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

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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
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 systemically 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) (Buhnemann 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 (Buhnemann 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 $2 \times 10^7$ 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 neovascularure 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 chemotactants. 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 (Reubinoff 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 intrapenumbra 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<sup>b</sup>-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,
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 (Honnou 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/Fk1 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 BSMCs 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-1β 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 (Schoberlein 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 extremely rich in 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 (Kozlowska 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 (Kozlowska 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 el 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-κB (NF-κB) 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, $\beta$-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.
Bioluminescence has been used 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 intrastriatally 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

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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-magnetite nanocluster (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 ($^{99m}$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). $^{99m}$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 $^{99m}$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 $^{99m}$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 $^{99m}$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 \textit{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 \textit{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 \textit{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
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-1α 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-1α and trophic/growth factors such as BDNF, GDNF, VEGF and its receptor F1K-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 (Ii 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

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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 rhodaminedextran  
GVHD  graft-versus-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

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Prog Neurobiol. Author manuscript; available in PMC 2014 May 29.


### Table 1

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

<table>
<thead>
<tr>
<th>Cell source</th>
<th>Cell type</th>
<th>Administration route</th>
<th>Administration time</th>
<th>Stroke model</th>
<th>Mechanisms</th>
<th>Therapeutic effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3 cell line</td>
<td>ESCs</td>
<td>IC</td>
<td>2 wk after MCAO</td>
<td>60-min MCAO in rats</td>
<td>Massive accumulation of stem cells in the infarcted area</td>
<td>NM</td>
<td>Hoehn et al. (2002)</td>
</tr>
<tr>
<td>Human</td>
<td>NSCs</td>
<td>IC</td>
<td>7 d after ischemia</td>
<td>Right MCA M1 segment occlusion in cynomolgus monkeys</td>
<td>Cell replacement</td>
<td>NM</td>
<td>Roitberg et al. (2006)</td>
</tr>
<tr>
<td>Syngenic</td>
<td>NSCs</td>
<td>IV</td>
<td>72 h after MCAO</td>
<td>45-min MCAO in mice</td>
<td>Lowered inflammation, glial scar formation and neuronal apoptotic death</td>
<td>Recovery improved</td>
<td>Bacigaluppi et al. (2009)</td>
</tr>
<tr>
<td>MHP36 cell line</td>
<td>NSCs</td>
<td>IC</td>
<td>3 d after ischemia</td>
<td>17-min bilateral common carotid artery occlusion in mice</td>
<td>NM</td>
<td>Reduced the extent of ischemic neuronal damage</td>
<td>Wong et al. (2005)</td>
</tr>
<tr>
<td>Mice</td>
<td>iPSCs</td>
<td>Subdural transplantation</td>
<td>Immediately after MCAO</td>
<td>1-h MCAO in rats</td>
<td>Decreased pro-inflammatory cytokines and increased anti-inflammatory cytokines</td>
<td>Decreased infarct size; Improved motor function</td>
<td>Chen et al. (2010b)</td>
</tr>
<tr>
<td>Human</td>
<td>iPSCs</td>
<td>IC</td>
<td>1 wk after MCAO in mice or 48 h after MCAO in rats</td>
<td>30-min MCAO in rats and mice; and permanent dMCAO + 30-min CCAO rat model by craniotomy</td>
<td>Cell replacement; Increased vascular endothelial growth factor levels and enhanced endogenous plasticity</td>
<td>Forepaw movements recovery improved</td>
<td>Oki et al. (2012)</td>
</tr>
<tr>
<td>Syngenic</td>
<td>BMSCs</td>
<td>IV</td>
<td>24 h or 7 d after MCAO</td>
<td>2-h MCAO in rats</td>
<td>Cell replacement</td>
<td>Neurological functional recovery improved</td>
<td>Chen et al. (2001b)</td>
</tr>
<tr>
<td>Syngenic</td>
<td>BMSCs</td>
<td>IA</td>
<td>1 d after MCAO</td>
<td>2-h MCAO in rats</td>
<td>Decreased glial wall thickness and increased axonal density</td>
<td>Functional deficits were improved for 1 year.</td>
<td>Shen et al. (2007)</td>
</tr>
<tr>
<td>Syngenic</td>
<td>MSCs</td>
<td>Intranasal</td>
<td>3 d after MCAO</td>
<td>1.5-h MCAO in neonatal rats</td>
<td>Increased cell proliferation and endogenous repair processes; induced cytokine and growth factor changes</td>
<td>Infarct size was reduced and motor deficits were improved.</td>
<td>van Velthoven et al. (2013)</td>
</tr>
<tr>
<td>Cell source</td>
<td>Cell type</td>
<td>Administration route</td>
<td>Administration time</td>
<td>Stroke model</td>
<td>Mechanisms</td>
<td>Therapeutic effects</td>
<td>Reference</td>
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<tr>
<td>Syngenic</td>
<td>BMSCs</td>
<td>IV</td>
<td>24 h after MCAO</td>
<td>2-h MCAO in T1DM rats</td>
<td>Increased BBB leakage; Cerebral artery neointimal formation; Increased angiogenin expression.</td>
<td>No functional improvement.</td>
<td>Chen et al. (2011b)</td>
</tr>
<tr>
<td>Human</td>
<td>Dental pulp-derived stem cells</td>
<td>IC</td>
<td>24 h after HI</td>
<td>Unilateral HI brain injury in P5 mice</td>
<td>Suppressed the expression of proinflammatory cytokines; Enhanced the expression of anti-inflammatory cytokines</td>
<td>Brain-tissue loss was reduced and neurological function was improved.</td>
<td>Yamagata et al. (2013)</td>
</tr>
<tr>
<td>Human</td>
<td>Adipose tissue Stromal cells</td>
<td>IC</td>
<td>1 d after MCAO</td>
<td>1.5-h MCAO in rats</td>
<td>Induced production of neurotrophic factors</td>
<td>Functional improvement</td>
<td>Kang et al. (2003)</td>
</tr>
<tr>
<td>Human</td>
<td>Umbilical cord blood cells</td>
<td>IV</td>
<td>24 h or 7 d after MCAO</td>
<td>2-h MCAO in rats</td>
<td>Cell replacement</td>
<td>Neurological functional recovery improved.</td>
<td>Chen et al. (2001c)</td>
</tr>
<tr>
<td>Human</td>
<td>Menstrual blood-derived stem cells</td>
<td>IC, IV</td>
<td>Within 2 h after MCAO</td>
<td>60-min MCAO in rats</td>
<td>Induced production of growth and trophic factors</td>
<td>Reduced cell death (in vitro); Behavioral impairments (in vivo)</td>
<td>Borlongan et al. (2010)</td>
</tr>
<tr>
<td>Human</td>
<td>Placental MSCs</td>
<td>IV</td>
<td>4 h after MCAO</td>
<td>2-h MCAO in rats</td>
<td>Induced production of growth and trophic factors</td>
<td>Decreased lesion volume; Promoted functional outcome</td>
<td>Chen et al. (2013b)</td>
</tr>
</tbody>
</table>

IC, intracerebral; IV, intravenous; IA, intra-arterial; ESCs, embryonic stem cell; 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>
<thead>
<tr>
<th>Patient number</th>
<th>Cell source</th>
<th>Cell type</th>
<th>Delivery route</th>
<th>Cell volume and administration time</th>
<th>Patient characteristics</th>
<th>Main outcomes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Immortalized cell line</td>
<td>LBS-neurons</td>
<td>IC</td>
<td>4 received 2 million cells and 8 received 2 or 6 million NT2N cells</td>
<td>Ischemic stroke (6 m-6 yr); Age: 44-75 yr</td>
<td>Feasible and function improved</td>
<td>Kondziolka et al. (2006)</td>
</tr>
<tr>
<td>18</td>
<td>Immortalized cell line</td>
<td>LBS-neurons</td>
<td>IC</td>
<td>Stroke (1-6 yr); Age: 18-75 yr; EES score: 0-10-45</td>
<td>Neurological events occurred and subdural hernia; Some patients had improved function</td>
<td>Study was terminated after 2 MCA</td>
<td>Kondziolka et al. (2000)</td>
</tr>
<tr>
<td>12</td>
<td>Pocine</td>
<td>LGE cells</td>
<td>IC</td>
<td>10^4-10^6 cells per needle tract; 5 needle tracks per patient</td>
<td>Ischemic stroke (6 m-6 yr); Age: 44-75 yr</td>
<td>Feasible and function improved</td>
<td>Bang et al. (2005)</td>
</tr>
<tr>
<td>5</td>
<td>Autologous</td>
<td>MSCs</td>
<td>IC</td>
<td>5 × 10^6 cells after isolation of bone marrow stem cells</td>
<td>Chronic stroke (1-10 yr); Age: 40-70 yr</td>
<td>Safe and neurological condition improved</td>
<td>Suarez-Monteagudo et al. (2009)</td>
</tr>
<tr>
<td>30</td>
<td>Autologous</td>
<td>MSCs</td>
<td>IV</td>
<td>5 × 10^7 cells twice: 4-5 (first boosting) and 7-9 wk (second boosting) after symptom onset</td>
<td>Acute ischemic lesions within MCA territory; Age: 30-75 yr; MCA infarct affecting the striatum</td>
<td>BI and mRS improved and safe</td>
<td>Bang et al. (2005)</td>
</tr>
<tr>
<td>5</td>
<td>Autologous</td>
<td>MSCs</td>
<td>IC</td>
<td>10^3-10^6 cells, 24 h after bone marrow aspiration</td>
<td>Chronic stroke (1-10 yr); Age: 40-70 yr</td>
<td>Safe and neurological condition improved</td>
<td>Suarez-Monteagudo et al. (2009)</td>
</tr>
<tr>
<td>12</td>
<td>Autologous</td>
<td>MSCs</td>
<td>IV</td>
<td>A mean of 50-60 × 10^6 cells after MSCs expansion</td>
<td>Acute ischemic stroke within MCA territory; Age: 50-70 yr; NIHSS ≤7</td>
<td>Only modest increase of FM and mRS and safe</td>
<td>Bhasin et al. (2011)</td>
</tr>
<tr>
<td>12</td>
<td>Autologous</td>
<td>MNCs</td>
<td>IV</td>
<td>Eight received 10 million cells/kg, and 2 received ≥7 million cells/kg between 24 and 72 h after stroke</td>
<td>Acute MCA ischemic stroke within 24-72 h of symptom onset; Age: 18-80 yr; NIHSS: 4-15</td>
<td>Daily rates of change in NIHSS and mRS, and median lesion volume reduced, safe</td>
<td>Battistella et al. (2011)</td>
</tr>
</tbody>
</table>

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

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.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Cell source</th>
<th>Cell type</th>
<th>Delivery route</th>
<th>Intervention</th>
<th>Inclusion criteria</th>
<th>Patient number</th>
<th>Design</th>
<th>Location</th>
<th>NCT identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>Allogenic</td>
<td>MSCs from adipose tissue</td>
<td>IV</td>
<td>1 million units/kg; within the first 2 wk after onset</td>
<td>Acute cerebral infarction (&lt;12h); 60–80yr; NIHSS 8–20</td>
<td>20</td>
<td>Randomized; placebo control; double blind</td>
<td>Spain</td>
<td>NCT01678534</td>
</tr>
<tr>
<td>IIA</td>
<td>Allogenic</td>
<td>Human placenta-derived cells (PDA001)</td>
<td>IV</td>
<td>2×10^8 cells (day 1) or 8×10^8 cells (days 1 and 8)</td>
<td>Ischemic stroke in MCA or PCA territory; 18–80yr; NIHSS 6–20</td>
<td>44</td>
<td>Randomized; placebo control; double blind</td>
<td>United States</td>
<td>NCT01310114</td>
</tr>
<tr>
<td>I/II</td>
<td>Allogenic</td>
<td>Adult BMSCs</td>
<td>IV</td>
<td>0.5–1.5 million cells/kg</td>
<td>Ischemic stroke (&gt;6m); &gt;18yr; NIHSS 6–20</td>
<td>35</td>
<td>Non-randomized; single group; open label</td>
<td>United States</td>
<td>NCT01297413</td>
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<tr>
<td>I/IIA</td>
<td>Allogenic</td>
<td>Modified stromal cells (SB621)</td>
<td>NM</td>
<td>2.5, 5.0, or 10 million cells once</td>
<td>Ischemic stroke in subcortical region of MCA or lenticulostriate artery with or without cortical involvement (6–36m); 18–75yr; NIHSS ≥7</td>
<td>18</td>
<td>Non-randomized; single group; open label</td>
<td>United States</td>
<td>NCT01287936</td>
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<tr>
<td>I</td>
<td>Autologous</td>
<td>BMSCs</td>
<td>IC</td>
<td>2–4 million cells per patient</td>
<td>Ischemia stroke or intracerebral hemorrhage patient (&gt;3 m); 40–70yr; NIHSS ≥7</td>
<td>30</td>
<td>Non-randomized; routine treatment control; open label</td>
<td>China</td>
<td>NCT01714167</td>
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<tr>
<td>I/I</td>
<td>Autologous</td>
<td>CD34+ stem cells</td>
<td>IA</td>
<td>NM</td>
<td>Acute stroke (&lt;7 d); 80–80yr; NIHSS ≥7</td>
<td>10</td>
<td>Non-randomized; single group; open label</td>
<td>United Kingdom</td>
<td>NCT00535197</td>
</tr>
<tr>
<td>I/I</td>
<td>Autologous</td>
<td>BMSCs+ EPCs</td>
<td>IV</td>
<td>2.5 million cells/kg twice in BMSCs or EPCs group; approximately 5wk after symptoms onset</td>
<td>Ischemic stroke within the MCA territory (&lt;7 d); 18–80yr; NIHSS ≥7</td>
<td>90</td>
<td>Randomized; placebo control; double blind</td>
<td>China</td>
<td>NCT01468064</td>
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<tr>
<td>I</td>
<td>Autologous</td>
<td>Peripheral HSCs</td>
<td>IA</td>
<td>4 million cells per patient</td>
<td>Internal carotid artery territory infarction (&lt;1 yr); 40–70yr; SSS &lt;40</td>
<td>40</td>
<td>Randomized; placebo control; open label</td>
<td>China</td>
<td>NCT01518231</td>
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<tr>
<td>I/II</td>
<td>Allogenic</td>
<td>MSCs</td>
<td>IV</td>
<td>2 million cells/kg; within 10 days of stroke</td>
<td>Acute cerebral ischemia (&lt;10 d); 20–80yr; MRS 3f</td>
<td>78</td>
<td>Randomized; plasmalyte-A control; double blind</td>
<td>Malaysia</td>
<td>NCT01091701</td>
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<tr>
<td>Phase</td>
<td>Cell source</td>
<td>Cell type</td>
<td>Delivery route</td>
<td>Intervention</td>
<td>Inclusion criteria</td>
<td>Patient number</td>
<td>Design</td>
<td>Location</td>
<td>NCT identifier</td>
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<tr>
<td>I</td>
<td>Autologous</td>
<td>OECs</td>
<td>IC</td>
<td>About 2–8x10^6 cells; after 1–2 m culture and expansion</td>
<td>Chronic stroke (6–60 m); 35–70yr; NIHSS 5–15</td>
<td>6</td>
<td>Randomized; crossover; single blind</td>
<td>Taiwan</td>
<td>NCT01327768</td>
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<tr>
<td>II</td>
<td>Autologous</td>
<td>BMSCs</td>
<td>IV</td>
<td>Single infusion</td>
<td>Stroke (1 wk–2 m); 30–70yr; NIHSS &gt;10</td>
<td>50</td>
<td>Randomized; routine treatment control; open label</td>
<td>Malaysia</td>
<td>NCT01461720</td>
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<tr>
<td>IIA</td>
<td>Autologous</td>
<td>MSCs</td>
<td>IV</td>
<td>A mixing of physiological salt solution/albumin 4% (volume &lt;100 ml); less than 6 wk after stroke</td>
<td>Right or left carotid ischemic stroke (&lt;14 d); 18–65yr; NIHSS &gt;2</td>
<td>30</td>
<td>Randomized; control without intervention nor placebo; open label</td>
<td>France</td>
<td>NCT00875654</td>
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<tr>
<td>I</td>
<td>Autologous</td>
<td>CTX0E03 neural stem cells</td>
<td>Intracranial</td>
<td>Single administration of increased dose level of 2.5, 10 or 20 million cells</td>
<td>Unilateral ischemic stroke involving subcortical white matter or basal ganglia (6 m–5yr); 60–85yr; NIHSS ≥6</td>
<td>12</td>
<td>Single group; open label</td>
<td>United Kingdom</td>
<td>NCT0151124</td>
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<tr>
<td>I</td>
<td>Autologous</td>
<td>Mononuclear bone marrow cells</td>
<td>IV</td>
<td>NM</td>
<td>Acute ischemic stroke; 18–83yr (if &gt;80yr; pre-MRS &lt;1); NIHSS 615 (R)/18 (L)</td>
<td>30</td>
<td>Single group; open label</td>
<td>United States</td>
<td>NCT00859014</td>
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<tr>
<td>II</td>
<td>Autologous</td>
<td>ALD-401 derived from bone marrow</td>
<td>IA</td>
<td>3 mL suspension of ALD-401; 13–19d after stroke</td>
<td>Acute MCA stroke (within 24h); 30–83 yr; NIHSS ≥2</td>
<td>100</td>
<td>Randomized; shame procedure control; double blind</td>
<td>United States</td>
<td>NCT01273337</td>
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<tr>
<td>I</td>
<td>Allogenic</td>
<td>CD34+ stem cell obtained from UCB</td>
<td>IC</td>
<td>Approximately 5 million</td>
<td>Ischemic stroke (6–60m); 35–70yr; NIHSS 5–15</td>
<td>6</td>
<td>Single group; open label</td>
<td>Taiwan</td>
<td>NCT01438593</td>
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<tr>
<td>I/I</td>
<td>Autologous</td>
<td>Adipose-derived stromal cells</td>
<td>Intra-carotid &amp; IV</td>
<td>NM</td>
<td>Ischemic stroke or hemorrhagic stroke; 18–80yr; NIHSS ≥6</td>
<td>10</td>
<td>Non-randomized; single group; open label</td>
<td>Mexico</td>
<td>NCT01453829</td>
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<tr>
<td>II</td>
<td>Autologous</td>
<td>MultiStem®</td>
<td>NM</td>
<td>Single infusion; 1–2 d post stroke</td>
<td>Cortical ischemic stroke; 18–79 yr; moderate to moderately severe stroke</td>
<td>140</td>
<td>Randomized; placebo control; double blind</td>
<td>United States</td>
<td>NCT01436487</td>
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<tr>
<td>III</td>
<td>Autologous</td>
<td>MSCs</td>
<td>IV</td>
<td>NM</td>
<td>Stroke (&lt; 90 d); 30–75 yr; NIHSS 6–21</td>
<td>60</td>
<td>Randomized; routine treatment control; open label</td>
<td>Korea</td>
<td>NCT01716481</td>
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<tr>
<td>Phase</td>
<td>Cell source</td>
<td>Cell type</td>
<td>Delivery route</td>
<td>Intervention</td>
<td>Inclusion criteria</td>
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<tr>
<td>NM</td>
<td>Autologous</td>
<td>CD34+ peripheral blood stem cells</td>
<td>IC</td>
<td>Stem cell therapy plus G-CSF</td>
<td>Chronic cerebral infarction in MCA territory (6 m-5 yr); &gt;30 yr; NIHSS 9–20</td>
<td>36</td>
<td>Case control; prospective</td>
<td>Taiwan</td>
<td>NCT01239602</td>
</tr>
</tbody>
</table>

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.