Published in final edited form as:
Neurobiol Dis. 2010 February ; 37(2): 275. doi:10.1016/j.nbd.2009.10.003.

Optimizing the Success of Cell Transplantation Therapy for Stroke

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Abstract

Stem cell transplantation has evolved as a promising experimental treatment approach for stroke. In this review, we address the major hurdles for successful translation from basic research into clinical applications and discuss possible strategies to overcome these issues. We summarize the results from present pre-clinical and clinical studies and focus on specific areas of current controversy and research: (i) the therapeutic time window for cell transplantation; (ii) the selection of patients likely to benefit from such a therapy; (iii) the optimal route of cell delivery to the ischemic brain; (iv) the most suitable cell types and sources; (v) the potential mechanisms of functional recovery after cell transplantation; and (vi) the development of imaging techniques to monitor cell therapy.

Keywords

Stroke; stem cells; transplantation; regenerative medicine; translational studies

Introduction

Stem cell transplantation offers an exciting new therapeutic avenue for stroke not only to prevent damage, which has been the focus of conventional therapeutic strategies, but also to actually repair the injured brain. Cell transplantation has shown much promise in experimental models of stroke with a diverse array of cell types including brain-, bone marrow-, and blood-derived progenitors reported to enhance functional recovery after ischemic (reviewed in Bliss et al., 2007) and hemorrhagic stroke (reviewed in Andres et al., 2008b). Such results led to early Phase I and II clinical trials (Table 1; reviewed in Andres et al., 2008a; Locatelli et al., 2009; Wechsler, 2009) using a cell line of immature neurons (hNT) derived from a human teratocarcinoma, fetal porcine cells, or autologous mesenchymal stem cells (MSCs). These studies focused on the safety and feasibility of cell transplantation therapy. No cell-related adverse effects were reported with the hNT (Kondziolka et al., 2005; Kondziolka et al., 2000) and MSC transplants (Bang et al., 2005). *However, 2 of the 5 patients receiving the porcine cells either developed seizures or aggravation of motor deficits (*Savitz et al., 2005*); the contribution of the cell therapy to these adverse effects is unclear. Conclusions about efficacy of the different treatments are difficult to draw due to small sample sizes for each trial. Moreover, these studies were not designed to determine whether treatment improved outcome; however, notable improvements were observed in some patients with stable chronic deficits in some of these studies.* Clearly, cell transplantation for stroke is still in its infancy and much

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more work is needed to make it a viable therapy. In this article we discuss critical inter-related issues that need to be addressed to optimize the success of this approach.

Therapeutic Time Window

A major promise of cell therapy is that it will open the therapeutic time window of intervention, thus benefiting a significantly larger patient population. The literature reports a wide range of successful stroke-to-transplantation intervals. The majority of pre-clinical studies have transplanted within the first 3 days after stroke and these have mostly used bone marrow- or blood-derived cells (reviewed in Bliss et al., 2007; Guzman et al., 2008a; Locatelli et al., 2009). This time window is already greater than the 3–6h window required for t-PA therapy, the only treatment for stroke that currently exists. Cell enhanced recovery has also been reported with sub-acute (1 week post-stroke) and chronic (> 3 weeks post-stroke) delivery of many cell types including neural cells (Borlongan et al., 1998; Chen et al., 2001; Daadi et al., 2008; Pollock et al., 2006; Shen et al., 2007b; Zhao et al., 2002). However, comparison of the results to identify an optimum time for transplantation is difficult as the studies used different models of stroke, cell types, methods of cell delivery, and behavioral tests to assess efficacy. This highlights the need for a more methodical and standardized approach to pre-clinical research so that direct comparisons can be made between individual studies.

The optimum time for transplantation may be dependent on the cell type used and their mechanism of action. If a treatment strategy focuses on neuroprotective mechanisms, acute delivery will be critical. If the cells act to enhance endogenous repair mechanisms (e.g. plasticity and angiogenesis), then sub-acute delivery would be pertinent as these events are more prevalent in the first few weeks after ischemia (Carmichael, 2006; Hayashi et al., 2003). The route of delivery (discussed later) may also dictate the timing of transplantation. Intravascular transplantation may require early administration as the cells use inflammatory signals to home to the injured brain (Guzman et al., 2008a; Guzman et al., 2008b; Park et al., 2009; Pluchino et al., 2005), although MSCs were also found in the brain after late intravascular delivery (1 month post-stroke) (Shen et al., 2007b). In contrast, intraparenchymal injection of cells would benefit from later delivery once the initial inflammatory response has subsided, as this affords greater cell survival (Grabowski et al., 1994; Kelly et al., 2004).

All of the aforementioned clinical trials opted for delivery of cells in the post-acute phase of stroke (see Table 1). How the timing of transplantation affected the outcome of these trials is not clear, but they at least demonstrate that delivery of cells at different times is feasible. Ultimately, once we have a better understanding of how the cells interact with the brain and vice versa, non-invasive imaging techniques could be used to determine the optimum time for transplantation on a patient-by-patient basis (as discussed further below).

Patient Selection

Choosing the right patient population for clinical trials will be vital to determine efficacy. In addition to the therapeutic time window discussed above, other fundamental issues still need to be addressed at the pre-clinical level.

Patient age and sex

Stroke is a heterogeneous disease that typically affects elderly patients with significant comorbidities such as atherosclerosis, hypertension and diabetes mellitus. In addition, men and women have different risk factors for stroke, exhibit different stroke pathologies, and respond differently to treatment (Bushnell, 2008; Lang and McCullough, 2008; Locatelli et al., 2009; Reeves et al., 2008; Wilson et al., 2008). However, most pre-clinical studies are performed in healthy, young adult, male laboratory animals, which fail to represent the complex human

pathology. As we move forward it will be critical to address these issues, as recommended by the pre-clinical STEPS (Stem cell Therapeutics as an Emerging Paradigm in Stroke) consortium (Borlongan et al., 2008; Wechsler et al., 2009). For example, a body of work from the Chopp group (Gao et al., 2005; Li et al., 2005; Zacharek et al., 2007) implies that MSC transplantation aids recovery in part by modulating the host astrocytic response to stroke, yet there are marked sex differences in how astrocytes respond after stroke (Cordeau et al., 2008), which questions whether MSCs would have the same efficacy in male and female rats. Similar issues pertain to the response of the aged brain to stroke; aged rats showed higher astrocyte reactivity, increased macrophage recruitment, and delayed neuronal death after hemorrhagic stroke, as compared to younger animals (Wasserman et al., 2008). Moreover, the extent of ischemic damage and blood-brain barrier breakdown increased with aging in female mice, whereas male animals showed opposite effects (Liu et al., 2009). In addition, as we move towards clinical trials, cell dosage becomes an important question in terms of efficacy and tolerance; these parameters may also have sex and age-related differences (Park et al., 2009) which must be taken into consideration when designing pre-clinical studies.

Lesion location and size

Lesion location and size will be important factors in determining patient suitability for cell therapy. The majority of pre-clinical studies show cell-enhanced recovery after striatal lesions (reviewed in Bliss et al., 2007; Guzman et al., 2008a; Hicks and Jolkkonen, 2009; Locatelli et al., 2009) although cell-induced improvements with cortical lesions are also reported (Hicks et al., 2009; Shyu et al., 2006; Zhao et al., 2002). However, not all studies find that cell therapy is effective (Hicks et al., 2008). Two groups report that neural progenitor cells (NPCs) improve recovery, but only if combined with enriched housing (Grabowski et al., 1995; Hicks et al., 2007), and we found very little effect of hNT cells in cortical stroke (Bliss et al., 2006) despite multiple studies showing efficacy of the same cells with striatal stroke (Borlongan et al., 1998; Saporta et al., 1999). Similarly, Makinen et al (Makinen et al., 2006) found no behavioral improvement after transplantation of human umbilical cord blood stem cells while other studies using similar cells, stroke model, and timing of transplantation did report recovery (Borlongan et al., 2004; Chen et al., 2001; Vendrame et al., 2004). Such ‘negative’ data, which are often not published, are important to consider, as they will help define the optimal conditions for cell transplant therapy. For example, do ‘negative’ studies use different behavior tests, or perhaps include animals with larger lesions while ‘positive’ studies exclude animals with large or very small lesions? More discussion of inclusion/exclusion criteria is required in the field (Dirnagl, 2006; Macleod et al., 2004). ‘Negative’ studies also highlight the need for a collaborative effort among multiple laboratories to confirm the efficacy of a particular stem/progenitor cell using the same study parameters (stroke model, timing of transplantation, behavior tests, rodent strain, age and gender) (Borlongan et al., 2008). Despite being expensive and very labor intensive, these confirmative studies bring an unprecedented degree of veracity that is essential for translation of cell therapy to the clinics.

Ischemic versus hemorrhagic stroke

The pathophysiology and mechanisms of recovery differ between ischemic and hemorrhagic strokes (Xi et al., 2006). For example, there is no salvageable penumbra with intracerebral hemorrhage (ICH) unlike ischemic stroke (Qureshi et al., 1999), and patients with ICH do not suffer from reperfusion injury with its burst of free radical production (Kleinig and Vink, 2009). Toxic blood breakdown products like thrombin, hemoglobin, and iron additionally contribute to neuronal damage after ICH (Hua et al., 2007; Wang et al., 2002). Therefore, it is plausible that hemorrhagic and ischemic stroke may respond differently to cell therapy and should be tested separately in clinical trials (Andres et al., 2008b; Wechsler et al., 2009).

Route of Cell Delivery

Functional recovery has been reported with intracerebral, intravascular, and intracerebroventricular delivery of cells (reviewed in Andres et al., 2008a; Bliss et al., 2007; Guzman et al., 2008a; Hicks and Jolkkonen, 2009), but the best route is not apparent. Intracerebral delivery results in more transplanted cells in the brain targeting the lesion compared to other delivery routes (Jin et al., 2005). It is speculated however, that intravascular delivery may be more appropriate for larger lesions as it could lead to wider distribution of cells around the ischemic area (Guzman et al., 2008a). But do the cells need to be near the lesion to be effective? In support of this, Guzman et al (Guzman et al., 2008b) found greater homing of CD49d+ NPCs to the brain after intraarterial injection compared to CD49d− cells, which correlated with increased recovery. However, it is unclear if increased homing is the critical parameter or whether the CD49d+ cells are more efficacious for other reasons. Many studies using systemic delivered cells find significant functional recovery with very few (Guzman et al., 2008a; Hicks and Jolkkonen, 2009; Li et al., 2002; Vendrame et al., 2004) or sometimes no cells (Borlongan et al., 2004) entering the brain. Even with intracerebral delivery, proximity of the graft to the lesion may not be important: Modo et al found equal functional recovery when cells were grafted in the ipsi- or contralesional hemispheres (Modo et al., 2002), and recent work from our lab revealed that the hNPCs exerted their major effect one week before they migrated to the lesion (Horie et al., 2009b). Thus, the need for transplanted cells to be near the lesion, or even in the brain requires further investigation.

Each route of delivery has safety issues. Intravascular delivery is less invasive than injection into the brain but raises concerns of cells sticking together creating microemboli, and cells homing to other organs. Intraarterial (intracarotid) administration is preferable to intravenous infusion, allowing first-pass delivery resulting in better targeting of cells to the brain (Fischer et al., 2009; Harting et al., 2009; Lappalainen et al., 2008) and fewer cells found in other organs (Guzman et al., 2008a; Hicks and Jolkkonen, 2009). However, Bang et al reported no adverse effects of intravenous infusion of MSCs in their clinical trial; *this was based on only 5 patients* (Bang et al., 2005). Intraparenchymal transplantation avoids this biodistribution issue, but is more invasive and often results in a physical mass of cells which itself could disrupt the healthy tissue. *The Phase I and II clinical trials with hNT cells transplanted into several striatal sites surrounding the lesion did report some adverse events in 4 out of 30 patients including a seizure and subdural hemorrhage; whether the transplantation surgery contributed to these events is unclear* (Kondziolka et al., 2005; Kondziolka et al., 2000). Transplantation into the lesion cavity is being investigated by other groups (Bible et al., 2009; Park et al., 2002). *The cavity presents a hostile inflammatory environment that lacks trophic support. As proximity to blood vessels and the extent of inflammation can influence graft survival (*Colton, 1995; Grabowski et al., 1994; Kelly et al., 2004*), it is probable that cells will need to be encapsulated or delivered within a scaffold to facilitate their survival in the cavity.* This strategy raises issues such as biocompatibility of the matrix material with the patient and the transplanted cells. Intracisternal and intraventricular delivery routes are also being tested (Kim et al., 2004; Li et al., 2006a; Zhang et al., 2003). In summary, the optimum route of human stem cell delivery has not been determined but will ultimately depend on the timing of delivery, the cell type used, and their mechanism of action.

Cell Type and Source

A variety of human cell types have been tested in experimental stroke (reviewed in Bliss et al., 2007): (1) neural stem/progenitor cells; (2) immortalized cell lines; and (3) hematopoietic/endothelial progenitors and stromal cells isolated from bone marrow, umbilical cord blood, peripheral blood, or adipose tissue. To become a useful therapeutic option, cells must show efficacy, have a large expansion capacity in culture to meet the eventual clinical demand, and

must meet strict criteria for stability and safety. We address this with respect to the different cell types below.

(1) Neural progenitor cells

NPCs have the potential to become neurons, astrocytes and oligodendrocytes, which might be advantageous given that stroke injury damages all three cell types. However, the involvement of cell replacement in functional recovery is not understood (discussed in the Mechanism section below). NPCs can be derived from several sources.

(a) Human Embryonic stem cell (hES)-derived NPCs—hES cells can be differentiated into NPCs by various methods (Daadi et al., 2008; Koch et al., 2009; Reubinoff et al., 2001; Studer, 2001; Zhang et al., 2001). Whether different protocols, or even if different hES lines result in distinguishable populations of NPCs is not understood, but enhanced recovery after transplantation into the stroke brain has been reported by several groups using different preparations of hES-derived NPCs (Daadi et al., 2008; Hicks et al., 2009; Ikeda et al., 2005; Theus et al., 2008). Moreover, integration into the host brain has also been reported (Buhnemann et al., 2006; Hayashi et al., 2006, Daadi et al,. 2009a). Although there are ethical concerns regarding the use of hES cells, these may eventually be overcome by the use of IPS cells (induced pluripotent stem cells), whereby somatic cells such as fibroblasts can be reprogrammed to become ES-like cells by the addition of 3 or 4 critical factors (Park et al., 2008; Takahashi et al., 2007).

An advantage of hES cells is their capacity to propagate in culture over many passages providing a virtually unlimited supply of NPCs, which is advantageous for clinical application. However, batch-to-batch variations in the resultant NPCs may be an issue. The price of this proliferative capacity is the tendency of hES cells to form tumors (Carson et al., 2006) and it is imperative that undifferentiated hES cells are removed from NPC preparations. Proof that hES-derived cells are safe may prove difficult in pre-clinical studies as Erdo et al (Erdo et al., 2003) showed that xenografts are less tumorigenic than allografts. Therefore, the true tumorigenic potential of human cells may not be realized until they are tested in patients. Yet, despite these concerns, Geron received FDA approval to use hES-derived NPCs in a clinical trial for acute spinal cord injury, although this has recently been put on hold pending FDA review of new non-clinical animal study data related to microscopic cyst formation in the regenerating injury site (www.geron.com/media/pressview.aspx?id=1188).

(b) Fetal-derived NPC—The first clinical trial (Phase I) using human fetal CNS-derived stem cells (HuCNS-SCs) was recently completed for Batten disease, a CNS lysosomal storage disease (Taupin, 2006). Although the results have not been published, it's been informally reported (Steiner et al., 2009; www.stemcellsinc.com/news/090608.html) that the HuCNS-SC transplantation was well tolerated, and autopsy of a patient who died of the disease showed evidence of donor cell survival in the brain for close to a year. We used similar cells in a cortical stroke model and found good survival and migration towards the lesion (Kelly et al., 2004), and recently demonstrated improved functional recovery with these cells (unpublished data). Ishibishi et al (Ishibashi et al., 2004) also found functional recovery with fetal derived NPCs. The tumorigenic potential of fetal-derived NPCs is less than hES-derived cells, although thorough characterization of the cells is vital (Amariglio et al., 2009; Jandial and Snyder, 2009). The more limited *in vitro* expansion potential of fetal-derived cells compared to hES cells may be problematic for adequate clinical supply. However, this will depend on the expansion characteristics inherent to each particular stem cell line.

(2) Cell lines

Several human neural cell lines are reported to elicit functional recovery after stroke (Borlongan et al., 1998; Chu et al., 2004; Jeong et al., 2003; Stroemer et al., 2008). These cell lines are immortalized, either because they originate from a teratocarcinoma (such as the hNT neurons; Andrews et al., 1984; Newman et al., 2005), or because they are transformed with an oncogene like myc; this is the case for the human fetal neural stem cell line ReN001 from ReNeuron (Stroemer et al., 2008) which is currently being considered for stroke clinical trials in the UK (www.reneuron.com). Being immortalized, these cell lines have the advantage of potentially unlimited expansion in culture. However, the risk of malignant transformation of immortalized cells remains an issue. Retinoic acid was used to differentiate the teratocarcinoma NT2 cell line into post-mitotic neurons (hNT cells) (Newman et al., 2005), and no signs of tumorigenicity were reported after a 2 year follow up in stroke patients (Kondziolka et al., 2005). In one transplanted stroke patient who died of a myocardial infarct 27 months after injection of the cells, autopsy revealed survival of the hNT cells with no deleterious effects or inflammation (despite only 2 months of immunosuppression) and no tumor formation (Nelson et al., 2002). ReNeuron took a different approach and created a conditionally immortalized cell line where c-myc is only active in the presence of tamoxifen (Stroemer et al., 2008); a successful Phase 1 clinical trial with these ReN001 cells will set precedence for such a strategy. *Another concern with immortalized cell lines is their potential divergence from neural stem/progenitor cells over time in culture as has been reported for the C17.2 immortalized mouse cell line (*Mi et al., 2005*). Therefore, strict characterization of immortalized lines will be essential to confirm the stability of such cell lines in a stem/progenitor state.*

(3) Blood, bone marrow, and adipose tissue-derived progenitor cells

The majority of stroke and progenitor cell transplantation studies employed non-neural cells: human bone marrow cells (HBMC), human umbilical cord blood cells (HUCBC), peripheral blood progenitor cells, and adipose tissue mesenchymal progenitor cells have all been reported to enhance recovery after stroke with intracerebral or intravascular delivery, and with acute (1 day), sub-acute (1 week), or chronic (1 month) delivery after stroke (Bliss et al., 2007; Guzman et al., 2008a; Hicks and Jolkkonen, 2009; Shen et al., 2007b). HBMC and HUCBC are composed of many cell types including hematopoietic and endothelial stem/progenitor cells (CD34+), MSCs (CD34−), and immature lymphocytes and monocytes (Erices et al., 2000; Newman et al., 2004; Nieda et al., 1997; Parr et al., 2007; Vendrame et al., 2004). It is not clear which cells are important to elicit recovery as enhanced function is reported with different cell populations (reviewed in Bliss et al., 2007). These cells lack the ethical issues associated with embryonic- and fetal-derived cells. They are easily obtained offering the potential of autologous transplants, obviating the need for immunosuppression regimes. Even with xenogenic transplants, MSCs are thought to be hypoimmunogenic, as they do not initiate T cell priming or humoral antibody production (Li et al., 2002; Li et al., 2006b; Ryan et al., 2005). However, these cells show poor survival when injected, due either to lack of trophic support or through triggering the innate immune system. Such poor survival may be a disadvantage of these cells, although functional recovery is sustained out to one year (Shen et al., 2007a). Another advantage of these cells is that they are already in clinical use for malignant and non-malignant disorders (Horwitz et al., 1999; Koc et al., 1999; Wakitani et al., 2004), and have been tested in a stroke clinical trial (Bang et al., 2005). Furthermore, *four additional stroke clinical trials are currently underway with these cells, and one additional trial approved* (Table 2).

Other cell safety and manufacturing issues

Most cells transplanted in experimental stroke models are a heterogeneous population. As discussed above, HBMCs and HUCBCs contain multiple cell types, as do NPCs, which are

composed of multipotent stem/progenitor cells in addition to cells already committed to a neuronal or glial fate. It is not understood which cell type, or if a certain ratio of cell types, in these heterogeneous populations are important for functional recovery. This is important to consider as we ride the wave of hope and hype from successful rodent transplant studies, as a sobering lesson can be learned from the Parkinson's field. Dyskinesia is a major side effect in Parkinson's patients that have undergone transplantation therapy. Recent work by Carlsson et al (Carlsson et al., 2009) revealed that the ratio of dopaminergic and serotoninergic grafted neurons play an important role in dyskinesia development, with an increasing ratio of serotonergic neurons being detrimental. Understanding the 'active population' will also be important for cell manufacturing as cell populations drift with time in culture and it will be necessary to monitor this to derive a clinically active product. Furthermore, cell karyotype must also be monitored as changes can occur with time in culture leading to aneuploidic cells.

Potential Mechanisms of Transplanted Cell-Mediated Recovery

It is valuable to understand how cell transplantation affects the host brain. This knowledge will help elucidate the mechanism of action of the transplanted cells, improve their efficacy, and perhaps more importantly, it will also highlight potential side effects. As questions of mechanism are addressed it will be key to investigate both the spatial and temporal effects of the transplanted cells as 'too much of a good thing can be bad', as will be discussed (Carmichael, 2009). It is likely that transplanted cells will have multiple modes of action which will be dependent on cell type, timing and route of administration.

Integration into the host brain

An attraction of NPCs cells is their potential to replace lost circuitry; however evidence for this is limited. Transplanted NPCs in a rat model of global ischemia (Toda et al., 2001) and hNT neurons in a model of traumatic brain injury (Zhang et al., 2005) have been reported to express synaptic proteins. In the ischemic brain, electron microscopy studies revealed that human NPCs form synapses with host circuits (Ishibashi et al., 2004, Daadi et al,. 2009a), and two groups demonstrated that hNPCs had electrophysiological properties characteristic of functional neurons (Buhnemann et al., 2006, Daadi et al,. 2009a). However, only very few synapses are seen, and recovery occurred too early to be attributable to newly formed neuronal connections (Englund et al., 2002; Song et al., 2002). Moreover, recovery is also reported with non-neuronal cells (e.g. MSCs). Together, this implies that neuronal replacement is not a major contributor to cell-induced recovery. Astrocytes play multiple roles in the brain including neuroprotection (Chen and Swanson, 2003; Panickar and Norenberg, 2005), regulation of synapse formation and activity (Allen and Barres, 2005), and involvement in the neurovascular unit which is important for blood brain barrier maintenance and coordinating blood supply with brain activity (Lok et al., 2007). Therefore, integration of astrocytes into the host brain will be as important as neuronal integration. White matter damage is another hallmark of stroke injury and replacement of lost oligodendrocytes to remyelinate axons would be beneficial. Remyelination by human NPCs was reported in spinal cord injury (Cummings et al., 2005), however, to date there are few reports of transplanted NPCs becoming oligodendrocytes in the ischemic brain (Daadi et al., 2008, Daadi et al., 2009a).

Neuroprotection

Acute cell delivery, within the 48h post-injury, often reduces lesion size and inhibits apoptosis in the penumbra, suggesting an important role for cell-induced neuroprotection in enhancing recovery (reviewed in Bliss et al., 2007; Guzman et al., 2008a; Hicks and Jolkkonen, 2009). A myriad of cells types elicit this effect, from NPCs, to bone marrow- and blood-derived cells. Common to all is the secretion of trophic factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), glial cell-derived neurotrophic factor (GDNF), and

brain-derived neurotrophic factor (BDNF) that are likely to contribute to this neuroprotective mechanism (Kurozumi et al., 2005; Llado et al., 2004).

Immunomodulation

Attenuation of stroke-induced inflammation is an emerging effect of transplanted cells. Intravenous injection of HUCBC (Vendrame et al., 2005) or human NPCs (Lee et al., 2008) reduced leukocyte numbers in the brain. Direct injection of human MSCs into the hippocampus after global ischemia downregulated many inflammatory and immune response genes and shifted the balance from a pro- to anti-inflammatory response (Ohtaki et al., 2008). These studies used acute cell delivery, and whether the results are a direct effect on the inflammatory response or a secondary effect attributable to a reduction in cell death is not clear. However, MSCs and NPCs can suppress T cell proliferation and modulate T cell induction *in vitro* possibly by releasing immunosuppressive cytokines and factors (Einstein et al., 2007; Nasef et al., 2007; Ryan et al., 2005; Tse et al., 2003; Yanez et al., 2006). Understanding the temporal effect of transplanted cells on inflammation will be critical as, like many therapeutic targets for stroke, inflammation has a dual role (Lo, 2008); during the acute phase, inflammation is detrimental mediating cell death, but in the recovery phase inflammation is beneficial for removal of cellular debris and neurovascular remodeling. Therefore, long-term attenuation of inflammation may not be advantageous.

Enhancing endogenous repair processes

There is mounting evidence that cell therapies can enhance many endogenous repair processes that occur after stroke.

(a) Vascular regeneration—Increased vascularization in the penumbra within a few days after stroke correlates with improved neurological recovery in patients (Krupinski et al., 1993; Senior, 2001) and offers a potential target for cell therapy. Transplanted cell-induced blood vessel formation has been reported with BMSCs, NPCs, HUCBCs and cells from human peripheral blood (Chen et al., 2003b; Horie et al., 2008; Shen et al., 2006; Shyu et al., 2006; Taguchi et al., 2004). The ability of transplanted cells to increase levels of angiogenic factors (e.g. VEGF, FGF, GDNF, BDNF) and chemoattractant factors (e.g. SDF-1), either by direct secretion of the factor or by inducing host expression (Chen et al., 2003b), is likely to be important to stimulate proliferation of existing vascular endothelial cells (angiogenesis) and mobilization and homing of endogenous endothelial progenitors (vasculogenesis). Understanding the spatial and temporal effect of the transplanted cells on the vasculature is critical as uncontrolled vascularization can be detrimental as observed in certain retinopathies (Aiello, 2000). Recent data from our group finds that transplanted NPCs enhance vascularization in the penumbra but not in healthy tissue, and that the initial increase in vessels is followed by vascular regression, which mirrors the vessel dynamics in control animals (Horie et al., 2008). This is important therapeutically as it demonstrates that NPCs affect the vasculature in a tightly controlled manner.

(b) Induction of host brain plasticity—An increase in endogenous brain structural plasticity and motor remapping after ischemia is postulated to underlie the spontaneous recovery seen after stroke (Carmichael, 2006; Carmichael, 2008; Dancause et al., 2005; Stroemer et al., 1995), and cell transplantation may enhance this. HUCBCs increased sprouting of nerve fibers from the contralateral to the ischemic hemisphere (Xiao et al., 2005), and our group has observed a similar phenomenon with fetal-derived NPCs (Horie et al., 2009a) and hES-derived NPCs (Daadi et al., 2009b). We have also observed that NPCs enhance dendritic branching in both the ipsi- and contralesional hemispheres (Horie et al., 2009a). Shen et al (Shen et al., 2006) reported increased synaptophysin expression after intravenous delivery of HBMCs, and we found that NPCs enhance synaptogenesis *in vitro*, which is partly mediated

by thrombospondin secretion. While these data are cause for hope, a note of caution must be taken from Hofstetter et al (Hofstetter et al., 2005) who found that NPCs grafted in a model of spinal cord injury induced aberrant axonal sprouting which was detrimental, leading to allodynia-like forepaw hypersensitivity.

(c) Recruitment of endogenous progenitors—Endogenous neurogenesis increases after stroke (Arvidsson et al., 2002; Jin et al., 2001). The function of this is not understood but may signify a natural repair mechanism of the brain that could be enhanced by transplanted cells. There is precedence for this with cord blood- and bone marrow-derived cells (Chen et al., 2003a; Taguchi et al., 2004). Moreover, MSC-treated rats demonstrated elevated oligodendrocyte precursors, which increased in concert with enhanced white matter areas (Li et al., 2005; Li et al., 2006b; Shen et al., 2006). In addition to local effects on the damaged tissue, transplanted cells may recruit progenitors from other tissues. For example, they could mobilize endogenous endothelial progenitors into circulation to enhance vascularization.

In vivo Monitoring of Cell Therapy

Clinical studies will benefit from non-invasive methods to monitor the transplanted cells. Several imaging techniques like magnetic resonance imaging (MRI) (Guzman et al., 2007; Yano et al., 2005), bioluminescence imaging (BLI) (Love et al., 2007), positron emission tomography (PET) (Love et al., 2007), and *in vivo* fluorescence microscopy (Yano et al., 2005) have been used to track transplanted stem cells *in vivo*. Tagging the cells with nanoparticles (such as superparamagnetic iron oxide (SPIO)) allows them to be monitored by MRI (Weissleder et al., 1997). Several studies have demonstrated the feasibility to longitudinally track transplanted stem cells in experimental models of stroke (Guzman et al., 2007; Hoehn et al., 2002; Modo et al., 2004) and in a clinical trial for traumatic brain injury (Zhu et al., 2006). However, as released iron oxide particles from dead cells give the same MR signal, MRI cannot assess graft survival, and dilution of SPIOs when cells proliferate may significantly affect longitudinal studies (Guzman et al., 2007; Yano et al., 2005). BLI overcomes this issue, as it requires expression of the luciferase reporter gene that is transfected or transduced into the cells prior to transplantation. After administration of the substrate D-luciferin, cells can be tracked by quantification of photon emission. Since this modality depends on an active enzyme, it allows assessment of transplanted cell viability. However, current BLI methods provide only two-dimensional images with low spatial resolution, and downregulation of luciferase expression would give a false negative result. Transplantation of cells harboring a PET reporter gene (Yaghoubi and Gambhir, 2006) is another approach to track cells *in vivo*. The detection threshold of PET is 7 log orders more sensitive than MRI (Gambhir et al., 2000), but the disadvantage is that PET has a low spatial resolution and lack of anatomical information. In the future, multimodal imaging, combining MRI, BLI, and PET imaging techniques, will be used to combine the strengths and off-set the limitations of each technique (Ray et al., 2007); combined BLI and PET has been used in clinical trials involving patients with recurrent gliomas (Jacobs et al., 2001).

In addition to monitoring the transplanted cells, *in vivo* imaging will also be necessary to monitor the response of the brain to cell therapy. Functional imaging studies including PET (to monitor metabolic activity), perfusion studies (to monitor neovascularization and blood flow), functional MRI (to monitor cerebral plasticity), diffusion-tensor imaging (to evaluate fiber tract integrity), and MRI tracking of macrophages that have phagocytosed ultrasmall SPIO particles (to monitor brain inflammation) (Dousset et al., 1999; Jander et al., 2007; Rausch et al., 2001; Wiart et al., 2007), will be useful surrogate clinical indicators of graft efficacy. Finally, as more is understood about the mechanism of action of the cells, *in vivo* imaging may be useful to predict, on a patient-by-patient basis, when the brain microenvironment is optimal for cell transplantation. For example, MRI and PET techniques

allow us to predict if a patient still has a penumbra (Baron, 2001; Schlaug et al., 1999). And, if enhancing vascularization is important for cell-mediated recovery it might be prudent to transplant when the angiogenic VEGF receptor is upregulated; we have recently demonstrated the feasibility of *in vivo* ^{64}Cu-DOTA-VEGF$_{121}$ PET imaging for investigating VEGFR expression kinetics (Cai et al., 2009).

Conclusions

The pre-clinical evidence shows great promise for cell transplantation as a therapy for stroke. While we can be cautiously optimistic about the reality of such a therapy, many fundamental questions related to the optimal patient (including age, sex, etiology, anatomic location and size of infarct, and medical history), the most appropriate cell type, cell dose, the timing of surgery, the route and site of delivery, the need for immunosuppression, and mechanism of action remain to be answered. Collaboration between basic scientists, clinicians, industry partners, and funding bodies is required to translate the potential of cell therapy into a reality in a timely, but safe and effective manner.

Acknowledgments

The authors would like to thank Hadar Keren-Gill for assistance with manuscript preparation. This work was supported by NIH NINDS grants RO1 NS27292, P01 NS37520 and R01 NS058784, the William Randolph Hearst Foundation, Bernard and Ronni Lacroute, Russell and Elizabeth Siegelman, and the Edward E. Hills Fund.

References

Aiello LP. Keeping in touch with angiogenesis. Nat Med 2000;6:379–81. [PubMed: 10742139]

Allen NJ, Barres BA. Signaling between glia and neurons: focus on synaptic plasticity. Curr Opin Neurobiol 2005;15:542–8. [PubMed: 16144764]

Amariglio N, Hirshberg A, Scheithauer BW, Cohen Y, Loewenthal R, Trakhtenbrot L, Paz N, Koren-Michowitz M, Waldman D, Leider-Trejo L, Toren A, Constantini S, Rechavi G. Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient. PLoS Med 2009;6:e1000029. [PubMed: 19226183]

Andres RH, Choi R, Steinberg GK, Guzman R. Potential of adult neural stem cells in stroke therapy. Regen Med 2008a;3:893–905. [PubMed: 18947311]

Andres RH, Guzman R, Ducray AD, Mordasini P, Gera A, Barth A, Widmer HR, Steinberg GK. Cell replacement therapy for intracerebral hemorrhage. Neurosurg Focus 2008b;24:E16. [PubMed: 18341392]

Andrews PW, Damjanov I, Simon D, Banting GS, Carlin C, Dracopoli NC, Fogh J. Pluripotent embryonal carcinoma clones derived from the human teratocarcinoma cell line Tera-2. Differentiation in vivo and in vitro. Lab Invest 1984;50:147–62. [PubMed: 6694356]

Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat Med 2002;8:963–70. [PubMed: 12161747]

Bang OY, Lee JS, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. Ann Neurol 2005;57:874–82. [PubMed: 15929052]

Baron JC. Mapping the ischaemic penumbra with PET: a new approach. Brain 2001;124:2–4. [PubMed: 11133782]

Bible E, Chau DY, Alexander MR, Price J, Shakesheff KM, Modo M. The support of neural stem cells transplanted into stroke-induced brain cavities by PLGA particles. Biomaterials 2009;30:2985–94. [PubMed: 19278723]

Bliss T, Guzman R, Daadi M, Steinberg GK. Cell transplantation therapy for stroke. Stroke 2007;38:817–826. [PubMed: 17261746]

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]

Borlongan CV, Chopp M, Steinberg GK, Bliss TM, Li Y, Lu M, Hess DC, Kondziolka D. Potential of stem/progenitor cells in treating stroke: the missing steps in translating cell therapy from laboratory to clinic. Regen Med 2008;3:249–50. [PubMed: 18462048]

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;35:2385–9. [PubMed: 15345799]

Borlongan CV, Tajima Y, Trojanowski JQ, Lee VM, Sanberg PR. Transplantation of cryopreserved human embryonal carcinoma-derived neurons (NT2N cells) promotes functional recovery in ischemic rats. Exp Neurol 1998;149:310–321. [PubMed: 9500961]

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;129:3238–48. [PubMed: 17018551]

Bushnell CD. Stroke and the female brain. Nat Clin Pract Neurol 2008;4:22–33. [PubMed: 18199994]

Cai W, Guzman R, Hsu AR, Wang H, Chen K, Sun G, Gera A, Choi R, Bliss T, He L, Li ZB, Maag AL, Hori N, Zhao H, Moseley M, Steinberg GK, Chen X. Positron emission tomography imaging of poststroke angiogenesis. Stroke 2009;40:270–7. [PubMed: 18948613]

Carlsson T, Carta M, Munoz A, Mattsson B, Winkler C, Kirik D, Bjorklund A. Impact of grafted serotonin and dopamine neurons on development of L-DOPA-induced dyskinesias in parkinsonian rats is determined by the extent of dopamine neuron degeneration. Brain 2009;132:319–35. [PubMed: 19039008]

Carmichael ST. Cellular and molecular mechanisms of neural repair after stroke: making waves. Ann Neurol 2006;59:735–742. [PubMed: 16634041]

Carmichael ST. Themes and strategies for studying the biology of stroke recovery in the poststroke epoch. Stroke 2008;39:1380–1388. [PubMed: 18309162]

Carmichael ST. Translating the frontiers of brain repair to treatments: Starting not to break the rules. Neurobiol Dis. 200910.1016/j.nbd.2009.09.005

Carson CT, Aigner S, Gage FH. Stem cells: the good, bad and barely in control. Nat Med 2006;12:1237–8. [PubMed: 17088886]

Chen J, Li Y, Katakowski M, Chen X, Wang L, Lu D, Lu M, Gautam SC, Chopp M. Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat. J Neurosci Res 2003a;73:778–86. [PubMed: 12949903]

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 2001;32:2682–2688. [PubMed: 11692034]

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 2003b;92:692–699. [PubMed: 12609969]

Chen Y, Swanson RA. Astrocytes and brain injury. J Cereb Blood Flow Metab 2003;23:137–49. [PubMed: 12571445]

Chu K, Kim M, Park KI, Jeong SW, Park HK, Jung KH, Lee ST, Kang L, Lee K, Park DK, Kim SU, Roh JK. Human neural stem cells improve sensorimotor deficits in the adult rat brain with experimental focal ischemia. Brain Res 2004;1016:145–153. [PubMed: 15246850]

Colton CK. Implantable biohybrid artificial organs. Cell Transplant 1995;4:415–36. [PubMed: 7582573]

Cordeau P Jr, Lalancette-Hebert M, Weng YC, Kriz J. Live imaging of neuroinflammation reveals sex and estrogen effects on astrocyte response to ischemic injury. Stroke 2008;39:935–42. [PubMed: 18258827]

Cummings BJ, Uchida N, Tamaki SJ, Salazar DL, Hooshmand M, Summers R, Gage FH, Anderson AJ. Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice. Proc Natl Acad Sci USA 2005;102:14069–74. [PubMed: 16172374]

Daadi MM, Li Z, Arac A, Grueter BA, Sofilos M, Malenka RC, Wu JC, Steinberg GK. Molecular and magnetic resonance imaging of human embryonic stem cell-derived neural stem cell grafts in ischemic rat brain. Molecular Therapy 2009a;17:1282–91. [PubMed: 19436269]

Daadi M, Arac A, Davis A, Maag AL, Batnagar R, Choi M, Sun GH, Steinberg GK. Grafts of human embryonic stem cell-derived neural stem cells promote neuroanatomical rewiring and connectivity with host in hypoxia ischemia model of neonates. Stroke 2009b;40(4):e169, P72.

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 2008;3:e1644. [PubMed: 18286199]

Dancause N, Barbay S, Frost SB, Plautz EJ, Chen D, Zoubina EV, Stowe AM, Nudo RJ. Extensive cortical rewiring after brain injury. J Neurosci 2005;25:10167–79. [PubMed: 16267224]

Dirnagl U. Bench to bedside: the quest for quality in experimental stroke research. J Cereb Blood Flow Metab 2006;26:1465–78. [PubMed: 16525413]

Dousset V, Delalande C, Ballarino L, Quesson B, Seilhan D, Coussemacq M, Thiaudiere E, Brochet B, Canioni P, Caille JM. In vivo macrophage activity imaging in the central nervous system detected by magnetic resonance. Magn Reson Med 1999;41:329–33. [PubMed: 10080281]

Einstein O, Fainstein N, Vaknin I, Mizrachi-Kol R, Reihartz E, Grigoriadis N, Lavon I, Baniyash M, Lassmann H, Ben-Hur T. Neural precursors attenuate autoimmune encephalomyelitis by peripheral immunosuppression. Ann Neurol 2007;61:209–18. [PubMed: 17187374]

Englund U, Bjorklund A, Wictorin K, Lindvall O, Kokaia M. Grafted neural stem cells develop into functional pyramidal neurons and integrate into host cortical circuitry. Proc Natl Acad Sci USA 2002;99:17089–94. [PubMed: 12471158]

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;23:780–5. [PubMed: 12843782]

Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. Br J Haematol 2000;109:235–42. [PubMed: 10848804]

Fischer UM, Harting MT, Jimenez F, Monzon-Posadas WO, Xue H, Savitz SI, Laine GA, Cox CS Jr. Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. Stem Cells Dev 2009;18:683–92. [PubMed: 19099374]

Gambhir SS, Herschman HR, Cherry SR, Barrio JR, Satyamurthy N, Toyokuni T, Phelps ME, Larson SM, Balatoni J, Finn R, Sadelain M, Tjuvajev J, Blasberg R. Imaging transgene expression with radionuclide imaging technologies. Neoplasia 2000;2:118–38. [PubMed: 10933072]

Gao Q, Li Y, Chopp M. Bone marrow stromal cells increase astrocyte survival via upregulation of phosphoinositide 3-kinase/threonine protein kinase and mitogen-activated protein kinase kinase/extracellular signal-regulated kinase pathways and stimulate astrocyte trophic factor gene expression after anaerobic insult. Neuroscience 2005;136:123–34. [PubMed: 16198497]

Grabowski M, Johansson BB, Brundin P. Survival of fetal neocortical grafts implanted in brain infarcts of adult rats: the influence of postlesion time and age of donor tissue. Exp Neurol 1994;127:126–36. [PubMed: 8200430]

Grabowski M, Sorensen JC, Mattsson B, Zimmer J, Johansson BB. Influence of an enriched environment and cortical grafting on functional outcome in brain infarcts of adult rats. Exp Neurol 1995;133:96–102. [PubMed: 7601267]

Guzman R, Choi R, Gera A, De Los Angeles A, Andres RH, Steinberg GK. Intravascular cell replacement therapy for stroke. Neurosurg Focus 2008a;24:E15. [PubMed: 18341391]

Guzman R, De Los Angeles A, Cheshier S, Choi R, Hoang S, Liauw J, Schaar B, Steinberg G. Intracarotid injection of fluorescence activated cell-sorted CD49d-positive neural stem cells improves targeted cell delivery and behavior after stroke in a mouse stroke model. Stroke 2008b;39:1300–6. [PubMed: 18309158]

Guzman R, Uchida N, Bliss TM, He D, Christopherson KK, Stellwagen D, Capela A, Greve J, Malenka RC, Moseley ME, Palmer TD, Steinberg GK. Long-term monitoring of transplanted human neural stem cells in developmental and pathological contexts with MRI. Proc Natl Acad Sci USA 2007;104:10211–6. [PubMed: 17553967]

Harting MT, Jimenez F, Xue H, Fischer UM, Baumgartner J, Dash PK, Cox CS. Intravenous mesenchymal stem cell therapy for traumatic brain injury. J Neurosurg 2009;110:1189–97. [PubMed: 19301973]

Hayashi J, Takagi Y, Fukuda H, Imazato T, Nishimura M, Fujimoto M, Takahashi J, Hashimoto N, Nozaki K. Primate embryonic stem cell-derived neuronal progenitors transplanted into ischemic brain. J Cereb Blood Flow Metab 2006;26:906–14. [PubMed: 16395293]

Hayashi T, Noshita N, Sugawara T, Chan PH. Temporal profile of angiogenesis and expression of related genes in the brain after ischemia. J Cereb Blood Flow Metab 2003;23:166–180. [PubMed: 12571448]

Hicks A, Jolkkonen J. Challenges and possibilities of intravascular cell therapy in stroke. Acta Neurobiol Exp (Wars) 2009;69:1–11. [PubMed: 19325636]

Hicks AU, Hewlett K, Windle V, Chernenko G, Ploughman M, Jolkkonen J, Weiss S, Corbett D. Enriched environment enhances transplanted subventricular zone stem cell migration and functional recovery after stroke. Neuroscience 2007;146:31–40. [PubMed: 17320299]

Hicks AU, Lappalainen RS, Narkilahti S, Suuronen R, Corbett D, Sivenius J, Hovatta O, Jolkkonen J. Transplantation of human embryonic stem cell-derived neural precursor cells and enriched environment after cortical stroke in rats: cell survival and functional recovery. Eur J Neurosci 2009;29:562–74. [PubMed: 19175403]

Hicks AU, MacLellan CL, Chernenko GA, Corbett D. Long-term assessment of enriched housing and subventricular zone derived cell transplantation after focal ischemia in rats. Brain Res 2008;1231:103–12. [PubMed: 18675262]

Hoehn M, Kustermann E, Blunk J, Wiedermann D, Trapp T, Wecker S, Focking M, Arnold H, Hescheler J, Fleischmann BK, Schwindt W, Buhrle C. Monitoring of implanted stem cell migration in vivo: a highly resolved in vivo magnetic resonance imaging investigation of experimental stroke in rat. Proc Natl Acad Sci USA 2002;99:16267–72. [PubMed: 12444255]

Hofstetter CP, Holmstrom NA, Lilja JA, Schweinhardt P, Hao J, Spenger C, Wiesenfeld-Hallin Z, Kurpad SN, Frisen J, Olson L. Allodynia limits the usefulness of intraspinal neural stem cell grafts; directed differentiation improves outcome. Nat Neurosci 2005;8:346–53. [PubMed: 15711542]

Horie N, Bliss T, Shichinohe H, Palmer T, Steinberg G. Ischemia-induced Angiogenesis Is Enhanced By hCNS-SCn Transplantation. Stroke 2008;39(2):658, 364.

Horie N, Andres RH, Keren-Gill H, Slikker W, Sun GH, McMillan E, Svendsen CN, Bliss TM, Steinberg GK. Transplanted human neural progenitor cells enhance endogenous dendritic plasticity and axonal sprouting after stroke in rat. Stroke 2009a;40(4):e150, 190.

Horie, N.; Pereira, MP.; Sun, GH.; Keren-Gill, H.; Jiang, K.; Encarnacion, A.; Shamloo, M.; Bliss, TM.; Steinberg, GK. Transplantation of human neural progenitor/stem cells enhances neovascularization and blood brain barrier integrity after stroke in rat. 2009b. http://strokeconference.org/sc_includes/pdfs/asaInitiativesAbstracts.pdf; ASA P2

Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE, Brenner MK. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med 1999;5:309–13. [PubMed: 10086387]

Hua Y, Keep RF, Hoff JT, Xi G. Brain injury after intracerebral hemorrhage: the role of thrombin and iron. Stroke 2007;38:759–62. [PubMed: 17261733]

Ikeda R, Kurokawa MS, Chiba S, Yoshikawa H, Ide M, Tadokoro M, Nito S, Nakatsuji N, Kondoh Y, Nagata K, Hashimoto T, Suzuki N. Transplantation of neural cells derived from retinoic acid-treated cynomolgus monkey embryonic stem cells successfully improved motor function of hemiplegic mice with experimental brain injury. Neurobiol Dis 2005;20:38–48. [PubMed: 16137565]

Ishibashi S, Sakaguchi M, Kuroiwa T, Yamasaki M, Kanemura Y, Shizuko I, Shimazaki T, Onodera M, Okano H, Mizusawa H. Human neural stem/progenitor cells, expanded in long-term neurosphere culture, promote functional recovery after focal ischemia in Mongolian gerbils. J Neurosci Res 2004;78:215–223. [PubMed: 15378509]

Jacobs A, Voges J, Reszka R, Lercher M, Gossmann A, Kracht L, Kaestle C, Wagner R, Wienhard K, Heiss WD. Positron-emission tomography of vector-mediated gene expression in gene therapy for gliomas. Lancet 2001;358:727–9. [PubMed: 11551583]

Jander S, Schroeter M, Saleh A. Imaging inflammation in acute brain ischemia. Stroke 2007;38:642–5. [PubMed: 17261707]

Jandial R, Snyder EY. A safer stem cell: on guard against cancer. Nat Med 2009;15:999–1001. [PubMed: 19734873]

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]

Jin K, Minami M, Lan JQ, Mao XO, Batteur S, Simon RP, Greenberg DA. Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. Proc Natl Acad Sci USA 2001;98:4710–5. [PubMed: 11296300]

Jin K, Sun Y, Xie L, Mao XO, Childs J, Peel A, Logvinova A, Banwait S, Greenberg DA. Comparison of ischemia-directed migration of neural precursor cells after intrastriatal, intraventricular, or intravenous transplantation in the rat. Neurobiol Dis 2005;18:366–74. [PubMed: 15686965]

Kelly S, Bliss TM, Shah AK, Sun GH, Ma M, Foo WC, Masel J, Yenari MA, Weissman IL, Uchida N, Palmer T, Steinberg GK. Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex. Proc Natl Acad Sci USA 2004;101:11839–11844. [PubMed: 15280535]

Kim DE, Schellingerhout D, Ishii K, Shah K, Weissleder R. Imaging of stem cell recruitment to ischemic infarcts in a murine model. Stroke 2004;35:952–7. [PubMed: 14988578]

Kleinig TJ, Vink R. Suppression of inflammation in ischemic and hemorrhagic stroke: therapeutic options. Curr Opin Neurol 2009;22:294–301. [PubMed: 19434798]

Koc ON, Peters C, Aubourg P, Raghavan S, Dyhouse S, DeGasperi R, Kolodny EH, Yoseph YB, Gerson SL, Lazarus HM, Caplan AI, Watkins PA, Krivit W. Bone marrow-derived mesenchymal stem cells remain host-derived despite successful hematopoietic engraftment after allogeneic transplantation in patients with lysosomal and peroxisomal storage diseases. Exp Hematol 1999;27:1675–81. [PubMed: 10560915]

Koch P, Opitz T, Steinbeck JA, Ladewig J, Brustle O. A rosette-type, self-renewing human ES cell-derived neural stem cell with potential for in vitro instruction and synaptic integration. Proc Natl Acad Sci USA 2009;106:3225–30. [PubMed: 19218428]

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. Neurology 2000;55:565–569. [PubMed: 10953194]

Krupinski J, Kaluza J, Kumar P, Wang M, Kumar S. Prognostic value of blood vessel density in ischaemic stroke. Lancet 1993;342:742. [PubMed: 8103843]

Kurozumi K, Nakamura K, Tamiya T, Kawano Y, Ishii K, Kobune M, Hirai S, Uchida H, Sasaki K, Ito Y, Kato K, Honmou O, Houkin K, Date I, Hamada H. Mesenchymal stem cells that produce neurotrophic factors reduce ischemic damage in the rat middle cerebral artery occlusion model. Mol Ther 2005;11:96–104. [PubMed: 15585410]

Lang JT, McCullough LD. Pathways to ischemic neuronal cell death: are sex differences relevant? J Transl Med 2008;6:33. [PubMed: 18573200]

Lappalainen RS, Narkilahti S, Huhtala T, Liimatainen T, Suuronen T, Narvanen A, Suuronen R, Hovatta O, Jolkkonen J. The SPECT imaging shows the accumulation of neural progenitor cells into internal organs after systemic administration in middle cerebral artery occlusion rats. Neurosci Lett 2008;440:246–50. [PubMed: 18572314]

Lee ST, Chu K, Jung KH, Kim SJ, Kim DH, Kang KM, Hong NH, Kim JH, Ban JJ, Park HK, Kim SU, Park CG, Lee SK, Kim M, Roh JK. Anti-inflammatory mechanism of intravascular neural stem cell transplantation in haemorrhagic stroke. Brain 2008;131:616–29. [PubMed: 18156155]

Li L, Jiang Q, Zhang L, Ding G, Wang L, Zhang R, Zhang ZG, Li Q, Ewing JR, Kapke A, Lu M, Chopp M. Ischemic cerebral tissue response to subventricular zone cell transplantation measured by iterative self-organizing data analysis technique algorithm. J Cereb Blood Flow Metab 2006a;26:1366–1377. [PubMed: 16511501]

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

Li Y, Chen J, Zhang CL, Wang L, Lu D, Katakowski M, Gao Q, Shen LH, Zhang J, Lu M, Chopp M. Gliosis and brain remodeling after treatment of stroke in rats with marrow stromal cells. Glia 2005;49:407–17. [PubMed: 15540231]

Li Y, McIntosh K, Chen J, Zhang C, Gao Q, Borneman J, Raginski K, Mitchell J, Shen L, Zhang J, Lu D, Chopp M. Allogeneic bone marrow stromal cells promote glial-axonal remodeling without immunologic sensitization after stroke in rats. Exp Neurol 2006b;198:313–25. [PubMed: 16455080]

Liu F, Yuan R, Benashski SE, McCullough LD. Changes in experimental stroke outcome across the life span. J Cereb Blood Flow Metab 2009;29:792–802. [PubMed: 19223913]

Llado J, Haenggeli C, Maragakis NJ, Snyder EY, Rothstein JD. Neural stem cells protect against glutamate-induced excitotoxicity and promote survival of injured motor neurons through the secretion of neurotrophic factors. Mol Cell Neurosci 2004;27:322–31. [PubMed: 15519246]

Lo EH. A new penumbra: transitioning from injury into repair after stroke. Nat Med 2008;14:497–500. [PubMed: 18463660]

Locatelli F, Bersano A, Ballabio E, Lanfranconi S, Papadimitriou D, Strazzer S, Bresolin N, Comi GP, Corti S. Stem cell therapy in stroke. Cell Mol Life Sci 2009;66:757–72. [PubMed: 18989624]

Lok J, Gupta P, Guo S, Kim WJ, Whalen MJ, van Leyen K, Lo EH. Cell-cell signaling in the neurovascular unit. Neurochem Res 2007;32:2032–2045. [PubMed: 17457674]

Love Z, Wang F, Dennis J, Awadallah A, Salem N, Lin Y, Weisenberger A, Majewski S, Gerson S, Lee Z. Imaging of mesenchymal stem cell transplant by bioluminescence and PET. J Nucl Med 2007;48:2011–20. [PubMed: 18006616]

Macleod MR, O'Collins T, Howells DW, Donnan GA. Pooling of animal experimental data reveals influence of study design and publication bias. Stroke 2004;35:1203–8. [PubMed: 15060322]

Makinen S, Kekarainen T, Nystedt J, Liimatainen T, Huhtala T, Narvanen A, Laine J, Jolkkonen J. Human umbilical cord blood cells do not improve sensorimotor or cognitive outcome following transient middle cerebral artery occlusion in rats. Brain Res 2006;1123:207–15. [PubMed: 17070789]

Mi R, Luo Y, Cai J, Limke TL, Rao MS, Hoke A. Immortalized neural stem cells differ from nonimmortalized cortical neurospheres and cerebellar granule cell progenitors. Exp Neurol 2005;194:301–19. [PubMed: 16022860]

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;21:311–7. [PubMed: 14741669]

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 2002;33:2270–2278. [PubMed: 12215598]

Nasef A, Mathieu N, Chapel A, Frick J, Francois S, Mazurier C, Boutarfa A, Bouchet S, Gorin NC, Thierry D, Fouillard L. Immunosuppressive effects of mesenchymal stem cells: involvement of HLA-G. Transplantation 2007;84:231–7. [PubMed: 17667815]

Nelson PT, Kondziolka D, Wechsler L, Goldstein S, Gebel J, DeCesare S, Elder EM, Zhang PJ, Jacobs A, McGrogan M, Lee VMY, Trojanowski JQ. Clonal human (hNT) neuron grafts for stroke therapy: neuropathology in a patient 27 months after implantation. Am J Pathol 2002;160:1201–06. [PubMed: 11943704]

Newman MB, Emerich DF, Borlongan CV, Sanberg CD, Sanberg PR. Use of human umbilical cord blood (HUCB) cells to repair the damaged brain. Curr Neurovasc Res 2004;1:269–81. [PubMed: 16181077]

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]

Nieda M, Nicol A, Denning-Kendall P, Sweetenham J, Bradley B, Hows J. Endothelial cell precursors are normal components of human umbilical cord blood. Br J Haematol 1997;98:775–7. [PubMed: 9332339]

Ohtaki H, Ylostalo JH, Foraker JE, Robinson AP, Reger RL, Shioda S, Prockop DJ. Stem/progenitor cells from bone marrow decrease neuronal death in global ischemia by modulation of inflammatory/immune responses. Proc Natl Acad Sci USA 2008;105:14638–43. [PubMed: 18794523]

Panickar KS, Norenberg MD. Astrocytes in cerebral ischemic injury: morphological and general considerations. Glia 2005;50:287–98. [PubMed: 15846806]

Park DH, Eve DJ, Musso J 3rd, Klasko SK, Cruz E, Borlongan CV, Sanberg PR. Inflammation and stem cell migration to the injured brain in higher organisms. Stem Cells Dev 2009;18:693–702. [PubMed: 19199787]

Park IH, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, Daley GQ. Reprogramming of human somatic cells to pluripotency with defined factors. Nature 2008;451:141–6. [PubMed: 18157115]

Park KI, Teng YD, Snyder EY. The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. Nat Biotechnol 2002;20:1111–7. [PubMed: 12379868]

Parr AM, Tator CH, Keating A. Bone marrow-derived mesenchymal stromal cells for the repair of central nervous system injury. Bone Marrow Transplant 2007;40:609–19. [PubMed: 17603514]

Pluchino S, Zanotti L, Rossi B, Brambilla E, Ottoboni L, Salani G, Martinello M, Cattalini A, Bergami A, Furlan R, Comi G, Constantin G, Martino G. Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. Nature 2005;436:266–71. [PubMed: 16015332]

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;199:143–55. [PubMed: 16464451]

Qureshi AI, Wilson DA, Hanley DF, Traystman RJ. No evidence for an ischemic penumbra in massive experimental intracerebral hemorrhage. Neurology 1999;52:266–72. [PubMed: 9932942]

Rausch M, Sauter A, Frohlich J, Neubacher U, Radu EW, Rudin M. Dynamic patterns of USPIO enhancement can be observed in macrophages after ischemic brain damage. Magn Reson Med 2001;46:1018–22. [PubMed: 11675656]

Ray P, Tsien R, Gambhir SS. Construction and validation of improved triple fusion reporter gene vectors for molecular imaging of living subjects. Cancer Res 2007;67:3085–93. [PubMed: 17409415]

Reeves MJ, Bushnell CD, Howard G, Gargano JW, Duncan PW, Lynch G, Khatiwoda A, Lisabeth L. Sex differences in stroke: epidemiology, clinical presentation, medical care, and outcomes. Lancet Neurol 2008;7:915–26. [PubMed: 18722812]

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;19:1134–40. [PubMed: 11731782]

Ryan JM, Barry FP, Murphy JM, Mahon BP. Mesenchymal stem cells avoid allogeneic rejection. J Inflamm (Lond) 2005;2:8. [PubMed: 16045800]

Saporta S, Borlongan CV, Sanberg PR. Neural transplantation of human neuroteratocarcinoma (hNT) neurons into ischemic rats. A quantitative dose-response analysis of cell survival and behavioral recovery. Neuroscience 1999;91:519–25. [PubMed: 10366009]

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;20:101–7. [PubMed: 15976503]

Schlaug G, Benfield A, Baird AE, Siewert B, Lovblad KO, Parker RA, Edelman RR, Warach S. The ischemic penumbra: operationally defined by diffusion and perfusion MRI. Neurology 1999;53:1528–37. [PubMed: 10534263]

Senior K. Angiogenesis and functional recovery demonstrated after minor stroke. Lancet 2001;358:817. [PubMed: 11564497]

Shen LH, Li Y, Chen J, Cui Y, Zhang C, Kapke A, Lu M, Savant-Bhonsale S, Chopp M. One-year follow-up after bone marrow stromal cell treatment in middle-aged female rats with stroke. Stroke 2007a; 38:2150–6. [PubMed: 17525391]

Shen LH, Li Y, Chen J, Zacharek A, Gao Q, Kapke A, Lu M, Raginski K, Vanguri P, Smith A, Chopp M. Therapeutic benefit of bone marrow stromal cells administered 1 month after stroke. J Cereb Blood Flow Metab 2007b;27:6–13. [PubMed: 16596121]

Shen LH, Li Y, Chen J, Zhang J, Vanguri P, Borneman J, Chopp M. Intracarotid transplantation of bone marrow stromal cells increases axon-myelin remodeling after stroke. Neuroscience 2006;137:393–399. [PubMed: 16298076]

Shyu WC, Lin SZ, Chiang MF, Su CY, Li H. Intracerebral peripheral blood stem cell (CD34+) implantation induces neuroplasticity by enhancing beta1 integrin-mediated angiogenesis in chronic stroke rats. J Neurosci 2006;26:3444–3453. [PubMed: 16571751]

Song HJ, Stevens CF, Gage FH. Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. Nat Neurosci 2002;5:438–45. [PubMed: 11953752]

Steiner, R.; Selden, N.; Huhn, SL.; Koch, T.; Al-Uzri, A.; Sikora, D.; Dean, S.; Penfield, J.; Sutcliffe, T.; Bammer, R.; Vogel, H.; Uchida, N. CNS transplantation of purified human neural stem cells in infantile and late-infantile neuronal ceroid lipofuscinoses: Resuts of the phase I trial. Proceedings of the 12th International Congress on Neuronal Ceroid Lipofuscinoses; Hamburg, Germany. 2009. p. 33

Stroemer P, Hope A, Patel S, Pollock K, Sinden J. Development of a human neural stem cell line for use in recovery from disability after stroke. Front Biosci 2008;13:2290–2. [PubMed: 17981710]

Stroemer RP, Kent TA, Hulsebosch CE. Neocortical neural sprouting, synaptogenesis, and behavioral recovery after neocortical infarction in rats. Stroke 1995;26:2135–44. [PubMed: 7482662]

Studer L. Stem cells with brainpower. Nat Biotechnol 2001;19:1117–8. [PubMed: 11731775]

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, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861–72. [PubMed: 18035408]

Taupin P. HuCNS-SC (StemCells). Curr Opin Mol Ther 2006;8:156–163. [PubMed: 16610769]

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 2008;210:656–70. [PubMed: 18279854]

Toda H, Takahashi J, Iwakami N, Kimura T, Hoki S, Mozumi-Kitamura K, Ono S, Hashimoto N. Grafting neural stem cells improved the impaired spatial recognition in ischemic rats. Neurosci Lett 2001;316:9–12. [PubMed: 11720766]

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;75:389–97. [PubMed: 12589164]

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;35:2390–5. [PubMed: 15322304]

Vendrame M, Gemma C, de Mesquita D, Collier L, Bickford PC, Sanberg CD, Sanberg PR, Pennypacker KR, Willing AE. Anti-inflammatory effects of human cord blood cells in a rat model of stroke. Stem Cells Dev 2005;14:595–604. [PubMed: 16305344]

Wakitani S, Mitsuoka T, Nakamura N, Toritsuka Y, Nakamura Y, Horibe S. Autologous bone marrow stromal cell transplantation for repair of full-thickness articular cartilage defects in human patellae: two case reports. Cell Transplant 2004;13:595–600. [PubMed: 15565871]

Wang X, Mori T, Sumii T, Lo EH. Hemoglobin-induced cytotoxicity in rat cerebral cortical neurons: caspase activation and oxidative stress. Stroke 2002;33:1882–8. [PubMed: 12105370]

Wasserman JK, Yang H, Schlichter LC. Glial responses, neuron death and lesion resolution after intracerebral hemorrhage in young vs. aged rats. Eur J Neurosci 2008;28:1316–28. [PubMed: 18973558]

Wechsler L, Steindler D, Borlongan C, Chopp M, Savitz S, Deans R, Caplan L, Hess D, Mays RW. 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;40:510–5. [PubMed: 19095993]

Wechsler LR. Clinical trials of stroke therapy: which cells, which patients? Stroke 2009;40:S149–51. [PubMed: 19064792]

Weissleder R, Cheng HC, Bogdanova A, Bogdanov A Jr. Magnetically labeled cells can be detected by MR imaging. J Magn Reson Imaging 1997;7:258–63. [PubMed: 9039625]

Wiart M, Davoust N, Pialat JB, Desestret V, Moucharrafie S, Cho TH, Mutin M, Langlois JB, Beuf O, Honnorat J, Nighoghossian N, Berthezene Y. MRI monitoring of neuroinflammation in mouse focal ischemia. Stroke 2007;38:131–7. [PubMed: 17122417]

Wilson AC, Clemente L, Liu T, Bowen RL, Meethal SV, Atwood CS. Reproductive hormones regulate the selective permeability of the blood-brain barrier. Biochim Biophys Acta 2008;1782:401–7. [PubMed: 18381207]

Xi G, Keep RF, Hoff JT. Mechanisms of brain injury after intracerebral haemorrhage. Lancet Neurol 2006;5:53–63. [PubMed: 16361023]

Xiao J, Nan Z, Motooka Y, Low WC. Transplantation of a novel cell line population of umbilical cord blood stem cells ameliorates neurological deficits associated with ischemic brain injury. Stem Cells Dev 2005;14:722–33. [PubMed: 16433627]

Yaghoubi SS, Gambhir SS. PET imaging of herpes simplex virus type 1 thymidine kinase (HSV1-tk) or mutant HSV1-sr39tk reporter gene expression in mice and humans using [18F]FHBG. Nat Protoc 2006;1:3069–75. [PubMed: 17406570]

Yanez R, Lamana ML, Garcia-Castro J, Colmenero I, Ramirez M, Bueren JA. Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus-host disease. Stem Cells 2006;24:2582–91. [PubMed: 16873762]

Yano S, Kuroda S, Shichinohe H, Hida K, Iwasaki Y. Do bone marrow stromal cells proliferate after transplantation into mice cerebral infarct? --a double labeling study. Brain Res 2005;1065:60–7. [PubMed: 16313889]

Zacharek A, Chen J, Cui X, Li A, Li Y, Roberts C, Feng Y, Gao Q, Chopp M. Angiopoietin1/Tie2 and VEGF/Flk1 induced by MSC treatment amplifies angiogenesis and vascular stabilization after stroke. J Cereb Blood Flow Metab 2007;27:1684–1691. [PubMed: 17356562]

Zhang C, Saatman KE, Royo NC, Soltesz KM, Millard M, Schouten JW, Motta M, Hoover RC, McMillan A, Watson DJ, Lee VM, Trojanowski JQ, McIntosh TK. Delayed transplantation of human neurons following brain injury in rats: a long-term graft survival and behavior study. J Neurotrauma 2005;22:1456–74. [PubMed: 16379583]

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;19:1129–33. [PubMed: 11731781]

Zhang ZG, Jiang Q, Zhang R, Zhang L, Wang L, Zhang L, Arniego P, Ho KL, Chopp M. Magnetic resonance imaging and neurosphere therapy of stroke in rat. Ann Neurol 2003;53:259–63. [PubMed: 12557295]

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]

Zhu J, Zhou L, XingWu F. Tracking neural stem cells in patients with brain trauma. N Engl J Med 2006;355:2376–8. [PubMed: 17135597]

Table 1

Completed clinical stem cell trials for stroke.

Study	Cell Type	Source	n	Delivery	Stroke	Location	Stroke Age	Results
Kondziolka et al., 2000	hNT	immortalized cell line	12	IC (1x)	IS IS	Striatum	6 months to 6 years	Feasibility proven. Adverse effects in 2 patients; unclear if related to transplant surgery
Kondziolka et al., 2005	hNT	immortalized cell line	18	IC (1x)	IS+HS IS+HS	Striatum	12 months to 6 years	Feasibility proven. Adverse effects in 2 patients; unclear if related to transplant surgery
Savitz et al., 2005	LGE	xeno/swine	5	IC (1x)	IS IS	Striatum	3 months to 10 years	Terminated by the FDA due to possible side effects
Bang et al., 2005	BMSC	autologous	5	IV (2x)	IS	Striatum+Cortex	4–5 weeks 7–9 weeks	Feasibility proven. No surgery- related adverse effects.

Abbreviations: LGE, lateral ganglionic eminence; BMSC, bone marrow-derived stem cells; IC, intracerebral; IV, intravenous; IS, ischemic stroke; HS, hemorrhagic stroke; FDA, Food and Drug Administration

Table 2

Current clinical cell transplantation trials for stroke.

Clinical Identifier & Clinical phase	Cell Type	Estimated enrollment	Time of delivery*	Route of delivery	Country
NCT00473057 Phase I	autologous bone marrow	10	3h – 90 d	intra-arterial	Brazil
NCT00859014 Phase I	autologous mononuclear bone marrow	10	24h – 72h	intravenous	USA
NCT00535197 Phase I/II	autologous CD34+ bone marrow	10	7d	intra-arterial	UK
NCT00950521 Phase II	autologous CD34+ peripheral blood	30	6 mo – 60 mo	intracerebral	China
NCT00875654 Phase II	autologous MSCs	30**	< 6wk	intravenous	France

Clinical identifier from Clinicaltrials.gov;

*time of delivery after stroke on-set;

**not yet recruiting