Stem Cell Transplantation Therapy for Multifaceted Therapeutic Benefits after Stroke

Ling Wei[1,2,3], **Zheng Z. Wei**[2], **Michael Qize Jiang**[2], **Osama Mohamad**[2], and **Shan Ping Yu**[1,2]

[1]Laboratories of Stem Cell Biology and Regenerative Medicine, Department of Neurology, Experimental Research Center and Neurological Disease Center, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China

[2]Department of Anesthesiology, Emory University School of Medicine, Atlanta, GA 30322, USA

[3]Department of Neurology, Emory University School of Medicine, Atlanta, GA 30322, USA

Abstract

One of the exciting advances in modern medicine and life science is cell-based neurovascular regeneration of damaged brain tissues and repair of neuronal structures. The progress in stem cell biology and creation of adult induced pluripotent stem (iPS) cells has significantly improved basic and pre-clinical research in disease mechanisms and generated enthusiasm for potential applications in the treatment of central nervous system (CNS) diseases including stroke. Endogenous neural stem cells and cultured stem cells are capable of self-renewal and give rise to virtually all types of cells essential for the makeup of neuronal structures. Meanwhile, stem cells and neural progenitor cells are well-known for their potential for trophic support after transplantation into the ischemic brain. Thus, stem cell-based therapies provide an attractive future for protecting and repairing damaged brain tissues after injury and in various disease states. Moreover, basic research on naïve and differentiated stem cells including iPS cells has markedly improved our understanding of cellular and molecular mechanisms of neurological disorders, and provides a platform for the discovery of novel drug targets. The latest advances indicate that combinatorial approaches using cell based therapy with additional treatments such as protective reagents, preconditioning strategies and rehabilitation therapy can significantly improve therapeutic benefits. In this review, we will discuss the characteristics of cell therapy in different ischemic models and the application of stem cells and progenitor cells as regenerative medicine for the treatment of stroke.

Corresponding author: Shan Ping Yu, MD/PhD, 101 Woodruff Circle, Woodruff Memorial Research Building Suite 620, Emory University School of Medicine Atlanta, GA 30322, USA, spyu@emory.edu.

Author Manuscript

Introduction

Stem cell therapy has been considered in basic and clinical stroke research fields to be a promising regenerative medical treatment and a more promising approach for brain injury induced by different types of strokes. Stem cells (either endogenous neural stem cells or induced pluripotent stem cells e.g. iPS cells) have the potential of replacing damaged cells in the brain. Cell replacement strategies have been proposed and tested in many stroke models across decades of research in animal models. In addition, multipotent and pluripotent stem cells have shown beneficial paracrine effects, which can reduce cell death and provide growth/trophic support to host cells and regenerative activities in the host brain.

Induction of iPS cells from somatic cells (e.g. fibroblasts) with transcriptional factors (Oct-3/4, Sox-2, c-Myc and Klf-4) has shown promising translational potential. Similar to embryonic stem (ES) cells, iPS cells can be expanded *in vitro* and induced into neurospheres, neural progenitors, and mature neurons. Neuronally-differentiating ES/iPS cells and ES/iPS-derived neural progenitors have been extensively tested in transplantation therapies for the treatment of stroke, traumatic brain injury (TBI), spinal cord injury (SCI) and neurodegenerative diseases. It is generally agreed that transplanted cells may provide morphological and functional benefits via multiple mechanisms including, but not limited to, cell replacement, trophic support, immunosuppression/anti-inflammation, stimulation of endogenous signaling for neural plasticity and regeneration, and regulatory interactions with endogenous cells such as astrocytes and oligodendrocytes (Horie *et al.*, 2015; McDonald and Howard, 2002; Volpe *et al.*, 2016; Yu *et al.*, 2013). Based on promising results and better understanding on the therapeutic mechanisms, translational studies and clinical trials using stem cells including mesenchymal stem cells (MSCs), umbilical cord stem cells (UCSCs), adipose-derived stem cells (ASCs), iPS cells, and many others are being tested for clinical applications.

In this review, we will focus on neural progenitor cells derived from ES, adult brain and iPS cells, and widely tested MSCs. Their mechanism of action and beneficial effects after stroke in animal models and human studies will be reviewed. Regarding transplantation therapy, we will discuss the efforts to improve the survival, neuronal differentiation and therapeutic potential of transplanted cells, post-transplantation cell distribution, and effects related to repairing the neurovascular unit. Cell delivery methods will be discussed highlighting the recent advances in non-invasive intranasal delivery of cells. We will also introduce functional and behavioral improvements after stem cell transplantation therapy.

Regenerative Medicine for the Treatment of Stroke

Stroke is the fifth leading cause of death and the number one cause of disability in the adult population in the United States (Mozaffarian *et al.*, 2015). With an average of one victim every 40 seconds, almost 795,000 individuals experience a stroke every year in the United States, accounting for approximately one out of every 18 deaths (Mozaffarian *et al.*, 2015; Roger *et al.*, 2012). Of all stroke cases, 87% are ischemic in nature and the rest are hemorrhagic. In ischemic stroke, a clot occludes a brain vessel (most commonly the middle cerebral artery or its branches) and blood flow to the brain region supplied by that vessel is

ceased, causing a cascade of pathological events associated with energy failure, acidosis, excessive glutamate release, elevated intracellular Ca^{2+} levels, generation of free radicals (especially after reperfusion), blood-brain-barrier (BBB) disruption, inflammation and eventually massive excitotoxic cell death composed of necrosis, apoptosis, autophagy and likely concurrent mixed forms of cell death involving hybrid mechanisms (Durukan and Tatlisumak, 2007; Li *et al.*, 2013a; Puyal *et al.*, 2013; Wei *et al.*, 2004; Xiao *et al.*, 2002). Hemorrhagic stroke, on the other hand, occurs when a blood vessel ruptures in the brain leading to intracranial hemorrhage (ICH) (Ferro, 2006). ICH, with the associated edema, makes the outcome of hemorrhagic stroke more serious than ischemic stroke. Approximately 10% of ischemic stroke victims and 38% of hemorrhagic stroke victims die within 30 days.

Despite an estimated cost of more than 70 billion dollars in 2010 (Roger *et al.*), stroke therapy remains very limited and dependent predominantly on supportive therapies. The only FDA-approved treatment for acute stroke patients is tissue plasminogen activator (t-PA). t-PA administered within the 4.5-hr treatment window breaks down the vessel clot, allowing reperfusion of the infarcted/penumbral area. Late t-PA administration, can cause intracranial hemorrhage and is thus contraindicated in hemorrhagic stroke. As such, t-PA is applicable for only about 2% of stroke patients who meet the criteria for t-PA treatment. Evidently, there is a critical and urgent need for effective therapies for acute as well as chronic stroke patients (Wahlgren *et al.*, 2008).

Many drugs that show significant neuroprotection in animal models have failed to replicate in human clinical trials. Erythropoietin (EPO), for example, has shown significant neuroprotection in animal models of stroke (Bernaudin *et al.*, 1999; Keogh *et al.*, 2007; Leist *et al.*, 2004; Li *et al.*, 2007a; Li *et al.*, 2007b; Prass *et al.*, 2003; Tsai *et al.*, 2006) and has even shown positive protection in a small clinical trial where t-PA was not used (Ehrenreich *et al.*, 2002). However, it failed to improve outcomes and may have detrimental effects in patients receiving systemic thrombolysis (Ehrenreich *et al.*, 2009). Mild and moderate hypothermia (therapeutic hypothermia) has provided significant neuroprotection in animal models of stroke (van der Worp *et al.*, 2007). In contrast, therapeutic hypothermia is considered one of very few therapies that had promising potential as a clinical treatment for stroke patients, based on consistent beneficial effects observed in animal models of ischemic and hemorrhage stroke and traumatic brain injury (TBI). Benefits have also been replicated in clinical settings for the treatment of heart ischemia after cardiovascular arrest, term neonates after hypoxic-ischemic encephalopathy (HIE), and children with TBI (Jeon, 2014; Lampe and Becker, 2011; Luehr and Etz, 2014; Pietrini *et al.*, 2012; Tissier *et al.*, 2012).

Stem Cells Are Pluripotent/Multipotent Cells Capable of Tissue Repair

Emerging research interests have been focused on protective and regenerative strategies using stem cells and neural progenitors cells in the sub-acute and chronic phases after stroke. Stem cells are defined as cells which can both self-renew and differentiate into other cell types. Pluripotent stem cells, such as ES and iPS cells, can differentiate into any cell type of the three germ layers. In contrast, multipotent stem cells, such as hematopoietic stem cells, have a more lineage-restricted differentiation potential. Undifferentiated pluripotent stem

cells can theoretically proliferate indefinitely *in vitro*, avoiding the senescence observed in cultures of adult multipotent stem cells (Gadalla *et al.*, 2015).

Since their first isolation in 1998 by Dr. Thomson (Thomson *et al.*, 1998), storms of ethical disputes have arisen around human ES cells. Due to the fact that they are derived from the inner cell mass of human blastocysts, human ES cells have raised many ethical concerns. Research progress and funding for human ES cells have been significantly hampered and thus, mainstream stem cell research has turned to alternative non-blastocyst derived cell sources. Along these same lines, different cocktails of transcriptions factors and small molecules have been developed whose expression or addition of various gene products can induce a pluripotent state in terminally differentiated somatic cells such as fibroblasts (Takahashi and Yamanaka, 2006; Yu *et al.*, 2007).

Ethical concerns and the fear of rejection when using allogeneic cells have prevented clinical trials using ES cells, but the introduction of iPS cells has spurred research into the personalized use of pluripotent cells as a therapy for neurodegenerative disorders (Crook *et al.*, 2015; Inoue *et al.*, 2014). These induced pluripotent cells can differentiate into cell types of the three germ layers. Recently, novel protocols have been developed for creating neurons or neural precursors directly from fibroblasts, astrocytes or other terminally differentiated cells while avoiding a pluripotent cell state (Caiazzo *et al.*; Lujan *et al.*, 2012; Marro *et al.*, 2011; Pfisterer *et al.*; Vierbuchen *et al.*).

Stem cell transplantation has proven to be beneficial in injury models such as myocardial infarction and heart failure, with promising work in animal models translating to success in human clinical trials (Lee and Terracciano; Wollert and Drexler; Yin *et al.*, 2014). In this review, we use the words "stem cells transplantation" to refer to all cell transplantation using stem cells and stem cell-derived neural progenitor cells. In fact, undifferentiated pluripotent cells such as ES cells are not used directly for transplantation because of their high risk of tumorigenesis. Meanwhile, transplantation of multipotent bone marrow stem cells (BMSCs) has been extensively evaluated for tumorigenesis but no tumor formation has been observed using these cells.

The clinical use of BMSCs in neurologic injury models, such as spinal cord injuries (Watanabe *et al.*, 2015; Yoon *et al.*, 2007) and neurodegenerative diseases such as multiple sclerosis (Ghobrial *et al.*, 2014; Su *et al.*, 2006), have demonstrated beneficial effects. It is unclear whether the observed improvements arise from cell engraftment or the replacement of damaged tissues. Considerable evidence has shown that bone marrow derived stem cells release trophic factors that show pro-survival and pro-regeneration effects in ischemic regions and promote endogenous repair mechanisms, particularly with regard to angiogenesis and neurogenesis. Furthermore, tissue engraftment in Parkinson's disease has provided a proof-of-concept for cell replacement (Hallett *et al.*, 2014; Lindvall and Kokaia, 2009), leading researchers to believe that stem cell therapies can eventually be used to replace damaged tissue resulting from neurological injuries and disorders.

In rodent models of focal ischemic stroke, endogenous neural stem cells in the sub-ventricular zone (SVZ) are recruited to the infarct site and form newborn neurons (Fig. 1).

However, the efficiency of this endogenous repair mechanism is very low and the number of new cells that can survive and differentiate into mature neurons is insufficient for effective repair of damaged brain tissue. Neural stem cells (NSCs) can be isolated from the adult brain and after proliferation and neural differentiation under culture conditions, differentiated neural progenitor cells (NPCs) can be transplanted into the ischemic brain (Fig. 1). These transplanted cells can differentiate into neural and vascular cells and, by secreting trophic factors, reduce cell death and increase endogenous neurogenesis and angiogenesis (Hess and Borlongan, 2008; Wei *et al.*, 2005b; Yu and Silva, 2008). Cells derived from the bone marrow (Chopp and Li, 2002) or umbilical cord blood (Taguchi *et al.*, 2004; Vendrame *et al.*, 2004) have also been used in rodent models of stroke. Improvements observed in such studies seem to have come primarily from trophic effects or enhancement of endogenous regeneration rather than replacement of damaged neuronal tissue. This idea is supported by the fact that functional improvements occur even when the homing of intravenously infused BMSCs is very low (~1%) so it appeared that cells did not need to cross the blood-brain barrier (BBB) to provide beneficial effects (Borlongan *et al.*, 2004).

Neural precursor cells that have been used in rodent models include conditionally immortalized cell lines derived from human fetal tissue (Pollock *et al.*, 2006; Stevanato *et al.*, 2009), carcinomas (Bliss *et al.*, 2006; Hara *et al.*, 2008), fetal neuronal stem cells (Zhang *et al.*, 2009a), mouse neural precursors derived from the post-stroke cortex (Nakagomi *et al.*, 2009), and precursors derived from mouse (Theus *et al.*, 2008a; Wei *et al.*, 2005; Yanagisawa *et al.*, 2006) or human (Daadi *et al.*, 2008b; Kim *et al.*, 2007b) embryonic stem cells. Undifferentiated pluripotent stem cells are normally treated with a neural induction protocol before transplantation to gain cell lineage direction as well as reduce the risk of tumorigenesis (Newman *et al.*, 2005). After neural induction, neural progenitor cells are either injected into the circulation (intravenously or intra-arterially) or directly into the ischemic brain (intracerebrally, intracranially, or intranasally) and have been shown to improve functional outcomes in rodent models of stroke.

Neuronal Differentiation of Stem Cells

In the absence of differentiation cues, pluripotent stem cells can remain in a state of continuous self-renewal in culture. Murine and human ES and iPS cells have the potential to differentiate into any of the three primary germ layers including the ectodermal layer responsible for neural development (Doetschman *et al.*, 1985). Neural progenitors can further differentiate into any of the neural cell types, including glial cells and neurons (Reubinoff *et al.*, 2001). The differentiation outcome greatly depends on the microenvironment in the cell culture (Watt and Hogan, 2000). Specific neuronal subtypes (dopaminergic, GABAergic, etc.) can be cultivated in a controlled microenvironment with specific substrates and media components. There are many different ways to differentiate mouse pluripotent cells into neurons depending on the neuronal subtype of interest (i.e. midbrain dopaminergic (Lee *et al.*, 2000; Maxwell and Li, 2005), serotonergic (Lee *et al.*, 2000; Lu *et al.*, 2016), forebrain (Jing *et al.*, 2011), radial glia (Glaser and Brustle, 2005; Gorris *et al.*, 2015).

Among different protocols for mouse stem cells, differentiation using the vitamin A derivative, retinoic acid (RA), is the most commonly used method (Bain *et al.*, 1995; Fraichard *et al.*, 1995; Francis and Wei, 2010; Guan *et al.*, 2001; Rohwedel *et al.*, 1999) (Fig. 1B). RA plays a role in the developing mammalian embryo and specifies the anterior-posterior body plan (Horschitz *et al.*, 2015; Kessel and Gruss, 1991). Anteriorly, RA induces *Hox* gene expression (Kessel and Gruss, 1991; Marshall *et al.*, 1992; Simeone *et al.*, 1991) and has specific effects on rhombomere development in the CNS (Marshall *et al.*, 1992). RA interacts with Cellular RA-Binding Proteins (CRABP) which bind to nuclear RA receptors. There are two families of RA receptors (RARs and RXRs) (Rohwedel *et al.*, 1999). RARs include RAR-α, RAR-β and RAR-γ. During neuroectodermal differentiation, RAR-α and RAR-β mRNA are upregulated suggesting that neural differentiation requires these receptor subtypes (Rohwedel *et al.*, 1999). The binding of RA to its receptor activates the transcription of downstream target genes leading to neural lineage selection. In pluripotent cell differentiation, RA induction activates neural-specific transcription factors, signaling molecules, and RA-inducible genes resulting in the production of different neuron sub-types such as GABAergic and glutamatergic neurons (Guan *et al.*, 2001). The RA neural induction method produces both glial cells and neurons. Neurons derived in this method show mature neuronal morphology, express mature neuronal markers such as neuronal nuclei (NeuN) and neurofilament and exhibit neuronal electrophysiology profiles that include mature sodium currents, potassium currents, and action potentials (Bain *et al.*, 1995).

Undifferentiated mouse ES and iPS cells are regularly maintained in a self-renewing, proliferating state in the presence of leukemia inhibitory factor (LIF) which suppresses differentiation and is required for the maintenance of pluripotency (Vidal *et al.*, 2015; Williams *et al.*, 1988). Neural induction using the "4+/4−" RA protocol takes eight days *in vitro*. After LIF withdrawal, cells are grown in suspension to form three dimensional spherical aggregates of cells known as embryonic bodies (EB). No RA is included in the medium for the first 4 days of EB culture (termed "4−"), but it is added in the last four days (termed "4+"). This treatment induces EBs to form neurospheres which are then dissociated and plated on an adherent substrate (laminin, Matrigel or fibronectin) to allow terminal neuronal differentiation and neurite extension. These cells will express neuronal markers such as the M subunit of neurofilament and class III β-tubulin protein within a few days. Most of the neurons derived in this fashion will express functional Na^+, K^+, and Ca^{2+} channels and fire action potentials (Bain *et al.*, 1995).

Neural cells can also be obtained from pluripotent stem cells through lineage selection (Guan *et al.*, 2001). In this method, differentiation into cell types of all three germs layers (ectoderm, mesoderm, and endoderm) occurs in the EBs (Guan *et al.*, 2001). Neuroectodermal cells are selected with serum withdrawal, as well as the inclusion of basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) to increase proliferation of neural progenitor cells. Neural differentiation factors and survival-promoting factors (SPF) (interleukin-1β, TGF-β, GDNF and many others) are then added for further differentiation of neural cells into specified neuronal cell types (Guan *et al.*, 2001).

There are also several other protocols for the neuronal differentiation of mouse ES and iPS cells. One particular protocol makes use of mouse stromal supportive cells for neuronal

induction (Barberi *et al.*, 2003). Murine bone marrow–derived stromal feeder MS5 cells (Itoh *et al.*, 1989) are mesenchymal stem cells that were originally derived to support the long-term growth and expansion of hematopoietic stem cells, but have also been found to induce a neuro-ectodermal lineage fate in mouse ES cells. In brief, undifferentiated murine ES cells are seeded at a very low density on MS5 cells in serum replacement and cultured for 7 days with bFGF added in the last two days. Co-culture of mouse ES with MS5 cells induces the expression of neural markers (Nestin and Musashi) as early as day 6. Further terminal differentiation can yield different neural subtypes (glial cells, dopaminergic, serotonergic, and GABAergic neurons) depending on the patterning factors used after neural induction. Although RA strongly induces neuronal formation in mouse ES cells, its use in transplantation therapy is unclear. Neuronal induction of the MS5 cell line produces cortical pyramidal neurons that connect with their correct targets *in vivo* after transplantation (Ideguchi *et al.*, 2010). These graft-to-host axonal connections, however, did not occur when cells were differentiated using RA. The MS5 protocol may thus be more appropriate for use in cell replacement strategies using murine cells.

Human ES and iPS Cell Differentiation

Human ES and iPS cells have the potential to differentiate into all human cell lineages including neurons, making them an attractive cell source for studying disease mechanisms and for drug screening as well as cell transplantation therapy in neurodegenerative diseases such as stroke (Fig. 2). As with mouse pluripotent cells, several approaches have been developed for the neural induction of human pluripotent cells (Suzuki and Vanderhaeghen, 2015). The neuralizing effect of RA, for example, is not the same between human and mouse pluripotent cells. However, like their mouse counterparts, human cells can be differentiated either as EBs in suspension or in adherent culture. We will discuss two differentiation protocols because of their widespread use and proven efficiency.

After the first isolation of human ES cells in 1998, efforts were focused on their neuronal differentiation based on the knowledge accumulated from the development of the human nervous system. One of the earliest efforts was led by Zhang and Thomson utilizing EB formation (Zhang *et al.*, 2001). In this protocol, human ES cell colonies are dissociated into smaller clusters of cells and grown in human ES cell media without bFGF in low adherence plates. When EBs form, they are grown for four days before being plated on tissue culture plastic in a neural induction media. After neural rosette formation (~8–10 days), rosettes are re-suspended and expanded as neurospheres in medium containing bFGF. Prior to neurosphere formation, cells in the rosette structures express neural markers such as Nestin and Musashi-1. After expansion, these cells are plated on laminin, with mature neuronal markers detectable within 7–10 days. Further differentiation will yield more neurons and, after longer periods in culture, glial cells. This protocol was one of the earliest methods used to generate neural cells from human ES cells and has successfully been applied to human iPS cells (Hu *et al.*, 2010).

Despite its high efficiency, the EB protocol usually involves several weeks of expansion to obtain large numbers of neural precursors before differentiation into neurons and glial cells. In an attempt to overcome this time limitation and avoid the heterogeneous signals

encountered by cells in aggregates, Chambers et. al. developed an adherent protocol for the neural differentiation of human ES and iPS cells that produces large numbers of neural precursors in approximately 15 days (Chambers *et al.*, 2009). In brief, human ES or iPS cells are dissociated into single cells and grown on Matrigel in the presence of Rho associated protein kinase (ROCK) inhibitor, which reduces apoptosis and increases human pluripotent cell survival after dissociation (Watanabe *et al.*, 2007). When they become confluent, the cells are grown in a knock-out serum replacement media for five days. This medium is supplemented with two inhibitors of the SMAD signaling pathway, Noggin and SB431542. The media is then sequentially changed to neural induction media (increasing amounts of N2 media) for a total of six days. Consequently, neural rosette-like structures are formed and can be further differentiated into neurons of different subtypes. Unlike the EB based protocol, this adherent protocol yields Pax-6 positive neural precursors in more than 80% of the cells, with little need to separate rosettes from cells that have differentiated into other lineages. When used with hiPS cells, the EB protocol had a significantly reduced efficiency and increased variability as compared to hES cells (Hu *et al.*, 2010). On the contrary, the dual inhibition protocol significantly promotes neural differentiation from multiple human ES and iPS cell lines, (Kim *et al.*, 2010) indicating more consistent results with this protocol. In our investigations, we have shown that Noggin can be replaced with the small molecule dorsomorphin in this protocol, greatly reducing the cost for neuronal differentiation of human cells (Drury-Stewart *et al.*, 2012). Dorsomorphin has been used to enhance neural differentiation in suspension cultures (Kim *et al.*, 2010; Morizane *et al.*, 2011) and has been shown to induce efficient neural differentiation alone in adherent culture (Zhou *et al.*, 2010).

Strategies to Enhance Cell Survival after Transplantation: Genetic Modification and Hypoxic Preconditioning

Genetic manipulation of transplanted cells has been tested for enhancing the cell survival after transplantation (Doeppner *et al.*, 2010; Wei *et al.*, 2005). Expression of pro-survival factors shows significant effects on enhancing graft viability after transplantation. For example, we reported in our early research that transplantation of ES cell-derived neural progenitor cells over-expressing the anti-apoptotic gene Bcl-2 survived much better than control cells after ischemic stroke in rats (Wei *et al.*, 2005). Compared to controls, Bcl-2 expressing progenitors also exhibited greater neuronal differentiation and better neurological and behavioral outcomes after stroke (Fig. 3) (Wei *et al.*, 2005). This approach of over-expressing pro-survival genes in transplanted cell has been actively investigated by a number of groups (Chen *et al.*, 2012; Leu *et al.*, 2010; Liu *et al.*, 2011). However, this type of genetic engineering requires the alteration of DNA sequences in stem cells, generally by inserting the gene(s) of interest into the genome. While this method can be highly efficient, manipulating the genome of stem cells increases the risk of tumorigenesis in a population of cells with an already established risk of tumor formation.

Other techniques have been developed to increase survival of stem cell grafts after transplantation. Instead of over-expressing exogenous genes in transplanted cells, we developed an alternative strategy stimulating endogenous mechanisms to promote the survival and regenerative properties of transplanted cells (Pignataro *et al.*, 2007; Wei *et al.*,

2005b). In cardiomyocyte ischemia, an early exposure of the heart to brief episodes of ischemia protected the myocardium against a later prolonged ischemic insult (Murry *et al.*, 1986). We showed that pretreatment of ES or iPS cell-derived neural progenitor cells in low oxygen condition (0.1–1% O2) for 8–12 hrs markedly enhanced their tolerance to OGD insult *in vitro* and after transplantation into the ischemic brain (Fig. 4). The pro-survival and an enhanced homing to the ischemic brain were later demonstrated using BMSCs (Theus *et al.*, 2008b; Wei *et al.*, 2012; Wei *et al.*, 2015; Yu *et al.*, 2013).

Numerous investigations have demonstrated the effectiveness of hypoxic/ischemic preconditioning in virtually all types of cells, organs, and in animals (Gross and Auchampach, 1992; Yu *et al.*, 2006). Preconditioning has been successfully applied in cell therapy to protect the transplanted cells from apoptosis and increase their survival after transplantation *in vivo*. Hypoxic preconditioning (HP) was performed by exposing cells to non-lethal hypoxia (0.1–1%) for certain hours before transplantation, which is very effective in increasing transplanted cell survival and improving overall functional recovery after stroke or myocardial infarction (MI) (Hu *et al.*, 2011b; Hu *et al.*, 2008; Sun *et al.*, 2015; Theus *et al.*, 2008a; Wei *et al.*, 2012). HP has also shown enhancement of cell differentiation in culture and after transplantation (Sart *et al.*, 2014; Sun *et al.*, 2015). Non-lethal exposure to hypoxic conditions is believed to activate the hypoxia-inducible factor 1α (HIF-1α) pathway, increasing expression of HIF-1α-dependent genes. Expression of a number of trophic factors is increased, including brain-derived and glial cell-derived neurotrophic factors (BDNF and GDNF), vascular endothelial growth factor (VEGF) and its receptor FIK-1, erythropoietin (EPO) and its receptor EPOR, and stromal derived factor-1 (SDF-1) and CXC chemokine receptor 4 (CXCR4) (Wei *et al.*, 2012) (Fig. 4). With a greater translational significance, these beneficial effects of HP have been demonstrated in iPS cell-derived neural progenitor cells (Fig. 5A and 5B).

Pharmacological preconditioning is an alternative way by exposing stem cells of pharmacological agents or trophic/growth factors to increase their *in vivo* survival. Diazoxide (Afzal *et al.*, 2010), minocycline (Sakata *et al.*, 2012), and SDF-1 (Pasha *et al.*, 2008) can all enhance stem cell survival after transplantation in stroke or MI animal models.

In Vitro and Animal Models for the Study of Ischemic Stroke

There are many *in vivo* and *in vitro* models used to study stroke and stem cell therapy. While successes in treating stroke in animal models have been unreliable when translated to human clinical trials, ischemic stroke models are necessary to understand the pathophysiology of stroke progression and provide initial information for the development of appropriate therapeutics. *In vitro* model of oxygen glucose deprivation (OGD) mimics the hypoxic and energy crisis that occur during acute ischemic injury. Hence, this model is widely accepted and tested for studies of cell death, mitochondrial dynamics, and reperfusion/reoxygenation injuries.

In vitro investigations using cell culture models allow the understanding of the basic cellular, molecular and biochemical mechanisms without the systemic influences of an *in vivo* model. The OGD technique, applied to cultures of pure primary neurons or mixed cultures

of glia and neurons, is the most commonly used *in vitro* model of stroke (Tornabene and Brodin, 2016). Depriving cultured neurons of oxygen and glucose supplies simulates, to a certain extent, *in vivo* ischemic conditions. Briefly, growth medium is replaced with a physiological solution like Ringer's solution and cultures are placed in an airtight chamber with low oxygen (0.1 – 1.0%). After certain duration of times (usually one to several hours depending on the degree of hypoxia and cell types), cells are then returned to their normal culture conditions. Cell death and survival are assessed after an appropriate "reperfusion" period, usually in 24 hours. Sometimes, cell death is measured after a prolonged time of OGD without "reperfusion". Cell death induced by different OGD episodes may be mediated or dominated by distinctive mechanisms, e.g. necrotic and apoptotic pathways (Jones *et al.*, 2004)(Agudo-López *et al.*, 2010). OGD can be used with different cell types, including ES cell-derived neural precursors and glial cells, to study their mechanisms of survival and differentiation under an ischemia-like condition. For example, researchers used OGD to study a nuclear translocator (ARNT) in ES cells in response to hypoxia (Maltepe *et al.*, 1997). *In vivo* models allow us to study stroke within the scope of a comprehensive biological system. Here, we will discuss some major *in vitro* and *in vivo* stroke models used in preclinical research.

Inducing stroke in animals allows researchers to reproduce stroke conditions *in vivo* together with the related systemic influences. This allows for the study of stroke pathology and pathophysiology and the exploration for optimal conditions (survival, timing, and location) for experimental cell transplantation therapies. Animal models for the study of ischemic stroke primarily vary by their methods of induction, location, duration, and, correspondingly, the severity of ischemic injury and functional deficits (Ingberg *et al.*, 2016; Molinari, 1988). Ischemic stroke models can be divided into two broad categories: focal and global ischemia. Focal ischemia is more commonly seen in clinical cases and widely tested in basic research. It is induced by an acute occlusion of specific vessels to cause damage in affected brain regions. Focal ischemia can be modeled with transient or permanent occlusions and the extent of damage can be small or large depending on the occlusion of the artery and the anatomy of cerebral vassal networks. The word "global ischemia" is used when all cerebral blood flow including that of the vertebral arteries cease. Clinically, global ischemia occurs during suffocation, cardiac arrest or after bilateral common carotid artery (CCA) occlusions. The middle cerebral artery (MCA) is the most commonly affected vessel in stroke patients. Accordingly, the most common models of ischemic stroke in rodents involve the occlusion of MCA. Here, we will discuss several models of ischemic stroke.

Focal Ischemic Stroke Models

Middle cerebral artery occlusion (MCAO) of rodents

The MCAO model is widely used and accepted because it most closely resembles human stroke conditions. The original method for inducing this stroke was the Tamura method for occluding the proximal MCA in the rat, causing ischemic damage in the cortex and basal ganglia (Tamura *et al.*, 1981a, b). This procedure requires an invasive subtemporal craniotomy. The intraluminal method, on the other hand, requires no craniotomy and is thus less invasive (Longa *et al.*, 1989). A neck incision is made and a nylon intraluminal suture is

introduced through the external carotid artery to occlude the MCA. This procedure is reversible and can be varied with a thicker (2–0) or thinner (4–0) suture thread (Longa *et al.*, 1989; O'Brien and Waltz, 1973). Both models induce relatively large brain injuries involving cortex and/or subcortical regions or even an entire half hemisphere.

Using MCAO models, stem cell transplantation studies have been extensively performed during the past 20 years. Neuroprotective effects of therapeutic benefits in reducing infarct volume, and improving functional outcomes during acute and chronic phases can be examined. It is difficult, however, to evaluate neurovascular damage and neuronal circuit reestablishment at structural and network levels in severe stroke models. To this end, small stroke modes are more suitable for morphological examinations of neural network structural repair.

Barrel cortex stroke and small stroke models

Barrel cortex stroke is a small focal stroke model, involving occlusions of distal branches of the MCA. According to American Heart Association, small strokes are common in clinical cases (~40% of total cases)(Lloyd-Jones *et al.*, 2010). In recent years, increasing attention has been paid to develop and study small stroke models (Liu *et al.*, 2012; Llovera *et al.*, 2014; Pevsner *et al.*, 2001). This shift appears not only due to clinical relevance, but also for the advantage of the feasibility of examining structural and neural network repair after a well controlled focal cerebral ischemia. Small ischemic strokes can be induced by ligations of the distal branches of MCA. A typical small stroke model in rats was initially reported by Ling Wei in 1995 (Liu *et al.*, 2012) (Fig. 6). In this model, the barrel cortex and its vasculature are visualized using intrinsic optical signal imaging based on the mechanism that stimulation of the contralateral whiskers increases the local cerebral blood flow (LCBF) in the barrel cortex. The distal branches of the MCA around the barrel cortex are identified and then ligated with 10-0 sutures. In recent years, variations of the small barrel stroke models have been reported. In the mouse, the main branch of the MCA is permanently occluded combined with transient bilateral occlusions of the CCA. This simplified surgery procedure is sufficient to reduce the local blood flow and cause infarct formation in the affected sensorimotor cortex. The infarct volume induced by this procedure is relatively larger than the original barrel cortex stroke; it retains all the advantages of a small stroke model but also allows for functional and behavioral tests of the sensorimotor cortex in rodents.

Since the whisker-thalamus-barrel cortex neuronal pathway and the relationship between the barrel cortex and whisker activity had been well defined (Woolsey *et al.*, 1996), the barrel cortex and the sensorimotor cortex stroke models are ideal for examining the intracortical and thalamo-cortical neuronal morphological connections and functional recovery. For example, the corner test can be used to test the functional deficit of affected whiskers after stroke (Langhauser *et al.*, 2014; Zhang *et al.*, 2002). In this test, when facing toward a 30° corner wall, normal control animals turn equally to the right or left whereas stroke animals turn more frequently toward the non-impaired side (ipsilateral to infarcted brain)(Zhang *et al.*, 2002). Moreover, our group has used whisker stimulation as a physical rehabilitation treatment during chronic phase of stroke. It was shown that the target-specific peripheral activity reduced cell death and increased trophic/growth factors in the peri-infarct region of

the cortex. Consequently, the physical therapy enhanced neurogenesis, angiogenesis, and functional recovery after ischemic stroke (Li *et al.*, 2011; Li *et al.*, 2008a; Whitaker *et al.*, 2007).

Other focal ischemic stroke models in rodents

There are several other ways to induce focal ischemia including the photothrombotic (Schmidt *et al.*, 2015), autologous clot (Jin *et al.*, 2014; Taupin and Gage, 2002), and endothelin-1 (Roome *et al.*, 2014) methods (Table 1). The photothrombotic method is a non-invasive method in which a photosensitive dye is intravenously injected into the animal (Chen *et al.*, 2004; De Ryck *et al.*, 1996; Eichenbaum *et al.*, 2002). To induce thrombotic occlusion, the distal MCA is irradiated by a laser to activate microthromboses. A significant strength of this model is that it allows MCA occlusion in awake animals. In the thromboembolic stroke model, the embolus is created by removing the arterial blood from a donor into tubing of predetermined size. The clots are formed by alternating the blood through different syringes with saline. In the technique described by Zhang and colleagues, the clot was introduced into the external carotid artery (ECA) to be lodged into the origin of the MCA (Zhang *et al.*, 1997). A drawback of thromboembolic models is the difficulty for precise control of reperfusion and larger variations in infarct formation (Wang *et al.*, 2001; Zhang *et al.*, 1997). In the Endothelin-1 model, the vasoconstrictor peptide Endothelin-1 (ET-1) is injected into the brain. This peptide reduces blood flow and induces ischemic injury (Windle *et al.*, 2006). This model can induce transient to semi-permanent focal ischemia, but reperfusion in this model still requires characterization (Biernaskie *et al.*, 2001). It was reported that systemic inflammation continues to impair reperfusion via endothelin dependent mechanisms (Murray *et al.*, 2014). ET-1 can be injected intracerebrally or topically applied onto the brain into an area of interest like the motor cortex (Windle *et al.*, 2006). It can also be injected next to the MCA to induce larger ischemic injury (Biernaskie *et al.*, 2001).

Global Ischemia Models

Two-vessel occlusion model

In this model, histopathological ischemic damage to the forebrain is induced by bilateral occlusion of the CCA in conjunction with systemic hypotension (down to 50 mm Hg) to attenuate forebrain blood flow in the animal (Pulsinelli and Brierley, 1979). Hypotension is induced by arterial or central venous exsanguinations along with CCA clamping. The occluding clamps are removed and the shed blood is then used to reperfuse the brain (Smith *et al.*, 1984). Injury to specific brain areas depends on the duration of exposure to the ischemic conditions. The hippocampus, caudate-putamen, and cerebral cortex can all be affected, depending on the duration of the ischemia (Smith *et al.*, 1984). This occlusion technique can produce a reversible and reliable forebrain ischemia.

Four-vessel occlusion model

This model is carried out in two stages. First, reversible clamps are loosely placed around the CCA and the vertebral arteries are electro-cauterized (Pulsinelli and Brierley, 1979). After 24 hours, the CCA clamps are tightened (Pulsinelli and Brierley, 1979). This results in

loss of body responsiveness, with approximately 75% of animals losing their righting reflex. Within minutes after CCA clamping, isoelectric activity appears on electroencephalography (EEG). Reperfusion is allowed when the clamps on the CCA are removed, at which point animal activity is restored. Histopathology reveals that neuronal damage occurs within 20 to 30 minutes of ischemia. Damage usually appears in the hippocampus, striatum, and posterior neocortex (Pulsinelli and Brierley, 1979). Unlike the two-vessel method, this method is only partly reversible because the vertebral arteries are cauterized. Another drawback to this model is that it has a higher risk of inducing seizures, which voids data if seizure is not the research target of the study.

Stem Cell/Neural Progenitor Transplantation in Animal Models

An appropriate selection of the animal model is important for reaching the goal of an investigation. For example, different stroke models may be suitable for investigations on neuroprotection or regeneration or neurovascular/neural network repair. An ischemic insult that damaged sub-cortical regions including the hippocampus and dentate gyrus may not be feasible for a study relying on the neurogenesis in the subgranular zone (SGZ). In addition to test the efficacy of cell therapies, one of the goals of preclinical cell transplantation studies is to optimize transplantation parameters so that the transplanted cells survive, migrate, differentiate and integrate with host cells appropriately *in vivo*. Two important parameters to be considered for transplanting any cell type are the administration route and the timing of delivery. These parameters may vary by cell type and the model of ischemic stroke. Before translating cell therapy into human patients, transplantation parameters need to be verified in different animal models. The STEPS committees have set out guidelines for preclinical studies pointing out the need for a well-characterized cell population, dose-response studies, and tests in different models of at least two species (de Mello *et al.*, 2015).

Intracerebral/Intracranial transplantation

Identifying a favorable microenvironment for stem cell transplantation allows for increased survival of the graft. Some have suggested that transplanting cells into the penumbra is a more successful approach because the penumbra provides a more favorable microenvironment for the graft (Johnston *et al.*, 2001; Theus *et al.*, 2008a; Wei *et al.*, 2005). An alternative for avoiding stem cell transplantation into the cytotoxic stroke core is to transplant cells into the hemisphere contralateral to the infarct (Modo *et al.*, 2002b). It was shown in rats that a greater percentage of cells survived in the contralateral somatosensory cortex and striatum compared to those from ipsilateral or intraventricular injections (Modo *et al.*, 2002b). This strategy may be useful based on the observation that endogenous neural stem cells and exogenous transplanted cells have the ability to migrate to the ischemic infarct region (Arvidsson *et al.*, 2002; Li *et al.*, 2008a; Modo *et al.*, 2004; Zhang *et al.*, 2009b).

Our recent investigation on the cell fate after stroke, however, provided a different rationale for selecting the transplantation site. We showed in the sensorimotor cortex stroke and several other stroke models that inside the ischemic core, normal or even higher levels of BDNF and VEGF remained for many days after stroke. Some NeuN-positive cells with

intact ultrastructural features of synapses and axons resided in the core 7–14 days post stroke. BrdU-positive but TUNEL-negative neuronal and endothelial cells were detected in the core where extensive extracellular matrix infrastructure developed. Active regenerative niche was identified inside the core many days after stroke. These data suggest that the ischemic core is an actively regulated brain region with residual and newly formed viable neuronal and vascular cells acutely and chronically after at least some types of ischemic strokes (Jiang *et al.*, 2016). The microenvironment of ischemic core several days and weeks after stroke provides certain cellular support, blood flow supply and strong trophic factor endorsements for cells to survive while the remaining and regenerating neurovascular infrastructure may be utilized as therapeutic targets for repairing damaged neurovascular unit and neuronal networks. With recent progress in priming transplanted cells using the preconditioning strategy, preconditioned cells are more tolerant and survive better even after transplantation into the ischemic core (Theus *et al.*, 2008a; Wei *et al.*, 2013; Wei *et al.*, 2015). It appears that for the purpose of tissue repair, the development of transplanted cells with greater survival and regenerative properties will have the advantage of implanting cells into the brain area(s) where they are needed (Fig. 5C).

Intravascular administration

Stem cells may also be administered systemically through the vasculature. While this strategy is less invasive and does not necessitate craniotomy, vascular administration adds the requirement that cells must cross the blood brain barrier and home to the brain injury site. Vascular delivery of cells can be either intravenous or intra-arterial. There are advantages and disadvantages to both methods. While less invasive than intra-arterial delivery, the intravenous route delivers the cells through the systemic and pulmonary circulations where cells are more likely to be entrapped in other organs (spleen, liver, and lungs). Intra-arterial delivery, on the other hand, circumvents the systemic circulation. Li and colleagues reported that 21% of cells entered the brain with intra-arterial administration compared to only 4% of cells with intravenous administration (Biernaskie *et al.*, 2001). Similarly, Guzman and colleagues reported that 1300 cells/mm^2 populate the infarct area after intra-arterial administration compared to only 74 cells/mm^2 after intravenous delivery (Guzman *et al.*, 2008). Bone marrow mesenchymal cells systemically infused into ischemic rats have been reported to migrate to the site of injury (Eglitis *et al.*, 1999; Samper *et al.*, 2013). Some reports also showed potential neuronal differentiation of the transplanted cells after intravascular administration (Chen *et al.*, 2001a). The homing of BMSCs to the brain lesion site, however, is generally very low (~1%) (Arbab *et al.*, 2012) and neuronal differentiation is uncertain or difficult for these multipotent cells although some recent work reported improved differentiation from these cells (Kitada, 2012). Intravenous injection of human neural stem cells into an ischemic rat led to incorporation of some cells into the ischemic infarct. However, transplanted stem cells were also trapped in other organs such as the kidney, lung, and spleen. Within the brain, transplanted neural stem/progenitor cells were also found in the cortex, along the corpus callosum, and in the hippocampus (Chu *et al.*, 2003).

Trophic factors may improve the homing of transplanted cells to the brain region. For example, it was demonstrated that the intravascular injection of bone marrow stem cells

coupled with subsequent injections of granulocyte-colony stimulating factor (G-CSF) and stem cell factor (SCF) increased cell homing to the brain and neuronal differentiation (Corti *et al.*, 2002; Piao *et al.*, 2009). With G-CSF and SCF, more transplanted bone marrow cells could arrive at the ischemic brain, suggesting that bone marrow-derived stem cell therapy supplemented with other neurotrophic factors is a potential strategy in the stroke treatment.

This is also supported by our observation that a parathyroid hormone (PTH) can mobilize stem cells from the bone marrow and promote the homing of these cells to the brain. We showed that after 6 days of PTH treatment, there was a significant increase in bone marrow derived CD-34/Fetal liver kinase-1 (Flk-1) positive endothelial progenitor cells (EPCs) in the peripheral blood. PTH treatment significantly increased the expression of trophic/ regenerative factors including VEGF, SDF-1, BDNF and Tie-1 in the brain peri-infarct region. Angiogenesis, assessed by co-labeled Glut-1 and BrdU vessels, was significantly increased in PTH-treated ischemic brain compared to vehicle controls. PTH treatment also promoted neuroblast migration from the subventricular zone (SVZ) and increased the number of newly formed neurons in the peri-infarct cortex (Wang *et al.*, 2014).

In any event, the route of intravenous administration has been effective in preclinical and clinical trials. In one study, IV administration of cord blood stem cells in rat stroke animals resulted in improvement in motor functional recovery as assessed by the Rota-rod test (Chen *et al.*, 2001b). Similarly, intra-arterial administration of mesenchymal stem cells improved the NSS score and performance in the adhesive-removal test of stroke rats (Biernaskie *et al.*, 2001). IV administration of autologous MSCs has already been shown to be safe and appears to be effective in small clinical trials in human stroke patients (Suarez-Monteagudo *et al.*, 2009; Wise *et al.*, 2014). The PTH-treated mice mentioned above showed significantly better sensorimotor functional recovery compared to stroke controls. It is possible that mobilizing endogenous bone marrow-derived stem cells/progenitor cells is an alternative regenerative treatment after ischemic stroke (Wang *et al.*, 2014).

Intranasal administration

The intranasal delivery route is a non-invasive method for bypassing the blood-brain barrier (BBB). It has been used clinically and in pre-clinical research to administer drugs, peptides/ proteins, and a variety of factors (Fig. 7A). In cell transplantation therapy, intranasal delivery of stem cells is a new avenue of investigation. Intranasal delivery offers two major advantages that show great clinical potential for the treatment of stroke. Transplanted cells essentially move from the nasal mucosa into the CNS through the cribriform plate and migrate into the brain parenchyma along the olfactory neural pathways and blood vessels (Fig. 7A). In this procedure, the membrane permeation enhancers (like hyaluronidase) are often needed (Lochhead and Thorne, 2011). It was shown that rat MSCs reached the brain only one hour after intranasal delivery through either the rostral migratory stream (RMS) or the cerebrospinal fluid (CSF) (Danielyan *et al.*, 2009). Complete reproducibility of cell distribution, however, still need verification and improvement for different cells.

We demonstrated for the first time that hypoxic preconditioning is a promising or even essential strategy for intranasal delivery of stem cells and neural progenitor cells (Hu *et al.*, 2011b) (Fig. 7B–7G). In focal ischemic stroke mice, intranasally delivered hypoxia

preconditioned (HP) BMSCs arrived at the ischemic cortex and deposited outside of vasculatures only 1.5 hrs after administration. In comparison to non-HP BMSCs prepared under normoxic condition (N-BMSCS), HP-treated BMSCs (HP-BMSCs) expressed high levels of genes associated with migratory activities, including the CXC chemokine receptor type 4 (CXCR4), matrix metalloproteinase 2 (MMP-2), and MMP-9. HP-BMSCs showed enhanced migratory activities *in vitro* and increased homing to the infarct cortex when compared with N-BMSCs. Three days after transplantation (four days after stroke), BMSC transplantation decreased cell death in the peri-infarct cortex. Mice receiving HP-BMSCs showed significantly reduced infarct volumes compared to stroke mice receiving N-BMSCs. HP-BMSC-treated mice performed much better than N-BMSC- and vehicle-treated animals (Wei *et al.*, 2013). Furthermore, we demonstrated in a neonatal stroke model of rats that intranasally delivered BMSCs showed beneficial effects on brain development after stroke and that rat pups receiving HP-BMSCs developed better sensorimotor activity, olfactory functional recovery, and performed better in social behavioral tests (Wei *et al.*, 2015). Recently, we showed that intranasal delivery of HP-BMSCs enhanced neurogenesis and angiogenesis after intracerebral hemorrhagic stroke in mice (Sun *et al.*, 2015).

Timing of Stem Cell Transplantation

The environment of the brain after stroke is constantly changing. Timing of stem cell delivery is an essential parameter to be considered before transplantation. Unfortunately, the optimal time for transplantation in animal models and consequently in patients is poorly defined. Stem cell transplantation has been performed in the order of hours to weeks after stroke induction depending mainly on the investigator. Early time transplantation (e.g. <24 hours) after stroke has been tested and some reported neuroprotective effects (Chu *et al.*, 2005a; Vendrame *et al.*, 2004). In an investigation on intrastriatal transplantation of human NSCs, it was shown that cells transplanted at 2 days after stroke survived better than those transplanted 6 weeks after stroke (Darsalia *et al.*, 2011). Transplanting greater numbers of grafted NSCs did not result in a greater number of surviving cells or increased neuronal differentiation. It is now widely believed that several days after the onset of stroke is an appropriate time window for cell transplantation therapy. Based on the understanding of the time course of excitotoxicity, brain edema, inflammatory reaction, and trophic factor expressions, we and many others have typically transplanted cells at 7 days after stroke, at which point the extracellular glutamate has returned to lower levels, brain edema subsided, and the microenvironment in the post-stroke brain has entered into a pro-regeneration stage (Schmidt and Minnerup, 2016). Coincidentally, reported clinical trials with autologous MSCs have been limited by the time needed to expand cells to the point that an appropriate cell numbers are available, which normally takes several days or longer (Honmou *et al.*, 2011; Lee *et al.*, 2010). Very delayed time points of cell transplantation (several months after stroke) are also clinically relevant, but few pre-clinical investigations have focused on the chronic phases. More research remains to be carried out to delineate the optimal strategies for cell transplantation for chronic stroke patients.

Mechanisms Underlying Stem Cell Therapy

Neuroprotective effect and reduction of infarct volume

Investigations on transplantation of neural stem/progenitor cells at early and sub-acute phases after stroke report neuroprotective effects of reducing infarct volume (Takahashi *et al.*, 2008; Wei *et al.*, 2012; Wei *et al.*, 2015). The infarct reducing effect can be observed in the acute phase after stroke. Using intracerebral injection and i.v. infusion, we and others have shown that BMSCs have the potential of reducing brain infarction and neuronal cell death and improving functional recovery after ischemic stroke. Since the homing of BMSCs to the brain tissues is very low after i.v. infusion, the mechanism of action of this treatment is likely due to increased diffusible trophic and growth factors such as BDNF, NGF and VEGF (Chau *et al.*, 2014; Sun *et al.*, 2015).

Endogenous repair mechanisms

Stem cell transplantation provides a supplement to the endogenous repair that may occur after ischemia. It is now generally recognized that the adult brain has the ability to regenerate neurons. As early as 1961, Smart first demonstrated that the adult mammalian brain showed neurogenic activity (Smart, 1961). It is apparent now that the adult brain has several niches for neurogenesis, particularly the subventricular zone (SVZ) lining the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus (Altman, 1962, 1969; Kaplan and Hinds, 1977; Kempermann *et al.*, 2004; Taupin and Gage, 2002). Under normal physiological conditions, neural progenitors from the SVZ migrate to the olfactory bulb (Kornack and Rakic, 2001) comprising the rostral migratory stream (RMS). These neural progenitors migrate in chains and differentiate into interneurons in the olfactory bulb where they functionally integrate themselves into the existing neural circuitry (Altman, 1969).

Several types of brain injuries including seizure, stroke, and traumatic brain injury can up-regulate progenitor proliferation (Ernst and Christie, 2006; Parent *et al.*, 1997; Rola *et al.*, 2006). Under ischemic conditions, proliferation of neural progenitors increases in the SGZ and the SVZ (Kuge *et al.*, 2009; Li *et al.*, 2008b; Li *et al.*, 2010). Neural progenitors from the SGZ do not seem to migrate to areas of injury after stroke, but those from the SVZ are diverted laterally from the RMS to the site of the injury. Neurogenesis peaks at 3 and 4 weeks after ischemic insult in the SVZ and the SGZ, respectively. Migration of progenitors from the SVZ to the site of injury peaks at around 4 weeks (Kuge *et al.*, 2009).

The adult brain's endogenous neurogenic machinery requires a coordinated effort between the SVZ, chemotactic factors, and the vasculature to replace dead or dying cells. The process of neuroblast migration relies greatly on the upregulation of chemotactic factors at the injury site and the physical scaffolding provided by blood vessels. Cells of the neurovascular unit (neurons, astrocytes, and endothelial cells) and cells recruited from the systemic circulation produce chemotactic factors including but not limited to SDF-1α, BDNF, and VEGF (Behar *et al.*, 1997; Imitola *et al.*, 2004; Pencea *et al.*, 2001; Robin *et al.*, 2006; Thored *et al.*, 2007; Wang *et al.*, 2007). Ohab and colleagues emphasized the importance of the neurovascular niche in which neural progenitors associate with vasculature to migrate to the site of

ischemic damage, showing that capillary endothelial cells in the infarct area release factors like SDF-1 and Angiopoietin-1 (Ang-1) (Ohab *et al.*, 2006). Under normal conditions, approximately half of the cells migrating to the olfactory bulb are in close proximity and contact with vasculature (Bovetti *et al.*, 2007) and this association continues when cells are diverted from the RMS. Progenitors migrate to the ischemic injury using blood vessels as a scaffold and chemotactic factors as a guide (Ohab *et al.*, 2006). SDF-1 in particular is a potent chemo-attractant, which is upregulated in the stroke infarct in a gradient fashion to guide migrating cells (Li *et al.*, 2008a). Migration from the SVZ to the infarct occurs in chains and as individual cells (Zhang *et al.*, 2009b). It was shown that neural progenitors extending from the border of the SVZ towards the infarcted striatum at a rate of 28.67±1.04 μm/h (Zhang *et al.*, 2009b). Doublecortin (DCX)-positive migrating progenitor cells move along with and cluster around the vasculature. It was observed that 35% of progenitors were within 5 μm of a blood vessel.

Nonetheless, the neurogenic activity induced new cells usually do not survive well during the migration and after arrival at the injury site (Capowski *et al.*, 2007; Daadi *et al.*, 2008a; Kim *et al.*, 2007a; Roy *et al.*, 2006; Yang *et al.*, 2008). The repair capability of newly formed neurons is very limited and does not suffice for an effective tissue repair after brain injury (Grégoire *et al.*, 2015). For example, it was calculated that about 80% of the newly born neurons die in the striatum stroke model and that endogenous regeneration only replaces about 0.2% of dead striatal neurons (Arvidsson *et al.*, 2002). Thus, the need for novel therapies to replace lost tissue is immense. Using the spatial and cell type specific optogenetic technique, we showed in a transgenic mouse expressing the light-gated channelrhodopsin-2 (ChR2) in glutamatergic neurons that optogenetic stimulation of the glutamatergic activity in the striatum triggered glutamate release into SVZ region, increased proliferation of SVZ neuroblasts mediated by AMPA receptor activation and Ca^{2+} increases in these cells. After stroke, optogenetic stimuli to the striatum for 8 days promoted cell proliferation and the migration of SVZ neuroblasts into the peri-infarct cortex with increased neuronal differentiation and improved long-term functional recovery. It was suggested that the striatum-SVZ neuronal regulation via a semi-phasic volume transmission mechanism may be a therapeutic target to directly promote SVZ neurogenesis after stroke(Song *et al.*, 2017)(Song *et al.*, 2017).

Trophic support and attenuation of inflammation

Adult and embryonic stem cells can enhance the survival of the surrounding tissue by providing trophic factor support. Trophic factors secreted by transplanted cells can provide trophic support to the injured parenchyma (Calió *et al.*, 2014; Dillon-Carter *et al.*, 2002; Johnston *et al.*, 2001). As an example, a large proportion of neural stem cells transplanted into the murine cochlea were found to express neurotrophins such as GDNF and BDNF. The release of these factors provided protection and support to the surrounding cells (Bang, 2016; Iguchi *et al.*, 2003).

Transplanted adult marrow-derived MSCs release cytokines and trophic or growth factors that have autocrine and paracrine effects (Caplan and Dennis, 2006; Haynesworth *et al.*, 1996; Malgieri *et al.*, 2010). MSCs have been shown to secrete colony-stimulating factor-1

(CSF-1), stem cell factor, bFGF, nerve growth factor (NGF), and VEGF (Chen *et al.*, 2002a; Labouyrie *et al.*, 1999; Laurenzi *et al.*, 1998; Majumdar *et al.*, 1998; Villars *et al.*, 2000). Such secreted factors reduce host cell death and attenuate inflammatory processes (Tse *et al.*, 2003). Interestingly, injecting BMSC-conditioned media into the brain after stroke led to functional benefits, indicating that trophic support is a major mechanism by which MSCs contribute to functional recovery (Caplan and Dennis, 2006; Chen *et al.*, 2002b; Li *et al.*, 2002).

Cell differentiation, replacement and integration after transplantation

Cell differentiation and integration after transplantation is an important aspect of cell therapy. Neural cells have a greater potential to functionally integrate into the existing host circuitry if they differentiate into the appropriate neuronal phenotype (Lam *et al.*, 2014). Several studies have examined the *in vivo* differentiation of transplanted stem cells (Fig. 8). In one systemic study, murine ES cell-derived neural precursors differentiated into different neuronal sub-types including cholinergic (1.4% of all grafted cells), serotonergic (1.8% of all grafted cells), GABAergic neurons (>50% of all grafted cells), and striatal neurons (1.4% of all grafted cells) after transplantation into the infarcted region. For the reason that is unclear, the percentage of excitatory glutamatergic neurons was not evaluated in this study. At 12 weeks after transplantation, 25% of the remaining transplanted cells were NeuN-positive while 8% were positive for glial fibrillary acidic protein (GFAP), a marker of astrocytes. Transplanted cells also exhibited voltage-gated Na^+, Ca^{2+} and K^+ currents. While continued proliferation after transplantation was detected, no tumors were detected at 4 or 12 weeks (Buhnemann *et al.*, 2006).

Another study reported that 30% of transplanted human fetal neural stem cells survived 1 month after stroke in the striatum (Darsalia *et al.*, 2007). Striatal-derived and cortical-derived neural stem cells from human fetal tissue were transplanted into the striatum of stroked rats. About 80% of the cells migrated from the original site of the transplant closer to the lesion. Unlike the aforementioned study with ES cell-derived progenitors, there was little proliferation (less than 1%) as measured by p-H3 and Ki67 staining. A high percentage of the cells differentiated into neurons expressing neural Hu protein D (HuD), calbindin, and parvalbumin, all markers of neuronal differentiation, but very few differentiated into astrocytes or oligodendrocytes (Darsalia *et al.*, 2007).

Combined Approach of Optogenetics and Stem Cell Therapy

Studying the integration of differentiated cells into the host structures after transplantation is essential for a long-term characterization of the therapy. Earlier studies used electrophysiological approaches to investigate whether transplanted stem cell-derived neural (NS) cells functionally integrate within the established host brain neuronal circuits (Opitz *et al.*, 2007; Scheffler *et al.*, 2003; Wernig *et al.*, 2004). However, electrophysiological stimulation and recording paradigms are cumbersome and lack temporal and spatial resolution for studying information processing in living multicellular networks. The advent of optogenetics has provided tools that allow *in vivo* probing and mapping of neuronal circuits with very high temporal and spatial resolution. These tools allow for accurate

control and observation of biological process within target cells using specific light stimulation (Nagel *et al.*, 2003; Nagel *et al.*, 2005). Optogenetics employ two classes of channels that transduce light of specific wavelengths into a biological response. When expressed in cells, these channels open in response to light of the proper wavelength and conduct ions altering the membrane potential. This leads to light-induced excitation (Channelrhodopsin-2, ChR2; 470 nm) or inhibition (halorhodopsin; 580 nm) of neurons with controlled frequency and timing. ChR2 and eNpHR can be genetically targeted to specific populations or sub-populations of cells (providing high spatial resolution), can control cell function on a millisecond scale (high temporal resolution down to a single action potential), and have proven to function in culture, acute brain slices, and living animals.

Optogenetic actuators has been used to accurately map brain circuits (Arenkiel *et al.*, 2007; Arrenberg *et al.*, 2009; Han *et al.*, 2009; Kastanenka and Landmesser, 2010; Kramer *et al.*, 2009), dissect pathways of learning and fear (Huber *et al.*, 2008; Johansen *et al.*, 2010), and understand the role of neural cells in pathology and physiology (Gourine *et al.*, 2010; Kravitz *et al.*, 2010). Stem cell therapy has also benefited from the exponential growth in the optogenetics field. Integrating optogenetics into cell based therapies through the over-expression of ChR2 in stem cell-derived neural precursors, it was demonstrated that ChR2 expressing human ES-cell derived neurons can receive excitatory and inhibitory post-synaptic current in acute brain slices (Weick *et al.*, 2010). In another study where ChR2 was over-expressed in mouse ES cells, optical excitation increased the expression of Nestin and β–III-tubulin in ChR2-ES cell-derived neural precursors and neurons, respectively (Stroh *et al.*, 2011). Optogenetic evidence showed that neural stem cell-derived dopaminergic neurons functionally integrate in the denervated striatum in an animal model of Parkinson's disease (Tonnesen *et al.*, 2011). This technology offers an invaluable set of tools for studying stem cell differentiation and *in vivo* integration after transplantation.

Limitations and Potential Adverse Effects

Immune responses in cell translantation therapy

Suppression of inflammation is an important consideration in cell-based treatment of neurological disorders. Cell therapies can modulate the host inflammatory response and establish a favorable microenvironment that prevents further endogenous cell death due to inflammation. By modulating the inflammatory processes, transplanted cells increase their own chances of survival. Otherwise, such processes can result in failure to survive or engraft, bleeding, altered immune responses and can reduce or even eliminate the therapeutic effects of transplantation (Strecker et al., 2016).

Inflammation may affect the ability of transplanted cells to replace damaged neurons. In a study on transplanted neurospheres derived from mouse ES cells into the striatum of allogeneic mice, some mice were treated with cyclosporine A to suppress the immune response. No difference was observed between the groups in graft size or cell survival, but fewer inflammatory cells were attracted to the graft in the cyclosporine A-treated animals (Ideguchi *et al.*, 2008). In addition, engrafted cells were more likely to be positive for NeuN and negative for GFAP in the cyclosporine A-treated animals, suggesting that the immune response created an environment that favored glial differentiation over neuronal

differentiation. In *in vitro* experiments, the addition of the inflammatory factor interleukin 6 (IL-6) to differentiating cells resulted in less cells positive for the β-III tubulin clone Tuj1 and more GFAP-positive cells, further suggesting that inflammation can suppress neuronal differentiation.

Much of the research on the suppression of inflammation by stem cell therapy has focused on MSCs, which have been shown to sup ress innate and adaptive immune responses (Broughton *et al.*, 2015). While there is a concern that the suppression of the immune system may lead to infection or tumor cell proliferation, there is a consensus that human trials should move forward using MSCs in the treatment of multiple sclerosis (MS) (Siatskas *et al.*). We observed that transplantation of BMSCs had a great effect of suppressing microglia activity in the brain(Wei *et al.*, 2012). MSCs can inhibit inflammation in multiple ways. *In vitro,* MSCs can reduce proliferation of allogeneic or even xenogeneic peripheral blood mononuclear cells (PBMCs) (Aggarwal and Pittenger, 2005) with specific effects noted on the proliferation of both T-lymphocytes (Augello *et al.*, 2005; Di Nicola *et al.*, 2002; Zappia *et al.*, 2005) and B-lymphocytes (Augello *et al.*, 2005; Corcione *et al.*, 2006). MSCs also reduce tumor necrosis factor alpha (TNF-α) in dendritic cells (DCs) and interferon gamma (INF-γ) in differentiating TH1 cells and natural killer cells (Aggarwal and Pittenger, 2005). They can also increase the anti-inflammatory interleukin IL-10 in DCs (Aggarwal and Pittenger, 2005) and macrophages (Nemeth *et al.*, 2009), as well as IL-4 in differentiating TH2 cells (Aggarwal and Pittenger, 2005). MSCs do not, however, seem to have any modulating effect on the *in vitro* activation of microglia (Tambuyzer *et al.*, 2009). *In vivo*, the immunosuppressive effects of MSCs are achieved regardless of how the cells are delivered, whether they are injected into the bloodstream, peritoneum, or parenchyma of the affected tissue (Siatskas *et al.*).

While most human transplantation studies have used autologous MSCs (Bang *et al.*, 2005b; Honmou *et al.*), the promising data with allogeneic MSCs suggests that these cells need not come from a genetically identical source. In fact, some clinical trials are using cells from allogenic sources. Additionally, protocols have been devised to derive MSCs from human ES cells (Barberi *et al.*, 2005; Lian *et al.*, 2007; Olivier *et al.*, 2006; Tan *et al.*). Like MSCs, these hES cell-derived cells can differentiate into bone, cartilage, and fat. In co-culture experiments, they can suppress lymphocyte proliferation and activation (Tan *et al.*). Interestingly, in a xenogenic transplant model using BALB/C mice with liver inflammation, undifferentiated hESCs induced antibody production, while MSCs derived from those cells did not (Tan *et al.*). This further supports the idea that MSCs are immune privileged. In addition, MSCs derived from hES cells reduced the infiltration of inflammatory cells to the injured liver area.

Another strategy to enhance cell-based therapies is co-transplantation of MSCs with neural progenitor cells (Liu *et al.*, 2014). Transplanted allogeneic ES cell-derived oligodendrocyte progenitors (OPCs) into a mouse model of MS with MSCs showed better survival of transplanted cells and greater levels of re-myelination were observed compared to the levels of OPCs alone (Cristofanilli *et al.*). Microglial infiltration was also reduced when MSCs were included, suggesting that syngeneic MSCs do not inhibit microglia. Given the

conflicting results, it is unclear whether or not allogeneic MSCs would work well in a co-transplant strategy.

Immune rejection

A potential hurdle in stem cell transplantation is the rejection of transplanted cells. Accumulating evidence suggests that culturing human ES cells on animal-derived substrates or with animal-derived media products increase their immunogenicity because of their increased expression of a non-human sialic acid residue, Neu5Gc (Byrne *et al.*, 2015; Martin *et al.*, 2005). This has pushed companies involved in the biotechnology of human ES cells to create media products and substrates that are fully defined and completely animal-product free. While clinical trials have been carried out with cells expanded in xenogenic serum without any reported ill effects from the animals products (Bang *et al.*, 2005b; Lee *et al.*, 2010), the removal of animal products is still preferred.

As with tissue and organ transplants, allogenic stem cells can be rejected and destroyed by the host immune system. Rejection is modulated by the expression and detection of unmatched human leukocyte antigen (HLA) proteins, specifically major histocompatibility (MHC) proteins (Litchfield *et al.*, 1997). Host T-lymphocytes recognize these proteins, primarily MHC-I, on the donor cells and sensitize the immune system, causing it to attack the transplanted cells. The brain has been historically seen as an immune privileged location, but even with allogenic cells or cells directly injected into the brain (Simpson, 2006; Sloan *et al.*, 1991), rejection might still occur and may require the use of immune suppression for continued benefit (Olanow *et al.*, 2003). On the other hand, an immune suppression treatment may show detrimental effect on endogenous healing. For example, some immune responses can be neuroprotective and necessary for endogenous neurogenesis (Beers *et al.*, 2008; Chiu *et al.*, 2008; Ziv *et al.*, 2006a; Ziv *et al.*, 2006b). Immune suppression may also decrease inflammatory signals that attract cells to the injury site (Ben-Hur *et al.*, 2003). Moreover, immune suppression may increase the risk of tumor formation (Dunn *et al.*, 2002; Dunn *et al.*, 2004). Because of these issues associated with long-term immune suppression (Marcen, 2009), the use of such suppression should be avoided in stem cell transplantation.

In some cases, short-term measurements of immune response to transplanted cells seem to suggest that immune suppression is not necessary and may even reduce graft survival (Modo *et al.*, 2002a). Some transplanted cells have been reported to survive via immune evasion rather than being immune privileged cells such as MSCs (Ankrum *et al.*, 2014). BMSCs and adult neural precursors have been shown to modulate the immune system, a functionality that might be lost with systemic immune suppression (Ben-Hur, 2008; Karussis *et al.*, 2008). However, the immune response can also be delayed as host antigen-presenting cells pick up on the foreign MHC peptides and sensitize the immune system to them (Drukker and Benvenisty, 2004; Gould and Auchincloss, 1999; Rogers and Lechler, 2001). The expression level of MHC proteins is a major determining factor in graft survival and cells expressing fewer mis-matched MHC proteins will likely demonstrate the best survival. Human ES cells express only very low levels of MHC-I in an undifferentiated state. However, MHC-I is upregulated when cells are differentiated and can be highly upregulated in undifferentiated or differentiated cells by interferons (Drukker *et al.*, 2002), which are expressed in

endogenous immune responses such as inflammation. It is possible to reduce MHC expression in transplanted cells (Hacke *et al.*, 2009), but genetic changes and viral vectors can introduce their own problems.

Tumor formation

One of the most prominent concerns in cell therapy is the propensity of transplanted cells to form tumors. Due to their cancerous origin, there are some fears that hNT cells can produce tumors, but the retinoic acid treatment used to obtain hNT cells from N2N cells seems to suppress their tumorigenicity (Maerz *et al.*, 1998; Newman *et al.*, 2005). A study has shown that iPS cells derived from B6 mouse embryonic fibroblasts formed teratomas that were immune-rejected after transplantation in B6 mice (Zhao *et al.*, 2011). On the contrary, ES cells derived from B6 mice form teratomas in B6 recipients without any evident immune rejection, suggesting a specific immune response to the iPS cells. Pluripotent cells, such as ES or iPS cells, can form teratomas or teratocarcinomas when implanted in a living host [16, 161]. These two terms (teratomas or teratocarcinomas) have been used interchangeably in the past, but standard definitions have begun to emerge (Blum and Benvenisty, 2009; Damjanov and Andrews, 2007; Lensch and Ince, 2007). Teratomas are benign tumors which contain tissues from all three germ layers and are believed to be formed from undifferentiated cells that remain karyotypically normal. As teratomas mature, they generally stop growing, but the damage done to the surrounding tissues can be fatal. Most of this damage is simply related to tissue displacement and pressure injuries. Teratocarcinomas, on the other hand, are believed to be formed from transformed or culture adapted pluripotent cells. These tumors contain highly proliferative undifferentiated cells known as embryonal carcinomas (ECs) and are aggressively malignant. ECs were first discovered in spontaneous testicular carcinomas (Stevens and Little, 1954) and are believed to be the malignant counterparts to ES cells. In fact, as ES cells become karyotypically abnormal in cell culture, they acquire many of the same mutations as those seen in ECs (Andrews *et al.*, 2005; Baker *et al.*, 2007; Harrison *et al.*, 2007). Human ES cells are routinely screened for karyotypic changes, but small additions and deletions within a chromosome, changes in mitochondrial DNA, and epigenetic changes that occur in culture can also select for populations of undifferentiated stem cells (Maitra *et al.*, 2005; Werbowetski-Ogilvie *et al.*, 2009). Screening for such small mutations will likely be necessary if ES cells are to be used in clinical settings.

iPS cells have also been shown to have a risk of tumorigenicity when transplants are contaminated with undifferentiated cells. The two major concerns for tumorigenicity of iPS cells have been mutagenesis at viral insertion sites and continued expression of exogenous genes used for reprogramming (Robbins *et al.*). c-Myc has been of particular concern as a possible oncogene (Okita *et al.*, 2007). In order to minimize these issues, transgene-free iPS cells have been created (Gonzalez *et al.*, 2009; Okita *et al.*, 2008; Sommer *et al.*, 2009; Stadtfeld *et al.*, 2008) and some protocols have found ways to induce pluripotency without the use of c-myc (Huangfu *et al.*, 2008; Nakagawa *et al.*, 2008). In addition, the tissue origin of iPS cells seems to play a role in tumorigenicity, with derivation from mouse embryonic fibroblasts or gastric epithelial cells mirroring ES cells and derivation from hepatocytes or tail fibroblasts both increasing the risk of tumor formation (Miura *et al.*, 2009). As with

teratocarcinomas, iPS cell tumorigenicity appears to be a result of cells that are resistant to differentiation, possibly because of incomplete reprogramming.

Interestingly, tumor formation from contamination of undifferentiated cells is dependent on the species of both the graft and host (Erdo *et al.*, 2003). When undifferentiated murine cells were xenotransplanted into a rat brain, tumors were small and relatively rare, occurring in 2 out of 22 animals. After transplantation to the contralateral hemisphere, these undifferentiated cells migrated to the ischemic infarct along the corpus callosum and differentiated into neurons. In mouse brain, however, undifferentiated mouse cells produced large malignant teratocarcinomas in the majority of animals, without migration or replacement of ischemic tissue. These tumors formed even after efficient neural differentiation in which a very small portion of cells remained pluripotent.

While xenotransplantation is unlikely as a viable cell therapy strategy in humans, these results do raise doubts about the applicability of xenotransplantation studies. Even if differentiation with a particular protocol is efficient enough to avoid tumor formation in xenotransplantation, a small number of contaminating cells may still induce tumorigenesis in homologous transplantation. Even very small numbers of contaminating undifferentiated ES cells caused tumors in nude mice (Lawrenz *et al.*, 2004), further suggesting that any safe cell therapy using pluripotent cells will have to avoid such contamination.

We have empirically determined from our work that if ES cells are efficiently pre-differentiated into progenitors *in vitro*, their risk of tumor formation is drastically reduced. Many protocols have been tested to optimize the homogeneity of the transplant. While a few transplantation studies reported tumor formation, most transplantation studies using progenitors found no tumorigenicity (Arnhold *et al.*, 2000; Brustle *et al.*, 1999; Reubinoff *et al.*, 2001; Theus *et al.*, 2008a; Wei *et al.*, 2005). Nonetheless, it is difficult to differentiate pluripotent stem cells with 100% efficiency. Alternatively, methods to remove any undifferentiated cells from the transplant are being developed. In some cases, cells are sorted by staining for a pluripotency marker (Shibata *et al.*, 2006) or by adding GFP-conjugated proteins that allow for the removal of undifferentiated cells (Eiges *et al.*, 2001). Other strategies would cause apoptosis through the use of ceramide analogues (Bieberich *et al.*, 2004) or survivin blockers (Blum *et al.*, 2009), both of which selectively target pluripotent cells. A "suicide gene" may be included in transplanted cells so that they can be induced to undergo apoptosis if a tumor begins (Jung *et al.*, 2007). With further progress in this area, it may be possible that the suicide gene will be turned on only in tumor-forming cells but not normally differentiating cells so the transplantation benefits will be achieved without the adverse consequence of tumor formation.

Stem Cells Transplantation in Stroke Clinical Trials

Human trials with stem cell transplantation for treating stroke are ongoing. An early study demonstrated that hNT human neuronal cells can engraft into the stroke region and survive for more than two years in a human patient (Nelson *et al.*, 2002). Other cell transplantation studies in Parkinson's disease have reported surviving cells up to 14 years after transplant (Hallett *et al.*, 2014). However, concerns about the cancerous origin and the possibility of

tumor formation with hNT cells persist and no organization appears to have moved into larger scale trials with this cell type. Bone marrow derived cells were shown to be promising in terms of safety and initial efficacy (Suarez-Monteagudo *et al.*, 2009). A number of Phase I and II clinical trials using cell populations derived from bone marrow have begun (Table 1). Early results suggest that IV injection of mesenchymal stem cells (MSCs) does not cause significant adverse effects and improves measures of function such as the Barthel Index (BI) (Bang *et al.*, 2005b), modified Rankin Scale (mRS) (Lee *et al.*, 2010), and National Institutes of Health Stroke Score (NIHSS) (Honmou *et al.*, 2011). Conflicting data also suggest that MSC injection may not improve functional outcomes (Prasad *et al.*, 2014). These studies used autologous MSCs that were expanded in culture before use. While no adverse effects from animal products were reported, cells expanded in autologous serum, leading to faster cell expansion and allaying fears of xenogeneic contamination [257].

A Phase 1 trial (ReNeuron trials) is currently being carried out with a conditionally immortalized fetal neural stem cell line (Pollock *et al.*, 2006; Stevanato *et al.*, 2009). No adverse effects have been reported to date. Although the ReNeuron trial is using neural stem cells, these cells are not expected to engraft/replace lost cells in the stroke region. StemCells, Inc., on the other hand, is currently testing a highly purified human neural stem cell product that they expect to engraft in the long-term. These cells are currently bring tested for spinal cord injury and neurodegenerative disorders, with plans for a stroke trial in the future.

Stem cell transplantation in stroke provides a promising avenue of exploration, but further studies are needed before such therapy becomes a reality. The stem cell therapies as an emerging paradigm in stroke meetings have brought together scientists, clinicians, regulators, and industry representatives to create guidelines for preclinical and clinical studies. The initial guidelines were published in 2009 (Therapies, 2009), followed by an update in early 2011 (Savitz *et al.*, 2011). A summary of the use of stem cells in stroke also concludes its potential as an emerging therapy in the near future (Liu *et al.*, 2014).

Stem Cells Transplantation in Stroke Combined with Other Disorders

As a clinically relevant issue, some translational studies have reported adverse effects of BMSC therapy such as atherosclerosis and arteriosclerosis in stroke diabetes animals (Chen *et al.*, 2011). Niaspan was shown to reduce these adverse effects of BMSCs in type one diabetic rats (Yan *et al.*, 2013). In future translational studies, efficacy to treat co-morbidities such as stroke and diabetes should be considered and tested.

Conclusions

The prospect of using stem cell therapy for stroke remains an exciting and promising avenue of exploration. While a great deal of preclinical work has increased knowledge and feasibility of possible cell transplantation therapies for CNS disorders including stroke, the ultimate goals of this research area remains to be satisfactorily achieved. The promise of stem cell therapy offers hope for both protection of neural tissue in the acute phase of stroke and replacement of lost tissues (either through direct replacement or enhancement of endogenous mechanisms) in the chronic phase. As research moves forward, new

technologies are consistently bring incorporated into the field. Prior to the derivation of iPS cells, it was difficult to imagine a way to amass enough HLA-matched neural precursor cells for an effective cell therapy, but now, there are many laboratories using these cells in preclinical studies. The recent exciting discovery of direct reprogramming adult somatic cells into mature and functional neurons provides another bright future with an alternative approach of repairing damaged brain tissues using endogenous resources without the need of exogenous cells (An *et al.*, 2016; Guo *et al.*, 2014). A number of clinical trials investigating stem cell therapy for stroke are now ongoing. Clinical challenges may include more complicated situations such as co-morbidities which can affect the efficacy and effectiveness of a cell therapy. It may take coordinated basic and clinical efforts before cell therapy can become a standard stroke treatment.

Acknowledgments

Abbreviation

Ang 1	Angiopoietin 1
BI	Barthel Index
bFGF	Basic fibroblast growth factor
BBB	Blood brain barrier
BMSCs	Bone marrow stem cells
BDNF	Brain-derived neurotrophic factors
CRABP	Cellular RA-Binding Proteins
ChR2	Channelrhodopsin-2
CXCR4	Chemokine receptor type 4
CSF-1	Colony-stimulating factor-1
CCA	Common carotid artery
DCX	Doublecortin
EEG	Electroencephalography
ET-1	Endothelin-1
ECs	Embryonal carcinomas
EB	Embryonic bodies
ES	Embryonic stem

EGF	Epidermal growth factor
EPO	Erythropoietin
ECA	External carotid artery
GRID	Gadolinium-RhodamIne Dextran
GDNF	Glial cell-derived neurotrophic factor
GFAP	Glial fibrillary acidic protein
G-CSF	Granulocyte-colony stimulating factor
HuD	Hu protein D
HLA	Human leukocyte antigen
HIE	Hypoxic-ischemic encephalopathy
HP	Hypoxia preconditioned
iPS cells	Induced pluripotent stem cells
IV	Injected intravenously
INF-γ	Interferon gamma
ICH	Intracranial hemorrhage
LIF	Leukemia inhibitory factor
LCBF	Local cerebral blood flow
MRI	Magnetic resonance imaging
MHC	Major histocompatibility
MSCs	Mesenchymal stem cells
MMP-2	Metalloproteinase 2
MCA	Middle cerebral artery
mRS	Modified Rankin Scale
MS	Multiple sclerosis
MI	Myocardial infarction
NIHSS	National Institutes of Health Stroke Score
NGF	Nerve growth factor
ARNT	Nuclear translocator
PBMCs	Peripheral blood mononuclear cells

OPCs	Oligodendrocyte progenitors
OGD	Oxygen-glucose deprivation
RA	Retinoic acid
RMS	Rostral migratory stream
SCF	Stem cell factor
SGZ	Subgranular zone
SVZ	Subventricular zone
SPF	Survival-promoting factors
t-PA	Tissue plasminogen activator
TBI	Traumatic brain injury
TNF-α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

References

Abeysinghe HC, Bokhari L, Quigley A, Choolani M, Chan J, Dusting GJ, Crook JM, Kobayashi NR, Roulston CL. Pre-differentiation of human neural stem cells into GABAergic neurons prior to transplant results in greater repopulation of the damaged brain and accelerates functional recovery after transient ischemic stroke. Stem Cell Res Ther. 2015; 6:186. [PubMed: 26420220]

Afzal MR, Haider H, Idris NM, Jiang S, Ahmed RP, Ashraf M. Preconditioning promotes survival and angiomyogenic potential of mesenchymal stem cells in the infarcted heart via NF-kappaB signaling. Antioxid Redox Signal. 2010; 12:693–702. [PubMed: 19751147]

Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood. 2005; 105:1815–1822. [PubMed: 15494428]

Agudo-López A, Miguel BG, Fernández I, Martínez AM. Involvement of mitochondria on neuroprotective effect of sphingosine-1-phosphate in cell death in an in vitro model of brain ischemia. Neuroscience letters. 2010; 470:130–133. [PubMed: 20045720]

Altman J. Are new neurons formed in the brains of adult mammals? Science. 1962; 135:1127–1128. [PubMed: 13860748]

Altman J. Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol. 1969; 137:433–457. [PubMed: 5361244]

An N, Xu H, Gao WQ, Yang H. Direct Conversion of Somatic Cells into Induced Neurons. Mol Neurobiol. 2016

Andrews PW, Matin MM, Bahrami AR, Damjanov I, Gokhale P, Draper JS. Embryonic stem (ES) cells and embryonal carcinoma (EC) cells: opposite sides of the same coin. Biochem Soc Trans. 2005; 33:1526–1530. [PubMed: 16246161]

Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. Nature biotechnology. 2014; 32:252–260.

Arbab AS, Thiffault C, Navia B, Victor SJ, Hong K, Zhang L, Jiang Q, Varma NR, Iskander A, Chopp M. Tracking of In-111-labeled human umbilical tissue-derived cells (hUTC) in a rat model of cerebral ischemia using SPECT imaging. BMC medical imaging. 2012; 12:1. [PubMed: 22248480]

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

Arenkiel BR, Peca J, Davison IG, Feliciano C, Deisseroth K, Augustine GJ, Ehlers MD, Feng G. In vivo light-induced activation of neural circuitry in transgenic mice expressing channelrhodopsin-2. Neuron. 2007; 54:205–218. [PubMed: 17442243]

Arnhold S, Lenartz D, Kruttwig K, Klinz FJ, Kolossov E, Hescheler J, Sturm V, Andressen C, Addicks K. Differentiation of green fluorescent protein-labeled embryonic stem cell-derived neural precursor cells into Thy-1-positive neurons and glia after transplantation into adult rat striatum. Journal of Neurosurgery. 2000; 93:1026–1032. [PubMed: 11117845]

Arrenberg AB, Del Bene F, Baier H. Optical control of zebrafish behavior with halorhodopsin. Proc Natl Acad Sci U S A. 2009; 106:17968–17973. [PubMed: 19805086]

Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat Med. 2002; 8:963–970. [PubMed: 12161747]

Augello A, Tasso R, Negrini SM, Amateis A, Indiveri F, Cancedda R, Pennesi G. Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. Eur J Immunol. 2005; 35:1482–1490. [PubMed: 15827960]

Bain G, Kitchens D, Yao M, Huettner JE, Gottlieb DI. Embryonic stem cells express neuronal properties in vitro. Dev Biol. 1995; 168:342–357. [PubMed: 7729574]

Baker DE, Harrison NJ, Maltby E, Smith K, Moore HD, Shaw PJ, Heath PR, Holden H, Andrews PW. Adaptation to culture of human embryonic stem cells and oncogenesis in vivo. Nat Biotechnol. 2007; 25:207–215. [PubMed: 17287758]

Bang OY. Clinical Trials of Adult Stem Cell Therapy in Patients with Ischemic Stroke. Journal of Clinical Neurology. 2016; 12:14–20. [PubMed: 26610894]

Bang OY, Lee JS, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. Ann Neurol. 2005a; 57:874–882. [PubMed: 15929052]

Bang OY, Lee JS, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. Annals of neurology. 2005b; 57:874–882. [PubMed: 15929052]

Barberi T, Klivenyi P, Calingasan NY, Lee H, Kawamata H, Loonam K, Perrier AL, Bruses J, Rubio ME, Topf N, Tabar V, Harrison NL, Beal MF, Moore MA, Studer L. Neural subtype specification of fertilization and nuclear transfer embryonic stem cells and application in parkinsonian mice. Nat Biotechnol. 2003; 21:1200–1207. [PubMed: 14502203]

Barberi T, Willis LM, Socci ND, Studer L. Derivation of multipotent mesenchymal precursors from human embryonic stem cells. PLoS Med. 2005; 2:e161. [PubMed: 15971941]

Battistella V, de Freitas GR, da Fonseca LM, Mercante D, Gutfilen B, Goldenberg RC, Dias JV, Kasai-Brunswick TH, Wajnberg E, Rosado-de-Castro PH, Alves-Leon SV, Mendez-Otero R, Andre C. Safety of autologous bone marrow mononuclear cell transplantation in patients with nonacute ischemic stroke. Regen Med. 2011; 6:45–52. [PubMed: 21175286]

Beers DR, Henkel JS, Zhao W, Wang J, Appel SH. CD4+ T cells support glial neuroprotection, slow disease progression, and modify glial morphology in an animal model of inherited ALS. Proc Natl Acad Sci U S A. 2008; 105:15558–15563. [PubMed: 18809917]

Behar TN, Dugich-Djordjevic MM, Li YX, Ma W, Somogyi R, Wen X, Brown E, Scott C, McKay RD, Barker JL. Neurotrophins stimulate chemotaxis of embryonic cortical neurons. Eur J Neurosci. 1997; 9:2561–2570. [PubMed: 9517461]

Ben-Hur T. Immunomodulation by neural stem cells. J Neurol Sci. 2008; 265:102–104. [PubMed: 17583749]

Ben-Hur T, Ben-Menachem O, Furer V, Einstein O, Mizrachi-Kol R, Grigoriadis N. Effects of proinflammatory cytokines on the growth, fate, and motility of multipotential neural precursor cells. Mol Cell Neurosci. 2003; 24:623–631. [PubMed: 14664813]

Bernaudin M, Marti HH, Roussel S, Divoux D, Nouvelot A, MacKenzie ET, Petit E. A potential role for erythropoietin in focal permanent cerebral ischemia in mice. J Cereb Blood Flow Metab. 1999; 19:643–651. [PubMed: 10366194]

Bieberich E, Silva J, Wang G, Krishnamurthy K, Condie BG. Selective apoptosis of pluripotent mouse and human stem cells by novel ceramide analogues prevents teratoma formation and enriches for neural precursors in ES cell-derived neural transplants. J Cell Biol. 2004; 167:723–734. [PubMed: 15545317]

Biernaskie J, Corbett D, Peeling J, Wells J, Lei H. A serial MR study of cerebral blood flow changes and lesion development following endothelin-1-induced ischemia in rats. Magn Reson Med. 2001; 46:827–830. [PubMed: 11590661]

Bliss TM, Kelly S, Shah AK, Foo WC, Kohli P, Stokes C, Sun GH, Ma M, Masel J, Kleppner SR, Schallert T, Palmer T, Steinberg GK. Transplantation of hNT neurons into the ischemic cortex: cell survival and effect on sensorimotor behavior. J Neurosci Res. 2006; 83:1004–1014. [PubMed: 16496370]

Blum B, Bar-Nur O, Golan-Lev T, Benvenisty N. The anti-apoptotic gene survivin contributes to teratoma formation by human embryonic stem cells. Nat Biotechnol. 2009; 27:281–287. [PubMed: 19252483]

Blum B, Benvenisty N. The tumorigenicity of diploid and aneuploid human pluripotent stem cells. Cell Cycle. 2009; 8:3822–3830. [PubMed: 19887907]

Boltze J, Forschler A, Nitzsche B, Waldmin D, Hoffmann A, Boltze CM, Dreyer AY, Goldammer A, Reischauer A, Hartig W, Geiger KD, Barthel H, Emmrich F, Gille U. Permanent middle cerebral artery occlusion in sheep: a novel large animal model of focal cerebral ischemia. J Cereb Blood Flow Metab. 2008; 28:1951–1964. [PubMed: 18698332]

Borlongan CV, Hadman M, Sanberg CD, Sanberg PR. Central nervous system entry of peripherally injected umbilical cord blood cells is not required for neuroprotection in stroke. Stroke. 2004; 35:2385–2389. [PubMed: 15345799]

Borlongan CV, Koutouzis TK, Jorden JR, Martinez R, Rodriguez AI, Poulos SG, Freeman TB, McKeown P, Cahill DW, Nishino H, Sanberg PR. Neural transplantation as an experimental treatment modality for cerebral ischemia. Neurosci Biobehav Rev. 1997; 21:79–90. [PubMed: 8994211]

Bovetti S, Hsieh YC, Bovolin P, Perroteau I, Kazunori T, Puche AC. Blood vessels form a scaffold for neuroblast migration in the adult olfactory bulb. J Neurosci. 2007; 27:5976–5980. [PubMed: 17537968]

Broughton BR, Lim R, Arumugam TV, Drummond GR, Wallace EM, Sobey CG. Post-stroke inflammation and the potential efficacy of novel stem cell therapies: focus on amnion epithelial cells. Developing stem cell-based therapies for neural repair. 2015

Brustle O, Jones KN, Learish RD, Karram K, Choudhary K, Wiestler OD, Duncan ID, McKay RD. Embryonic stem cell-derived glial precursors: a source of myelinating transplants. Science. 1999; 285:754–756. [PubMed: 10427001]

Buhnemann C, Scholz A, Bernreuther C, Malik CY, Braun H, Schachner M, Reymann KG, Dihne M. Neuronal differentiation of transplanted embryonic stem cell-derived precursors in stroke lesions of adult rats. Brain. 2006; 129:3238–3248. [PubMed: 17018551]

Burns TC, Verfaillie CM, Low WC. Stem cells for ischemic brain injury: a critical review. J Comp Neurol. 2009; 515:125–144. [PubMed: 19399885]

Byrne G, Lin Y, Du Z, Kogelberg H, McGregor C. Xenogeneic glycans: Human Antibody Reactivity and Their Impact on Xenograft Rejection. The Journal of Heart and Lung Transplantation. 2015; 34:S271–S272.

Caiazzo M, Dell'anno MT, Dvoretskova E, Lazarevic D, Taverna S, Leo D, Sotnikova TD, Menegon A, Roncaglia P, Colciago G, Russo G, Carninci P, Pezzoli G, Gainetdinov RR, Gustincich S, Dityatev A, Broccoli V. Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. Nature.

Calió ML, Marinho DS, Ko GM, Ribeiro RR, Carbonel AF, Oyama LM, Ormanji M, Guirao TP, Calió PL, Reis LA. Transplantation of bone marrow mesenchymal stem cells decreases oxidative stress, apoptosis, and hippocampal damage in brain of a spontaneous stroke model. Free Radical Biology and Medicine. 2014; 70:141–154. [PubMed: 24525001]

Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. J Cell Biochem. 2006; 98:1076–1084. [PubMed: 16619257]

Capowski EE, Schneider BL, Ebert AD, Seehus CR, Szulc J, Zufferey R, Aebischer P, Svendsen CN. Lentiviral vector-mediated genetic modification of human neural progenitor cells for ex vivo gene therapy. J Neurosci Methods. 2007; 163:338–349. [PubMed: 17397931]

Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat Biotechnol. 2009; 27:275–280. [PubMed: 19252484]

Chau MJ, Deveau TC, Song M, Gu X, Chen D, Wei L. iPSC Transplantation increases regeneration and functional recovery after ischemic stroke in neonatal rats. Stem Cells. 2014; 32:3075–3087. [PubMed: 25132189]

Chen F, Suzuki Y, Nagai N, Peeters R, Sun X, Coudyzer W, Marchal G, Ni Y. Rat cerebral ischemia induced with photochemical occlusion of proximal middle cerebral artery: a stroke model for MR imaging research. MAGMA. 2004; 17:103–108. [PubMed: 15517468]

Chen J, Chen J, Chen S, Zhang C, Zhang L, Xiao X, Das A, Zhao Y, Yuan B, Morris M. Transfusion of CXCR4-primed endothelial progenitor cells reduces cerebral ischemic damage and promotes repair in db/db diabetic mice. PLoS One. 2012; 7:e50105. [PubMed: 23185548]

Chen J, Li Y, Wang L, Zhang Z, Lu D, Lu M, Chopp M. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. Stroke. 2001a; 32:1005–1011. [PubMed: 11283404]

Chen J, Sanberg PR, Li Y, Wang L, Lu M, Willing AE, Sanchez-Ramos J, Chopp M. Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. Stroke. 2001b; 32:2682–2688. [PubMed: 11692034]

Chen J, Ye X, Yan T, Zhang C, Yang XP, Cui X, Cui Y, Zacharek A, Roberts C, Liu X, Dai X, Lu M, Chopp M. Adverse effects of bone marrow stromal cell treatment of stroke in diabetic rats. Stroke. 2011; 42:3551–3558. [PubMed: 21940967]

Chen J, Zhang ZG, Li Y, Wang L, Xu YX, Gautam SC, Lu M, Zhu Z, Chopp M. Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. Circ Res. 2003; 92:692–699. [PubMed: 12609969]

Chen ST, Hsu CY, Hogan EL, Maricq H, Balentine JD. A model of focal ischemic stroke in the rat: reproducible extensive cortical infarction. Stroke. 1986; 17:738–743. [PubMed: 2943059]

Chen X, Katakowski M, Li Y, Lu D, Wang L, Zhang L, Chen J, Xu Y, Gautam S, Mahmood A, Chopp M. Human bone marrow stromal cell cultures conditioned by traumatic brain tissue extracts: growth factor production. J Neurosci Res. 2002a; 69:687–691. [PubMed: 12210835]

Chen X, Li Y, Wang L, Katakowski M, Zhang L, Chen J, Xu Y, Gautam SC, Chopp M. Ischemic rat brain extracts induce human marrow stromal cell growth factor production. Neuropathology. 2002b; 22:275–279. [PubMed: 12564767]

Chiu IM, Chen A, Zheng Y, Kosaras B, Tsiftsoglou SA, Vartanian TK, Brown RH Jr, Carroll MC. T lymphocytes potentiate endogenous neuroprotective inflammation in a mouse model of ALS. Proc Natl Acad Sci U S A. 2008; 105:17913–17918. [PubMed: 18997009]

Chopp M, Li Y. Treatment of neural injury with marrow stromal cells. Lancet Neurol. 2002; 1:92–100. [PubMed: 12849513]

Chu K, Kim M, Jeong SW, Kim SU, Yoon BW. Human neural stem cells can migrate, differentiate, and integrate after intravenous transplantation in adult rats with transient forebrain ischemia. Neurosci Lett. 2003; 343:129–133. [PubMed: 12759181]

Chu K, Park KI, Lee ST, Jung KH, Ko SY, Kang L, Sinn DI, Lee YS, Kim SU, Kim M. Combined treatment of vascular endothelial growth factor and human neural stem cells in experimental focal cerebral ischemia. Neuroscience Res. 2005a; 53:384–390.

Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, Risso M, Gualandi F, Mancardi GL, Pistoia V, Uccelli A. Human mesenchymal stem cells modulate B-cell functions. Blood. 2006; 107:367–372. [PubMed: 16141348]

Corti S, Locatelli F, Strazzer S, Salani S, Del Bo R, Soligo D, Bossolasco P, Bresolin N, Scarlato G, Comi GP. Modulated generation of neuronal cells from bone marrow by expansion and mobilization of circulating stem cells with in vivo cytokine treatment. Exp Neurol. 2002; 177:443–452. [PubMed: 12429190]

Cristofanilli M, Harris VK, Zigelbaum A, Goossens AM, Lu A, Rosenthal H, Sadiq SA. Mesenchymal Stem Cells Enhance the Engraftment and Myelinating Ability of Allogeneic Oligodendrocyte Progenitors in Dysmyelinated Mice. Stem Cells Dev.

Crook JM, Wallace G, Tomaskovic-Crook E. The potential of induced pluripotent stem cells in models of neurological disorders: implications on future therapy. Expert Review of Neurotherapeutics. 2015; 15:295–304. [PubMed: 25664599]

Daadi MM, Maag AL, Steinberg GK. Adherent self-renewable human embryonic stem cell-derived neural stem cell line: functional engraftment in experimental stroke model. PLoS One. 2008a; 3:e1644. [PubMed: 18286199]

Damjanov I, Andrews PW. The terminology of teratocarcinomas and teratomas. Nat Biotechnol. 2007; 25:1212. discussion 1212. [PubMed: 17989665]

Danielyan L, Schafer R, von Ameln-Mayerhofer A, Buadze M, Geisler J, Klopfer T, Burkhardt U, Proksch B, Verleysdonk S, Ayturan M, Buniatian GH, Gleiter CH, Frey WH 2nd. Intranasal delivery of cells to the brain. Eur J Cell Biol. 2009; 88:315–324. [PubMed: 19324456]

Darsalia V, Allison SJ, Cusulin C, Monni E, Kuzdas D, Kallur T, Lindvall O, Kokaia Z. Cell number and timing of transplantation determine survival of human neural stem cell grafts in stroke-damaged rat brain. J Cereb Blood Flow Metab. 2011; 31:235–242. [PubMed: 20531461]

Darsalia V, Kallur T, Kokaia Z. Survival, migration and neuronal differentiation of human fetal striatal and cortical neural stem cells grafted in stroke-damaged rat striatum. Eur J Neurosci. 2007; 26:605–614. [PubMed: 17686040]

de Mello RF, de Souza Santos I, Alencar AP, Benseñor IM, Lotufo PA, Goulart AC. Major Depression as a Predictor of Poor Long-Term Survival in a Brazilian Stroke Cohort (Study of Stroke Mortality and Morbidity in Adults) EMMA study. Journal of Stroke and Cerebrovascular Diseases. 2016; 25(3):618–25. [PubMed: 26725125]

De Ryck M, Keersmaekers R, Duytschaever H, Claes C, Clincke G, Janssen M, Van Reet G. Lubeluzole protects sensorimotor function and reduces infarct size in a photochemical stroke model in rats. J Pharmacol Exp Ther. 1996; 279:748–758. [PubMed: 8930180]

Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S, Gianni AM. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood. 2002; 99:3838–3843. [PubMed: 11986244]

Dillon-Carter O, Johnston RE, Borlongan CV, Truckenmiller ME, Coggiano M, Freed WJ. T155g-immortalized kidney cells produce growth factors and reduce sequelae of cerebral ischemia. Cell Transplant. 2002; 11:251–259. [PubMed: 12075990]

Doeppner TR, El Aanbouri M, Dietz GP, Weise J, Schwarting S, Bähr M. Transplantation of TAT-Bcl-x L-transduced neural precursor cells: Long-term neuroprotection after stroke. Neurobiology of disease. 2010; 40:265–276. [PubMed: 20554038]

Doetschman TC, Eistetter H, Katz M, Schmidt W, Kemler R. The in vitro development of blastocyst-derived embryonic stem cell lines: formation of visceral yolk sac, blood islands and myocardium. J Embryol Exp Morphol. 1985; 87:27–45. [PubMed: 3897439]

Drukker M, Benvenisty N. The immunogenicity of human embryonic stem-derived cells. Trends Biotechnol. 2004; 22:136–141. [PubMed: 15036864]

Drukker M, Katz G, Urbach A, Schuldiner M, Markel G, Itskovitz-Eldor J, Reubinoff B, Mandelboim O, Benvenisty N. Characterization of the expression of MHC proteins in human embryonic stem cells. Proc Natl Acad Sci U S A. 2002; 99:9864–9869. [PubMed: 12114532]

Drury-Stewart D, Song M, Mohamad O, Guo Y, Gu X, Chen D, Wei L. Highly efficient differentiation of neural precursors from human embryonic stem cells and benefits of transplantation after ischemic stroke in mice. Stem Cell Res Ther. 2013; 4:93. [PubMed: 23928330]

Drury-Stewart D, Song M, Mohamad O, Yu SP, Wei L. Small Molecule Promoted Adherent and Feeder Free Differentiation of Functional Neurons from Human Embryonic and Induced Pluripotent Stem Cells. J Stem Cells. 2012; 6:1–7.

Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002; 3:991–998. [PubMed: 12407406]

Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity. 2004; 21:137–148. [PubMed: 15308095]

Durukan A, Tatlisumak T. Acute ischemic stroke: overview of major experimental rodent models, pathophysiology, and therapy of focal cerebral ischemia. Pharmacol Biochem Behav. 2007; 87:179–197. [PubMed: 17521716]

Eglitis MA, Dawson D, Park KW, Mouradian MM. Targeting of marrow-derived astrocytes to the ischemic brain. Neuroreport. 1999; 10:1289–1292. [PubMed: 10363941]

Ehrenreich H, Hasselblatt M, Dembowski C, Cepek L, Lewczuk P, Stiefel M, Rustenbeck HH, Breiter N, Jacob S, Knerlich F, Bohn M, Poser W, Ruther E, Kochen M, Gefeller O, Gleiter C, Wessel TC, De Ryck M, Itri L, Prange H, Cerami A, Brines M, Siren AL. Erythropoietin therapy for acute stroke is both safe and beneficial. Mol Med. 2002; 8:495–505. [PubMed: 12435860]

Ehrenreich H, Weissenborn K, Prange H, Schneider D, Weimar C, Wartenberg K, Schellinger PD, Bohn M, Becker H, Wegrzyn M, Jahnig P, Herrmann M, Knauth M, Bahr M, Heide W, Wagner A, Schwab S, Reichmann H, Schwendemann G, Dengler R, Kastrup A, Bartels C. Recombinant human erythropoietin in the treatment of acute ischemic stroke. Stroke. 2009; 40:e647–656. [PubMed: 19834012]

Eichenbaum JW, Pevsner PH, Pivawer G, Kleinman GM, Chiriboga L, Stern A, Rosenbach A, Iannuzzi K, Miller DC. A murine photochemical stroke model with histologic correlates of apoptotic and nonapoptotic mechanisms. J Pharmacol Toxicol Methods. 2002; 47:67–71. [PubMed: 12459144]

Eiges R, Schuldiner M, Drukker M, Yanuka O, Itskovitz-Eldor J, Benvenisty N. Establishment of human embryonic stem cell-transfected clones carrying a marker for undifferentiated cells. Curr Biol. 2001; 11:514–518. [PubMed: 11413002]

Erdo F, Buhrle C, Blunk J, Hoehn M, Xia Y, Fleischmann B, Focking M, Kustermann E, Kolossov E, Hescheler J, Hossmann KA, Trapp T. Host-dependent tumorigenesis of embryonic stem cell transplantation in experimental stroke. J Cereb Blood Flow Metab. 2003; 23:780–785. [PubMed: 12843782]

Ernst C, Christie BR. Temporally specific proliferation events are induced in the hippocampus following acute focal injury. J Neurosci Res. 2006; 83:349–361. [PubMed: 16342206]

Fan X, Qiu J, Yu Z, Dai H, Singhal AB, Lo EH, Wang X. A rat model of studying tissue-type plasminogen activator thrombolysis in ischemic stroke with diabetes. Stroke. 2012; 43:567–570. [PubMed: 22052516]

Ferro JM. Update on intracerebral haemorrhage. J Neurol. 2006; 253:985–999. [PubMed: 16680558]

Fraichard A, Chassande O, Bilbaut G, Dehay C, Savatier P, Samarut J. In vitro differentiation of embryonic stem cells into glial cells and functional neurons. J Cell Sci. 1995; 108(Pt 10):3181–3188. [PubMed: 7593279]

Francis KR, Wei L. Human embryonic stem cell neural differentiation and enhanced cell survival promoted by hypoxic preconditioning. Cell Death Dis. 2010; 1:e22. [PubMed: 21364630]

Friedrich MA, Martins MP, Araujo MD, Klamt C, Vedolin L, Garicochea B, Raupp EF, Sartori El Ammar J, Machado DC, Costa JC, Nogueira RG, Rosado-de-Castro PH, Mendez-Otero R, Freitas GR. Intra-arterial infusion of autologous bone marrow mononuclear cells in patients with moderate to severe middle cerebral artery acute ischemic stroke. Cell Transplant 21 Suppl. 2012; 1:S13–21.

Fukuda Y, Horie N, Satoh K, Yamaguchi S, Morofuji Y, Hiu T, Izumo T, Hayashi K, Nishida N, Nagata I. Intra-arterial transplantation of low-dose stem cells provides functional recovery without adverse effects after stroke. Cell Mol Neurobiol. 2015; 35:399–406. [PubMed: 25398358]

Gadalla SM, Wang T, Haagenson M, Spellman SR, Lee SJ, Williams KM, Wong JY, De Vivo I, Savage SA. Association between donor leukocyte telomere length and survival after unrelated allogeneic hematopoietic cell transplantation for severe aplastic anemia. Jama. 2015; 313:594–602. [PubMed: 25668263]

Ghobrial G, Haas C, Maulucci C, Lepore A, Fischer I. Promising Advances in Targeted Cellular Based Therapies: Treatment Update in Spinal Cord Injury. J Stem Cell Res Ther. 2014; 4:2.

Glaser T, Brustle O. Retinoic acid induction of ES-cell-derived neurons: the radial glia connection. Trends Neurosci. 2005; 28:397–400. [PubMed: 15955577]

Gonzalez F, Barragan Monasterio M, Tiscornia G, Montserrat Pulido N, Vassena R, Batlle Morera L, Rodriguez Piza I, Izpisua Belmonte JC. Generation of mouse-induced pluripotent stem cells by transient expression of a single nonviral polycistronic vector. Proc Natl Acad Sci U S A. 2009; 106:8918–8922. [PubMed: 19458047]

Gorris R, Fischer J, Erwes KL, Kesavan J, Peterson DA, Alexander M, Nöthen MM, Peitz M, Quandel T, Karus M. Pluripotent stem cell - derived radial glia - like cells as stable intermediate for efficient generation of human oligodendrocytes. Glia. 2015; 63:2152–2167. [PubMed: 26123132]

Gould DS, Auchincloss H Jr. Direct and indirect recognition: the role of MHC antigens in graft rejection. Immunol Today. 1999; 20:77–82. [PubMed: 10098326]

Gourine AV, Kasymov V, Marina N, Tang F, Figueiredo MF, Lane S, Teschemacher AG, Spyer KM, Deisseroth K, Kasparov S. Astrocytes control breathing through pH-dependent release of ATP. Science. 2010; 329:571–575. [PubMed: 20647426]

Grégoire CA, Goldenstein BL, Floriddia EM, Barnabé - Heider F, Fernandes KJ. Endogenous neural stem cell responses to stroke and spinal cord injury. Glia. 2015; 63:1469–1482. [PubMed: 25921491]

Gross GJ, Auchampach JA. Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. Circ Res. 1992; 70:223–233. [PubMed: 1310443]

Guan K, Chang H, Rolletschek A, Wobus AM. Embryonic stem cell-derived neurogenesis. Retinoic acid induction and lineage selection of neuronal cells. Cell and Tissue Res. 2001; 305:171–176. [PubMed: 11545254]

Guo Z, Zhang L, Wu Z, Chen Y, Wang F, Chen G. In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease model. Cell Stem Cell. 2014; 14:188–202. [PubMed: 24360883]

Guzman R, De Los Angeles A, Cheshier S, Choi R, Hoang S, Liauw J, Schaar B, Steinberg G. Intracarotid injection of fluorescence activated cell-sorted CD49d-positive neural stem cells improves targeted cell delivery and behavior after stroke in a mouse stroke model. Stroke. 2008; 39:1300–1306. [PubMed: 18309158]

Hacke K, Falahati R, Flebbe-Rehwaldt L, Kasahara N, Gaensler KM. Suppression of HLA expression by lentivirus-mediated gene transfer of siRNA cassettes and in vivo chemoselection to enhance hematopoietic stem cell transplantation. Immunol Res. 2009; 44:112–126. [PubMed: 19048410]

Hallett PJ, Cooper O, Sadi D, Robertson H, Mendez I, Isacson O. Long-term health of dopaminergic neuron transplants in Parkinson's disease patients. Cell reports. 2014; 7:1755–1761. [PubMed: 24910427]

Han X, Qian X, Bernstein JG, Zhou HH, Franzesi GT, Stern P, Bronson RT, Graybiel AM, Desimone R, Boyden ES. Millisecond-timescale optical control of neural dynamics in the nonhuman primate brain. Neuron. 2009; 62:191–198. [PubMed: 19409264]

Hara K, Yasuhara T, Maki M, Matsukawa N, Masuda T, Yu SJ, Ali M, Yu G, Xu L, Kim SU, Hess DC, Borlongan CV. Neural progenitor NT2N cell lines from teratocarcinoma for transplantation therapy in stroke. Prog Neurobiol. 2008; 85:318–334. [PubMed: 18514379]

Harrison NJ, Baker D, Andrews PW. Culture adaptation of embryonic stem cells echoes germ cell malignancy. Int J Androl. 2007; 30:275–281. discussion 281. [PubMed: 17488340]

Haynesworth SE, Baber MA, Caplan AI. Cytokine expression by human marrow-derived mesenchymal progenitor cells in vitro: effects of dexamethasone and IL-1 alpha. J Cell Physiol. 1996; 166:585–592. [PubMed: 8600162]

Hess DC, Borlongan CV. Cell-based therapy in ischemic stroke. Expert Rev Neurother. 2008; 8:1193–1201. [PubMed: 18671663]

Honmou O, Houkin K, Matsunaga T, Niitsu Y, Ishiai S, Onodera R, Waxman SG, Kocsis JD. Intravenous administration of auto serum-expanded autologous mesenchymal stem cells in stroke. Brain. 2011; 134:1790–1807. [PubMed: 21493695]

Horie N, Hiu T, Nagata I. Stem Cell Transplantation Enhances Endogenous Brain Repair after Experimental Stroke. Neurol Med Chir (Tokyo) 55 Suppl. 2015; 1:107–112.

Horschitz S, Matthäus F, Groß A, Rosner J, Galach M, Greffrath W, Treede RD, Utikal J, Schloss P, Meyer-Lindenberg A. Impact of preconditioning with retinoic acid during early development on morphological and functional characteristics of human induced pluripotent stem cell-derived neurons. Stem Cell Res. 2015; 15:30–41. [PubMed: 26001168]

Hu BY, Weick JP, Yu J, Ma LX, Zhang XQ, Thomson JA, Zhang SC. Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. Proc Natl Acad Sci U S A. 2010; 107:4335–4340. [PubMed: 20160098]

Hu X, Wei L, Taylor TM, Wei J, Zhou X, Wang JA, Yu SP. Hypoxic preconditioning enhances bone marrow mesenchymal stem cell migration via Kv2. 1 channel and FAK activation. American Journal of Physiology-Cell Physiol. 2011a; 301:C362–C372.

Hu X, Wei L, Taylor TM, Wei J, Zhou X, Wang JA, Yu SP. Hypoxic preconditioning enhances bone marrow mesenchymal stem cell migration via Kv2.1 channel and FAK activation. Am J Physiol Cell Physiol. 2011b; 301:C362–372. [PubMed: 21562308]

Hu X, Yu SP, Fraser JL, Lu Z, Ogle ME, Wang JA, Wei L. Transplantation of hypoxia-preconditioned mesenchymal stem cells improves infarcted heart function via enhanced survival of implanted cells and angiogenesis. J Thorac Cardiovasc Surg. 2008; 135:799–808. [PubMed: 18374759]

Huangfu D, Osafune K, Maehr R, Guo W, Eijkelenboom A, Chen S, Muhlestein W, Melton DA. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. Nat Biotechnol. 2008; 26:1269–1275. [PubMed: 18849973]

Huber D, Petreanu L, Ghitani N, Ranade S, Hromadka T, Mainen Z, Svoboda K. Sparse optical microstimulation in barrel cortex drives learned behaviour in freely moving mice. Nature. 2008; 451:61–64. [PubMed: 18094685]

Ideguchi M, Palmer TD, Recht LD, Weimann JM. Murine embryonic stem cell-derived pyramidal neurons integrate into the cerebral cortex and appropriately project axons to subcortical targets. J Neurosci. 2010; 30:894–904. [PubMed: 20089898]

Ideguchi M, Shinoyama M, Gomi M, Hayashi H, Hashimoto N, Takahashi J. Immune or inflammatory response by the host brain suppresses neuronal differentiation of transplanted ES cell-derived neural precursor cells. J Neurosci Res. 2008; 86:1936–1943. [PubMed: 18335525]

Iguchi F, Nakagawa T, Tateya I, Kim TS, Endo T, Taniguchi Z, Naito Y, Ito J. Trophic support of mouse inner ear by neural stem cell transplantation. Neuroreport. 2003; 14:77–80. [PubMed: 12544835]

Imitola J, Raddassi K, Park KI, Mueller FJ, Nieto M, Teng YD, Frenkel D, Li J, Sidman RL, Walsh CA, Snyder EY, Khoury SJ. Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. Proc Natl Acad Sci U S A. 2004; 101:18117–18122. [PubMed: 15608062]

Ingberg E, Dock H, Theodorsson E, Theodorsson A, Strom JO. Method parameters' impact on mortality and variability in mouse stroke experiments: a meta-analysis. 2016; 6:21086.

Inoue H, Nagata N, Kurokawa H, Yamanaka S. iPS cells: a game changer for future medicine. The EMBO journal. 2014; 33:409–417. [PubMed: 24500035]

Itoh K, Tezuka H, Sakoda H, Konno M, Nagata K, Uchiyama T, Uchino H, Mori KJ. Reproducible establishment of hemopoietic supportive stromal cell lines from murine bone marrow. Exp Hematol. 1989; 17:145–153. [PubMed: 2783573]

Jäger C, Glaab E, Michelucci A, Binz TM, Köglsberger S, Garcia P, Trezzi JP, Ghelfi J, Balling R, Buttini M. The Mouse Brain Metabolome: Region-Specific Signatures and Response to Excitotoxic Neuronal Injury. Amer J Pathol. 2015; 185:1699–1712. [PubMed: 25934215]

Jeon SB. Therapeutic Hypothermia in the Intensive Care Unit. J Neurocritical Care. 2014; 7:6–15.

Jeong SW, Chu K, Jung KH, Kim SU, Kim M, Roh JK. Human neural stem cell transplantation promotes functional recovery in rats with experimental intracerebral hemorrhage. Stroke. 2003; 34:2258–2263. [PubMed: 12881607]

Jiang MQ, Zhao YY, Cao W, Wei ZZ, Gu X, Wei L, Yu SP. Long-term survival and regeneration of neuronal and vasculature cells inside the core region after ischemic stroke in adult mice. Brain Pathol. 2016 Epub ahead of print.

Jin R, Zhu X, Li G. Embolic middle cerebral artery occlusion (MCAO) for ischemic stroke with homologous blood clots in rats. JoVE (Journal of Visualized Experiments). 2014:e51956–e51956.

Jing Y, Machon O, Hampl A, Dvorak P, Xing Y, Krauss S. In vitro differentiation of mouse embryonic stem cells into neurons of the dorsal forebrain. Cell Mol Neurobiol. 2011; 31:715–727. [PubMed: 21424551]

Johansen JP, Hamanaka H, Monfils MH, Behnia R, Deisseroth K, Blair HT, LeDoux JE. Optical activation of lateral amygdala pyramidal cells instructs associative fear learning. Proc Natl Acad Sci U S A. 2010; 107:12692–12697. [PubMed: 20615999]

Johnston RE, Dillon-Carter O, Freed WJ, Borlongan CV. Trophic factor secreting kidney cell lines: in vitro characterization and functional effects following transplantation in ischemic rats. Brain Res. 2001; 900:268–276. [PubMed: 11334807]

Jones PA, May GR, McLuckie JA, Iwashita A, Sharkey J. Apoptosis is not an invariable component of in vitro models of cortical cerebral ischaemia. Cell Res. 2004; 14:241–250. [PubMed: 15225418]

Jung J, Hackett NR, Pergolizzi RG, Pierre-Destine L, Krause A, Crystal RG. Ablation of tumor-derived stem cells transplanted to the central nervous system by genetic modification of embryonic stem cells with a suicide gene. Hum Gene Ther. 2007; 18:1182–1192. [PubMed: 18021021]

Kalladka D, Sinden J, Pollock K, Haig C, McLean J, Smith W, McConnachie A, Santosh C, Bath PM, Dunn L, Muir KW. Human neural stem cells in patients with chronic ischaemic stroke (PISCES): a phase 1, first-in-man study. Lancet. 2016; 388:787–796. [PubMed: 27497862]

Kaplan MS, Hinds JW. Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. Science. 1977; 197:1092–1094. [PubMed: 887941]

Karussis D, Kassis I, Kurkalli BG, Slavin S. Immunomodulation and neuroprotection with mesenchymal bone marrow stem cells (MSCs): a proposed treatment for multiple sclerosis and other neuroimmunological/neurodegenerative diseases. J Neurol Sci. 2008; 265:131–135. [PubMed: 17610906]

Kastanenka KV, Landmesser LT. In vivo activation of channelrhodopsin-2 reveals that normal patterns of spontaneous activity are required for motoneuron guidance and maintenance of guidance molecules. J Neurosci. 2010; 30:10575–10585. [PubMed: 20686000]

Kempermann G, Wiskott L, Gage FH. Functional significance of adult neurogenesis. Curr Opin Neurobiol. 2004; 14:186–191. [PubMed: 15082323]

Keogh CL, Yu SP, Wei L. The effect of recombinant human erythropoietin on neurovasculature repair after focal ischemic stroke in neonatal rats. J Pharmacol Exp Ther. 2007; 322:521–528. [PubMed: 17494864]

Kessel M, Gruss P. Homeotic transformations of murine vertebrae and concomitant alteration of Hox codes induced by retinoic acid. Cell. 1991; 67:89–104. [PubMed: 1680565]

Kim DY, Park SH, Lee SU, Choi DH, Park HW, Paek SH, Shin HY, Kim EY, Park SP, Lim JH. Effect of human embryonic stem cell-derived neuronal precursor cell transplantation into the cerebral infarct model of rat with exercise. Neurosci Res. 2007a; 58:164–175. [PubMed: 17408791]

Kim DS, Lee JS, Leem JW, Huh YJ, Kim JY, Kim HS, Park IH, Daley GQ, Hwang DY, Kim DW. Robust enhancement of neural differentiation from human ES and iPS cells regardless of their innate difference in differentiation propensity. Stem Cell Rev. 2010; 6:270–281. [PubMed: 20376579]

Kitada M. Mesenchymal cell populations: development of the induction systems for Schwann cells and neuronal cells and finding the unique stem cell population. Anat Sci Int. 2012; 87:24–44. [PubMed: 22237924]

Kondziolka D, Steinberg GK, Wechsler L, Meltzer CC, Elder E, Gebel J, Decesare S, Jovin T, Zafonte R, Lebowitz J, Flickinger JC, Tong D, Marks MP, Jamieson C, Luu D, Bell-Stephens T, Teraoka J. Neurotransplantation for patients with subcortical motor stroke: a phase 2 randomized trial. J Neurosurg. 2005; 103:38–45. [PubMed: 16121971]

Kondziolka D, Wechsler L, Goldstein S, Meltzer C, Thulborn KR, Gebel J, Jannetta P, DeCesare S, Elder EM, McGrogan M, Reitman MA, Bynum L. Transplantation of cultured human neuronal cells for patients with stroke. Neurol. 2000; 55:565–569.

Kornack DR, Rakic P. The generation, migration, and differentiation of olfactory neurons in the adult primate brain. Proc Natl Acad Sci USA. 2001; 98:4752–4757. [PubMed: 11296302]

Kramer RH, Fortin DL, Trauner D. New photochemical tools for controlling neuronal activity. Curr Opin Neurobiol. 2009; 19:544–552. [PubMed: 19828309]

Kravitz AV, Freeze BS, Parker PR, Kay K, Thwin MT, Deisseroth K, Kreitzer AC. Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. Nature. 2010; 466:622–626. [PubMed: 20613723]

Kuge A, Takemura S, Kokubo Y, Sato S, Goto K, Kayama T. Temporal profile of neurogenesis in the subventricular zone, dentate gyrus and cerebral cortex following transient focal cerebral ischemia. Neurol Res. 2009; 31:969–976. [PubMed: 19138475]

Kurozumi K, Nakamura K, Tamiya T, Kawano Y, Kobune M, Hirai S, Uchida H, Sasaki K, Ito Y, Kato K, Honmou O, Houkin K, Date I, Hamada H. BDNF gene-modified mesenchymal stem cells promote functional recovery and reduce infarct size in the rat middle cerebral artery occlusion model. Mol Ther. 2004; 9:189–197. [PubMed: 14759803]

Labouyrie E, Dubus P, Groppi A, Mahon FX, Ferrer J, Parrens M, Reiffers J, de Mascarel A, Merlio JP. Expression of neurotrophins and their receptors in human bone marrow. Am J Pathol. 1999; 154:405–415. [PubMed: 10027399]

Lam J, Lowry WE, Carmichael ST, Segura T. Delivery of iPS - NPCs to the Stroke Cavity within a Hyaluronic Acid Matrix Promotes the Differentiation of Transplanted Cells. Advanced Functional Mat. 2014; 24:7053–7062.

Lampe JW, Becker LB. State of the art in therapeutic hypothermia. Annual review of medicine. 2011; 62:79.

Langhauser F, Kraft P, Göb E, Leinweber J, Schuhmann MK, Lorenz K, Gelderblom M, Bittner S, Meuth SG, Wiendl H. Blocking of α4 integrin does not protect from acute ischemic stroke in mice. Stroke. 2014; 45:1799–1806. [PubMed: 24743435]

Laurenzi MA, Beccari T, Stenke L, Sjolinder M, Stinchi S, Lindgren JA. Expression of mRNA encoding neurotrophins and neurotrophin receptors in human granulocytes and bone marrow cells--enhanced neurotrophin-4 expression induced by LTB4. J Leukoc Biol. 1998; 64:228–234. [PubMed: 9715263]

Lawrenz B, Schiller H, Willbold E, Ruediger M, Muhs A, Esser S. Highly sensitive biosafety model for stem-cell-derived grafts. Cytotherapy. 2004; 6:212–222. [PubMed: 15203978]

Lee HJ, Kim KS, Kim EJ, Choi HB, Lee KH, Park IH, Ko Y, Jeong SW, Kim SU. Brain transplantation of immortalized human neural stem cells promotes functional recovery in mouse intracerebral hemorrhage stroke model. Stem Cells. 2007; 25:1204–1212. [PubMed: 17218400]

Lee J, Terracciano CM. Cell therapy for cardiac repair. Br Med Bull.

Lee JS, Hong JM, Moon GJ, Lee PH, Ahn YH, Bang OY. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. Stem Cells. 2010; 28:1099–1106. [PubMed: 20506226]

Lee SH, Lumelsky N, Studer L, Auerbach JM, McKay RD. Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. Nat Biotechnol. 2000; 18:675–679. [PubMed: 10835609]

Leist M, Ghezzi P, Grasso G, Bianchi R, Villa P, Fratelli M, Savino C, Bianchi M, Nielsen J, Gerwien J, Kallunki P, Larsen AK, Helboe L, Christensen S, Pedersen LO, Nielsen M, Torup L, Sager T, Sfacteria A, Erbayraktar S, Erbayraktar Z, Gokmen N, Yilmaz O, Cerami-Hand C, Xie QW, Coleman T, Cerami A, Brines M. Derivatives of erythropoietin that are tissue protective but not erythropoietic. Science. 2004; 305:239–242. [PubMed: 15247477]

Lensch MW, Ince TA. The terminology of teratocarcinomas and teratomas. Nat Biotechnol. 2007; 25:1211. author reply 1211–1212. [PubMed: 17989663]

Leu S, Lin Y-C, Yuen C-M, Yen C-H, Kao Y-H, Sun C-K, Yip H-K. Adipose-derived mesenchymal stem cells markedly attenuate brain infarct size and improve neurological function in rats. J Transl Med. 2010; 8:63. [PubMed: 20584315]

Li WL, Fraser JL, Shan PY, Zhu J, Jiang YJ, Wei L. The role of VEGF/VEGFR2 signaling in peripheral stimulation-induced cerebral neurovascular regeneration after ischemic stroke in mice. Exper Brain Res. 2011; 214:503–513. [PubMed: 21922279]

Li WL, Yu S, Chen D, Yu S, Jiang YJ, Genetta T, Wei L. The regulatory role of NF-κB in autophagy-like cell death after focal cerebral ischemia in mice. Neurosci. 2013a; 244:16–30.

Li WL, Yu SP, Ogle ME, Ding XS, Wei L. Enhanced neurogenesis and cell migration following focal ischemia and peripheral stimulation in mice. Developmental Neurobiol. 2008a; 68:1474–1486.

Li Y, Chen J, Chen XG, Wang L, Gautam SC, Xu YX, Katakowski M, Zhang LJ, Lu M, Janakiraman N, Chopp M. Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. Neurology. 2002; 59:514–523. [PubMed: 12196642]

Li Y, Lu Z, Keogh CL, Yu SP, Wei L. Erythropoietin-induced neurovascular protection, angiogenesis, and cerebral blood flow restoration after focal ischemia in mice. J Cereb Blood Flow Metab. 2007a; 27:1043–1054. [PubMed: 17077815]

Li Y, Lu ZY, Ogle M, Wei L. Erythropoietin prevents blood brain barrier damage induced by focal cerebral ischemia in mice. Neurochem Res. 2007b; 32:2132–2141. [PubMed: 17562165]

Li Y, Yu S, Mohamad O, Genetta T, Wei L. Sublethal Transient Global Ischemia Stimulates Migration of Neuroblasts and Neurogenesis in Mice. Translational Stroke Res. 2010; 1:184–196.

Li ZM, Zhang ZT, Guo CJ, Geng FY, Qiang F, Wang LX. Autologous bone marrow mononuclear cell implantation for intracerebral hemorrhage-a prospective clinical observation. Clin Neurol Neurosurg. 2013b; 115:72–76. [PubMed: 22657095]

Lian Q, Lye E, Suan Yeo K, Khia Way Tan E, Salto-Tellez M, Liu TM, Palanisamy N, El Oakley RM, Lee EH, Lim B, Lim SK. Derivation of clinically compliant MSCs from CD105+, CD24-differentiated human ESCs. Stem Cells. 2007; 25:425–436. [PubMed: 17053208]

Lindvall O, Kokaia Z. Prospects of stem cell therapy for replacing dopamine neurons in Parkinson's disease. Trends Pharmacol Sci. 2009; 30:260–267. [PubMed: 19362379]

Liska MG, Crowley MG, Nguyen H, Borlongan CV. Biobridge concept in stem cell therapy for ischemic stroke. J Neurosurg Sci. 2017; 61:173–179. [PubMed: 27406955]

Litchfield TM, Whiteley SJ, Yee KT, Tyers P, Usherwood EJ, Nash AA, Lund RD. Characterisation of the immune response in a neural xenograft rejection paradigm. J Neuroimmunol. 1997; 73:135–144. [PubMed: 9058769]

Liu N, Zhang Y, Fan L, Yuan M, Du H, Cheng R, Liu D, Lin F. Effects of transplantation with bone marrow-derived mesenchymal stem cells modified by Survivin on experimental stroke in rats. J Transl Med. 2011; 9:105–106. [PubMed: 21733181]

Liu S, Hu WX, Zu QQ, Lu SS, Xu XQ, Sun L, Zhou WZ, Shi HB. A novel embolic stroke model resembling lacunar infarction following proximal middle cerebral artery occlusion in beagle dogs. Journal of neuroscience methods. 2012; 209:90–96. [PubMed: 22722089]

Liu X, Ye R, Yan T, Yu SP, Wei L, Xu G, Fan X, Jiang Y, Stetler RA, Liu G. Cell based therapies for ischemic stroke: from basic science to bedside. Progress in neurobiology. 2014; 115:92–115. [PubMed: 24333397]

Llovera G, Roth S, Plesnila N, Veltkamp R, Liesz A. Modeling stroke in mice: Permanent coagulation of the distal middle cerebral artery. JoVE (Journal of Visualized Experiments). 2014:e51729–e51729. [PubMed: 25145316]

Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, Ferguson TB, Ford E, Furie K, Gillespie C. Heart disease and stroke statistics—2010 update A report from the American Heart Association. Circulation. 2010; 121:e46–e215. [PubMed: 20019324]

Lochhead JJ, Thorne RG. Intranasal delivery of biologics to the central nervous system. Adv Drug Deliv Rev. 2011

Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke. 1989; 20:84–91. [PubMed: 2643202]

Lu J, Zhong X, Liu H, Hao L, Huang CTL, Sherafat MA, Jones J, Ayala M, Li L, Zhang SC. Generation of serotonin neurons from human pluripotent stem cells. Nature biotechnology. 2016; 34:89–94.

Luehr M, Etz CD. Moderate-to-mild hypothermia may not be sufficient to protect the spinal cord during aortic arch surgery. European Journal of Cardio-Thoracic Surgery. 2014; 45:767–767. [PubMed: 23832835]

Lujan E, Chanda S, Ahlenius H, Sudhof TC, Wernig M. Direct conversion of mouse fibroblasts to self-renewing, tripotent neural precursor cells. Proc Natl Acad Sci U S A. 2012; 109(7):2527–32. [PubMed: 22308465]

Luo W, Wang Z, Li P, Zeng S, Luo Q. A modified mini-stroke model with region-directed reperfusion in rat cortex. J Cereb Blood Flow Metab. 2008; 28:973–983. [PubMed: 18073774]

Mackie AR, Losordo DW. CD34-positive stem cells: in the treatment of heart and vascular disease in human beings. Tex Heart Inst J. 2011; 38:474–485. [PubMed: 22163120]

Maerz WJ, Baselga J, Reuter VE, Mellado B, Myers ML, Bosl GJ, Spinella MJ, Dmitrovsky E. FGF4 dissociates anti-tumorigenic from differentiation signals of retinoic acid in human embryonal carcinomas. Oncogene. 1998; 17:761–767. [PubMed: 9715278]

Maitra A, Arking DE, Shivapurkar N, Ikeda M, Stastny V, Kassauei K, Sui G, Cutler DJ, Liu Y, Brimble SN, Noaksson K, Hyllner J, Schulz TC, Zeng X, Freed WJ, Crook J, Abraham S, Colman A, Sartipy P, Matsui S, Carpenter M, Gazdar AF, Rao M, Chakravarti A. Genomic alterations in cultured human embryonic stem cells. Nat Genet. 2005; 37:1099–1103. [PubMed: 16142235]

Majumdar MK, Thiede MA, Mosca JD, Moorman M, Gerson SL. Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (MSCs) and stromal cells. J Cell Physiol. 1998; 176:57–66. [PubMed: 9618145]

Malgieri A, Kantzari E, Patrizi MP, Gambardella S. Bone marrow and umbilical cord blood human mesenchymal stem cells: state of the art. Int J Clin Exp Med. 2010; 3:248–269. [PubMed: 21072260]

Maltepe E, Schmidt JV, Baunoch D, Bradfield CA, Simon MC. Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. Nature. 1997; 386:403–407. [PubMed: 9121557]

Marcen R. Immunosuppressive drugs in kidney transplantation: impact on patient survival, and incidence of cardiovascular disease, malignancy and infection. Drugs. 2009; 69:2227–2243. [PubMed: 19852526]

Marro S, Pang ZP, Yang N, Tsai MC, Qu K, Chang HY, Sudhof TC, Wernig M. Direct lineage conversion of terminally differentiated hepatocytes to functional neurons. Cell Stem Cell. 2011; 9:374–382. [PubMed: 21962918]

Marshall H, Nonchev S, Sham MH, Muchamore I, Lumsden A, Krumlauf R. Retinoic acid alters hindbrain Hox code and induces transformation of rhombomeres 2/3 into a 4/5 identity. Nature. 1992; 360:737–741. [PubMed: 1361214]

Martin MJ, Muotri A, Gage F, Varki A. Human embryonic stem cells express an immunogenic nonhuman sialic acid. Nat Med. 2005; 11:228–232. [PubMed: 15685172]

Maxwell SL, Li M. Midbrain dopaminergic development in vivo and in vitro from embryonic stem cells. J Anat. 2005; 207:209–218. [PubMed: 16185245]

McDonald JW, Howard MJ. Repairing the damaged spinal cord: a summary of our early success with embryonic stem cell transplantation and remyelination. Prog Brain Res. 2002; 137:299–309. [PubMed: 12449097]

Miura K, Okada Y, Aoi T, Okada A, Takahashi K, Okita K, Nakagawa M, Koyanagi M, Tanabe K, Ohnuki M, Ogawa D, Ikeda E, Okano H, Yamanaka S. Variation in the safety of induced pluripotent stem cell lines. Nat Biotechnol. 2009; 27:743–745. [PubMed: 19590502]

Modo M, Mellodew K, Cash D, Fraser SE, Meade TJ, Price J, Williams SC. Mapping transplanted stem cell migration after a stroke: a serial, in vivo magnetic resonance imaging study. Neuroimage. 2004; 21:311–317. [PubMed: 14741669]

Modo M, Rezaie P, Heuschling P, Patel S, Male DK, Hodges H. Transplantation of neural stem cells in a rat model of stroke: assessment of short-term graft survival and acute host immunological response. Brain Res. 2002a; 958:70–82. [PubMed: 12468031]

Modo M, Stroemer RP, Tang E, Patel S, Hodges H. Effects of implantation site of stem cell grafts on behavioral recovery from stroke damage. Stroke. 2002b; 33:2270–2278. [PubMed: 12215598]

Molinari GF. Why model strokes? Stroke. 1988; 19:1195–1197. [PubMed: 3270338]

Moniche F, Escudero I, Zapata-Arriaza E, Usero-Ruiz M, Prieto-Leon M, de la Torre J, Gamero MA, Tamayo JA, Ochoa-Sepulveda JJ, Maestre J, Carmona M, Pinero P, Calderon-Cabrera C, Jimenez MD, Gonzalez A, Montaner J. Intra-arterial bone marrow mononuclear cells (BM-MNCs) transplantation in acute ischemic stroke (IBIS trial): protocol of a phase II, randomized, dose-finding, controlled multicenter trial. Int J Stroke. 2015; 10:1149–1152. [PubMed: 26044701]

Morizane A, Doi D, Kikuchi T, Nishimura K, Takahashi J. Small-molecule inhibitors of bone morphogenic protein and activin/nodal signals promote highly efficient neural induction from human pluripotent stem cells. J Neurosci Res. 2011; 89:117–126. [PubMed: 21162120]

Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Després J-P, Fullerton HJ. Heart Disease and Stroke Statistics—2016 Update A Report From the American Heart Association. Circulation, CIR. 2015 0000000000000350.

Murray KN, Girard S, Holmes WM, Parkes LM, Williams SR, Parry-Jones AR, Allan SM. Systemic inflammation impairs tissue reperfusion through endothelin-dependent mechanisms in cerebral ischemia. Stroke. 2014; 45:3412–3419. [PubMed: 25228257]

Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation. 1986; 74:1124–1136. [PubMed: 3769170]

Nagai A, Kim WK, Lee HJ, Jeong HS, Kim KS, Hong SH, Park IH, Kim SU. Multilineage potential of stable human mesenchymal stem cell line derived from fetal marrow. PLoS One. 2007; 2:e1272. [PubMed: 18060066]

Nagasawa H, Kogure K. Correlation between cerebral blood flow and histologic changes in a new rat model of middle cerebral artery occlusion. Stroke. 1989; 20:1037–1043. [PubMed: 2756535]

Nagel G, Szellas T, Huhn W, Kateriya S, Adeishvili N, Berthold P, Ollig D, Hegemann P, Bamberg E. Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. Proc Natl Acad Sci U S A. 2003; 100:13940–13945. [PubMed: 14615590]

Nagel G, Szellas T, Kateriya S, Adeishvili N, Hegemann P, Bamberg E. Channelrhodopsins: directly light-gated cation channels. Biochem Soc Trans. 2005; 33:863–866. [PubMed: 16042615]

Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, Okita K, Mochiduki Y, Takizawa N, Yamanaka S. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat Biotechnol. 2008; 26:101–106. [PubMed: 18059259]

Nakagomi N, Nakagomi T, Kubo S, Nakano-Doi A, Saino O, Takata M, Yoshikawa H, Stern DM, Matsuyama T, Taguchi A. Endothelial cells support survival, proliferation, and neuronal differentiation of transplanted adult ischemia-induced neural stem/progenitor cells after cerebral infarction. Stem Cells. 2009; 27:2185–2195. [PubMed: 19557831]

Nakazaki M, Sasaki M, Kataoka-Sasaki Y, Oka S, Namioka T, Namioka A, Onodera R, Suzuki J, Sasaki Y, Nagahama H, Mikami T, Wanibuchi M, Kocsis JD, Honmou O. Intravenous infusion of mesenchymal stem cells inhibits intracranial hemorrhage after recombinant tissue plasminogen activator therapy for transient middle cerebral artery occlusion in rats. J Neurosurg. 2017:1–10.

Nelson PT, Kondziolka D, Wechsler L, Goldstein S, Gebel J, DeCesare S, Elder EM, Zhang PJ, Jacobs A, McGrogan M, Lee VM, Trojanowski JQ. Clonal human (hNT) neuron grafts for stroke therapy: neuropathology in a patient 27 months after implantation. Am J Pathol. 2002; 160:1201–1206. [PubMed: 11943704]

Nemeth K, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, Robey PG, Leelahavanichkul K, Koller BH, Brown JM, Hu X, Jelinek I, Star RA, Mezey E. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med. 2009; 15:42–49. [PubMed: 19098906]

Newman MB, Misiuta I, Willing AE, Zigova T, Karl RC, Borlongan CV, Sanberg PR. Tumorigenicity issues of embryonic carcinoma-derived stem cells: relevance to surgical trials using NT2 and hNT neural cells. Stem Cells Dev. 2005; 14:29–43. [PubMed: 15725742]

O'Brien MD, Waltz AG. Transorbital approach for occluding the middle cerebral artery without craniectomy. Stroke. 1973; 4:201–206. [PubMed: 4702307]

Ohab JJ, Fleming S, Blesch A, Carmichael ST. A neurovascular niche for neurogenesis after stroke. J Neurosci. 2006; 26:13007–13016. [PubMed: 17167090]

Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature. 2007; 448:313–317. [PubMed: 17554338]

Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. Science. 2008; 322:949–953. [PubMed: 18845712]

Olanow CW, Goetz CG, Kordower JH, Stoessl AJ, Sossi V, Brin MF, Shannon KM, Nauert GM, Perl DP, Godbold J, Freeman TB. A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. Ann Neurol. 2003; 54:403–414. [PubMed: 12953276]

Olivier EN, Rybicki AC, Bouhassira EE. Differentiation of human embryonic stem cells into bipotent mesenchymal stem cells. Stem Cells. 2006; 24:1914–1922. [PubMed: 16644919]

Opitz T, Scheffler B, Steinfarz B, Schmandt T, Brustle O. Electrophysiological evaluation of engrafted stem cell-derived neurons. Nat Protoc. 2007; 2:1603–1613. [PubMed: 17585301]

Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. J Neurosci. 1997; 17:3727–3738. [PubMed: 9133393]

Pasha Z, Wang Y, Sheikh R, Zhang D, Zhao T, Ashraf M. Preconditioning enhances cell survival and differentiation of stem cells during transplantation in infarcted myocardium. Cardiovasc Res. 2008; 77:134–142. [PubMed: 18006467]

Pencea V, Bingaman KD, Wiegand SJ, Luskin MB. Infusion of brain-derived neurotrophic factor into the lateral ventricle of the adult rat leads to new neurons in the parenchyma of the striatum, septum, thalamus, and hypothalamus. Journal of Neuroscience. 2001; 21:6706–6717. [PubMed: 11517260]

Pevsner PH, Eichenbaum JW, Miller DC, Pivawer G, Eichenbaum KD, Stern A, Zakian KL, Koutcher JA. A photothrombotic model of small early ischemic infarcts in the rat brain with histologic and MRI correlation. Journal of pharmacological and toxicological methods. 2001; 45:227–233. [PubMed: 11755387]

Pfisterer U, Kirkeby A, Torper O, Wood J, Nelander J, Dufour A, Bjorklund A, Lindvall O, Jakobsson J, Parmar M. Direct conversion of human fibroblasts to dopaminergic neurons. Proc Natl Acad Sci U S A. 108:10343–10348.

Piao CS, Gonzalez-Toledo ME, Xue YQ, Duan WM, Terao S, Granger DN, Kelley RE, Zhao LR. The role of stem cell factor and granulocyte-colony stimulating factor in brain repair during chronic stroke. J Cereb Blood Flow Metab. 2009; 29:759–770. [PubMed: 19209180]

Pietrini D, Piastra M, Luca E, Mancino A, Conti G, Cavaliere F, De Luca D. Neuroprotection and hypothermia in infants and children. Current drug targets. 2012; 13:925–935. [PubMed: 22512392]

Pignataro G, Studer FE, Wilz A, Simon RP, Boison D. Neuroprotection in ischemic mouse brain induced by stem cell-derived brain implants. Journal of Cerebral Blood Flow & Metabolism. 2007; 27:919–927. [PubMed: 17119544]

Pollock K, Stroemer P, Patel S, Stevanato L, Hope A, Miljan E, Dong Z, Hodges H, Price J, Sinden JD. A conditionally immortal clonal stem cell line from human cortical neuroepithelium for the treatment of ischemic stroke. Exp Neurol. 2006; 199:143–155. [PubMed: 16464451]

Prasad K, Sharma A, Garg A, Mohanty S, Bhatnagar S, Johri S, Singh KK, Nair V, Sarkar RS, Gorthi SP. Intravenous Autologous Bone Marrow Mononuclear Stem Cell Therapy for Ischemic Stroke A Multicentric, Randomized Trial. Stroke. 2014; 45:3618–3624. [PubMed: 25378424]

Prass K, Scharff A, Ruscher K, Lowl D, Muselmann C, Victorov I, Kapinya K, Dirnagl U, Meisel A. Hypoxia-induced stroke tolerance in the mouse is mediated by erythropoietin. Stroke. 2003; 34:1981–1986. [PubMed: 12829864]

Pulsinelli WA, Brierley JB. A new model of bilateral hemispheric ischemia in the unanesthetized rat. Stroke. 1979; 10:267–272. [PubMed: 37614]

Puyal J, Ginet V, Clarke PG. Multiple interacting cell death mechanisms in the mediation of excitotoxicity and ischemic brain damage: a challenge for neuroprotection. Progress in neurobiology. 2013; 105:24–48. [PubMed: 23567504]

Reubinoff BE, Itsykson P, Turetsky T, Pera MF, Reinhartz E, Itzik A, Ben-Hur T. Neural progenitors from human embryonic stem cells. Nat Biotechnol. 2001; 19:1134–1140. [PubMed: 11731782]

Robbins RD, Prasain N, Maier BF, Yoder MC, Mirmira RG. Inducible pluripotent stem cells: not quite ready for prime time? Curr Opin Organ Transplant. 15:61–67.

Robin AM, Zhang ZG, Wang L, Zhang RL, Katakowski M, Zhang L, Wang Y, Zhang C, Chopp M. Stromal cell-derived factor 1alpha mediates neural progenitor cell motility after focal cerebral ischemia. J Cereb Blood Flow Metab. 2006; 26:125–134. [PubMed: 15959456]

Robinson MJ, Macrae IM, Todd M, Reid JL, McCulloch J. Reduction of local cerebral blood flow to pathological levels by endothelin-1 applied to the middle cerebral artery in the rat. Neurosci Lett. 1990; 118:269–272. [PubMed: 2274283]

Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, Carnethon MR, Dai S, de Simone G, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Greenlund KJ, Hailpern SM, Heit JA, Ho

PM, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, McDermott MM, Meigs JB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Rosamond WD, Sorlie PD, Stafford RS, Turan TN, Turner MB, Wong ND, Wylie-Rosett J. Heart disease and stroke statistics—2011 update: a report from the American Heart Association. Circulation. 123:e18–e209. [PubMed: 21160056]

Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Heart disease and stroke statistics--2012 update: a report from the american heart association. Circulation. 2012; 125:e2–e220. [PubMed: 22179539]

Rogers NJ, Lechler RI. Allorecognition. Am J Transplant. 2001; 1:97–102. [PubMed: 12099369]

Rohwedel J, Guan K, Wobus AM. Induction of cellular differentiation by retinoic acid in vitro. Cells Tissues Organs. 1999; 165:190–202. [PubMed: 10592391]

Rola R, Mizumatsu S, Otsuka S, Morhardt DR, Noble-Haeusslein LJ, Fishman K, Potts MB, Fike JR. Alterations in hippocampal neurogenesis following traumatic brain injury in mice. Exp Neurol. 2006; 202:189–199. [PubMed: 16876159]

Roome RB, Bartlett RF, Jeffers M, Xiong J, Corbett D, Vanderluit JL. A reproducible Endothelin-1 model of forelimb motor cortex stroke in the mouse. Journal of neuroscience methods. 2014; 233:34–44. [PubMed: 24915635]

Rosenblum WI, El-Sabban F. Platelet aggregation in the cerebral microcirculation: effect of aspirin and other agents. Circ Res. 1977; 40:320–328. [PubMed: 13943]

Roy NS, Cleren C, Singh SK, Yang L, Beal MF, Goldman SA. Functional engraftment of human ES cell–derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. Nature medicine. 2006; 12:1259–1268.

Sakata H, Niizuma K, Yoshioka H, Kim GS, Jung JE, Katsu M, Narasimhan P, Maier CM, Nishiyama Y, Chan PH. Minocycline-preconditioned neural stem cells enhance neuroprotection after ischemic stroke in rats. J Neurosci. 2012; 32:3462–3473. [PubMed: 22399769]

Samper E, Diez-Juan A, Montero J, Sepúlveda P. Cardiac cell therapy: boosting mesenchymal stem cells effects. Stem Cell Reviews and Reports. 2013; 9:266–280. [PubMed: 22350458]

Sart S, Ma T, Li Y. Preconditioning stem cells for in vivo delivery. BioResearch open access. 2014; 3:137–149. [PubMed: 25126478]

Savitz SI, Chopp M, Deans R, Carmichael ST, Phinney D, Wechsler L. Stem Cell Therapy as an Emerging Paradigm for Stroke (STEPS) II. Stroke. 2011; 42:825–829. [PubMed: 21273569]

Savitz SI, Dinsmore J, Wu J, Henderson GV, Stieg P, Caplan LR. Neurotransplantation of fetal porcine cells in patients with basal ganglia infarcts: a preliminary safety and feasibility study. Cerebrovasc Dis. 2005; 20:101–107.

Scheffler B, Schmandt T, Schroder W, Steinfarz B, Husseini L, Wellmer J, Seifert G, Karram K, Beck H, Blumcke I, Wiestler OD, Steinhauser C, Brustle O. Functional network integration of embryonic stem cell-derived astrocytes in hippocampal slice cultures. Development. 2003; 130:5533–5541. [PubMed: 14530298]

Schmidt A, Diederich K, Strecker JK, Geng B, Hoppen M, Duning T, Schäbitz WR, Minnerup J. Progressive Cognitive Deficits in a Mouse Model of Recurrent Photothrombotic Stroke. Stroke. 2015; 46:1127–1131. [PubMed: 25744521]

Schmidt A, Minnerup J. Promoting recovery from ischemic stroke. Expert Rev Neurotherapeutics. 2016; 16(2):173–86.

Sharkey J, Ritchie IM, Kelly PA. Perivascular microapplication of endothelin-1: a new model of focal cerebral ischaemia in the rat. J Cereb Blood Flow Metab. 1993; 13:865–871. [PubMed: 8360292]

Shibata H, Ageyama N, Tanaka Y, Kishi Y, Sasaki K, Nakamura S, Muramatsu S, Hayashi S, Kitano Y, Terao K, Hanazono Y. Improved safety of hematopoietic transplantation with monkey embryonic stem cells in the allogeneic setting. Stem Cells. 2006; 24:1450–1457. [PubMed: 16456125]

Siatskas C, Payne NL, Short MA, Bernard CC. A consensus statement addressing mesenchymal stem cell transplantation for multiple sclerosis: it's time! Stem Cell Rev. 6:500–506. [PubMed: 20665128]

Simeone A, Acampora D, Nigro V, Faiella A, D'Esposito M, Stornaiuolo A, Mavilio F, Boncinelli E. Differential regulation by retinoic acid of the homeobox genes of the four HOX loci in human embryonal carcinoma cells. Mech Dev. 1991; 33:215–227. [PubMed: 1677812]

Simpson E. A historical perspective on immunological privilege. Immunol Rev. 2006; 213:12–22. [PubMed: 16972893]

Sloan DJ, Wood MJ, Charlton HM. The immune response to intracerebral neural grafts. Trends Neurosci. 1991; 14:341–346. [PubMed: 1721738]

Smart I. Subependymal Layer of Mouse Brain and Its Cell Production as Shown by Radioautography after Thymidine-H3 Injection. J Comp Neurol. 1961; 116:325.

Smith ML, Auer RN, Siesjo BK. The density and distribution of ischemic brain injury in the rat following 2–10 min of forebrain ischemia. Acta Neuropathologica. 1984; 64:319–332. [PubMed: 6507048]

Sommer CA, Stadtfeld M, Murphy GJ, Hochedlinger K, Kotton DN, Mostoslavsky G. Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. Stem Cells. 2009; 27:543–549. [PubMed: 19096035]

Song M, Yu SP. Ionic regulation of cell volume changes and cell death after ischemic stroke. Translational stroke research. 2014; 5:17–27. [PubMed: 24323733]

Song M, Yu SP, Mohamad O, Cao W, Wei ZZ, Gu X, Jiang MQ, Wei L. Optogenetic stimulation of glutamatergic neuronal activity in the striatum enhances neurogenesis in the subventricular zone of normal and stroke mice. Neurobiol Dis. 2017; 98:9–24. [PubMed: 27884724]

Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. Science. 2008; 322:945–949. [PubMed: 18818365]

Stevanato L, Corteling RL, Stroemer P, Hope A, Heward J, Miljan EA, Sinden JD. c-MycERTAM transgene silencing in a genetically modified human neural stem cell line implanted into MCAo rodent brain. BMC Neurosci. 2009; 10:86. [PubMed: 19622162]

Stevens LC, Little CC. Spontaneous Testicular Teratomas in an Inbred Strain of Mice. Proc Natl Acad Sci U S A. 1954; 40:1080–1087. [PubMed: 16578442]

Strecker JK, Olk J, Hoppen M, Gess B, Diederich K, Schmidt A, Schäbitz WR, Schilling M, Minnerup J. Combining Growth Factor and Bone Marrow Cell Therapy Induces Bleeding and Alters Immune Response After Stroke in Mice. Stroke. 2016; 47:852–862. [PubMed: 26839353]

Stroh A, Tsai HC, Wang LP, Zhang F, Kressel J, Aravanis A, Santhanam N, Deisseroth K, Konnerth A, Schneider MB. Tracking stem cell differentiation in the setting of automated optogenetic stimulation. Stem Cells. 2011; 29:78–88. [PubMed: 21280159]

Su L, Xu J, Ji BX, Wan SG, Lu CY, Dong HQ, Yu YY, Lu DP. Autologous peripheral blood stem cell transplantation for severe multiple sclerosis. Int J Hematol. 2006; 84:276–281. [PubMed: 17050205]

Suarez-Monteagudo C, Hernandez-Ramirez P, Alvarez-Gonzalez L, Garcia-Maeso I, de la Cuetara-Bernal K, Castillo-Diaz L, Bringas-Vega ML, Martinez-Aching G, Morales-Chacon LM, Baez-Martin MM, Sanchez-Catasus C, Carballo-Barreda M, Rodriguez-Rojas R, Gomez-Fernandez L, Alberti-Amador E, Macias-Abraham C, Balea ED, Rosales LC, Del Valle Perez L, Ferrer BB, Gonzalez RM, Bergado JA. Autologous bone marrow stem cell neurotransplantation in stroke patients. An open study. Restor Neurol Neurosci. 2009; 27:151–161. [PubMed: 19531871]

Sun J, Wei ZZ, Gu X, Zhang JY, Zhang Y, Li J, Wei L. Intranasal delivery of hypoxia-preconditioned bone marrow-derived mesenchymal stem cells enhanced regenerative effects after intracerebral hemorrhagic stroke in mice. Experimental neurology. 2015; 272:78–87. [PubMed: 25797577]

Suzuki IK, Vanderhaeghen P. Is this a brain which I see before me? Modeling human neural development with pluripotent stem cells. Development. 2015; 142:3138–3150. [PubMed: 26395142]

Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H, Yoshikawa H, Tsukamoto Y, Iso H, Fujimori Y, Stern DM, Naritomi H, Matsuyama T. Administration of CD34+ cells after stroke enhances

neurogenesis via angiogenesis in a mouse model. J Clin Invest. 2004; 114:330–338. [PubMed: 15286799]

Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006; 126:663–676. [PubMed: 16904174]

Takahashi K, Yasuhara T, Shingo T, Muraoka K, Kameda M, Takeuchi A, Yano A, Kurozumi K, Agari T, Miyoshi Y, Kinugasa K, Date I. Embryonic neural stem cells transplanted in middle cerebral artery occlusion model of rats demonstrated potent therapeutic effects, compared to adult neural stem cells. Brain Res. 2008; 1234:172–182. [PubMed: 18703033]

Tambuyzer BR, Bergwerf I, De Vocht N, Reekmans K, Daans J, Jorens PG, Goossens H, Ysebaert DK, Chatterjee S, Van Marck E, Berneman ZN, Ponsaerts P. Allogeneic stromal cell implantation in brain tissue leads to robust microglial activation. Immunol Cell Biol. 2009; 87:267–273. [PubMed: 19290016]

Tamura A, Graham DI, McCulloch J, Teasdale GM. Focal cerebral ischaemia in the rat: 1. Description of technique and early neuropathological consequences following middle cerebral artery occlusion. J Cereb Blood Flow Metab. 1981a; 1:53–60. [PubMed: 7328138]

Tamura A, Graham DI, McCulloch J, Teasdale GM. Focal cerebral ischaemia in the rat: 2. Regional cerebral blood flow determined by [14C]iodoantipyrine autoradiography following middle cerebral artery occlusion. J Cereb Blood Flow Metab. 1981b; 1:61–69. [PubMed: 7328139]

Tan Z, Su ZY, Wu RR, Gu B, Liu YK, Zhao XL, Zhang M. Immunomodulative effects of mesenchymal stem cells derived from human embryonic stem cells in vivo and in vitro. J Zhejiang Univ Sci B. 12:18–27.

Taupin P, Gage FH. Adult neurogenesis and neural stem cells of the central nervous system in mammals. J Neurosci Res. 2002; 69:745–749. [PubMed: 12205667]

Therapies SC. Stem Cell Therapies as an Emerging Paradigm in Stroke (STEPS): bridging basic and clinical science for cellular and neurogenic factor therapy in treating stroke. Stroke. 2009; 40:510–515. [PubMed: 19095993]

Theus MH, Wei L, Cui L, Francis K, Hu X, Keogh C, Yu SP. In vitro hypoxic preconditioning of embryonic stem cells as a strategy of promoting cell survival and functional benefits after transplantation into the ischemic rat brain. Exp Neurol. 2008a; 210:656–670. [PubMed: 18279854]

Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science. 1998; 282:1145–1147. [PubMed: 9804556]

Thored P, Wood J, Arvidsson A, Cammenga J, Kokaia Z, Lindvall O. Long-term neuroblast migration along blood vessels in an area with transient angiogenesis and increased vascularization after stroke. Stroke. 2007; 38:3032–3039. [PubMed: 17901386]

Tissier R, Ghaleh B, Cohen MV, Downey JM, Berdeaux A. Myocardial protection with mild hypothermia. Cardiovascular research. 2012; 94:217–225. [PubMed: 22131353]

Tonnesen J, Parish CL, Sorensen AT, Andersson A, Lundberg C, Deisseroth K, Arenas E, Lindvall O, Kokaia M. Functional integration of grafted neural stem cell-derived dopaminergic neurons monitored by optogenetics in an in vitro Parkinson model. PLoS One. 2011; 6:e17560. [PubMed: 21394212]

Tornabene E, Brodin B. Stroke and Drug Delivery In Vitro Models of the Ischemic Blood-Brain Barrier. Journal of pharmaceutical sciences. 2016; 105:398–405. [PubMed: 26869407]

Toyoda K. Epidemiology and registry studies of stroke in Japan. J Stroke. 2013; 15:21–26. [PubMed: 24324936]

Toyoshima A, Yasuhara T, Kameda M, Morimoto J, Takeuchi H, Wang F, Sasaki T, Sasada S, Shinko A, Wakamori T, Okazaki M, Kondo A, Agari T, Borlongan CV, Date I. Intra-Arterial Transplantation of Allogeneic Mesenchymal Stem Cells Mounts Neuroprotective Effects in a Transient Ischemic Stroke Model in Rats: Analyses of Therapeutic Time Window and Its Mechanisms. PLoS One. 2015; 10:e0127302. [PubMed: 26075717]

Tsai PT, Ohab JJ, Kertesz N, Groszer M, Matter C, Gao J, Liu X, Wu H, Carmichael ST. A critical role of erythropoietin receptor in neurogenesis and post-stroke recovery. J Neurosci. 2006; 26:1269–1274. [PubMed: 16436614]

Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC. Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. Transplantation. 2003; 75:389–397. [PubMed: 12589164]

van der Worp HB, Sena ES, Donnan GA, Howells DW, Macleod MR. Hypothermia in animal models of acute ischaemic stroke: a systematic review and meta-analysis. Brain. 2007; 130:3063–3074. [PubMed: 17478443]

Veizovic T, Beech JS, Stroemer RP, Watson WP, Hodges H. Resolution of stroke deficits following contralateral grafts of conditionally immortal neuroepithelial stem cells. Stroke. 2001; 32:1012–1019. [PubMed: 11283405]

Vendrame M, Cassady J, Newcomb J, Butler T, Pennypacker KR, Zigova T, Sanberg CD, Sanberg PR, Willing AE. Infusion of human umbilical cord blood cells in a rat model of stroke dose-dependently rescues behavioral deficits and reduces infarct volume. Stroke. 2004; 35:2390–2395. [PubMed: 15322304]

Vidal SE, Stadtfeld M, Apostolou E. F-Class Cells: New Routes and Destinations for Induced Pluripotency. Cell stem cell. 2015; 16:9–10. [PubMed: 25575077]

Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Sudhof TC, Wernig M. Direct conversion of fibroblasts to functional neurons by defined factors. Nature. 463:1035–1041.

Villars F, Bordenave L, Bareille R, Amedee J. Effect of human endothelial cells on human bone marrow stromal cell phenotype: role of VEGF? J Cell Biochem. 2000; 79:672–685. [PubMed: 10996857]

Volpe G, Bernstock JD, Peruzzotti-Jametti L, Pluchino S. Modulation of host immune responses following non-hematopoietic stem cell transplantation: Translational implications in progressive multiple sclerosis. J Neuroimmunol. 2016; (16):30312–5. pii: S0165-5728. Epub ahead of print. DOI: 10.1016/j.jneuroim.2016.12.005

Wahlgren N, Ahmed N, Davalos A, Hacke W, Millan M, Muir K, Roine RO, Toni D, Lees KR. Thrombolysis with alteplase 3–4.5 h after acute ischaemic stroke (SITS-ISTR): an observational study. Lancet. 2008; 372:1303–1309. [PubMed: 18790527]

Wang CX, Yang T, Shuaib A. An improved version of embolic model of brain ischemic injury in the rat. J Neurosci Methods. 2001; 109:147–151. [PubMed: 11513949]

Wang LL, Chen D, Lee J, Gu X, Alaaeddine G, Li J, Wei L, Yu SP. Mobilization of endogenous bone marrow derived endothelial progenitor cells and therapeutic potential of parathyroid hormone after ischemic stroke in mice. PLoS One. 2014; 9:e87284. [PubMed: 24503654]

Wang Y, Jin K, Mao XO, Xie L, Banwait S, Marti HH, Greenberg DA. VEGF-overexpressing transgenic mice show enhanced post-ischemic neurogenesis and neuromigration. J Neurosci Res. 2007; 85:740–747. [PubMed: 17243175]

Watanabe K, Ueno M, Kamiya D, Nishiyama A, Matsumura M, Wataya T, Takahashi JB, Nishikawa S, Muguruma K, Sasai Y. A ROCK inhibitor permits survival of dissociated human embryonic stem cells. Nat Biotechnol. 2007; 25:681–686. [PubMed: 17529971]

Watanabe S, Uchida K, Nakajima H, Matsuo H, Sugita D, Yoshida A, Honjoh K, Johnson WE, Baba H. Early Transplantation of Mesenchymal Stem Cells After Spinal Cord Injury Relieves Pain Hypersensitivity Through Suppression of Pain - Related Signaling Cascades and Reduced Inflammatory Cell Recruitment. Stem Cells. 2015; 33:1902–1914. [PubMed: 25809552]

Watson BD, Dietrich WD, Busto R, Wachtel MS, Ginsberg MD. Induction of reproducible brain infarction by photochemically initiated thrombosis. Ann Neurol. 1985; 17:497–504. [PubMed: 4004172]

Watt FM, Hogan BL. Out of Eden: stem cells and their niches. Science. 2000; 287:1427–1430. [PubMed: 10688781]

Wei L, Cui L, Snider BJ, Rivkin M, Yu SS, Lee CS, Adams LD, Gottlieb DI, Johnson EM Jr, Yu SP, Choi DW. Transplantation of embryonic stem cells overexpressing Bcl-2 promotes functional recovery after transient cerebral ischemia. Neurobiol Dis. 2005; 19:183–193. [PubMed: 15837573]

Wei L, Fraser JL, Lu ZY, Hu X, Yu SP. Transplantation of hypoxia preconditioned bone marrow mesenchymal stem cells enhances angiogenesis and neurogenesis after cerebral ischemia in rats. Neurobiol Dis. 2012; 46(3):635–45. [PubMed: 22426403]

Wei L, Keogh CL, Whitaker VR, Theus MH, Yu SP. Angiogenesis and stem cell transplantation as potential treatments of cerebral ischemic stroke. Pathophysiology. 2005b; 12:47–62. [PubMed: 15927824]

Wei L, Rovainen CM, Woolsey TA. Ministrokes in rat barrel cortex. Stroke. 1995; 26:1459–1462. [PubMed: 7631353]

Wei L, Ying DJ, Cui L, Langsdorf J, Yu SP. Necrosis, apoptosis and hybrid death in the cortex and thalamus after barrel cortex ischemia in rats. Brain research. 2004; 1022:54–61. [PubMed: 15353213]

Wei N, Yu SP, Gu X, Taylor TM, Song D, Liu XF, Wei L. Delayed intranasal delivery of hypoxic-preconditioned bone marrow mesenchymal stem cells enhanced cell homing and therapeutic benefits after ischemic stroke in mice. Cell Transplant. 2013; 22:977–991. [PubMed: 23031629]

Wei ZZ, Gu X, Ferdinand A, Lee JH, Ji X, Ji XM, Yu SP, Wei L. Intranasal delivery of bone marrow mesenchymal stem cells improved neurovascular regeneration and rescued neuropsychiatric deficits after neonatal stroke in rats. Cell transplantation. 2015; 24:391–402. [PubMed: 25647744]

Weick JP, Johnson MA, Skroch SP, Williams JC, Deisseroth K, Zhang SC. Functional control of transplantable human ESC-derived neurons via optogenetic targeting. Stem Cells. 2010; 28:2008–2016. [PubMed: 20827747]

Werbowetski-Ogilvie TE, Bosse M, Stewart M, Schnerch A, Ramos-Mejia V, Rouleau A, Wynder T, Smith MJ, Dingwall S, Carter T, Williams C, Harris C, Dolling J, Wynder C, Boreham D, Bhatia M. Characterization of human embryonic stem cells with features of neoplastic progression. Nat Biotechnol. 2009; 27:91–97. [PubMed: 19122652]

Wernig M, Benninger F, Schmandt T, Rade M, Tucker KL, Bussow H, Beck H, Brustle O. Functional integration of embryonic stem cell-derived neurons in vivo. J Neurosci. 2004; 24:5258–5268. [PubMed: 15175396]

Whitaker VR, Cui L, Miller S, Yu SP, Wei L. Whisker stimulation enhances angiogenesis in the barrel cortex following focal ischemia in mice. J Cerebr Blood F Met. 2007; 27:57–68.

Williams RL, Hilton DJ, Pease S, Willson TA, Stewart CL, Gearing DP, Wagner EF, Metcalf D, Nicola NA, Gough NM. Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells. Nature. 1988; 336:684–687. [PubMed: 3143916]

Windle V, Szymanska A, Granter-Button S, White C, Buist R, Peeling J, Corbett D. An analysis of four different methods of producing focal cerebral ischemia with endothelin-1 in the rat. Exp Neurol. 2006; 201:324–334. [PubMed: 16740259]

Wise AF, Williams TM, Kiewiet MB, Payne NL, Siatskas C, Samuel CS, Ricardo SD. Human mesenchymal stem cells alter macrophage phenotype and promote regeneration via homing to the kidney following ischemia-reperfusion injury. American Journal of Physiology-Renal Physiology. 2014; 306:F1222–F1235. [PubMed: 24623144]

Wollert KC, Drexler H. Cell therapy for the treatment of coronary heart disease: a critical appraisal. Nat Rev Cardiol.

Woolsey TA, Rovainen CM, Cox SB, Henegar MH, Liang GE, Liu D, Moskalenko YE, Sui J, Wei L. Neuronal units linked to microvascular modules in cerebral cortex: response elements for imaging the brain. Cerebral Cortex. 1996; 6:647–660. [PubMed: 8921201]

Xiao AY, Wei L, Xia S, Rothman S, Yu SP. Ionic mechanism of ouabain-induced concurrent apoptosis and necrosis in individual cultured cortical neurons. The Journal of neuroscience. 2002; 22:1350–1362. [PubMed: 11850462]

Yan T, Ye X, Chopp M, Zacharek A, Ning R, Venkat P, Roberts C, Lu M, Chen J. Niaspan attenuates the adverse effects of bone marrow stromal cell treatment of stroke in type one diabetic rats. PLoS One. 2013; 8:e81199. [PubMed: 24303036]

Yanagisawa D, Qi M, Kim DH, Kitamura Y, Inden M, Tsuchiya D, Takata K, Taniguchi T, Yoshimoto K, Shimohama S, Akaike A, Sumi S, Inoue K. Improvement of focal ischemia-induced rat dopaminergic dysfunction by striatal transplantation of mouse embryonic stem cells. Neurosci Lett. 2006; 407:74–79. [PubMed: 16959414]

Yang D, Zhang ZJ, Oldenburg M, Ayala M, Zhang SC. Human Embryonic Stem Cell - Derived Dopaminergic Neurons Reverse Functional Deficit in Parkinsonian Rats. Stem Cells. 2008; 26:55–63. [PubMed: 17951220]

Yin F, Battiwalla M, Ito S, Feng X, Chinian F, Melenhorst JJ, Koklanaris E, Sabatino M, Stroncek D, Samsel L. Bone marrow mesenchymal stromal cells to treat tissue damage in allogeneic stem cell transplant recipients: correlation of biological markers with clinical responses. Stem Cells. 2014; 32:1278–1288. [PubMed: 24452962]

Yoon SH, Shim YS, Park YH, Chung JK, Nam JH, Kim MO, Park HC, Park SR, Min BH, Kim EY, Choi BH, Park H, Ha Y. Complete spinal cord injury treatment using autologous bone marrow cell transplantation and bone marrow stimulation with granulocyte macrophage-colony stimulating factor: Phase I/II clinical trial. Stem Cells. 2007; 25:2066–2073. [PubMed: 17464087]

Yu D, Silva GA. Stem cell sources and therapeutic approaches for central nervous system and neural retinal disorders. Neurosurg Focus. 2008; 24:E11.

Yu HM, Zhi JL, Cui Y, Tang EH, Sun SN, Feng JQ, Chen PX. Role of the JAK-STAT pathway in protection of hydrogen peroxide preconditioning against apoptosis induced by oxidative stress in PC12 cells. Apoptosis. 2006; 11:931–941. [PubMed: 16547593]

Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007; 318:1917–1920. [PubMed: 18029452]

Yu SP, Wei Z, Wei L. Preconditioning strategy in stem cell transplantation therapy. Translational stroke research. 2013; 4:76–88. [PubMed: 23914259]

Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, Gerdoni E, Giunti D, Ceravolo A, Cazzanti F, Frassoni F, Mancardi G, Uccelli A. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood. 2005; 106:1755–1761. [PubMed: 15905186]

Zhang L, Schallert T, Zhang ZG, Jiang Q, Arniego P, Li Q, Lu M, Chopp M. A test for detecting long-term sensorimotor dysfunction in the mouse after focal cerebral ischemia. J Neurosci Methods. 2002; 117:207–214. [PubMed: 12100987]

Zhang P, Li J, Liu Y, Chen X, Kang Q. Transplanted human embryonic neural stem cells survive, migrate, differentiate and increase endogenous nestin expression in adult rat cortical peri-infarction zone. Neuropathology. 2009a

Zhang RL, Chopp M, Gregg SR, Toh Y, Roberts C, Letourneau Y, Buller B, Jia L, SPND, Zhang ZG. Patterns and dynamics of subventricular zone neuroblast migration in the ischemic striatum of the adult mouse. J Cereb Blood Flow Metab. 2009b; 29:1240–1250. [PubMed: 19436318]

Zhang RL, Chopp M, Zhang ZG, Jiang Q, Ewing JR. A rat model of focal embolic cerebral ischemia. Brain Res. 1997; 766:83–92. [PubMed: 9359590]

Zhang SC, Wernig M, Duncan ID, Brustle O, Thomson JA. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. Nat Biotechnol. 2001; 19:1129–1133. [PubMed: 11731781]

Zhao LR, Duan WM, Reyes M, Keene CD, Verfaillie CM, Low WC. Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats. Exp Neurol. 2002; 174:11–20. [PubMed: 11869029]

Zhao T, Zhang ZN, Rong Z, Xu Y. Immunogenicity of induced pluripotent stem cells. Nature. 2011; 474:212–215. [PubMed: 21572395]

Zhou J, Su P, Li D, Tsang S, Duan E, Wang F. High-efficiency induction of neural conversion in human ESCs and human induced pluripotent stem cells with a single chemical inhibitor of transforming growth factor beta superfamily receptors. Stem Cells. 2010; 28:1741–1750. [PubMed: 20734356]

Ziv Y, Avidan H, Pluchino S, Martino G, Schwartz M. Synergy between immune cells and adult neural stem/progenitor cells promotes functional recovery from spinal cord injury. Proc Natl Acad Sci U S A. 2006a; 103:13174–13179. [PubMed: 16938843]

Ziv Y, Ron N, Butovsky O, Landa G, Sudai E, Greenberg N, Cohen H, Kipnis J, Schwartz M. Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. Nat Neurosci. 2006b; 9:268–275. [PubMed: 16415867]

Highlights

- A comprehensive review of cell-based regeneration therapy for ischemic stroke.

- Summary of different stem cells in the transplantation therapy.

- Summary of different stroke models for regenerative research.

- Introduction of the mechanism of action of transplanted cells.

- Introduction of strategies for promoting cell survival and regenerative properties.

- Introduction of innovative approaches in cell transplantation and translational research.
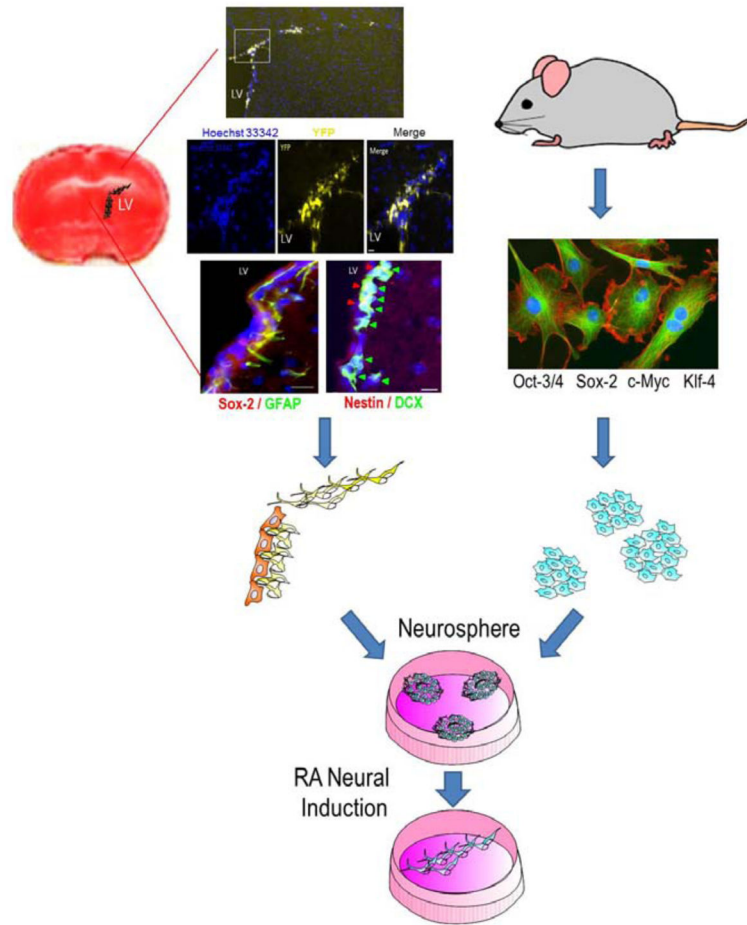
Figure 1. Neural stem cells from the SVZ and induced pluripotent stem cells from somatic cells
The brain coronal section with TTC staining (red) shows the SVZ region.
Immunohistochemical images show multiple markers for neural stem cells in the lateral
ventricle SVZ 14 days after an ischemic insult. Using Nestin-Cre-ER^{T2}/Rosa-YFP
transgenic mice, the YFP+ cells indicate neural stem cells from the SVZ. Neural progenitors
were DCX+ and Nestin+ while neural stem cells were GFAP+ and SOX-2+. Hoechst
staining (blue) shows cell nuclei in the lateral ventricle SVZ. These neural stem/progenitors
cells can be isolated, cultured and differentiated into neurons. Induced pluripotent stem (iPS)
cells can be induced from rodents or humans using a set of reprogramming factors such as
Oct-3/4, Sox2, cMyc, and Klf4. iPS cells can also be expanded and differentiated into
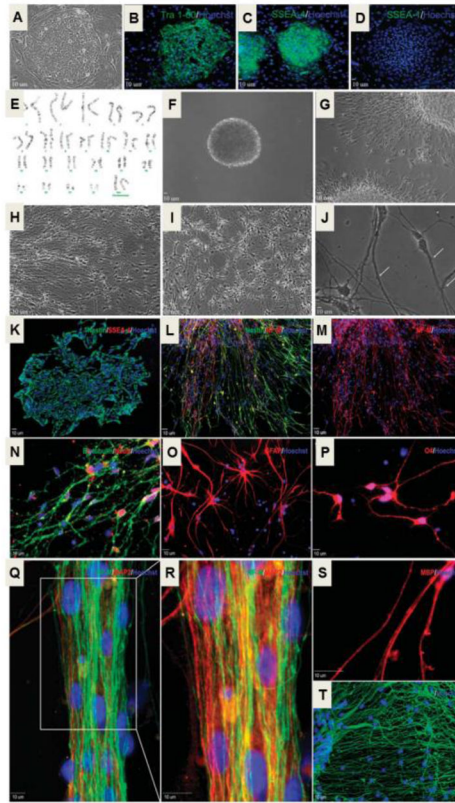different cell types including neurons.

Figure 2. Neural gene expression in differentiating human ES cell-derived neural progenitor cells
Neural differentiation of UCO6 human ES cells (hESCs). **A.** Typical colony of hESCs (passage 70). **B**–**D.** Colonies express pluripotent markers TRA-1-60 (**B**) and SSEA4 (**C**), but are negative for SSEA-1 (**D**). **E.** Normal karyotypic analysis of passage 75 UCO6 colonies. **F.** Twenty-eight day old floating neurospheres (~200 μm in size). **G**–**J.** After beginning terminal differentiation, cells exhibit increasingly neuronal morphology after 1 day (**G**), 7 days (**H**) and 14 days (**I**). Cells exhibit significant neurite extensions and many dendritic spines form (white arrows) after 21 days of terminal differentiation (**J**). **K.** Cells exhibit high expression of the neural precursor marker Nestin (green) and very low expression of SSEA-4 (red) 1 day after cell adhesion. **L** and **M.** Three days into terminal differentiation, Nestin (green) positive, elongated cells begin to express medium chain neurofilament (NF-M; red). **N.** Seven days after plating, the neuronal markers βIII-tubulin (green) and NeuN (red) were highly expressed. **O.** Expression of the astrocyte marker GFAP is observed after 7 days. **P.** Very few O4-positive immunoreactive cells were found in these cells. **Q** and **R.** Extensive NF-M (green) and microtubule-associate protein-2 (MAP2; red) immunoreactive projections were present in cells of at 21 days into differentiation. **S.** Twenty eight days after plating, some projections are positive for myelin-binding protein (MBP), indicating axonal myelination. **T.** Extensive beds of GFAP-positive astrocytes can also be observed after 28 days into terminal differentiation. All blue staining indicates Hoechst-positive nuclei. Adopted from Figures 1 and 2 in Francis and Wei, 2010 in compliance with the journal's copyright policy.
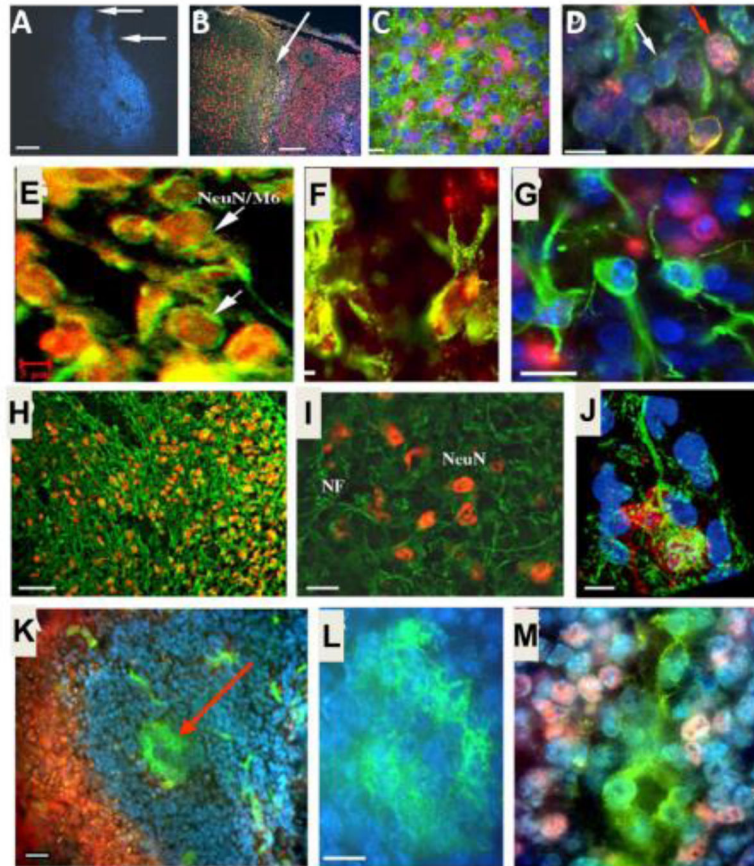
Figure 3. ES cell survival and differentiation after transplantation into the ischemic brain
Immunohistochemical staining 7 days after intracranial transplantation (14 days post-ischemia). **A**. Distribution of transplanted ES cells in the post-ischemic basal ganglia revealed by pre-labeled Hoechst (blue) staining. **B**. Distribution of transplanted ES cells in the post-ischemic cortex. Hoechst-positive ES cells (blue) were present next to undamaged tissue (arrow and the area to the left). NeuN (red) staining shows endogenous neurons in non-ischemic cortical region (left of the arrow) and differentiated neuron-like cells in the ischemic region (right of the arrow). The NeuN staining on the right appears pink in color due to overlap with the blue Hoechst 33342 staining. **C**. Mouse cell-specific antibodies M2 and M6 (both green) and Hoechst 33342 staining (blue) verified the transplant origin of cells surviving 7 days in the rat host ischemic cortex. NeuN staining (pink) identified differentiated neuronal cells. **D**. Higher magnification of a confocal image shows the double labeling of Hoechst (blue) and mouse antibody M2/M6 (green) that confirms murine origin of these cells (white arrow). The triple-labeled cells with the additional staining of NeuN (pink) identify ES cell-derived neurons (red arrow). **E**. Confocal images of NeuN staining (red), mouse antibody M6 staining (green), and overlapped double staining (yellow) show neuronal cells originated from transplanted ES cells in a transplantation area. Scale bar equals 800, 200, 20, 10 and 5 μm, in A, B, C, D and E, respectively. **F.** and **G.** Differentiations of transplanted ES cells in the host ischemic cortex and striatum. ES cells were labeled with BrdU or Hoechst 33342 prior to transplantation. The double labeling of

BrdU (red) and GFAP (green) identified differentiated astrocytes of ES cell origin (**F**). An image taken from (**G**) an 8-μm section shows Hoechst-prelabeled ES cells double stained either with NeuN (red) or GFAP (green), consistent with ES cell differentiation into neurons and astrocytes. Scale bar = 20 μm. **H–J**. Dendrite growth after ES cell transplantation in the ischemic cortex. NeuN-positive cells and apical dendrite distribution in the ipsilateral cortex. Seven weeks after ES cell transplantation, NeuN and NF double immunostaining in the ischemic core region of the ipsilateral cortex under an inverted fluorescence microscope. Many NeuN-positive cells (yellow or orange) were surrounded by NF labeled processes (green). A confocal image of NeuN/NF-positive ES cell in the 8-Am-thick slice of ischemic cortex. The cell body was positive to NeuN staining (red), cell processes were positive to NF staining (green). The Hoechst labeling (blue) showed several transplanted ES cells. Scale bar = 60 μm in **H**, 20 μm in **I**, 10 μm in **J**. **K–M**. ES cell-derived Glut-1-positive cells in the ischemic region. Seven days after transplantation, the ischemic core was filled with ES cells (Hoechst-positive, blue) and contained Glut-1-positive vascular-like structures of endothelial cells (green; arrow). Scale bar = 50 μm. Enlarged view of a vascular structure in **K** (**L**), positively stained with Glut-1 (green). Scale bar = 10 Am. Glut-1-positive endothelial cells (green) were also co-labeled with Hoechst (blue), verifying their origination from transplanted ES cells (**M**). The pink color is from neighboring NeuN-positive cells. Images were taken using an inverted fluorescence microscope. Adopted from Figures in Wei et al., 2005 in compliance with the journal's copyright policy.
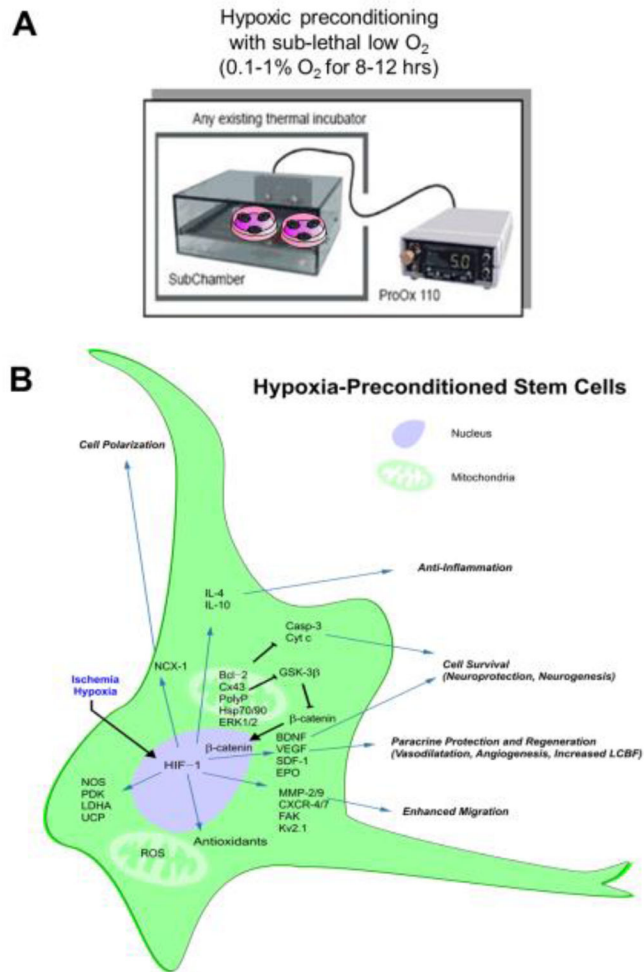
Figure 4. Hypoxic preconditioning of stem cells or neural progeinitor cells and the mechanis of action

A. Neuronally-differentiated cells or BMSCs were subjected to hypoxic preconditioning priming treatment before to be used for cell therapy. Cells were placed in a hypoxic chamber (0.1–1% O2) for 8–12 hrs before transplantation. **B**. Potential mechanisms underlying the beneficial effects of hypoxic preconditioning. The hypoxic preconditioning strategy was designed to mimic and utilize endogenous protective mechanisms to promote neuroprotection, tissue regeneration and brain function recovery. Hypoxic preconditioning directly induces HIF-1α/β upregulation that increases BDNF, SDF-1, VEGF, EPO and many other genes which can stimulate neurogenesis, angiogenesis, vasodilatation and increase cell survival. HIF-1 expression regulates antioxidants, survival signals and other genes related to cell adhesion, polarization, migration, and anti-inflammatory responses. Abbreviation in the figure: BDNF, brain-derived neurotrophic factor; Casp, caspase; Cx43, connexin-43; CXCR, CXC chemokine receptor; Cyt c, cytochrome c; EPO, erythropoietin; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; GSK-3β, glycogen synthase kinase-3β; HIF-1, hypoxia-inducible factor-1; Hsp, heat shock protein; IL-4, interleukin-4; IL-10, interleukin-10; MMP, matrix metalloproteinase; NCX-1, sodium-calcium exchanger-1; NOS, nitric oxide synthase; PDK, pyruvate dehydrogenase kinase; polyP, polyphosphate;

ROS, reactive oxygen species; SDF-1, stromal-derived factor-1; UCP, uncoupling protein; VEGF, vascular endothelial
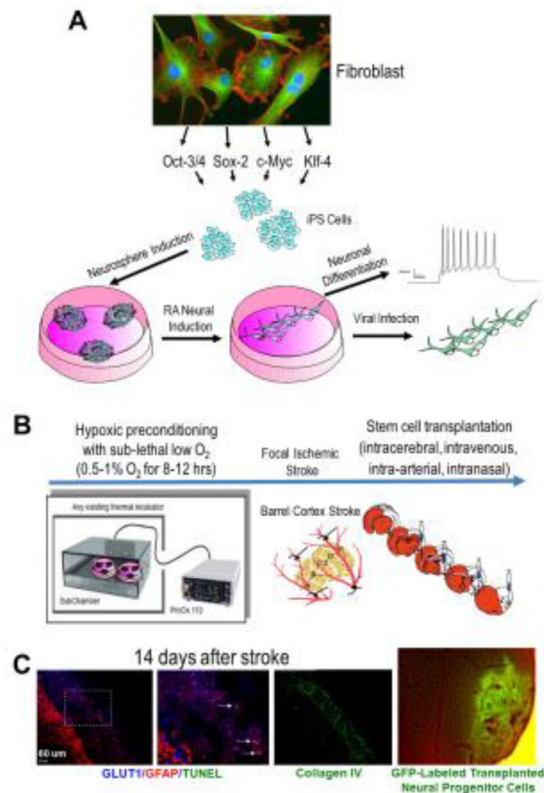
Figure 5. Neuronal differentiation of iPS cells with hypoxic preconditioning and transplantation into the ischemic brain

A. Somatic cells such as fibroblasts can be reprogrammed into pluripotent stem cells with specific transcription genes. These cells can be differentiated into mature and fucntional neurons, firing repetitive actiona potentials and can be manipulated with gene modification. **B**. At the later stage of neural induction, cells are subjected to non-lethal hypoxic preconditioning treatment and then ready for translantation into the ischemic brain. **C**. Demonstration of implanted cells in the ischemic core and peri-infect region. Immunohistochemial staining show remaining and/or regenerated vasculartures (GLUT1 (blue) and Collagen IV (green) staining) and the formation of a glia score (GAFP, red) surrounding the ischemic core 14 days after sensorimotor cortex stroke. Green flourescence GFP labeled iPS-derived neural progenitor cells were implanted into the ischemic core and peri-infarct regions;. these hypoxic preconditioned cells survived well several weeks after transplantation.
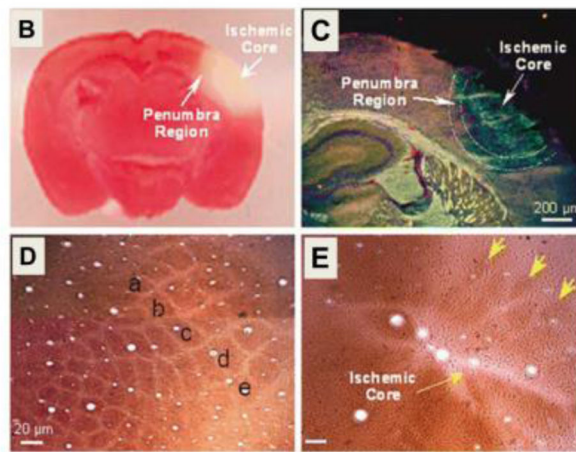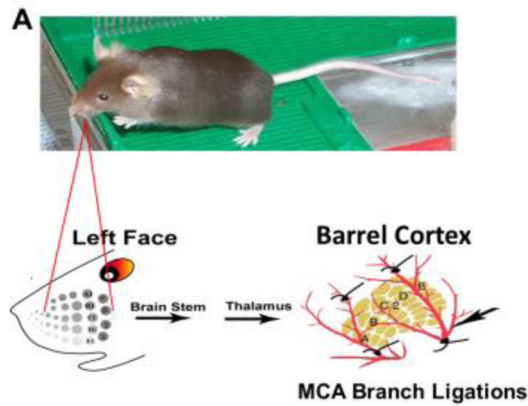
Figure 6. Small stroke model of barrel cortex ischemia in rodents
A. Focal ischemic insult to the barrel cortex is induced by permanent occlusion of the distal branches of the right or left middle cerebral artery combined with transient ligations of bilateral common carotid arteries. The barrel cortex is identified by the optical imaging in responding to whisker stimulation. **B.** Ischemia-induced barrel cortex infarct formation and cell death. Middle cerebral artery branch ligations induced a focal ischemic infarct and cell death in the barrel cortex. Selective damage occurred to the right barrel cortex region shown as a negative (white) area in triphenyl tetrazolium staining (red) 24 hrs after ischemia. The pink area between the normal cortex and ischemic core represents the bordering penumbra area. **C.** In the immunohistochemical image, Glut-1 staining (red) shows vascular endothelial cells in the penumbra and normal brain tissues. NeuN (blue) indicates neurons. Ischemic core shows the autofluorescence associated with damaged tissue. **D** and **E.** Cytochrome oxidase staining revealed the barrel column structures in the context of a normal cortex (**D**) and undergone ischemic damage (**E**). The lowercase letters "a"–"e" in image **D** illustrate the characteristic pattern of barrel distribution in the cortex. Fourteen days after ischemia, most of the barrel column structures disappear except a few barrel-like clumps (arrowhead) preserved in the peripheral region of the barrel cortex (**E**).
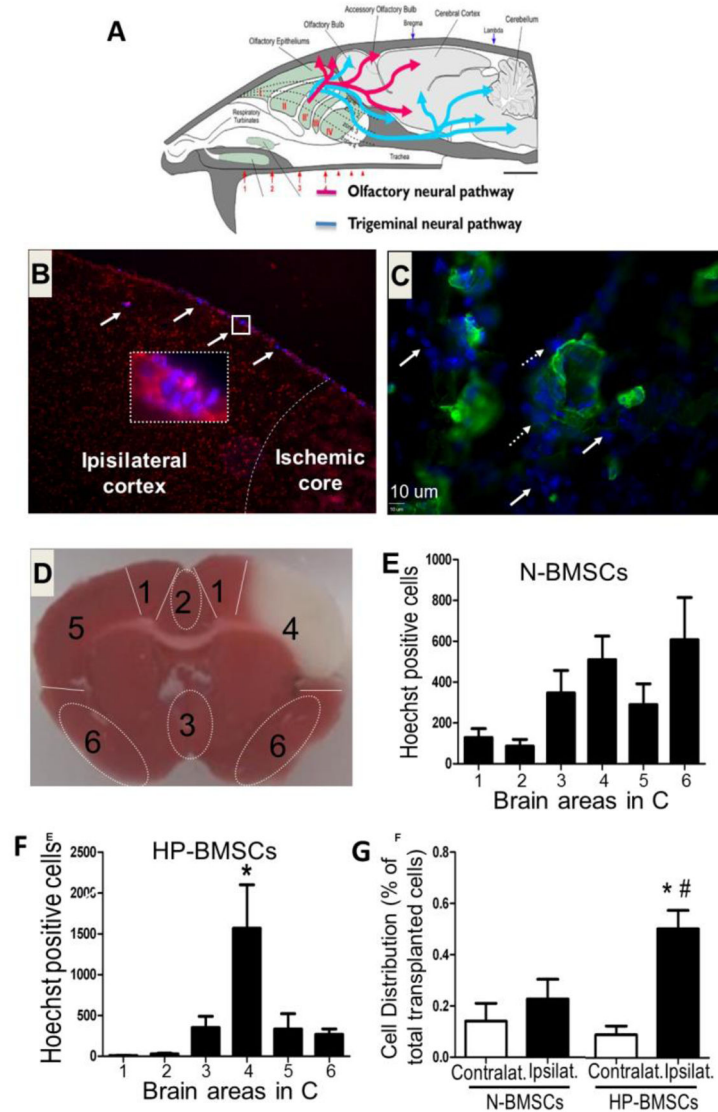
Figure 7. Distribution of BMSCs after intranasal delivery into the ischemic brain and the effect of hypoxic preconditioning

The intranasal administration is an established method for drug delivery. **A**. The drawing illustrates how therapeutics may bypass the BBB via from the intranasal space and distribute in brain regions. **B**. Immunohistochemical staining reveals the distribution of transplanted cells after intranasal delivery of BMSCs in a stroke mouse brain. BMSCs (blue color from pre-labeled Hoechst 33342) were seen in the cortex near the ischemic core. The morphology of pre-labeled cells can be better seen under higher magnification (400 X, magenta cells, inserts images). **C**. collagen IV (green) was used to show brain blood vessels. Transplanted cells (blue) were found inside vessels, lining vessels (dashed arrows), or deposited outside the vessels (arrows). **D.** Distribution of BMSCs in different brain regions was measured and quantified by counting Hoechst 33342 dye positive cells in different brain regions. The assigned number for each brain area is shown in the brain section stained with TTC. Area 4 is the ischemic core region. **E** and **F**. N-BMSCs or HP-BMSCs (1 X 10^6) were delivered via

intranasal administration 24 hrs after stroke. One and half hrs later, host brain cells (propidium iodide, PI, red, see B) and transplanted BMSCs (Hoechst 33342, blue) were counted. N-BMSCs showed a random distribution pattern (E) while HP-BMSCs (F) selectively home to the ischemic cortex (area 4 in D). **G**. The bar graphs summarize the percentage of cells that reached the ischemic brain region 1.5 hrs after intranasal delivery compared to all the cells found in the other brain region. Significantly higher percentage of HP-BMSCs was seen in the ispilateral side of the brain. N=6, *. $p<0.05$ vs. HP-BMSC in contralateral brain, #. $p<0.05$ vs. N-BMSC in the ispilateral brain (I think * and # need to be clarified). Revised from Figures in Wei *et al.*, 2013 in compliance with the journal's copyright policy.
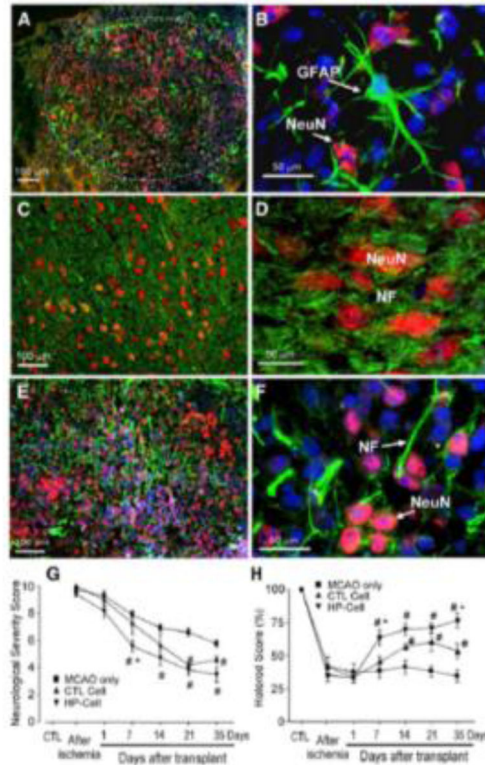
Figure 8. Neural differentiation of transplantation of ES-NPCs and functional benefits after stroke

Neural differentiation of transplanted HP-ES-NPCs was examined 14 days after transplantation into the ischemic rat brain. **A.** Hoechst 33342 staining (blue) illustrated transplanted HP-ES-NPCs in the ischemic region. HP-ES-NPCs differentiated in vivo into NeuN-positive cells (red) and GFAP-positive (green) astrocytes. The circular dotted line demarcates the transplantation area. **B.** High magnification image showing co-localization of NeuN (red) or GFAP (green) with Hoechst 33342 (blue). **C–F.** NeuN-positive cells and neurite distribution in the contralateral cortex (**C** and **D**) and ipsilateral cortex (**E** and **F**). In the contralateral non-ischemic cortex, normal neurons were stained with NeuN, appearing red/orange. Neuronal processes were labeled with neurofilament (NF, green). In the ipsilateral cortex, NeuN or NF double immunostaining with pre-labeled Hoechst 33342 (blue) was apparent under a fluorescence microscope. Many NeuN-positive cells (red or pink when co-labeled with Hoechst 33342) were surrounded by NF-labeled processes (green), although the distribution of these processes was less organized compared to the contralateral side. Note the substantially smaller ES-derived NeuN-positive cells in **E** and **F** compared to the native cells in **C** and **D**. Neurological and motor functions were examined after transplantation with no-HP control progenitor cells and HP-treated ES-NPCs. **G.** Neurological Severity Score was tested up to 35 days after transplantation. Normal non-ischemic rats have a NSS near zero while the NSS for rats several days after stroke was 9–10. Transplantation of either control cells or HP-progenitor cells improved functional recovery 21 days after ischemia while animals without ES cell transplantation had limited recovery. HP-ES-NPC transplantation, however, afforded significant functional recovery as

early as 7 days after transplantation while at this time rats that received control cells showed no difference from culture media group. **H.** Cell transplantation enhanced motor ability after stroke. Scores were compared to baseline data generated before stroke and are represented as % of control. Animals receiving HP-ES-NPCs had accelerated motor function recovery; at day 7 and 35 after transplantation the testing score was significantly better than that of medium and control cell groups. #. $P < 0.05$ compared with MCAO only; *$P < 0.05$ compared with control cell transplant; n=5–8 rats per group. Adopted from Figure 8 in Theus *et al.*, 2008a in compliance with the journal's copyright policy.

Table 1

Focal ischemic stroke models.

Model	Surgical procedures	Species/Ages	Major pathological change	Reference
Photothrombosis	intravascular photocoagulation and inducing occlusion of the irradiated vessels with secondary tissue ischemia	Rat/Adult	Localized focal injury that may depend on injection site. Massive T cell and macrophage infiltration, controlled lesion in cortical areas, fast blood-brain barrier disruption and vasogenic edema	(Rosenblum and El-Sabban, 1977); (Watson *et al.*, 1985)
MCA Occlusion	thrombembolic MCAO: injecting clots that were formed in vitro or by endovascular instillation of thrombin for in situ clotting	Rat/Adult/Neonate Mouse/Adult Dogs/Adult Sheep/Adult Monkey/ Adult	Severe stroke model can cause half hemisphere damage in suture MCAO; Vascular endothelial damage, platelet activation and subsequent thrombotic vessel occlusion	(Tamura *et al.*, 1981a); (Nagasawa and Kogure, 1989); (Chen *et al.*, 1986); (Boltze *et al.*, 2008); (Fan *et al.*, 2012)
Endothelin Stroke	cortical application of ET-1	Rat/Adult	Necrotic, apoptotic and hypertrophic neurons and neurons with pyknosis in surrounding penumbra	(Robinson *et al.*, 1990); Masaki et al (1992); Macrae et al. (1993); (Sharkey *et al.*, 1993); Fuxe et al. (1997)
Barrel Cortex Stroke	permanently ligating 3 to 6 branches of MCA, temporarily occluding CCA	Mouse/6–8 wk; Rat/Adult/Neonate	First ischemic model specifically targeting the barrel cortex and suitable for whisker functional studies; Infarct formation in rodent somatosensory barrel cortex, vascular endothelial damage, and platelet activation	(Wei *et al.*, 1995); (Luo *et al.*, 2008); (Jiang *et al.*, 2016)

Abbreviation in the Table: BBB, blood-brain barrier; BMSC, bone marrow mesenchymal stem cell; CCA, common carotid artery; ES, embryonic stem cells; ET-1, endothelin-1; iPS, induced pluripotent stem cells; LCBF, local cerebral blood flow; MCA, middle cerebral artery; NSC, neural stem cells; PN, postnatal.

Table 2

Cell transplantation therapy research in ischemic and hemorrhage stroke models.

Administration route	Initiation time point	Cell type/Dose	Species/Model	Outcome	Mechanism	Reference
Intracerebral	1 months	NT2N line/0.8m	Rat/tMCAO	Motor function recovery	Biobridge, cell replacement, induced growth and trophic support	(Borlongan et al., 1997; Liska et al., 2017)
	7 days	hBMSC	Rat/tMCAO	Sensorimotor recovery	Induced growth and trophic support	(Zhao et al., 2002); (Burns et al., 2009); (Kurozumi et al., 2004)
	14 days	MHP36 line/0.2m/8 μl	Rat/tMCAO	Sensorimotor recovery	Cell replacement	(Veizovic et al., 2001); (Modo et al., 2002b)
	7 days	hBMSC	Rat/ICH	Sensorimotor recovery	Induced growth and trophic factors	(Nagai et al., 2007)
	7 days	hNSC/	Mouse/ICH	Motor function recovery	Cell replacement	(Lee et al., 2007)
	7 days	hNSC/0.8m/2 μl	Rat/Endothelin Stroke	Motor function recovery	Cell replacement	(Abeysinghe et al., 2015)
	7 days	hES/0.2m/4 μl	Mouse/Barrel Stroke	Sensorimotor recovery	Cell replacement	(Drury-Stewart et al., 2013)
	7 days	rESC/0.1m	Rat/MCAO	Survival and differentiation of grafts	Cell replacement	(Buhnemann et al., 2006)
Intracranial	7 days	miPS/0.4m/4 μl	Rat/Barrel Stroke	Sensorimotor recovery	Cell replacement	(Chau et al., 2014)
Intravenous		rBMSC/1m/1 ml	Rat/tMCAO	Reducing hemorrhagic transformation, motor function recovery	Inhibit endothelial dysfunction	(Nakazaki et al., 2017)
	24 hrs	hUCBC/	Rat/tMCAO	Sensorimotor recovery	Cell replacement	(Chen et al., 2001b)
	24 hrs	rMSC/3m	Rat/tMCAO	Sensorimotor recovery	Reducing cell death, induced growth and trophic factors	(Chen et al., 2003)
	-	hNSC	Rat	-	Cell replacement	; (Chu et al., 2005b)
		hNSC/5m/500 μl	Rat/ICH	Sensorimotor recovery	-	(Jeong et al., 2003)
Intra-arterial	1 hrs	rBMSC/1m/1 ml	Rat/tMCAO	Reducing infarct	Induced growth and trophic factors	(Toyoshima et al., 2015)
	24 hrs	hBMSC/1,000	Rat/tMCAO	Sensorimotor recovery	Reducing inflammation	(Fukuda et al., 2015)
Intranasal	6 hrs	rBMSC/1m/100 μl	Rat/Barrel Stroke	Reducing infarct, sensorimotor recovery, improved olfactory functions, social and neuropsychiatric benefits	Induced growth and trophic factors	(Wei et al., 2015)

Abbreviation in the Table: BMSC, bone marrow mesenchymal stem cell; ES, embryonic stem cells; ICH, intracerebral hemorrhage; iPS, induced pluripotent stem cells; m indicates million cells; MCAO, middle cerebral artery occlusion; NSC, neural stem cells; UCBC, umbilical cord blood cells.

Table 3

Stroke clinical trials using stem cell therapy.

Trials	Initiation year	Country/Region	Cell source/Dose	Administration route	Population	Outcome	Status	Reference
Safety			NT2/D1	Intracerebral	Basal ganglia stroke	Feasible	Completed	(Kondziolka et al., 2000),(Nelson et al., 2002)
	2001	USA	NT2/D1	Intracerebral	Stroke patients	Feasible with small risk of seizure	Completed	(Kondziolka et al., 2005)
			MSC	Intravenous	MCA			(Bang et al., 2005a)
	2005	USA	ES	Intracerebral	Ischemic stroke patients	2/5 patients showed improvements	Terminated	(Savitz et al., 2005)
	2008	India	BMMNC	Intrathecal	Stroke patients		Completed	NCT02065778
	2009	Cuba	BMSC	Intracerebral	Stroke patients	Good tolerance and safety	Completed	(Suarez-Monteagudo et al., 2009)
	2010	Brazil	BMMNC	Intra-arterial	Nonacute ischemic stroke	Feasible and safe	Completed	(Battistella et al., 2011)
	2011	Japan	MSC	Intravenous	Stroke patients	Feasible and safe	Completed	(Honmou et al., 2011)
	2012	Hong Kong	UCBMC	Intracranial	Stroke in the middle cerebral artery territory and stable hemiplegia or hemiparesis	N/A	Completed	NCT01673932
	2012	China	BMSC	Intracerebral	Ischemia stroke or ICH patient	No adverse effects observed; 87% functional/neurological improvement	Completed	(Li et al., 2013b)
	2012	Brazil	BMMNC	Intra-arterial	MCA acute ischemic stroke	Safe	Completed	(Friedrich et al., 2012)
	2010	France	MSC	Intravenous	Carotid ischemic stroke patients		Ongoing	NCT00875654
	2010	UK	NSC	Intracranial	Stroke patients		Ongoing	(Kalladka et al., 2016)
	2011	Taiwan	OEC	Intracerebral	Thromboembolic Stroke		Recruiting	NCT01327768
	2012	China	HSC	Intra-arterial	Internal carotid artery territory infarction	N/A	Recruiting	NCT01518231
	2014	Spain	BMMNC/2m/kg or 5m/kg	Intra-arterial	Moderate-to-severe acute ischemic stroke patients	Appears to be safe; 30% clinical improvement at 90 days	Recruiting	(Moniche et al., 2015)
	2014	Mexico	NSC	Intravenous	Ischemic stroke		Ongoing	www.Novastem.mx
	2014	China	NSC	Intracerebral	Chronic ischemia stroke		Completed	www.Neuralstem.com

Trials	Initiation year	Country/Region	Cell source/Dose	Administration route	Population	Outcome	Status	Reference
	2014	China	EPC	Intravenous	Chronic ischemia stroke		Recruiting	NCT01468064
	2015	China	UCMSC/20m	Intravenous	ICH		Ongoing	NCT02283879
	2016	China	UCMSC	Intravenous	Intracerebral ischemic stroke		-	NCT02580019
	2016	China	MSC	Intracerebral	ICH		Recruiting	NCT02767817
	2016	USA	BMSC	Intravenous/Intranasal	functional damage to CNS/PNS		Recruiting	NCT02795052
	2016	Taiwan	ADSC	Intracerebral	Stroke patients		-	NCT02813512
Efficacy	2008	Japan	BMMNC/25 ml	Intravenous	Stroke patients		Completed	(Toyoda, 2013)
	2009	Taiwan	CD34+ Stem Cell	Intracerebral	Chronic stroke adult patient		Completed	(Mackie a.,nd Losordo, 2011); NCT01438593, NCT01249287
	2011	China	BMSC, EPC/2.5m/kg/100 ml	Intravenous	Ischemic lesion within the MCA territory		Ongoing	NCT01468064
	2011	USA	BMSC/2.5m 5.0m or 10m	Intracranial	Chronic stroke patients	No serious adverse events attributable and significant improvements in motor impairment	Completed	www.san-bio.com
	2014	UK	NSC	Intracerebral	Stroke patient Phase II	Strongly positive results for 12 months; Well-tolerated/no serious adverse events	Ongoing	www.reneuron.com; NCT02117635
	2014	India	BMMNC	Intravenous	Ischemic stroke Phase II	Safe but no beneficial effect	Recruiting	(Prasad *et al.*, 2014); NCT02245698
	2016	US/UK	MultiStem cells	Intravenous	Ischemic stroke Phase II	Excellent; 12-month functional improvement	Completed	www.athersys.com
	2016	Europe	ADSC/1m/kg	Intravenous	Hemispheric ischemic stroke		Recruiting	NCT02849613
Effectiveness	2013	China	MSC	Intrathecal	Cerebral palsy		Recruiting	NCT01929434

This Table may not include all current clinical trials. The reference list includes published papers, searchable websites or ClinicalTrials.gov Identifiers. Abbreviation in the Table: ASC, Adipose-derived Stromal Cells; BMSC, bone marrow mesenchymal stem cell; EPC, endothelial progenitor cells; ES, embryonic stem cells; ICH, intracerebral hemorrhage; iPS, induced pluripotent stem cells; m indicates million cells; MCAO, middle cerebral artery occlusion; NSC, neural stem cells; UCBMC, umbilical cord blood mononuclear cells; UCMSC, umbilical cord mesenchymal stem cells.