranasal delivery to the central nervous system: mechanisms and experimental considerations. *Journal of Pharmaceutical Sciences*. 2010; 99:1654–1673. [PubMed: 19877171]
- Ding J, Cheng Y, Gao S, Chen J. Effects of nerve growth factor and Nogginmodified bone marrow stromal cells on stroke in rats. *Journal of Neuroscience Research*. 2011; 89:222–230. [PubMed: 21162129]
- Donega V, van Velthoven CT, Nijboer CH, van Bel F, Kas MJ, Kavelaars A, Heijnen CJ. Intranasal mesenchymal stem cell treatment for neonatal brain damage: long-term cognitive and sensorimotor improvement. *PLoS ONE*. 2013; 8:e51253. [PubMed: 23300948]
- Eaves CJ, Cashman JD, Kay RJ, Dougherty GJ, Otsuka T, Gaboury LA, Hogge DE, Lansdorp PM, Eaves AC, Humphries RK. Mechanisms that regulate the cell cycle status of very primitive hematopoietic cells in long-term human marrow cultures. II. Analysis of positive and negative regulators produced by stromal cells within the adherent layer. *Blood*. 1991; 78:110–117. [PubMed: 1712638]

- Egashira Y, Sugitani S, Suzuki Y, Mishihiro K, Tsuruma K, Shimazawa M, Yoshimura S, Iwama T, Hara H. The conditioned medium of murine and human adipose-derived stem cells exerts neuroprotective effects against experimental stroke model. *Brain Research*. 2012; 1461:87–95. [PubMed: 22608076]
- Einstein O, Fainstein N, Vaknin I, Mizrahi-Kol R, Reihartz E, Grigoriadis N, Lavon I, Baniyash M, Lassmann H, Ben-Hur T. Neural precursors attenuate autoimmune encephalomyelitis by peripheral immunosuppression. *Annals of Neurology*. 2007; 61:209–218. [PubMed: 17187374]
- Eiraku M, Watanabe K, Matsuo-Takasaki M, Kawada M, Yonemura S, Matsumura M, Wataya T, Nishiyama A, Muguruma K, Sasai Y. Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. *Cell Stem Cell*. 2008; 3:519–532. [PubMed: 18983967]
- Emborg ME, Liu Y, Xi J, Zhang X, Yin Y, Lu J, Joers V, Swanson C, Holden JE, Zhang SC. Induced pluripotent stem cell-derived neural cells survive and mature in the nonhuman primate brain. *Cell Reports*. 2013; 3:646–650. [PubMed: 23499447]
- Erdo F, Buhrlé C, Blunk J, Hoehn M, Xia Y, Fleischmann B, Focking M, Kustermann E, Kolossov E, Hescheler J, Hossmann KA, Trapp T. Host-dependent tumorigenesis of embryonic stem cell transplantation in experimental stroke. *Journal of Cerebral Blood Flow and Metabolism*. 2003; 23:780–785. [PubMed: 12843782]
- Ergul A, Alhusban A, Fagan SC. Angiogenesis: a harmonized target for recovery after stroke. *Stroke*. 2012; 43:2270–2274. [PubMed: 22618382]
- Feigin VL, Lawes CM, Bennett DA, Barker-Collo SL, Parag V. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. *Lancet Neurology*. 2009; 8:355–369. [PubMed: 19233729]
- Fischer UM, Harting MT, Jimenez F, Monzon-Posadas WO, Xue H, Savitz SI, Laine GA, Cox CS Jr. Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. *Stem Cells and Development*. 2009; 18:683–692. [PubMed: 19099374]
- Fisher M. New approaches to neuroprotective drug development. *Stroke*. 2011; 42:S24–S27. [PubMed: 21164111]
- Fisher M, Feuerstein G, Howells DW, Hurn PD, Kent TA, Savitz SI, Lo EH. Update of the stroke therapy academic industry roundtable preclinical recommendations. *Stroke*. 2009; 40:2244–2250. [PubMed: 19246690]
- Fraichard A, Chassande O, Bilbaut G, Dehay C, Savatier P, Samarut J. In vitro differentiation of embryonic stem cells into glial cells and functional neurons. *Journal of Cell Science*. 1995; 108:3181–3188. [PubMed: 7593279]
- Francis KR, Wei L. Human embryonic stem cell neural differentiation and enhanced cell survival promoted by hypoxic preconditioning. *Cell Death & Disease*. 2010; 1:e22. [PubMed: 21364630]
- Franco EC, Cardoso MM, Gouveia A, Pereira A, Gomes-Leal W. Modulation of microglial activation enhances neuroprotection and functional recovery derived from bone marrow mononuclear cell transplantation after cortical ischemia. *Neuroscience Research*. 2012; 73:122–132. [PubMed: 22465414]
- Frenkel D, Solomon B. Filamentous phage as vector-mediated antibody delivery to the brain. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; 99:5675–5679. [PubMed: 11960022]
- Friedrich MA, Martins MP, Araujo MD, Klamt C, Vedolin L, Garicochea B, Raupp EF, Sartori EI, Ammar J, Machado DC, Costa JC, Nogueira RG, Rosado-de-Castro PH, Mendez-Otero R, Freitas GR. Intra-arterial infusion of autologous bone marrow mononuclear cells in patients with moderate to severe middle cerebral artery acute ischemic stroke. *Cell Transplantation*. 2012; 21(Suppl 1):S13–S21. [PubMed: 22507676]
- Fujimoto M, Hayashi H, Takagi Y, Hayase M, Marumo T, Gomi M, Nishimura M, Kataoka H, Takahashi J, Hashimoto N, Nozaki K, Miyamoto S. Transplantation of telencephalic neural progenitors induced from embryonic stem cells into subacute phase of focal cerebral ischemia. *Laboratory Investigation*. 2012; 92:522–531. [PubMed: 22330341]

- Fukuchi Y, Nakajima H, Sugiyama D, Hirose I, Kitamura T, Tsuji K. Human placenta-derived cells have mesenchymal stem/progenitor cell potential. *Stem Cells*. 2004; 22:649–658. [PubMed: 15342929]
- Ginsberg MD. Neuroprotection for ischemic stroke: past, present and future. *Neuropharmacology*. 2008; 55:363–389. [PubMed: 18308347]
- Glover LE, Tajiri N, Weinbren NL, Ishikawa H, Shinozuka K, Kaneko Y, Watterson DM, Borlongan CV. A step-up approach for cell therapy in stroke: translational hurdles of bone marrow-derived stem cells. *Translational Stroke Research*. 2012; 3:90–98. [PubMed: 22389662]
- Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Magid D, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER, Moy CS, Mussolino ME, Nichol G, Paynter NP, Schreiner PJ, Sorlie PD, Stein J, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Heart disease and stroke statistics – 2013 update: a report from the American Heart Association. *Circulation*. 2013; 127:e6–e245. [PubMed: 23239837]
- Goldman SA, Chen Z. Perivascular instruction of cell genesis and fate in the adult brain. *Nature Neuroscience*. 2011; 14:1382–1389.
- Gomi M, Takagi Y, Morizane A, Doi D, Nishimura M, Miyamoto S, Takahashi J. Functional recovery of the murine brain ischemia model using human induced pluripotent stem cell-derived telencephalic progenitors. *Brain Research*. 2012; 1459:52–60. [PubMed: 22572083]
- Gornicka-Pawlak el B, Janowski M, Habich A, Jablonska A, Drela K, Kozłowska H, Lukomska B, Sypecka J, Domanska-Janik K. Systemic treatment of focal brain injury in the rat by human umbilical cord blood cells being at different level of neural commitment. *Acta Neurobiologiae Experimentalis*. 2011; 71:46–64. [PubMed: 21499326]
- Greenberg DA, Jin K. Vascular endothelial growth factors (VEGFs) and stroke. *Cellular and Molecular Life Sciences*. 2013; 70:1753–1761. [PubMed: 23475070]
- Grotta JC, Jacobs TP, Koroshetz WJ, Moskowitz MA. Stroke program review group: an interim report. *Stroke*. 2008; 39:1364–1370. [PubMed: 18309142]
- Guzman R, Bliss T, De Los Angeles A, Moseley M, Palmer T, Steinberg G. Neural progenitor cells transplanted into the uninjured brain undergo targeted migration after stroke onset. *Journal of Neuroscience Research*. 2008a; 86:873–882. [PubMed: 17975825]
- Guzman R, Choi R, Gera A, De Los Angeles A, Andres RH, Steinberg GK. Intravascular cell replacement therapy for stroke. *Neurosurgical Focus*. 2008b; 24:E15. [PubMed: 18341391]
- Guzman R, De Los Angeles A, Cheshier S, Choi R, Hoang S, Liauw J, Schaar B, Steinberg G. Intracarotid injection of fluorescence activated cell-sorted CD49d-positive neural stem cells improves targeted cell delivery and behavior after stroke in a mouse stroke model. *Stroke*. 2008c; 39:1300–1306. [PubMed: 18309158]
- Guzman R, Uchida N, Bliss TM, He D, Christopherson KK, Stellwagen D, Capela A, Greve J, Malenka RC, Moseley ME, Palmer TD, Steinberg GK. Long-term monitoring of transplanted human neural stem cells in developmental and pathological contexts with MRI. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:10211–10216. [PubMed: 17553967]
- Han IK, Kim MY, Byun HM, Hwang TS, Kim JM, Hwang KW, Park TG, Jung WW, Chun T, Jeong GJ, Oh YK. Enhanced brain targeting efficiency of intranasally administered plasmid DNA: an alternative route for brain gene therapy. *Journal of Molecular Medicine*. 2007; 85:75–83. [PubMed: 17089096]
- Hayashi J, Takagi Y, Fukuda H, Imazato T, Nishimura M, Fujimoto M, Takahashi J, Hashimoto N, Nozaki K. Primate embryonic stem cell-derived neuronal progenitors transplanted into ischemic brain. *Journal of Cerebral Blood Flow and Metabolism*. 2006; 26:906–914. [PubMed: 16395293]
- Hayashi T, Noshita N, Sugawara T, Chan PH. Temporal profile of angiogenesis and expression of related genes in the brain after ischemia. *Journal of Cerebral Blood Flow and Metabolism*. 2003; 23:166–180. [PubMed: 12571448]

- He A, Jiang Y, Gui C, Sun Y, Li J, Wang JA. The antiapoptotic effect of mesenchymal stem cell transplantation on ischemic myocardium is enhanced by anoxic preconditioning. *Canadian Journal of Cardiology*. 2009; 25:353–358. [PubMed: 19536376]
- Hedlund M, Stenqvist AC, Nagaeva O, Kjellberg L, Wulff M, Baranov V, Mincheva-Nilsson L. Human placenta expresses and secretes NKG2D ligands via exosomes that down-modulate the cognate receptor expression: evidence for immunosuppressive function. *Journal of Immunology*. 2009; 183:340–351.
- Henning RJ, Abu-Ali H, Balis JU, Morgan MB, Willing AE, Sanberg PR. Human umbilical cord blood mononuclear cells for the treatment of acute myocardial infarction. *Cell Transplantation*. 2004; 13:729–739. [PubMed: 15690974]
- Henning RJ, Burgos JD, Vasko M, Alvarado F, Sanberg CD, Sanberg PR, Morgan MB. Human cord blood cells and myocardial infarction: effect of dose and route of administration on infarct size. *Cell Transplantation*. 2007; 16:907–917. [PubMed: 18293889]
- Hess DC, Borlongan CV. Stem cells and neurological diseases. *Cell Proliferation*. 2008; 41(Suppl 1): 94–114. [PubMed: 18181951]
- Hicks AU, Lappalainen RS, Narkilahti S, Suuronen R, Corbett D, Sivenius J, Hovatta O, Jolkonen J. Transplantation of human embryonic stem cell-derived neural precursor cells and enriched environment after cortical stroke in rats: cell survival and functional recovery. *European Journal of Neuroscience*. 2009; 29:562–574. [PubMed: 19175403]
- Hirko AC, Dallsen R, Jomura S, Xu Y. Modulation of inflammatory responses after global ischemia by transplanted umbilical cord matrix stem cells. *Stem Cells*. 2008; 26:2893–2901. [PubMed: 18719227]
- Hoehn M, Kustermann E, Blunk J, Wiedermann D, Trapp T, Wecker S, Focking M, Arnold H, Hescheler J, Fleischmann BK, Schwandt W, Buhle C. Monitoring of implanted stem cell migration in vivo: a highly resolved in vivo magnetic resonance imaging investigation of experimental stroke in rat. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; 99:16267–16272. [PubMed: 12444255]
- Hoehn M, Wiedermann D, Justicia C, Ramos-Cabrer P, Kruttwig K, Farr T, Himmelreich U. Cell tracking using magnetic resonance imaging. *Journal of Physiology*. 2007; 584:25–30. [PubMed: 17690140]
- Honmou O, Houkin K, Matsunaga T, Niitsu Y, Ishiai S, Onodera R, Waxman SG, Kocsis JD. Intravenous administration of auto serum-expanded autologous mesenchymal stem cells in stroke. *Brain*. 2011; 134:1790–1807. [PubMed: 21493695]
- Honmou O, Onodera R, Sasaki M, Waxman SG, Kocsis JD. Mesenchymal stem cells: therapeutic outlook for stroke. *Trends in Molecular Medicine*. 2012; 18:292–297. [PubMed: 22459358]
- Horita Y, Honmou O, Harada K, Houkin K, Hamada H, Kocsis JD. Intravenous administration of glial cell line-derived neurotrophic factor gene-modified human mesenchymal stem cells protects against injury in a cerebral ischemia model in the adult rat. *Journal of Neuroscience Research*. 2006; 84:1495–1504. [PubMed: 16998918]
- Hu X, Wei L, Taylor TM, Wei J, Zhou X, Wang JA, Yu SP. Hypoxic preconditioning enhances bone marrow mesenchymal stem cell migration via Kv2.1 channel and FAK activation. *American Journal of Physiology Cell Physiology*. 2011; 301:C362–C372. [PubMed: 21562308]
- Huang W, Mo X, Qin C, Zheng J, Liang Z, Zhang C. Transplantation of differentiated bone marrow stromal cells promotes motor functional recovery in rats with stroke. *Neurological Research*. 2013; 35:320–328. [PubMed: 23485057]
- Idris NM, Ashraf M, Ahmed RP, Shujia J, Haider KH. Activation of IL-11/STAT3 pathway in preconditioned human skeletal myoblasts blocks apoptotic cascade under oxidant stress. *Regenerative Medicine*. 2012; 7:47–57. [PubMed: 22168497]
- Igura K, Zhang X, Takahashi K, Mitsuru A, Yamaguchi S, Takashi TA. Isolation and characterization of mesenchymal progenitor cells from chorionic villi of human placenta. *Cytotherapy*. 2004; 6:543–553. [PubMed: 15770794]
- Ii M, Nishimura H, Iwakura A, Wecker A, Eaton E, Asahara T, Losordo DW. Endothelial progenitor cells are rapidly recruited to myocardium and mediate protective effect of ischemic

preconditioning via “imported” nitric oxide synthase activity. *Circulation*. 2005; 111:1114–1120. [PubMed: 15723985]

- Ii M, Nishimura H, Sekiguchi H, Kamei N, Yokoyama A, Horii M, Asahara T. Concurrent vasculogenesis and neurogenesis from adult neural stem cells. *Circulation Research*. 2009; 105:860–868. [PubMed: 19762683]
- Inoue T, Sugiyama M, Hattori H, Wakita H, Wakabayashi T, Ueda M. Stem cells from human exfoliated deciduous tooth-derived conditioned medium enhance recovery of focal cerebral ischemia in rats. *Tissue Engineering Part A*. 2013; 19:24–29. [PubMed: 22839964]
- Ishibashi S, Sakaguchi M, Kuroiwa T, Yamasaki M, Kanemura Y, Shizuko I, Shimazaki T, Onodera M, Okano H, Mizusawa H. Human neural stem/progenitor cells, expanded in long-term neurosphere culture, promote functional recovery after focal ischemia in Mongolian gerbils. *Journal of Neuroscience Research*. 2004; 78:215–223. [PubMed: 15378509]
- Ito D, Okano H, Suzuki N. Accelerating progress in induced pluripotent stem cell research for neurological diseases. *Annals of Neurology*. 2012; 72:167–174. [PubMed: 22926850]
- Jaiswal JK, Simon SM. Imaging single events at the cell membrane. *Nature Chemical Biology*. 2007; 3:92–98.
- Jat PS, Noble MD, Ataliotis P, Tanaka Y, Yannoutsos N, Larsen L, Kioussis D. Direct derivation of conditionally immortal cell lines from an H-2Kb-tsA58 transgenic mouse. *Proceedings of the National Academy of Sciences of the United States of America*. 1991; 88:5096–5100. [PubMed: 1711218]
- Jendelova P, Herynek V, DeCroos J, Glogarova K, Andersson B, Hajek M, Sykova E. Imaging the fate of implanted bone marrow stromal cells labeled with superparamagnetic nanoparticles. *Magnetic Resonance in Medicine*. 2003; 50:767–776. [PubMed: 14523963]
- Jensen MB, Yan H, Krishnaney-Davison R, Al Sawaf A, Zhang SC. Survival and differentiation of transplanted neural stem cells derived from human induced pluripotent stem cells in a rat stroke model. *Journal of Stroke and Cerebrovascular Diseases*. 2013; 22:304–308. [PubMed: 22078778]
- Jiang B, Xiao W, Shi Y, Liu M, Xiao X. Heat shock pretreatment inhibited the release of Smac/DIABLO from mitochondria and apoptosis induced by hydrogen peroxide in cardiomyocytes and C2C12 myogenic cells. *Cell Stress & Chaperones*. 2005; 10:252–262. [PubMed: 16184770]
- Jiang M, Lv L, Ji H, Yang X, Zhu W, Cai L, Gu X, Chai C, Huang S, Sun J, Dong Q. Induction of pluripotent stem cells transplantation therapy for ischemic stroke. *Molecular and Cellular Biochemistry*. 2011a; 354:67–75. [PubMed: 21465238]
- Jiang Y, Wei N, Zhu J, Zhai D, Wu L, Chen M, Xu G, Liu X. A new approach with less damage: intranasal delivery of tetracycline-inducible replication-defective herpes simplex virus type-1 vector to brain. *Neuroscience*. 2012a; 201:96–104. [PubMed: 22101000]
- Jiang Y, Zhu J, Xu G, Liu X. Intranasal delivery of stem cells to the brain. *Expert Opinion on Drug Delivery*. 2011b; 8:623–632. [PubMed: 21417782]
- Jiang Y, Zhu W, Zhu J, Wu L, Xu G, Liu X. Feasibility of delivering mesenchymal stem cells via catheter to the proximal end of lesion artery in patients with stroke in the territory of middle cerebral artery. *Cell Transplantation*. 2012b
- Jin K, Mao X, Xie L, Greenberg RB, Peng B, Moore A, Greenberg MB, Greenberg DA. Delayed transplantation of human neural precursor cells improves outcome from focal cerebral ischemia in aged rats. *Aging Cell*. 2010; 9:1076–1083. [PubMed: 20883527]
- Jin K, Minami M, Lan JQ, Mao XO, Batteur S, Simon RP, Greenberg DA. Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. *Proceedings of the National Academy of Sciences of the United States of America*. 2001; 98:4710–4715. [PubMed: 11296300]
- Jin K, Xie L, Mao X, Greenberg MB, Moore A, Peng B, Greenberg RB, Greenberg DA. Effect of human neural precursor cell transplantation on endogenous neurogenesis after focal cerebral ischemia in the rat. *Brain Research*. 2011; 1374:56–62. [PubMed: 21167824]
- Johnston SC, Mendis S, Mathers CD. Global variation in stroke burden and mortality: estimates from monitoring, surveillance, and modelling. *Lancet Neurology*. 2009; 8:345–354. [PubMed: 19233730]

- Kamiya N, Ueda M, Igarashi H, Nishiyama Y, Suda S, Inaba T, Katayama Y. Intra-arterial transplantation of bone marrow mononuclear cells immediately after reperfusion decreases brain injury after focal ischemia in rats. *Life Sciences*. 2008; 83:433–437. [PubMed: 18727931]
- Kang SK, Lee DH, Bae YC, Kim HK, Baik SY, Jung JS. Improvement of neurological deficits by intracerebral transplantation of human adipose tissue-derived stromal cells after cerebral ischemia in rats. *Experimental Neurology*. 2003; 183:355–366. [PubMed: 14552877]
- Kawabori M, Kuroda S, Sugiyama T, Ito M, Shichinohe H, Houkin K, Kuge Y, Tamaki N. 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. [PubMed: 22007875]
- Kawai H, Yamashita T, Ohta Y, Deguchi K, Nagotani S, Zhang X, Ikeda Y, Matsuura T, Abe K. Tridermal tumorigenesis of induced pluripotent stem cells transplanted in ischemic brain. *Journal of Cerebral Blood Flow and Metabolism*. 2010; 30:1487–1493. [PubMed: 20216552]
- Kelly S, Bliss TM, Shah AK, Sun GH, Ma M, Foo WC, Masel J, Yenari MA, Weissman IL, Uchida N, Palmer T, Steinberg GK. Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:11839–11844. [PubMed: 15280535]
- Kim DE, Schellingerhout D, Ishii K, Shah K, Weissleder R. Imaging of stem cell recruitment to ischemic infarcts in a murine model. *Stroke*. 2004; 35:952–957. [PubMed: 14988578]
- Kim JH, Oh AY, Choi YM, Ku SY, Kim YY, Lee NJ, Sepac A, Bosnjak ZJ. Isoflurane decreases death of human embryonic stem cell-derived, transcriptional marker Nkx2.5(+) cardiac progenitor cells. *Acta Anaesthesiologica Scandinavica*. 2011; 55:1124–1131. [PubMed: 22092211]
- Kim SS, Yoo SW, Park TS, Ahn SC, Jeong HS, Kim JW, Chang DY, Cho KG, Kim SU, Huh Y, Lee JE, Lee SY, Lee YD, Suh-Kim H. Neural induction with neurogenin1 increases the therapeutic effects of mesenchymal stem cells in the ischemic brain. *Stem Cells*. 2008; 26:2217–2228. [PubMed: 18617687]
- Kinnaird T, Stabile E, Burnett MS, Epstein SE. Bone marrow-derived cells for enhancing collateral development: mechanisms, animal data, and initial clinical experiences. *Circulation Research*. 2004a; 95:354–363. [PubMed: 15321945]
- Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, Epstein SE. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. *Circulation Research*. 2004b; 94:678–685. [PubMed: 14739163]
- Kobayashi T, Ahlenius H, Thored P, Kobayashi R, Kokaia Z, Lindvall O. Intracerebral infusion of glial cell line-derived neurotrophic factor promotes striatal neurogenesis after stroke in adult rats. *Stroke*. 2006; 37:2361–2367. [PubMed: 16873711]
- Kocsis JD, Honmou O. Bone marrow stem cells in experimental stroke. *Progress in Brain Research*. 2012; 201:79–98. [PubMed: 23186711]
- Koh W, Sheng CT, Tan B, Lee QY, Kuznetsov V, Kiang LS, Tanavde V. Analysis of deep sequencing microRNA expression profile from human embryonic stem cells derived mesenchymal stem cells reveals possible role of let-7 microRNA family in downstream targeting of hepatic nuclear factor 4 alpha. *BMC Genomics*. 2010; 11(Suppl 1):S6. [PubMed: 20158877]
- Kokaia Z, Martino G, Schwartz M, Lindvall O. Cross-talk between neural stem cells and immune cells: the key to better brain repair? *Nature Neuroscience*. 2012; 15:1078–1087.
- Kondo-Nakamura M, Shintani-Ishida K, Uemura K, Yoshida K. Brief exposure to carbon monoxide preconditions cardiomyogenic cells against apoptosis in ischemia–reperfusion. *Biochemical and Biophysical Research Communications*. 2010; 393:449–454. [PubMed: 20152804]
- Kondziolka D, Steinberg GK, Wechsler L, Meltzer CC, Elder E, Gebel J, Decesare S, Jovin T, Zafonte R, Lebowitz J, Flickinger JC, Tong D, Marks MP, Jamieson C, Luu D, Bell-Stephens T, Teraoka J. Neurotransplantation for patients with subcortical motor stroke: a phase 2 randomized trial. *Journal of Neurosurgery*. 2005; 103:38–45. [PubMed: 16121971]

- Kondziolka D, Wechsler L, Goldstein S, Meltzer C, Thulborn KR, Gebel J, Jannetta P, DeCesare S, Elder EM, McGrogan M, Reitman MA, Bynum L. Transplantation of cultured human neuronal cells for patients with stroke. *Neurology*. 2000; 55:565–569. [PubMed: 10953194]
- Kozłowska H, Jablonka J, Janowski M, Jurga M, Kossut M, Domanska-Janik K. Transplantation of a novel human cord blood-derived neural-like stem cell line in a rat model of cortical infarct. *Stem Cells and Development*. 2007; 16:481–488. [PubMed: 17610378]
- Kranz A, Wagner DC, Kamprad M, Scholz M, Schmidt UR, Nitzsche F, Aberman Z, Emmrich F, Riegelsberger UM, Boltze J. Transplantation of placenta-derived mesenchymal stromal cells upon experimental stroke in rats. *Brain Research*. 2010; 1315:128–136. [PubMed: 20004649]
- Krupinski J, Kaluza J, Kumar P, Kumar S, Wang JM. Role of angiogenesis in patients with cerebral ischemic stroke. *Stroke*. 1994; 25:1794–1798. [PubMed: 7521076]
- Kurozumi K, Nakamura K, Tamiya T, Kawano Y, Ishii K, Kobune M, Hirai S, Uchida H, Sasaki K, Ito Y, Kato K, Honmou O, Houkin K, Date I, Hamada H. Mesenchymal stem cells that produce neurotrophic factors reduce ischemic damage in the rat middle cerebral artery occlusion model. *Molecular Therapy*. 2005; 11:96–104. [PubMed: 15585410]
- Kurozumi K, Nakamura K, Tamiya T, Kawano Y, Kobune M, Hirai S, Uchida H, Sasaki K, Ito Y, Kato K, Honmou O, Houkin K, Date I, Hamada H. BDNF gene-modified mesenchymal stem cells promote functional recovery and reduce infarct size in the rat middle cerebral artery occlusion model. *Molecular Therapy*. 2004; 9:189–197. [PubMed: 14759803]
- Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, Salto-Tellez M, Timmers L, Lee CN, El Oakley RM, Pasterkamp G, de Kleijn DP, Lim SK. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. *Stem Cell Research*. 2010; 4:214–222. [PubMed: 20138817]
- Lappalainen RS, Narkilahti S, Huhtala T, Liimatainen T, Suuronen T, Narvanen A, Suuronen R, Hovatta O, Jolkkonen J. The SPECT imaging shows the accumulation of neural progenitor cells into internal organs after systemic administration in middle cerebral artery occlusion rats. *Neuroscience Letters*. 2008; 440:246–250. [PubMed: 18572314]
- Lee HJ, Kim KS, Kim EJ, Choi HB, Lee KH, Park IH, Ko Y, Jeong SW, Kim SU. Brain transplantation of immortalized human neural stem cells promotes functional recovery in mouse intracerebral hemorrhage stroke model. *Stem Cells*. 2007a; 25:1204–1212. [PubMed: 17218400]
- Lee HJ, Kim KS, Park IH, Kim SU. Human neural stem cells overexpressing VEGF provide neuroprotection, angiogenesis and functional recovery in mouse stroke model. *PLoS ONE*. 2007b; 2:e156. [PubMed: 17225860]
- 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. [PubMed: 20506226]
- Lee SH, Lumelsky N, Studer L, Auerbach JM, McKay RD. Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. *Nature Biotechnology*. 2000; 18:675–679.
- Lee ST, Chu K, Jung KH, Kim SJ, Kim DH, Kang KM, Hong NH, Kim JH, Ban JJ, Park HK, Kim SU, Park CG, Lee SK, Kim M, Roh JK. Antiinflammatory mechanism of intravascular neural stem cell transplantation in haemorrhagic stroke. *Brain*. 2008; 131:616–629. [PubMed: 18156155]
- Leonardo CC, Hall AA, Collier LA, Ajmo CT Jr, Willing AE, Pennypacker KR. Human umbilical cord blood cell therapy blocks the morphological change and recruitment of CD11b-expressing, isolectin-binding proinflammatory cells after middle cerebral artery occlusion. *Journal of Neuroscience Research*. 2010; 88:1213–1222. [PubMed: 19998484]
- Leong WK, Henshall TL, Arthur A, Kremer KL, Lewis MD, Helps SC, Field J, Hamilton-Bruce MA, Warming S, Manavis J, Vink R, Gronthos S, Koblar SA. Human adult dental pulp stem cells enhance poststroke functional recovery through non-neural replacement mechanisms. *Stem Cells Translational Medicine*. 2012; 1:177–187. [PubMed: 23197777]
- Li F, Ambrosini G, Chu EY, Plescia J, Tognin S, Marchisio PC, Altieri DC. Control of apoptosis and mitotic spindle checkpoint by survivin. *Nature*. 1998; 396:580–584. [PubMed: 9859993]

- Li JH, Zhang N, Wang JA. Improved anti-apoptotic and anti-remodeling potency of bone marrow mesenchymal stem cells by anoxic pre-conditioning in diabetic cardiomyopathy. *Journal of Endocrinological Investigation*. 2008; 31:103–110. [PubMed: 18362500]
- Li L, Jiang Q, Ding G, Zhang L, Zhang ZG, Li Q, Panda S, Lu M, Ewing JR, Chopp M. Effects of administration route on migration and distribution of neural progenitor cells transplanted into rats with focal cerebral ischemia, an MRI study. *Journal of Cerebral Blood Flow and Metabolism*. 2010; 30:653–662. [PubMed: 19888287]
- Li L, Zeng H, Chen JX. Apelin-13 increases myocardial progenitor cells and improves repair postmyocardial infarction. *American Journal of Physiology Heart and Circulatory Physiology*. 2012; 303:H605–H618. [PubMed: 22752632]
- Li Y, Chen J, Chen XG, Wang L, Gautam SC, Xu YX, Katakowski M, Zhang LJ, Lu M, Janakiraman N, Chopp M. Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. *Neurology*. 2002; 59:514–523. [PubMed: 12196642]
- Li Y, Chen J, Chopp M. Adult bone marrow transplantation after stroke in adult rats. *Cell Transplantation*. 2001a; 10:31–40. [PubMed: 11294470]
- Li Y, Chen J, Wang L, Lu M, Chopp M. Treatment of stroke in rat with intracarotid administration of marrow stromal cells. *Neurology*. 2001b; 56:1666–1672. [PubMed: 11425931]
- Li Y, Chen J, Zhang CL, Wang L, Lu D, Katakowski M, Gao Q, Shen LH, Zhang J, Lu M, Chopp M. Gliosis and brain remodeling after treatment of stroke in rats with marrow stromal cells. *Glia*. 2005; 49:407–417. [PubMed: 15540231]
- Li Y, Chopp M, Chen J, Wang L, Gautam SC, Xu YX, Zhang Z. Intrastriatal transplantation of bone marrow nonhematopoietic cells improves functional recovery after stroke in adult mice. *Journal of Cerebral Blood Flow and Metabolism*. 2000; 20:1311–1319. [PubMed: 10994853]
- Li Y, Lu Z, Keogh CL, Yu SP, Wei L. Erythropoietin-induced neurovascular protection, angiogenesis, and cerebral blood flow restoration after focal ischemia in mice. *Journal of Cerebral Blood Flow and Metabolism*. 2007; 27:1043–1054. [PubMed: 17077815]
- Li Y, McIntosh K, Chen J, Zhang C, Gao Q, Borneman J, Raginski K, Mitchell J, Shen L, Zhang J, Lu D, Chopp M. Allogeneic bone marrow stromal cells promote glial-axonal remodeling without immunologic sensitization after stroke in rats. *Experimental Neurology*. 2006; 198:313–325. [PubMed: 16455080]
- Lim JY, Jeong CH, Jun JA, Kim SM, Ryu CH, Hou Y, Oh W, Chang JW, Jeun SS. 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 Research & Therapy*. 2011; 2:38. [PubMed: 21939558]
- Liu H, Honmou O, Harada K, Nakamura K, Houkin K, Hamada H, Kocsis JD. Neuroprotection by PlGF gene-modified human mesenchymal stem cells after cerebral ischaemia. *Brain*. 2006; 129:2734–2745. [PubMed: 16901914]
- Liu N, Deguchi K, Yamashita T, Liu W, Ikeda Y, Abe K. Intracerebral transplantation of bone marrow stromal cells ameliorates tissue plasminogen activator-induced brain damage after cerebral ischemia in mice detected by in vivo and ex vivo optical imaging. *Journal of Neuroscience Research*. 2012a; 90:2086–2093. [PubMed: 22791305]
- Liu N, Zhang Y, Fan L, Yuan M, Du H, Cheng R, Liu D, Lin F. Effects of transplantation with bone marrow-derived mesenchymal stem cells modified by Survivin on experimental stroke in rats. *Journal of Translational Medicine*. 2011; 9:105. [PubMed: 21733181]
- Liu X. Clinical trials of intranasal delivery for treating neurological disorders – a critical review. *Expert Opinion on Drug Delivery*. 2011; 8:1681–1690. [PubMed: 22097907]
- Liu X. Beyond the time window of intravenous thrombolysis: standing by or by stenting. *Interventional Neurology*. 2012; 1:3–15.
- Liu YP, Seckin H, Izci Y, Du ZW, Yan YP, Baskaya MK. Neuroprotective effects of mesenchymal stem cells derived from human embryonic stem cells in transient focal cerebral ischemia in rats. *Journal of Cerebral Blood Flow and Metabolism*. 2009; 29:780–791. [PubMed: 19209181]
- Liu Z, Li Y, Qu R, Shen L, Gao Q, Zhang X, Lu M, Savant-Bhonsale S, Borneman J, Chopp M. Axonal sprouting into the denervated spinal cord and synaptic and postsynaptic protein

expression in the spinal cord after transplantation of bone marrow stromal cell in stroke rats. *Brain Research*. 2007; 1149:172–180. [PubMed: 17362881]

- Liu Z, Li Y, Zhang L, Xin H, Cui Y, Hanson LR, Frey WH 2nd, Chopp M. Subacute intranasal administration of tissue plasminogen activator increases functional recovery and axonal remodeling after stroke in rats. *Neurobiology of Disease*. 2012b; 45:804–809. [PubMed: 22115941]
- Lu D, Sanberg PR, Mahmood A, Li Y, Wang L, Sanchez-Ramos J, Chopp M. Intravenous administration of human umbilical cord blood reduces neurological deficit in the rat after traumatic brain injury. *Cell Transplantation*. 2002; 11:275–281. [PubMed: 12075993]
- Lu G, Ashraf M, Haider KH. Insulin-like growth factor-1 preconditioning accentuates intrinsic survival mechanism in stem cells to resist ischemic injury by orchestrating protein kinase Calpha-ERK1/2 activation. *Antioxidants & Redox Signaling*. 2012; 16:217–227. [PubMed: 21923556]
- Lu T, Jiang Y, Zhou Z, Yue X, Wei N, Chen Z, Ma M, Xu G, Liu X. Intranasal ginsenoside Rb1 targets the brain and ameliorates cerebral ischemia/reperfusion injury in rats. *Biological & Pharmaceutical Bulletin*. 2011; 34:1319–1324. [PubMed: 21804225]
- Ma YP, Ma MM, Ge S, Guo RB, Zhang HJ, Frey WH 2nd, Xu GL, Liu XF. Intranasally delivered TGF-beta1 enters brain and regulates gene expressions of its receptors in rats. *Brain Research Bulletin*. 2007; 74:271–277. [PubMed: 17720549]
- Majumdar MK, Thiede MA, Mosca JD, Moorman M, Gerson SL. Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (MSCs) and stromal cells. *Journal of Cellular Physiology*. 1998; 176:57–66. [PubMed: 9618145]
- Makinen S, Kekarainen T, Nystedt J, Liimatainen T, Huhtala T, Narvanen A, Laine J, Jolkkonen J. Human umbilical cord blood cells do not improve sensorimotor or cognitive outcome following transient middle cerebral artery occlusion in rats. *Brain Research*. 2006; 1123:207–215. [PubMed: 17070789]
- Manley NC, Steinberg GK. Tracking stem cells for cellular therapy in stroke. *Current Pharmaceutical Design*. 2012; 18:3685–3693. [PubMed: 22571604]
- Martino G, Franklin RJ, Baron Van Evercooren A, Kerr DA. Stem cell transplantation in multiple sclerosis: current status and future prospects. *Nature Reviews Neurology*. 2010; 6:247–255.
- Martino G, Pluchino S. The therapeutic potential of neural stem cells. *Nature Reviews Neuroscience*. 2006; 7:395–406.
- Martino G, Pluchino S, Bonfanti L, Schwartz M. Brain regeneration in physiology and pathology: the immune signature driving therapeutic plasticity of neural stem cells. *Physiological Reviews*. 2011; 91:1281–1304. [PubMed: 22013212]
- Matsuda-Hashii Y, Takai K, Ohta H, Fujisaki H, Tokimasa S, Osugi Y, Ozono K, Matsumoto K, Nakamura T, Hara J. Hepatocyte growth factor plays roles in the induction and autocrine maintenance of bone marrow stromal cell IL-11, SDF-1 alpha, and stem cell factor. *Experimental Hematology*. 2004; 32:955–961. [PubMed: 15504551]
- McGuckin CP, Jurga M, Miller AM, Sarnowska A, Wiedner M, Boyle NT, Lynch MA, Jablonska A, Drela K, Lukomska B, Domanska-Janik K, Kenner L, Moriggl R, Degoul O, Perruisseau-Carrier C, Forraz N. Ischemic brain injury: a consortium analysis of key factors involved in mesenchymal stem cell-mediated inflammatory reduction. *Archives of Biochemistry and Biophysics*. 2013; 534:88–97. [PubMed: 23466243]
- Mehler MF, Rozental R, Dougherty M, Spray DC, Kessler JA. Cytokine regulation of neuronal differentiation of hippocampal progenitor cells. *Nature*. 1993; 362:62–65. [PubMed: 8383296]
- Meng S, Cao J, Wang L, Zhou Q, Li Y, Shen C, Zhang X, Wang C. MicroRNA 107 partly inhibits endothelial progenitor cells differentiation via HIF-1beta. *PLoS ONE*. 2012; 7:e40323. [PubMed: 22792280]
- Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ, Sundaresan G, Wu AM, Gambhir SS, Weiss S. Quantum dots for live cells, in vivo imaging, and diagnostics. *Science*. 2005; 307:538–544. [PubMed: 15681376]
- Miki Y, Nonoguchi N, Ikeda N, Coffin RS, Kuroiwa T, Miyatake S. Vascular endothelial growth factor gene-transferred bone marrow stromal cells engineered with a herpes simplex virus type 1

- vector can improve neurological deficits and reduce infarction volume in rat brain ischemia. *Neurosurgery*. 2007; 61:586–594. (discussion 594-585). [PubMed: 17881973]
- Mine Y, Tatarishvili J, Oki K, Monni E, Kokaia Z, Lindvall O. Grafted human neural stem cells enhance several steps of endogenous neurogenesis and improve behavioral recovery after middle cerebral artery occlusion in rats. *Neurobiology of Disease*. 2013; 52:191–203. [PubMed: 23276704]
- Mir O, Savitz SI. Stem cell therapy in stroke treatment: is it a viable option? *Expert Review of Neurotherapeutics*. 2013; 13:119–121. [PubMed: 23368796]
- Misra V, Ritchie MM, Stone LL, Low WC, Janardhan V. Stem cell therapy in ischemic stroke: role of IV and intra-arterial therapy. *Neurology*. 2012; 79:S207–S212. [PubMed: 23008400]
- Mitkari B, Kerkela E, Nystedt J, Korhonen M, Mikkonen V, Huhtala T, Jolkonen J. Intra-arterial infusion of human bone marrow-derived mesenchymal stem cells results in transient localization in the brain after cerebral ischemia in rats. *Experimental Neurology*. 2013; 239:158–162. [PubMed: 23059455]
- Miura K, Okada Y, Aoi T, Okada A, Takahashi K, Okita K, Nakagawa M, Koyanagi M, Tanabe K, Ohnuki M, Ogawa D, Ikeda E, Okano H, Yamanaka S. Variation in the safety of induced pluripotent stem cell lines. *Nature Biotechnology*. 2009; 27:743–745.
- Modo M, Beech JS, Meade TJ, Williams SC, Price J. A chronic 1 year assessment of MRI contrast agent-labelled neural stem cell transplants in stroke. *Neuroimage*. 2009; 47(Suppl 2):T133–T142. [PubMed: 18634886]
- Modo M, Cash D, Mellodew K, Williams SC, Fraser SE, Meade TJ, Price J, Hodges H. Tracking transplanted stem cell migration using bifunctional, contrast agent-enhanced, magnetic resonance imaging. *Neuroimage*. 2002a; 17:803–811. [PubMed: 12377155]
- Modo M, Mellodew K, Cash D, Fraser SE, Meade TJ, Price J, Williams SC. Mapping transplanted stem cell migration after a stroke: a serial, in vivo magnetic resonance imaging study. *Neuroimage*. 2004; 21:311–317. [PubMed: 14741669]
- Modo M, Rezaie P, Heuschling P, Patel S, Male DK, Hodges H. Transplantation of neural stem cells in a rat model of stroke: assessment of short-term graft survival and acute host immunological response. *Brain Research*. 2002b; 958:70–82. [PubMed: 12468031]
- Modo M, Stroemer RP, Tang E, Patel S, Hodges H. Effects of implantation site of stem cell grafts on behavioral recovery from stroke damage. *Stroke*. 2002c; 33:2270–2278. [PubMed: 12215598]
- Moniche F, Gonzalez A, Gonzalez-Marcos JR, Carmona M, Pinero P, Espigado I, Garcia-Solis D, Cayuela A, Montaner J, Boada C, Rosell A, Jimenez MD, Mayol A, Gil-Peralta A. Intra-arterial bone marrow mononuclear cells in ischemic stroke: a pilot clinical trial. *Stroke*. 2012; 43:2242–2244. [PubMed: 22764211]
- Morgado JM, Pratas R, Laranjeira P, Henriques A, Crespo I, Regateiro F, Paiva A. The phenotypical and functional characteristics of cord blood monocytes and CD14($_{\text{low}}$)/CD16(+) dendritic cells can be relevant to the development of cellular immune responses after transplantation. *Transplant Immunology*. 2008; 19:55–63. [PubMed: 18346638]
- Nagai N, Kawao N, Okada K, Okumoto K, Teramura T, Ueshima S, Umemura K, Matsuo O. Systemic transplantation of embryonic stem cells accelerates brain lesion decrease and angiogenesis. *Neuroreport*. 2010; 21:575–579. [PubMed: 20431496]
- Nakano-Doi A, Nakagomi T, Fujikawa M, Nakagomi N, Kubo S, Lu S, Yoshikawa H, Soma T, Taguchi A, Matsuyama T. Bone marrow mononuclear cells promote proliferation of endogenous neural stem cells through vascular niches after cerebral infarction. *Stem Cells*. 2010; 28:1292–1302. [PubMed: 20517983]
- Neuhoff S, Moers J, Rieks M, Grunwald T, Jensen A, Dermietzel R, Meier C. Proliferation, differentiation, and cytokine secretion of human umbilical cord blood-derived mononuclear cells in vitro. *Experimental Hematology*. 2007; 35:1119–1131. [PubMed: 17588481]
- Niagara MI, Haider H, Jiang S, Ashraf M. Pharmacologically preconditioned skeletal myoblasts are resistant to oxidative stress and promote angiomyogenesis via release of paracrine factors in the infarcted heart. *Circulation Research*. 2007; 100:545–555. [PubMed: 17234963]
- Nikolic WV, Hou H, Town T, Zhu Y, Giunta B, Sanberg CD, Zeng J, Luo D, Ehrhart J, Mori T, Sanberg PR, Tan J. Peripherally administered human umbilical cord blood cells reduce

- parenchymal and vascular beta-amyloid deposits in Alzheimer mice. *Stem Cells and Development*. 2008; 17:423–439. [PubMed: 18366296]
- Nishishita T, Ouchi K, Zhang X, Inoue M, Inazawa T, Yoshiura K, Kuwabara K, Nakaoka T, Watanabe N, Igura K, Takahashi TA, Yamashita N. A potential pro-angiogenic cell therapy with human placenta-derived mesenchymal cells. *Biochemical and Biophysical Research Communications*. 2004; 325:24–31. [PubMed: 15522196]
- Nystedt J, Makinen S, Laine J, Jolkkonen J. Human cord blood CD34+ cells and behavioral recovery following focal cerebral ischemia in rats. *Acta Neurobiologiae Experimentalis*. 2006; 66:293–300. [PubMed: 17265691]
- O'Collins VE, Macleod MR, Donnan GA, Horky LL, van der Worp BH, Howells DW. 1,026 experimental treatments in acute stroke. *Annals of Neurology*. 2006; 59:467–477. [PubMed: 16453316]
- Ogle ME, Gu X, Espinera AR, Wei L. Inhibition of prolyl hydroxylases by dimethyloxaloylglycine after stroke reduces ischemic brain injury and requires hypoxia inducible factor-1alpha. *Neurobiology of Disease*. 2012; 45:733–742. [PubMed: 22061780]
- Ohab JJ, Fleming S, Blesch A, Carmichael ST. A neurovascular niche for neurogenesis after stroke. *Journal of Neuroscience*. 2006; 26:13007–13016. [PubMed: 17167090]
- Okazaki T, Magaki T, Takeda M, Kajiura Y, Hanaya R, Sugiyama K, Arita K, Nishimura M, Kato Y, Kurisu K. Intravenous administration of bone marrow stromal cells increases survivin and Bcl-2 protein expression and improves sensorimotor function following ischemia in rats. *Neuroscience Letters*. 2008; 430:109–114. [PubMed: 18053646]
- Oki K, Tatarishvili J, Wood J, Koch P, Wattananit S, Mine Y, Monni E, Tornero D, Ahlenius H, Ladewig J, Brustle O, Lindvall O, Kokaia Z. Human-induced pluripotent stem cells form functional neurons and improve recovery after grafting in stroke-damaged brain. *Stem Cells*. 2012; 30:1120–1133. [PubMed: 22495829]
- Onda T, Honmou O, Harada K, Houkin K, Hamada H, Kocsis JD. Therapeutic benefits by human mesenchymal stem cells (hMSCs) and Ang-1 gene-modified hMSCs after cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism*. 2008; 28:329–340. [PubMed: 17637706]
- Ou Y, Yu S, Kaneko Y, Tajiri N, Bae EC, Chheda SH, Stahl CE, Yang T, Fang L, Hu K, Borlongan CV, Yu G. Intravenous infusion of GDNF gene-modified human umbilical cord blood CD34+ cells protects against cerebral ischemic injury in spontaneously hypertensive rats. *Brain Research*. 2010; 1366:217–225. [PubMed: 20888805]
- Oyamada N, Itoh H, Sone M, Yamahara K, Miyashita K, Park K, Taura D, Inuzuka M, Sonoyama T, Tsujimoto H, Fukunaga Y, Tamura N, Nakao K. Transplantation of vascular cells derived from human embryonic stem cells contributes to vascular regeneration after stroke in mice. *Journal of Translational Medicine*. 2008; 6:54. [PubMed: 18823569]
- Pang ZP, Yang N, Vierbuchen T, Ostermeier A, Fuentes DR, Yang TQ, Citri A, Sebastiano V, Marro S, Sudhof TC, Wernig M. Induction of human neuronal cells by defined transcription factors. *Nature*. 2011; 476:220–223. [PubMed: 21617644]
- Park KI, Himes BT, Stieg PE, Tessler A, Fischer I, Snyder EY. Neural stem cells may be uniquely suited for combined gene therapy and cell replacement: evidence from engraftment of neurotrophin-3-expressing stem cells in hypoxic-ischemic brain injury. *Experimental Neurology*. 2006; 199:179–190. [PubMed: 16714016]
- Park S, Koh SE, Maeng S, Lee WD, Lim J, Lee YJ. Neural progenitors generated from the mesenchymal stem cells of first-trimester human placenta matured in the hypoxic-ischemic rat brain and mediated restoration of locomotor activity. *Placenta*. 2011; 32:269–276. [PubMed: 21300404]
- Parody R, Martino R, Rovira M, Vazquez L, Vazquez MJ, de la Camara R, Blazquez C, Fernandez-Aviles F, Carreras E, Salavert M, Jarque I, Martin C, Martinez F, Lopez J, Torres A, Sierra J, Sanz GF. Severe infections after unrelated donor allogeneic hematopoietic stem cell transplantation in adults: comparison of cord blood transplantation with peripheral blood and bone marrow transplantation. *Biology of Blood and Marrow Transplantation*. 2006; 12:734–748. [PubMed: 16785063]

- Pasha Z, Wang Y, Sheikh R, Zhang D, Zhao T, Ashraf M. Preconditioning enhances cell survival and differentiation of stem cells during transplantation in infarcted myocardium. *Cardiovascular Research*. 2008; 77:134–142. [PubMed: 18006467]
- Patkar S, Tate R, Modo M, Plevin R, Carswell HV. Conditionally immortalised neural stem cells promote functional recovery and brain plasticity after transient focal cerebral ischaemia in mice. *Stem Cell Research*. 2012; 8:14–25. [PubMed: 22099017]
- Payen E, Leboulch P. Advances in stem cell transplantation and gene therapy in the beta-hemoglobinopathies. *American Society of Hematology Education Program*. 2012; 2012:276–283.
- Pendharkar AV, Chua JY, Andres RH, Wang N, Gaeta X, Wang H, De A, Choi R, Chen S, Rutt BK, Gambhir SS, Guzman R. Biodistribution of neural stem cells after intravascular therapy for hypoxic-ischemia. *Stroke*. 2010; 41:2064–2070. [PubMed: 20616329]
- Peterson KM, Aly A, Lerman A, Lerman LO, Rodriguez-Porcel M. Improved survival of mesenchymal stromal cell after hypoxia preconditioning: role of oxidative stress. *Life Sciences*. 2011; 88:65–73. [PubMed: 21062632]
- Pluchino S, Gritti A, Blezer E, Amadio S, Brambilla E, Borsellino G, Cossetti C, Del Carro U, Comi G, Hart B, Vescovi A, Martino G. Human neural stem cells ameliorate autoimmune encephalomyelitis in non-human primates. *Annals of Neurology*. 2009; 66:343–354. [PubMed: 19798728]
- Pluchino S, Zanotti L, Rossi B, Brambilla E, Ottoboni L, Salani G, Martinello M, Cattalini A, Bergami A, Furlan R, Comi G, Constantin G, Martino G. Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. *Nature*. 2005; 436:266–271. [PubMed: 16015332]
- Polentes J, Jendelova P, Cailleret M, Braun H, Romanyuk N, Tropel P, Brenot M, Itier V, Seminatore C, Baldauf K, Turnovcova K, Jirak D, Teletin M, Come J, Tournois J, Reymann K, Sykova E, Viville S, Onteniente B. Human induced pluripotent stem cells improve stroke outcome and reduce secondary degeneration in the recipient brain. *Cell Transplantation*. 2012; 21:2587–2602. [PubMed: 22889472]
- Polezhaev LV, Alexandrova MA. Transplantation of embryonic brain tissue into the brain of adult rats after hypoxic hypoxia. *Journal für Hirnforschung*. 1984; 25:99–106. [PubMed: 6725943]
- Polezhaev LV, Alexandrova MA, Vitvitsky VN, Girman SV, Golovina IL. Morphological, biochemical and physiological changes in brain nervous tissue of adult intact and hypoxia-subjected rats after transplantation of embryonic nervous tissue. *Journal für Hirnforschung*. 1985; 26:281–289. [PubMed: 4031487]
- Pollock K, Stroemer P, Patel S, Stevanato L, Hope A, Miljan E, Dong Z, Hodges H, Price J, Sinden JD. A conditionally immortal clonal stem cell line from human cortical neuroepithelium for the treatment of ischemic stroke. *Experimental Neurology*. 2006; 199:143–155. [PubMed: 16464451]
- Ponte AL, Marais E, Gallay N, Langonne A, Delorme B, Herault O, Charbord P, Domenech J. 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. [PubMed: 17395768]
- Portmann-Lanz CB, Schoebedein A, Huber A, Sager R, Malek A, Holzgreve W, Surbek DV. Placental mesenchymal stem cells as potential autologous graft for pre- and perinatal neuroregeneration. *American Journal of Obstetrics and Gynecology*. 2006; 194:664–673. [PubMed: 16522395]
- Prasad K, Mohanty S, Bhatia R, Srivastava MV, Garg A, Srivastava A, Goyal V, Tripathi M, Kumar A, Bal C, Vij A, Mishra NK. Autologous intravenous bone marrow mononuclear cell therapy for patients with subacute ischaemic stroke: a pilot study. *Indian Journal of Medical Research*. 2012; 136:221–228. [PubMed: 22960888]
- Prather WR, Toren A, Meiron M, Ofir R, Tschope C, Horwitz EM. The role of placental-derived adherent stromal cell (PLX-PAD) in the treatment of critical limb ischemia. *Cytotherapy*. 2009; 11:427–434. [PubMed: 19526389]
- Raber J, Fan Y, Matsumori Y, Liu Z, Weinstein PR, Fike JR, Liu J. Irradiation attenuates neurogenesis and exacerbates ischemia-induced deficits. *Annals of Neurology*. 2004; 55:381–389. [PubMed: 14991816]

- Rabinovich SS, Seledtsov VI, Banul NV, Poveshchenko OV, Senyukov VV, Astrakov SV, Samarin DM, Taraban VY. Cell therapy of brain stroke. *Bulletin of Experimental Biology and Medicine*. 2005; 139:126–128. [PubMed: 16142294]
- Reitz M, Demestre M, Sedlacik J, Meissner H, Fiehler J, Kim SU, Westphal M, Schmidt NO. Intranasal delivery of neural stem/progenitor cells: a noninvasive passage to target intracerebral glioma. *Stem Cells Translational Medicine*. 2012; 1:866–873. [PubMed: 23283548]
- Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A. Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. *Nature Biotechnology*. 2000; 18:399–404.
- Riess P, Zhang C, Saatman KE, Laurer HL, Longhi LG, Raghupathi R, Lenzlinger PM, Lifshitz J, Boockvar J, Neugebauer E, Snyder EY, McIntosh TK. Transplanted neural stem cells survive, differentiate, and improve neurological motor function after experimental traumatic brain injury. *Neurosurgery*. 2002; 51:1043–1052. (discussion 1052–1044). [PubMed: 12234415]
- Rocha V, Gluckman E. Clinical use of umbilical cord blood hematopoietic stem cells. *Biology of Blood and Marrow Transplantation*. 2006; 12:34–41. [PubMed: 16399582]
- Roitberg BZ, Mangubat E, Chen EY, Sugaya K, Thulborn KR, Kordower JH, Pawar A, Konecny T, Emborg ME. Survival and early differentiation of human neural stem cells transplanted in a nonhuman primate model of stroke. *Journal of Neurosurgery*. 2006; 105:96–102. [PubMed: 16871883]
- Rosado-de-Castro PH, Schmidt FdaR, Battistella V, Lopes de Souza SA, Gutfilen B, Goldenberg RC, Kasai-Brunswick TH, Vairo L, Silva RM, Wajnberg E, Alvarenga Americano do Brasil PE, Gasparetto EL, Maiolino A, Alves-Leon SV, Andre C, Mendez-Otero R, Rodriguez de Freitas G, Barbosa da Fonseca LM. Biodistribution of bone marrow mononuclear cells after intraarterial or intravenous transplantation in subacute stroke patients. *Regenerative Medicine*. 2013; 8:145–155. [PubMed: 23477395]
- Rosenblum S, Wang N, Smith TN, Pendharkar AV, Chua JY, Birk H, Guzman R. Timing of intra-arterial neural stem cell transplantation after hypoxia–ischemia influences cell engraftment, survival, and differentiation. *Stroke*. 2012; 43:1624–1631. [PubMed: 22535265]
- Roura S, Pujal JM, Bayes-Genis A. Umbilical cord blood for cardiovascular cell therapy: from promise to fact. *Annals of the New York Academy of Sciences*. 2012; 1254:66–70. [PubMed: 22548571]
- Rowe DD, Leonardo CC, Hall AA, Shahaduzzaman MD, Collier LA, Willing AE, Pennypacker KR. Cord blood administration induces oligodendrocyte survival through alterations in gene expression. *Brain Research*. 2010; 17:172–188. [PubMed: 20883670]
- Rowe DD, Leonardo CC, Recio JA, Collier LA, Willing AE, Pennypacker KR. Human umbilical cord blood cells protect oligodendrocytes from brain ischemia through Akt signal transduction. *Journal of Biological Chemistry*. 2012; 287:4177–4187. [PubMed: 22158864]
- Rubinstein P, Carrier C, Scaradavou A, Kurtzberg J, Adamson J, Migliaccio AR, Berkowitz RL, Cabbad M, Dobrila NL, Taylor PE, Rosenfield RE, Stevens CE. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *New England Journal of Medicine*. 1998; 339:1565–1577. [PubMed: 9828244]
- Sahota P, Savitz SI. Investigational therapies for ischemic stroke: neuroprotection and neurorecovery. *Neurotherapeutics*. 2011; 8:434–451. [PubMed: 21604061]
- Sakata H, Niizuma K, Wakai T, Narasimhan P, Maier CM, Chan PH. Neural stem cells genetically modified to overexpress Cu/Zn-superoxide dismutase enhance amelioration of ischemic stroke in mice. *Stroke*. 2012; 43:2423–2429. [PubMed: 22713489]
- Sanberg PR, Eve DJ, Metcalf C, Borlongan CV. Advantages and challenges of alternative sources of adult-derived stem cells for brain repair in stroke. *Progress in brain research*. 2012; 201:99–117. [PubMed: 23186712]
- Sanchez-Ramos J, Song S, Cardozo-Pelaez F, Hazzi C, Stedeford T, Willing A, Freeman TB, Saporta S, Janssen W, Patel N, Cooper DR, Sanberg PR. Adult bone marrow stromal cells differentiate into neural cells in vitro. *Experimental Neurology*. 2000; 164:247–256. [PubMed: 10915564]
- Savitz SI, Chopp M, Deans R, Carmichael ST, Phinney D, Wechsler L. Stem cell therapy as an emerging paradigm for stroke (STEPS) II. *Stroke*. 2011a; 42:825–829. [PubMed: 21273569]

- Savitz SI, Dinsmore J, Wu J, Henderson GV, Stieg P, Caplan LR. Neurotransplantation of fetal porcine cells in patients with basal ganglia infarcts: a preliminary safety and feasibility study. *Cerebrovascular Diseases*. 2005; 20:101–107. [PubMed: 15976503]
- Savitz SI, Misra V, Kasam M, Juneja H, Cox CS, Alderman S, Aisiku I, Kar S, Gee A, Grotta JC. Intravenous autologous bone marrow mononuclear cells for ischemic stroke. *Annals of Neurology*. 2011b; 70:59–69. [PubMed: 21786299]
- Savitz SI, Rosenbaum DM, Dinsmore JH, Wechsler LR, Caplan LR. Cell transplantation for stroke. *Annals of Neurology*. 2002; 52:266–275. [PubMed: 12205638]
- Schoeberlein A, Mueller M, Reinhart U, Sager R, Messerli M, Surbek DV. Homing of placenta-derived mesenchymal stem cells after perinatal intracerebral transplantation in a rat model. *American Journal of Obstetrics and Gynecology*. 2011; 205:277.e1–277.e6. [PubMed: 22071064]
- Seyed Jafari SS, Ali Aghaei A, Asadi-Shekaari M, Nematollahi-Mahani SN, Sheibani V. Investigating the effects of adult neural stem cell transplantation by lumbar puncture in transient cerebral ischemia. *Neuroscience Letters*. 2011; 495:1–5. [PubMed: 21333715]
- Shapiro EM, Sharer K, Skrtic S, Koretsky AP. In vivo detection of single cells by MRI. *Magnetic Resonance in Medicine*. 2006; 55:242–249. [PubMed: 16416426]
- Sheikh AM, Nagai A, Wakabayashi K, Narantuya D, Kobayashi S, Yamaguchi S, Kim SU. Mesenchymal stem cell transplantation modulates neuroinflammation in focal cerebral ischemia: contribution of fractalkine and IL-5. *Neurobiology of Disease*. 2011; 41:717–724. [PubMed: 21168500]
- Shen CC, Lin CH, Yang YC, Chiao MT, Cheng WY, Ko JL. Intravenous implanted neural stem cells migrate to injury site, reduce infarct volume, and improve behavior after cerebral ischemia. *Current Neurovascular Research*. 2010a; 7:167–179. [PubMed: 20560882]
- Shen LH, Li Y, Chen J, Cui Y, Zhang C, Kapke A, Lu M, Savant-Bhonsale S, Chopp M. One-year follow-up after bone marrow stromal cell treatment in middle-aged female rats with stroke. *Stroke*. 2007; 38:2150–2156. [PubMed: 17525391]
- Shen LH, Li Y, Chen J, Zhang J, Vanguri P, Borneman J, Chopp M. Intracarotid transplantation of bone marrow stromal cells increases axonmyelin remodeling after stroke. *Neuroscience*. 2006; 137:393–399. [PubMed: 16298076]
- 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*. 2010b; 58:1074–1081. [PubMed: 20468049]
- Shen LH, Xin H, Li Y, Zhang RL, Cui Y, Zhang L, Lu M, Zhang ZG, Chopp M. Endogenous tissue plasminogen activator mediates bone marrow stromal cell-induced neurite remodeling after stroke in mice. *Stroke*. 2011; 42:459–464. [PubMed: 21212396]
- Shichinohe H, Kuroda S, Lee JB, Nishimura G, Yano S, Seki T, Ikeda J, Tamura M, Iwasaki Y. In vivo tracking of bone marrow stromal cells transplanted into mice cerebral infarct by fluorescence optical imaging. *Brain Research Brain Research Protocols*. 2004; 13:166–175. [PubMed: 15296854]
- Shin S, Sung BJ, Cho YS, Kim HJ, Ha NC, Hwang JI, Chung CW, Jung YK, Oh BH. An anti-apoptotic protein human survivin is a direct inhibitor of caspase-3 and -7. *Biochemistry*. 2001; 40:1117–1123. [PubMed: 11170436]
- Shyu WC, Lin SZ, Chiang MF, Su CY, Li H. Intracerebral peripheral blood stem cell (CD34+) implantation induces neuroplasticity by enhancing beta1 integrin-mediated angiogenesis in chronic stroke rats. *Journal of Neuroscience*. 2006; 26:3444–3453. [PubMed: 16571751]
- Sideri A, Neokleous N, Brunet De La Grange P, Guerton B, Le Bousse Kerdilles MC, Uzan G, Peste-Tsilimidos C, Gluckman E. An overview of the progress on double umbilical cord blood transplantation. *Haematologica*. 2011; 96:1213–1220. [PubMed: 21546497]
- Smith EJ, Stroemer RP, Gorenkova N, Nakajima M, Crum WR, Tang E, Stevanato L, Sinden JD, Modo M. Implantation site and lesion topology determine efficacy of a human neural stem cell line in a rat model of chronic stroke. *Stem Cells*. 2012; 30:785–796. [PubMed: 22213183]
- Smith HK, Gavins FN. The potential of stem cell therapy for stroke: is PISCES the sign? *FASEB Journal*. 2012; 26:2239–2252. [PubMed: 22426119]

- Song M, Mohamad O, Gu X, Wei L, Yu SP. Restoration of intracortical and thalamocortical circuits after transplantation of bone marrow mesenchymal stem cells into the ischemic brain of mice. *Cell Transplantation*. 2012
- Stenderup K, Justesen J, Clausen C, Kassem M. Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. *Bone*. 2003; 33:919–926. [PubMed: 14678851]
- Stephan RP, Reilly CR, Witte PL. Impaired ability of bone marrow stromal cells to support B-lymphopoiesis with age. *Blood*. 1998; 91:75–88. [PubMed: 9414271]
- Stroemer P, Patel S, Hope A, Oliveira C, Pollock K, Sinden J. The neural stem cell line CTX0E03 promotes behavioral recovery and endogenous neurogenesis after experimental stroke in a dose-dependent fashion. *Neurorehabilitation and Neural Repair*. 2009; 23:895–909. [PubMed: 19633272]
- Stubbs SL, Hsiao ST, Peshavariya HM, Lim SY, Dusting GJ, Dilley RJ. Hypoxic preconditioning enhances survival of human adipose-derived stem cells and conditions endothelial cells in vitro. *Stem Cells and Development*. 2012; 21:1887–1896. [PubMed: 22165914]
- Suarez-Montegudo C, Hernandez-Ramirez P, Alvarez-Gonzalez L, Garcia-Maeso I, de la Cuetara-Bernal K, Castillo-Diaz L, Bringas-Vega ML, Martinez-Aching G, Morales-Chacon LM, Baez-Martin MM, Sanchez-Catasus C, Carballo-Barreda M, Rodriguez-Rojas R, Gomez-Fernandez L, Alberti-Amador E, Macias-Abraham C, Balea ED, Rosales LC, Del Valle Perez L, Ferrer BB, Gonzalez RM, Bergado JA. Autologous bone marrow stem cell neurotransplantation in stroke patients. An open study. *Restorative Neurology and Neuroscience*. 2009; 27:151–161. [PubMed: 19531871]
- Sugiyama T, Kuroda S, Osanai T, Shichinohe H, Kuge Y, Ito M, Kawabori M, Iwasaki Y. Near-infrared fluorescence labeling allows noninvasive tracking of bone marrow stromal cells transplanted into rat infarct brain. *Neurosurgery*. 2011; 68:1036–1047. (discussion 1047). [PubMed: 21221028]
- Sutton EJ, Henning TD, Pichler BJ, Bremer C, Daldrop-Link HE. Cell tracking with optical imaging. *European Radiology*. 2008; 18:2021–2032. [PubMed: 18506449]
- Szabolcs P, Niedzwiecki D. Immune reconstitution after unrelated cord blood transplantation. *Cytotherapy*. 2007; 9:111–122. [PubMed: 17453963]
- Tae-Hoon L, Yoon-Seok L. Transplantation of mouse embryonic stem cell after middle cerebral artery occlusion. *Acta Cirúrgica Brasileira*. 2012; 27:333–339. [PubMed: 22534809]
- Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H, Yoshikawa H, Tsukamoto Y, Iso H, Fujimori Y, Stern DM, Naritomi H, Matsuyama T. Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. *Journal of Clinical Investigation*. 2004; 114:330–338. [PubMed: 15286799]
- Takagi Y, Nishimura M, Morizane A, Takahashi J, Nozaki K, Hayashi J, Hashimoto N. Survival and differentiation of neural progenitor cells derived from embryonic stem cells and transplanted into ischemic brain. *Journal of Neurosurgery*. 2005; 103:304–310. [PubMed: 16175861]
- Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007a; 131:861–872. [PubMed: 18035408]
- Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126:663–676. [PubMed: 16904174]
- Takahashi K, Yasuhara T, Shingo T, Muraoka K, Kameda M, Takeuchi A, Yano A, Kurozumi K, Agari T, Miyoshi Y, Kinugasa K, Date I. Embryonic neural stem cells transplanted in middle cerebral artery occlusion model of rats demonstrated potent therapeutic effects, compared to adult neural stem cells. *Brain Research*. 2008; 1234:172–182. [PubMed: 18703033]
- Takahashi S, Ooi J, Tomonari A, Konuma T, Tsukada N, Oiwa-Monna M, Fukuno K, Uchiyama M, Takasugi K, Iseki T, Tojo A, Yamaguchi T, Asano S. Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. *Blood*. 2007b; 109:1322–1330. [PubMed: 17038536]

- Tang YL, Zhao Q, Qin X, Shen L, Cheng L, Ge J, Phillips MI. Paracrine action enhances the effects of autologous mesenchymal stem cell transplantation on vascular regeneration in rat model of myocardial infarction. *Annals of Thoracic Surgery*. 2005; 80:229–236. (discussion 236–227). [PubMed: 15975372]
- Tate CC, Fonck C, McGrogan M, Case CC. Human mesenchymal stromal cells and their derivative, SB623 cells, rescue neural cells via trophic support following in vitro ischemia. *Cell Transplantation*. 2010; 19:973–984. [PubMed: 20350349]
- 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. [PubMed: 19095993]
- Theus MH, Wei L, Cui L, Francis K, Hu X, Keogh C, Yu SP. In vitro hypoxic preconditioning of embryonic stem cells as a strategy of promoting cell survival and functional benefits after transplantation into the ischemic rat brain. *Experimental Neurology*. 2008; 210:656–670. [PubMed: 18279854]
- Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998; 282:1145–1147. [PubMed: 9804556]
- Tilkorn DJ, Davies EM, Keramidas E, Dingle AM, Gerrand YW, Taylor CJ, Han XL, Palmer JA, Penington AJ, Mitchell CA, Morrison WA, Dusing GJ, Mitchell GM. The in vitro preconditioning of myoblasts to enhance subsequent survival in an in vivo tissue engineering chamber model. *Biomaterials*. 2012; 33:3868–3879. [PubMed: 22369961]
- Toda H, Takahashi J, Iwakami N, Kimura T, Hoki S, Mozumi-Kitamura K, Ono S, Hashimoto N. Grafting neural stem cells improved the impaired spatial recognition in ischemic rats. *Neuroscience Letters*. 2001; 316:9–12. [PubMed: 11720766]
- Tohill M, Mantovani C, Wiberg M, Terenghi G. Rat bone marrow mesenchymal stem cells express glial markers and stimulate nerve regeneration. *Neuroscience Letters*. 2004; 362:200–203. [PubMed: 15158014]
- Toyama K, Honmou O, Harada K, Suzuki J, Houkin K, Hamada H, Kocsis JD. Therapeutic benefits of angiogenic gene-modified human mesenchymal stem cells after cerebral ischemia. *Experimental Neurology*. 2009; 216:47–55. [PubMed: 19094989]
- Tymianski M. Can molecular and cellular neuroprotection be translated into therapies for patients? Yes, but not the way we tried it before. *Stroke*. 2010; 41:S87–S90. [PubMed: 20876514]
- Valable S, Montaner J, Bellail A, Berezowski V, Brillault J, Cecchelli R, Divoux D, Mackenzie ET, Bernaudin M, Roussel S, Petit E. VEGF-induced BBB permeability is associated with an MMP-9 activity increase in cerebral ischemia: both effects decreased by Ang-1. *Journal of Cerebral Blood Flow and Metabolism*. 2005; 25:1491–1504. [PubMed: 15902195]
- Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nature Cell Biology*. 2007; 9:654–659.
- van Velthoven CT, Kavelaars A, Heijnen CJ. Mesenchymal stem cells as a treatment for neonatal ischemic brain damage. *Pediatric Research*. 2012a; 71:474–481. [PubMed: 22430383]
- van Velthoven CT, Kavelaars A, van Bel F, Heijnen CJ. Repeated mesenchymal stem cell treatment after neonatal hypoxia–ischemia has distinct effects on formation and maturation of new neurons and oligodendrocytes leading to restoration of damage, corticospinal motor tract activity, and sensorimotor function. *Journal of Neuroscience*. 2010; 30:9603–9611. [PubMed: 20631189]
- van Velthoven CT, Sheldon RA, Kavelaars A, Derugin N, Vexler ZS, Willemen HL, Maas M, Heijnen CJ, Ferriero DM. Mesenchymal stem cell transplantation attenuates brain injury after neonatal stroke. *Stroke*. 2013; 44:1426–1432. [PubMed: 23539530]
- van Velthoven CT, van de Looij Y, Kavelaars A, Zijlstra J, van Bel F, Huppi PS, Sizonenko S, Heijnen CJ. Mesenchymal stem cells restore cortical rewiring after neonatal ischemia in mice. *Annals of Neurology*. 2012b; 71:785–796. [PubMed: 22718545]
- Vandeputte C, Thomas D, Dresselaers T, Crabbe A, Verfaillie C, Baekelandt V, Van Laere K, Himmelreich U. Characterization of the inflammatory response in a photothrombotic stroke

- model by MRI: implications for stem cell transplantation. *Molecular Imaging and Biology*. 2011; 13:663–671. [PubMed: 20700767]
- Veizovic T, Beech JS, Stroemer RP, Watson WP, Hodges H. Resolution of stroke deficits following contralateral grafts of conditionally immortal neuroepithelial stem cells. *Stroke*. 2001; 32:1012–1019. [PubMed: 11283405]
- Vendrame M, Cassady J, Newcomb J, Butler T, Pennypacker KR, Zigova T, Sanberg CD, Sanberg PR, Willing AE. Infusion of human umbilical cord blood cells in a rat model of stroke dose-dependently rescues behavioral deficits and reduces infarct volume. *Stroke*. 2004; 35:2390–2395. [PubMed: 15322304]
- Vendrame M, Gemma C, de Mesquita D, Collier L, Bickford PC, Sanberg CD, Sanberg PR, Pennypacker KR, Willing AE. Anti-inflammatory effects of human cord blood cells in a rat model of stroke. *Stem Cells and Development*. 2005; 14:595–604. [PubMed: 16305344]
- Vlassov AV, Magdaleno S, Setterquist R, Conrad R. Exosomes: current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. *Biochimica et Biophysica Acta*. 2012; 1820:940–948. [PubMed: 22503788]
- Walczak P, Zhang J, Gilad AA, Kedziorek DA, Ruiz-Cabello J, Young RG, Pittenger MF, van Zijl PC, Huang J, Bulte JW. Dual-modality monitoring of targeted intraarterial delivery of mesenchymal stem cells after transient ischemia. *Stroke*. 2008; 39:1569–1574. [PubMed: 18323495]
- Wang J, Chao F, Han F, Zhang G, Xi Q, Li J, Jiang H, Yu G, Tian M, Zhang H. PET demonstrates functional recovery after transplantation of induced pluripotent stem cells in a rat model of cerebral ischemic injury. *Journal of Nuclear Medicine*. 2013; 54:785–792. [PubMed: 23503731]
- Wang JA, He A, Hu X, Jiang Y, Sun Y, Jiang J, Gui C, Wang Y, Chen H. Anoxic preconditioning: a way to enhance the cardioprotection of mesenchymal stem cells. *International Journal of Cardiology*. 2009; 133:410–412. [PubMed: 18234368]
- Wang Y, Xu F, Zhang C, Lei D, Tang Y, Xu H, Zhang Z, Lu H, Du X, Yang GY. High MR sensitive fluorescent magnetite nanocluster for stem cell tracking in ischemic mouse brain. *Nanomedicine*. 2011; 7:1009–1019. [PubMed: 21530678]
- Warrier S, Haridas N, Bhonde R. Inherent propensity of amnion-derived mesenchymal stem cells towards endothelial lineage: vascularization from an avascular tissue. *Placenta*. 2012; 33:850–858. [PubMed: 22840296]
- Wei L, Cui L, Snider BJ, Rivkin M, Yu SS, Lee CS, Adams LD, Gottlieb DI, Johnson EM Jr, Yu SP, Choi DW. Transplantation of embryonic stem cells overexpressing Bcl-2 promotes functional recovery after transient cerebral ischemia. *Neurobiology of Disease*. 2005; 19:183–193. [PubMed: 15837573]
- Wei L, Fraser JL, Lu ZY, Hu X, Yu SP. Transplantation of hypoxia preconditioned bone marrow mesenchymal stem cells enhances angiogenesis and neurogenesis after cerebral ischemia in rats. *Neurobiology of Disease*. 2012; 46:635–645. [PubMed: 22426403]
- Wei N, Yu SP, Gu X, Taylor TM, Song D, Liu XF, Wei L. Delayed intranasal delivery of hypoxic-preconditioned bone marrow mesenchymal stem cells enhanced cell homing and therapeutic benefits after ischemic stroke in mice. *Cell Transplantation*. 2013; 22:977–991. [PubMed: 23031629]
- Wei X, Du Z, Zhao L, Feng D, Wei G, He Y, Tan J, Lee WH, Hampel H, Dodel R, Johnstone BH, March KL, Farlow MR, Du Y. IFATS collection: the conditioned media of adipose stromal cells protect against hypoxia–ischemia-induced brain damage in neonatal rats. *Stem Cells*. 2009; 27:478–488. [PubMed: 19023032]
- Welling MM, Duijvestein M, Signore A, van der Weerd L. In vivo biodistribution of stem cells using molecular nuclear medicine imaging. *Journal of Cellular Physiology*. 2011; 226:1444–1452. [PubMed: 21413018]
- Wichterle H, Lieberam I, Porter JA, Jessell TM. Directed differentiation of embryonic stem cells into motor neurons. *Cell*. 2002; 110:385–397. [PubMed: 12176325]
- Williams RW, Herrup K. The control of neuron number. *Annual Review of Neuroscience*. 1988; 11:423–453.
- Wong AM, Hodges H, Horsburgh K. Neural stem cell grafts reduce the extent of neuronal damage in a mouse model of global ischaemia. *Brain Research*. 2005; 1063:140–150. [PubMed: 16289485]

- Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells*. 2007; 25:2648–2659. [PubMed: 17615264]
- Xie X, Sun A, Zhu W, Huang Z, Hu X, Jia J, Zou Y, Ge J. Transplantation of mesenchymal stem cells preconditioned with hydrogen sulfide enhances repair of myocardial infarction in rats. *Tohoku Journal of Experimental Medicine*. 2012; 226:29–36. [PubMed: 22186034]
- Xin H, Li Y, Buller B, Katakowski M, Zhang Y, Wang X, Shang X, Zhang ZG, Chopp M. Exosome-mediated transfer of miR-133b from multipotent mesenchymal stromal cells to neural cells contributes to neurite outgrowth. *Stem Cells*. 2012; 30:1556–1564. [PubMed: 22605481]
- Xin H, Li Y, Liu Z, Wang X, Shang X, Cui Y, Gang Zhang Z, Chopp M. Mir-133b promotes neural plasticity and functional recovery after treatment of stroke with multipotent mesenchymal stromal cells in rats via transfer of exosome-enriched extracellular particles. *Stem Cells*. 2013
- Xin H, Li Y, Shen LH, Liu X, Wang X, Zhang J, Pourabdollah-Nejad DS, Zhang C, Zhang L, Jiang H, Zhang ZG, Chopp M. Increasing tPA activity in astrocytes induced by multipotent mesenchymal stromal cells facilitate neurite outgrowth after stroke in the mouse. *PLoS ONE*. 2010; 5:e9027. [PubMed: 20140248]
- Yamagata M, Yamamoto A, Kako E, Kaneko N, Matsubara K, Sakai K, Sawamoto K, Ueda M. Human dental pulp-derived stem cells protect against hypoxic–ischemic brain injury in neonatal mice. *Stroke*. 2013; 44:551–554. [PubMed: 23238858]
- Yamashita T, Kawai H, Tian F, Ohta Y, Abe K. Tumorigenic development of induced pluripotent stem cells in ischemic mouse brain. *Cell Transplantation*. 2011; 20:883–891. [PubMed: 21054935]
- Yan F, Yao Y, Chen L, Li Y, Sheng Z, Ma G. Hypoxic preconditioning improves survival of cardiac progenitor cells: role of stromal cell derived factor-1alpha-CXCR4 axis. *PLoS ONE*. 2012; 7:e37948. [PubMed: 22815687]
- Yanagisawa D, Qi M, Kim DH, Kitamura Y, Inden M, Tsuchiya D, Takata K, Taniguchi T, Yoshimoto K, Shimohama S, Akaike A, Sumi S, Inoue K. Improvement of focal ischemia-induced rat dopaminergic dysfunction by striatal transplantation of mouse embryonic stem cells. *Neuroscience Letters*. 2006; 407:74–79. [PubMed: 16959414]
- Yang M, Baranov E, Moossa AR, Penman S, Hoffman RM. Visualizing gene expression by whole-body fluorescence imaging. *Proceedings of the National Academy of Sciences of the United States of America*. 2000; 97:12278–12282. [PubMed: 11050247]
- Yang T, Tsang KS, Poon WS, Ng HK. Neurotrophism of bone marrow stromal cells to embryonic stem cells: noncontact induction and transplantation to a mouse ischemic stroke model. *Cell Transplantation*. 2009; 18:391–404. [PubMed: 19622227]
- Yao Y, Zhang F, Wang L, Zhang G, Wang Z, Chen J, Gao X. Lipopolysaccharide preconditioning enhances the efficacy of mesenchymal stem cells transplantation in a rat model of acute myocardial infarction. *Journal of Biomedical Science*. 2009; 16:74. [PubMed: 19691857]
- Yarygin KN, Kholodenko IV, Konieva AA, Burunova VV, Tairova RT, Gubsky LV, Cheglakov IB, Pirogov YA, Yarygin VN, Skvortsova VI. Mechanisms of positive effects of transplantation of human placental mesenchymal stem cells on recovery of rats after experimental ischemic stroke. *Bulletin of Experimental Biology and Medicine*. 2009; 148:862–868. [PubMed: 21116492]
- Yasuhara T, Hara K, Maki M, Xu L, Yu G, Ali MM, Masuda T, Yu SJ, Bae EK, Hayashi T, Matsukawa N, Kaneko Y, Kuzmin-Nichols N, Ellovitch S, Cruz EL, Klasko SK, Sanberg CD, Sanberg PR, Borlongan CV. Mannitol facilitates neurotrophic factor up-regulation and behavioural recovery in neonatal hypoxic–ischaemic rats with human umbilical cord blood grafts. *Journal of Cellular and Molecular Medicine*. 2010; 14:914–921. [PubMed: 20569276]
- Ye R, Kong X, Yang Q, Zhang Y, Han J, Li P, Xiong L, Zhao G. Ginsenoside Rd in experimental stroke: superior neuroprotective efficacy with a wide therapeutic window. *Neurotherapeutics*. 2011; 8:515–525. [PubMed: 21647765]
- Ye R, Yang Q, Kong X, Li N, Zhang Y, Han J, Xiong L, Liu X, Zhao G. Sevoflurane preconditioning improves mitochondrial function and long-term neurologic sequelae after transient cerebral ischemia: role of mitochondrial permeability transition. *Critical Care Medicine*. 2012; 40:2685–2693. [PubMed: 22732280]
- Ye R, Zhao G, Liu X. Ginsenoside Rd for acute ischemic stroke: translating from bench to bedside. *Expert Review of Neurotherapeutics*. 2013; 13:603–613. [PubMed: 23738998]

- Yoo KH, Jang IK, Lee MW, Kim HE, Yang MS, Eom Y, Lee JE, Kim YJ, Yang SK, Jung HL, Sung KW, Kim CW, Koo HH. Comparison of immunomodulatory properties of mesenchymal stem cells derived from adult human tissues. *Cellular Immunology*. 2009; 259:150–156. [PubMed: 19608159]
- Yu SP, Wei Z, Wei L. Preconditioning strategy in stem cell transplantation therapy. *Translational Stroke Research*. 2013; 4:76–88. [PubMed: 23914259]
- Yu X, Chen D, Zhang Y, Wu X, Huang Z, Zhou H, Zhang Z. Overexpression of CXCR4 in mesenchymal stem cells promotes migration, neuroprotection and angiogenesis in a rat model of stroke. *Journal of The Neurological Sciences*. 2012; 316:141–149. [PubMed: 22280945]
- Zacharek A, Chen J, Cui X, Li A, Li Y, Roberts C, Feng Y, Gao Q, Chopp M. Angiopoietin1/Tie2 and VEGF/Flk1 induced by MSC treatment amplifies angiogenesis and vascular stabilization after stroke. *Journal of Cerebral Blood Flow and Metabolism*. 2007; 27:1684–1691. [PubMed: 17356562]
- Zawadzka M, Lukasiuk K, Machaj EK, Pojda Z, Kaminska B. Lack of migration and neurological benefits after infusion of umbilical cord blood cells in ischemic brain injury. *Acta Neurobiologiae Experimentalis*. 2009; 69:46–51. [PubMed: 19325640]
- Zemani F, Silvestre JS, Fauvel-Lafeve F, Bruel A, Vilar J, Bieche I, Laurendeau I, Galy-Fauroux I, Fischer AM, Boisson-Vidal C. Ex vivo priming of endothelial progenitor cells with SDF-1 before transplantation could increase their proangiogenic potential. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2008; 28:644–650.
- Zhang J, Chen GH, Wang YW, Zhao J, Duan HF, Liao LM, Zhang XZ, Chen YD, Chen H. Hydrogen peroxide preconditioning enhances the therapeutic efficacy of Wharton's Jelly mesenchymal stem cells after myocardial infarction. *Chinese Medical Journal*. 2012a; 125:3472–3478. [PubMed: 23044308]
- Zhang L, Li Y, Romanko M, Kramer BC, Gosiewska A, Chopp M, Hong K. Different routes of administration of human umbilical tissue-derived cells improve functional recovery in the rat after focal cerebral ischemia. *Brain Research*. 2012b; 1489:104–112. [PubMed: 23063717]
- 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*. 2011a; 42:1437–1444. [PubMed: 21493915]
- Zhang P, Li J, Liu Y, Chen X, Kang Q. Transplanted human embryonic neural stem cells survive, migrate, differentiate and increase endogenous nestin expression in adult rat cortical peri-infarction zone. *Neuropathology*. 2009a; 29:410–421. [PubMed: 19170896]
- Zhang P, Li J, Liu Y, Chen X, Kang Q, Zhao J, Li W. Human neural stem cell transplantation attenuates apoptosis and improves neurological functions after cerebral ischemia in rats. *Acta Anaesthesiologica Scandinavica*. 2009b; 53:1184–1191. [PubMed: 19650809]
- Zhang P, Li J, Liu Y, Chen X, Lu H, Kang Q, Li W, Gao M. Human embryonic neural stem cell transplantation increases subventricular zone cell proliferation and promotes peri-infarct angiogenesis after focal cerebral ischemia. *Neuropathology*. 2011b; 31:384–391. [PubMed: 21175862]
- Zhang ZG, Chopp M. Neurorestorative therapies for stroke: underlying mechanisms and translation to the clinic. *Lancet Neurology*. 2009; 8:491–500. [PubMed: 19375666]
- Zhang ZG, Zhang L, Jiang Q, Zhang R, Davies K, Powers C, Bruggen N, Chopp M. VEGF enhances angiogenesis and promotes blood–brain barrier leakage in the ischemic brain. *Journal of Clinical Investigation*. 2000; 106:829–838. [PubMed: 11018070]
- Zhao C, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. *Cell*. 2008; 132:645–660. [PubMed: 18295581]
- Zhao H, Yenari MA, Cheng D, Sapolsky RM, Steinberg GK. Bcl-2 overexpression protects against neuron loss within the ischemic margin following experimental stroke and inhibits cytochrome c translocation and caspase-3 activity. *Journal of Neurochemistry*. 2003; 85:1026–1036. [PubMed: 12716434]
- 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. *Experimental Neurology*. 2002; 174:11–20. [PubMed: 11869029]

- Zhao MZ, Nonoguchi N, Ikeda N, Watanabe T, Furutama D, Miyazawa D, Funakoshi H, Kajimoto Y, Nakamura T, Dezawa M, Shibata MA, Otsuki Y, Coffin RS, Liu WD, Kuroiwa T, Miyatake S. Novel therapeutic strategy for stroke in rats by bone marrow stromal cells and ex vivo HGF gene transfer with HSV-1 vector. *Journal of Cerebral Blood Flow and Metabolism*. 2006; 26:1176–1188. [PubMed: 16421510]
- Zhu W, Mao Y, Zhao Y, Zhou LF, Wang Y, Zhu JH, Zhu Y, Yang GY. Transplantation of vascular endothelial growth factor-transfected neural stem cells into the rat brain provides neuroprotection after transient focal cerebral ischemia. *Neurosurgery*. 2005; 57:325–333. (discussion 325-333). [PubMed: 16094163]

Table 1

Representative experimental studies of various cell-based therapies for stroke treatment.

Cell source	Cell type	Administration route	Administration time	Stroke model	Mechanisms	Therapeutic effects	Reference
D3 cell line	ESCs	IC	2 wk after MCAO	60-min MCAO in rats	Massive accumulation of stem cells in the infarcted area	NM	Hoehn et al. (2002)
Human	NSCs	IC	7 d after ischemia	Right MCA M ₁ segment occlusion in cynomolgus monkeys	Cell replacement	NM	Roitberg et al. (2006)
Syngenic	NSCs	IV	72 h after MCAO	45-min MCAO in mice	Lowered inflammation, glial scar formation and neuronal apoptotic death	Recovery improved	Bacigaluppi et al. (2009)
MHP36 cell line	NSCs	IC	3 d after ischemia	17-min bilateral common carotid artery occlusion in mice	NM	Reduced the extent of ischemic neuronal damage	Wong et al. (2005)
Mice	iPSCs	Subdural transplantation	Immediately after MCAO	1-h MCAO in rats	Decreased pro-inflammatory cytokines and increased anti-inflammatory cytokines	Decreased infarct size; Improved motor function	Chen et al. (2010b)
Human	iPSCs	IC	1 wk after MCAO in mice or 48 h after MCAO in rats	30-min MCAO in rats and mice; and permanent dMCAO + 30-min CCAO rat model by craniotomy	Cell replacement; Increased vascular endothelial growth factor levels and enhanced endogenous plasticity	Forepaw movements recovery improved	Oki et al. (2012)
Syngenic	BMSCs	IV	24 h or 7 d after MCAO	2-h MCAO in rats	Cell replacement	Neurological functional recovery improved	Chen et al. (2001b)
Syngenic	BMSCs	IA	1 d after MCAO	2-h MCAO in rats	Decreased glial wall thickness and increased axonal density	Functional deficits were improved for 1 year.	Shen et al. (2007)
Syngenic	MSCs	Intranasal	3 d after MCAO	1.5-h MCAO in neonatal rats	Increased cell proliferation and endogenous repair processes; induced cytokine and growth factor changes	Infarct size was reduced and motor deficits were improved.	van Velthoven et al. (2013)

Cell source	Cell type	Administration route	Administration time	Stroke model	Mechanisms	Therapeutic effects	Reference
Syngenic	BMSCs	IV	24 h after MCAO	2-h MCAO in T1DM rats	Increased BBB leakage; Cerebral artery neointimal formation; Increased angiogenic expression.	No functional improvement.	Chen et al. (2011b)
Human	Dental pulp-derived stem cells	IC	24 h after HI	Unilateral HI brain injury in P5 mice	Suppressed the expression of proinflammatory cytokines; Enhanced the expression of anti-inflammatory cytokines	Brain-tissue loss was reduced and neurological function was improved.	Yamagata et al. (2013)
Human	Adipose tissue Stromal cells	IC	1 d after MCAO	1.5-h MCAO in rats	Induced production of neurotrophic factors	Functional improvement	Kang et al. (2003)
Human	Umbilical cord blood cells	IV	24 h or 7 d after MCAO	2-h MCAO in rats	Cell replacement	Neurological functional recovery improved.	Chen et al. (2001c)
Human	Menstrual blood-derived stem cells	IC, IV	Within 2 h after MCAO	60-min MCAO in rats	Induced production of growth and trophic factors	Reduced cell death (<i>in vitro</i>); Behavioral impairments (<i>in vivo</i>)	Borlongan et al. (2010)
Human PDA001 cells	Placental MSCs	IV	4 h after MCAO	2-h MCAO in rats	Induced production of growth and trophic factors	Decreased lesion volume; Promoted functional outcome	Chen et al. (2013b)

IC, intracerebral; IV, intravenous; IA, intra-arterial; ESCs, embryonic stem cells; MSCs, mesenchymal stem cells; NSCs, neural stem cells; BMSCs, bone marrow-derived stem cells; iPSCs, induced pluripotent stem cells; NM, not mentioned; BBB, blood-brain barrier; MCAO, middle cerebral artery occlusion; CCAO, common carotid artery occlusion; HI, hypoxia-ischemia.

Table 2

Completed clinical trials of cell-based therapies for ischemic stroke.

Cell source	Cell type	Delivery route	Cell volume and administration time	Patient characteristics	Patient number	Main outcomes	Reference
Immortalized cell line	LBS-neurons	IC	4 received 2 million cells and 8 received 2 or 6 million cells after cells preparation	Ischemic stroke (6 m-6 yr); Age: 44–75 yr.	12	Feasible and function improved	Kondziolka et al. (2000)
Immortalized cell line	LBS-neurons	IC	5 or 10 million NT2N cells	Stroke (1–6 yr); Age: 18–75 yr; ESS motor score: 10–45.	18	Neurological events occurred (seizure, syncope and subdural hematoma); Some patients had improved function	Kondziolka et al. (2005)
Porcine	LGE cells	IC	10×10^6 cells per needle tract; 5 needle tracts per patient	Age: 18–70 yr; Ischemic stroke (3 m-10 yr); MCA infarct affecting the striatum.	5	Study was terminated after 2 SAEs	Savitz et al. (2005)
Autologous	MSCs	IV	5×10^7 cells twice: 4–5 (first boosting) and 7–9 wk (second boosting) after symptom onset	Acute cerebral infarction within 7 d of the onset of symptoms; Age: 30–75 yr; NIHSS 7.	30	BI and mRS improved and safe	Bang et al. (2005)
Autologous	MSCs	IC	$1.4-55 \times 10^6$ cells, 24 h after isolation of bone marrow stem cells	Chronic stroke (1–10 yr); Age: 40–70 yr.	5	Safe and neurological condition improved	Suarez-Monteagudo et al. (2009)
Autologous	MSCs	IV	5×10^7 cells for 4 wk after bone marrow aspiration; the same amount cells for 2 wk after initial boosting	Acute ischemic lesions within MCA territory; Age: 30–75 yr; mNIHSS 7.	85	Safe and function improvement	Lee et al. (2010)
Autologous	MSCs	IV	A mean of $50-60 \times 10^6$ cells after MSCs expansion	Chronic stroke (3 m-1 yr); Motor strength of hand muscles of at least 2, and NIHSS 4–15; Age: 20–60 yr.	12	Only modest increase of FM and mBI and safe	Bhasin et al. (2011)
Autologous	MSCs	IV	$0.6-1.6 \times 10^8$ cells during 36–133 d post-stroke	Stroke onset within 6 m; Age: 20–75 yr; mRS 3	12	Daily rates of change in NIHSS increased and mean lesion volume reduced; safe	Honnou et al. (2011)
Autologous	MNCs	IA	$1-5 \times 10^8$ cells after bone marrow aspiration, cell separation and labeling	MCA ischemic stroke within 90 d of symptom onset; Age: 18–75 yr; NIHSS: 4–17	6	No worsening in the neurological scales	Battistella et al. (2011)
Autologous	MNCs	IV	Eight received 10 million cells/kg, and 2 received 7 million cells/kg between 24 and 72 h after stroke	Acute MCA ischemic stroke within 24–72 h; Age: 18–80 yr; NIHSS: 6–20.	10	Median NIHSS and mRS reduced but two patients had infarct expansion, one died	Savitz et al. (2011b)

Cell source	Cell type	Delivery route	Cell volume and administration time	Patient characteristics	Patient number	Main outcomes	Reference
Autologous	MNCs	IA	$5.1-60 \times 10^7$ cells during 3-7 d after stroke onset	Moderate to severe acute MCA infarcts; Age: 18-80 yr; NIHSS >8.	20	Safe; Clinical improvement and good outcomes occurred	Friedrich et al. (2012)
Autologous	MNCs	IV	40×10^6 cells within 4 h of aspiration	Ischemic stroke involving anterior circulation within 7-30 d; Age: 18-80 yr; GCS >8; BI 50; NIHSS 7.	11	Safe and favorable clinical outcome occurred	Prasad et al. (2012)
Autologous	MNCs and MSCs	IV	$50-60 \times 10^6$ cells in 250 ml saline with the time of 2-3 h	Chronic stroke (3 m-2 yr); Age: 18-63 yr; NIHSS: 4-15.	40	Safe and mBI improved	Bhasin et al. (2013)
Allogenic	UCMSCs	IA	One single dose of 2×10^7 UCMSCs was infused within 20 min	Stroke within 3 m; Age <60 yr.	4	Muscle strength and mRS improved; safe	Jiang et al. (2012b)
Autologous	MNCs	IA	Mean BM-MNCs of 1.59×10^8 were injected between 5 and 9 d after stroke	Severe MCA territory stroke; Age: 18-80 yr; NIHSS 8.	20	Isolate partial seizure was found; no function improvements at 180 d	Moniche et al. (2012)

LBS, Layton Bio-Science Inc.; LGE, lateral ganglionic eminence; IC, intracerebral; IV, intravenous; IA, intra-arterial; MSCs, mesenchymal stem cells; MNCs, mononuclear cells; UCMSCs, umbilical cord mesenchymal stem cells; ESS, European Stroke Scale; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Score; FM, Fugl Meyer; mBI, modified Barthel index; GCS, Glasgow Coma Scale; MCA, middle cerebral artery.

Table 3

Current ongoing clinical trials of cell-based therapies for ischemic stroke.

Phase	Cell source	Cell type	Delivery route	Intervention	Inclusion criteria	Patient number	Design	Location	NCT identifier
II	Allogenic	MSCs from adipose tissue	IV	1 million units/kg; within the first 2 wk after onset	Acute cerebral infarction (<12h); 60–80yr; NIHSS 8–20	20	Randomized; placebo control; double blind	Spain	NCT01678534
IIA	Allogenic	Human placenta-derived cells (PDA001)	IV	2×10 ⁸ cells (day 1 or day 1 and day 8); 8×10 ⁸ cells (days 1 and 8)	Ischemic stroke in MCA or PCA territory; 18–80yr; NIHSS 6–20	44	Randomized; placebo control; double blind	United States	NCT01310114
I/II	Allogenic	Adult BMSCs	IV	0.5–1.5 million cells/kg	Ischemic stroke (>6m); >18yr; NIHSS 6–20	35	Non-randomized; single group; open label	United States	NCT01297413
I/IIA	Allogenic	Modified stromal cells (SB623)	NM	2.5, 5.0, or 10 million cells once	Ischemic stroke in subcortical region of MCA or lenticulostriate artery with or without cortical involvement (6–36m); 18–75yr; NIHSS >7	18	Non-randomized; single group; open label	United States	NCT01287936
I	Autologous	BMSCs	IC	2–4 million cells per patient	Ischemia stroke or intracerebral hemorrhage patient (>3 m); 40–70yr; NIHSS >7	30	Non-randomized; routine treatment control; open label	China	NCT01714167
I/II	Autologous	CD34+ stem cells	IA	NM	Acute stroke (<7 d); 30–80yr; NIHSS >7	10	Non-randomized; single group; open label	United Kingdom	NCT00535197
I/II	Autologous	BMSCs+ EPCs	IV	2.5 million cells/kg twice in BMSCs or EPCs group; approximately 5wk after symptoms onset	Ischemic stroke within the MCA territory (<7 d); 18–80yr; NIHSS >7	90	Randomized; placebo control; double blind	China	NCT01468064
I	Autologous	Peripheral HSCs	IA	4 million cells per patient	Internal carotid artery territory infarction (<1 yr); 40–70yr; SSS <40	40	Randomized; placebo control; open label	China	NCT01518231
I/II	Allogenic	MSCs	IV	2 million cells/kg; within 10 days of stroke	Acute cerebral ischemia (<10 d); 20–80yr; MRS 4	78	Randomized; plasmalyte-A control; double blind	Malaysia	NCT01091701

Phase	Cell source	Cell type	Delivery route	Intervention	Inclusion criteria	Patient number	Design	Location	NCT identifier
I	Autologous	OECs	IC	About 2–8×10 ⁶ cells; after 1–2 m culture and expansion	Chronic stroke (6–60 m); 35–70yr; NIHSS 5–15	6	Randomized; crossover; single blind	Taiwan	NCT01327768
II	Autologous	BMSCs	IV	Single infusion	Stroke (1 wk–2 m); 30–70yr; NIHSS >10	50	Randomized; routine treatment control; open label	Malaysia	NCT01461720
IIA	Autologous	MSCs	IV	A mixing of physiological salt solution/albumin 4% (volume <100 mL); less than 6 wk after stroke	Right or left carotid ischemic stroke (<14 d); 18–65yr; NIHSS >2	30	Randomized; control without intervention nor placebo; open label	France	NCT00875654
I	Autologous	CTX0E03 neural stem cells	Intracranial	Single administration of increased dose level of 2, 5, 10 or 20 million cells	Unilateral ischemic stroke involving subcortical white matter or basal ganglia (6 m–5yr); 60–85yr; NIHSS 6	12	Single group; open label	United Kingdom	NCT01151124
I	Autologous	Mononuclear bone marrow cells	IV	NM	Acute ischemic stroke; 18–83yr (if >80yr, pre-MRS <1); NIHSS 615 (R)/ 18 (L)	30	Single group; open label	United States	NCT00859014
II	Autologous	ALD-401 derived from bone marrow	IA	3 mL suspension of ALD-401; 13–19d after stroke	Acute MCA stroke (within 24h); 30–83 yr; NIHSS 22	100	Randomized; shame procedure control; double blind	United States	NCT01273337
I	Allogenic	CD34+ stem cell obtained from UCB	IC	Approximately 5 million	Ischemic stroke (6–60m); 35–70yr; NIHSS 5–15	6	Single group; open label	Taiwan	NCT01438593
I/II	Autologous	Adipose-derived stromal cells	Intra-carotid & IV	NM	Ischemic stroke or hemorrhagic stroke; 18–80yr; NIHSS 8	10	Non-randomized; single group; open label	Mexico	NCT01453829
II	Autologous	MultiStem®	NM	Single infusion; 1–2 d post stroke	Cortical ischemic stroke; 18–79 yr; moderate to moderately severe stroke	140	Randomized; placebo control; double blind	United States	NCT01436487
III	Autologous	MSCs	IV	NM	Stroke (< 90 d); 30–75 yr; NIHSS 6–21	60	Randomized; routine treatment control; open label	Korea	NCT01716481

Phase	Cell source	Cell type	Delivery route	Intervention	Inclusion criteria	Patient number	Design	Location	NCT identifier
NM	Autologous	CD34+ peripheral blood stem cells	IC	Stem cell therapy plus G-CSF	Chronic cerebral infarction in MCA territory (6 m-5 yr); >30 yr; NIHSS 9-20	36	Case control; prospective	Taiwan	NCT01239602

IV, intravenous; IA, intra-arterial; IC, intracerebral; NM, not mentioned; NIHSS, National Institutes of Health Stroke Scale; MSCs, mesenchymal stem cells; OECs, olfactory ensheathing cells; EPCs, endothelial progenitor cells; BMSCs, bone marrow-derived stem cells; HSCs, hematopoietic stem cells; MCA, middle cerebral artery; PCA, posterior cerebral artery.