Cell. Mol. Life Sci. 66 (2009) 757 – 772
1420-682X/09/050757-16
DOI 10.1007/s00018-008-8346-1

Cellular and Molecular Life Sciences

Review

Stem cell therapy in stroke

F. Locatelli[a], A. Bersano[b], E. Ballabio[b], S. Lanfranconi[b], D. Papadimitriou[b], S. Strazzer[a], N. Bresolin[a,b,c], G. P. Comi[b,c] and S. Corti[b,c,*]

[a] IRCCS Eugenio Medea, Bosisio Parini, Lecco (Italy)
[b] Dino Ferrari Centre, Department of Neurological Sciences, University of Milan, IRCCS Foundation Ospedale Maggiore Policlinico, Mangiagalli and Regina Elena, Padiglione Ponti, Via Francesco Sforza 35, 20122 Milan, Italy, Fax number: +39-(0)25-032-04-30, e-mail: stefania.corti@unimi.it
[c] Centre of Excellence on Neurodegenerative Diseases, University of Milan, Milan (Italy)

Received 23 June 2008; received after revision 24 September 2008; accepted 30 September 2008
Online First 8 November 2008

Abstract. Recent work has focused on cell transplantation as a therapeutic option following ischemic stroke, based on animal studies showing that cells transplanted to the brain not only survive, but also lead to functional improvement. Neural degeneration after ischemia is not selective but involves different neuronal populations, as well as glial and endothelial cell types. In models of stroke, the principal mechanism by which any improvement has been observed, has been attributed to the release of trophic factors, possibly promoting endogenous repair mechanisms, reducing cell death and stimulating neurogenesis and angiogenesis. Initial human studies indicate that stem cell therapy may be technically feasible in stroke patients, however, issues still need to be addressed for use in human subjects.

Keywords. Stem cell therapy, stroke, preclinical and clinical studies.

Introduction

Cerebrovascular diseases are a major cause of death and disability worldwide. Some interventions during the acute phase of stroke such as thrombolytic agents have been recognized to improve the outcome including survival and residual disability. However, once cell damage from stroke is established, little can be done to restore pre-stroke conditions. Recently cell transplantation has been introduced, on the basis of emerging animal studies showing that cells transplanted to the brain not only survive but also lead to functional improvement in different neurodegenerative diseases models [1]. Transplanted cells have been hypothesized to be effective not only by cell replacement within the damaged host tissue, but also by providing trophic and neuroprotective support as well as immunomodulatory mediators.

Anatomy

Despite stroke injury being focal, the neuronal degeneration in stroke is not selective but involves different neuronal populations, glial cell types and endothelial cells. Moreover, stroke may also affect both white and grey matter and disrupt various anatomical pathways that need to be restored.
Most experimental studies are conducted using a middle cerebral artery (MCA) stroke model that presents mostly striatum and, in a minor part, cortex damage. Only a few authors have investigated cell therapy for cortex infarcts [2, 3] and still there are not

* Corresponding author.

any conclusive results on the possibility of restoring cortical damage and thereby memory and behavioural functions. It can be argued that infarcts associated with cortical involvement are larger and require the reestablishment of essential connections.

Timing of transplantation

The optimal time for cell engraftment after stroke is not yet well defined, because of the dynamic modifications of the ischemic lesion's environment over time [4]. Excitotoxic neurotransmitters, free radicals as well as proinflammatory mediators are released in acute phase [5]. The activated inflammatory response, leading to microglial reaction, together with apoptosis limit both the growth and survival of transplanted cells and endogenous neurogenesis [6, 7]. Otherwise, the increased release during the stroke acute phase of cytokines and neurotrophic factors such as granulocyte colony stimulating factor (G-CSF) [8] potentially could favour cell implant survival and growth. In experimental stroke, it has been observed that during the first 2–3 weeks and even longer, the peri-infarct cortex upregulates gene expression related to the modulation of neuronal growth, involving increased expression of cytoskeletal proteins, angiogenesis, cell proliferation, differentiation and migration from subventricular zone (SVZ) [9]. On the other hand, transplantation subsequent to the acute phase, encounters difficulties due to the hostile lesion environment generated from the scar tissue formation. Stroke spontaneous recovery depends on brain's plasticity in terms of replacement of afferent and efferent connections and synaptogenesis, which occur early after stroke and last for months or years. There is evidence that these endogenous repair mechanisms can be enhanced by transplantation. Some authors [10, 11] described, within an experimental stroke rat, an increased synaptophysin expression in the penumbra area after intravenous administration of human bone marrow stromal cells (hBMSCs). In conclusion, it is rational to delay transplantation until neurological deficits reach a plateau and any further spontaneous recovery is unlikely.

Vascular supply

Vascular supply in the infarct area is crucial to support graft survival. In the ischemic border, angiogenesis involving vascular sprouting from mature endothelial cells of pre-existing blood vessels, creates a hospitable microenvironment for neuronal plasticity, leading to functional recovery [12]. Greater microvessel density in the ischemic border correlates with longer survival in stroke patients [13]. Moreover, increased spontaneous vascularisation during neurological recovery in the site of penumbra, has been described and could represent a potential target for cell therapy [14, 15]. Thus, angiogenesis and neurogenesis seem to be coupled processes in the brain after stroke [16, 17]. Neuroblasts from the SVZ migrate to the ischemic boundary where angiogenesis is active. Indeed, neuroblast migration is closely related to blood vessels [16]. In addition, cerebral endothelial cells, activated by ischemia, increase neural progenitor cell (NPC) proliferation and neuronal differentiation, demonstrated *in vivo* [18] and *in vitro* [17]. In this latter work, activated NPCs isolated from the ischemic SVZ in coculture with endothelial cells are shown to promote in vitro angiogenesis via secretion of vascular endothelial growth factor (VEGF) [17] while the blocking of VEGFR2 with an antagonist abolishes both neurogenesis and angiogenesis [17]. Some authors described the critical role of haematopoietic-derived-endothelial progenitor cells (EPCs). Taguchi et al. demonstrated that the systemic administration of EPCs of human CD34-positive cells in an animal model accelerated the neo-vascularisation in the cerebral ischemic zone 48 hours after stroke [19]. Bone marrow derived cells have also been shown to participate in neo-vascularisation processes in adult brain of ischemic mice [20, 21]. Moreover, transplanted stem cells have been involved in the release of endogenous growth factors stimulating vasculogenesis [22]. In addition, direct incorporation of transplanted cells within newly formed blood vessels has also been described [23].

Route and site of implantation

The route of cell administration is another key point in stem cell transplantation. Several studies reported functional recovery in stroke animal models and in humans by using different ways of delivery (intracerebral, intraventricular or intravenous) [19, 24, 25]. Cell injection into the fluid-filled cavity of a chronic infarct facilitates cell migration [26]. Despite the fact that cells target the lesions and induce a similar recovery independently from the way of administration [27], a wider number of cells were detected near the lesion when using the intracerebral route [27]. In the acute period, it may be more reasonable to inject cells into the penumbral area, in the presence of a viable environment [28]. Alternatively, transplantation could be performed far from the infarct area, for instance at the contralateral side, characterized by healthy and well vascularised surroundings [29].

Modo et al. demonstrated that after stroke, both intact and damaged hemispheres attract grafted stem cells, suggesting the activation of repair processes both locally and within the contralateral motor pathways [30]. Intravenous administration even though less invasive, raises a cell migration specificity issue. Overall, the best route of transplantation still needs to be established considering the specific cell type or the mechanism of action underlying the beneficial effect [31].

In vivo monitoring

In vivo monitoring of stem cells after grafting is essential for the follow up of their migrational dynamics and differentiation processes. Clinical studies require non-invasive methods to monitor the transplanted cells [31]. On this topic some authors have observed cell migration, engraftment and differentiation using the non-invasive approach of *in vivo* magnetic resonance imaging (MRI) [32]. Recently, a very innovative field was introduced focusing on the development of responsive contrast agents, targeted to be incorporated into the cells. As these agents can be activated by specifically selected enzymes turned on only under precise cell conditions, this may successfully lead to the detection of a functional cell status through the imaging of gene expression reporters or enzymatic activity. Another innovative approach involves the use of transgenic cell lines for transplantation, which possess their own inbuilt MR contrast agent under specific gene control, like the iron storage protein, ferritin [33, 34]. Neural and non-neural stem and progenitor cells have been magnetically labelled using dextran-coated superparamagnetic iron oxide (SPIO) particles and tracked by MRI [32, 35, 36]. Embryonic stem cells (ESCs) marked with SPIO nanoparticles were recently transplanted either intracerebrally or intravenously in models of stroke and spinal cord injury. Migration of donor cells was detected by MRI after more than 30 days. At histology, prussian blue staining confirmed a large number of iron-positive cells within the lesions, while the lesions themselves were significantly smaller *vs.* controls [37]. Nonetheless, possible confounding factors leading to misinterpretations of MRI data should be taken into consideration, like the intrinsic MRI signal of blood vessels that must be distinguished from labelled cells, macrophages phagocytosing transplanted cells, bleedings, signal loss and transfer of contrast agents to host cells [38]. Recently, mesenchymal stem cells (MSCs) were labelled by SPIO as well and injected intra-arterial or intravenously in an animal model of stroke. In this work cell infusion was monitored in real time by transcranial laser Doppler flowmetry, while cellular delivery was assessed by MRI *in vivo* detecting labelled MSCs only after intra-arterial delivery. Intra-arterial injection determined high cerebral engraftment rates associated with impeded cerebral blood flow [39]. Human MSCs (hMSCs) were also labelled with ferumoxides (Feridex) protamine sulfate complexes and transplanted into a rodent model of MCA occlusion. Donor cells were visualized by MRI up to 10 weeks following the engraft, demonstrating a great migration capacity and a pathotropism [40]. In another study, immortalized hBMSCs were transfected with a standard contrast agent Gd-DTPA using the cellular labelling substance Effectene and were transplanted into the rat ischemic brain. Migration and homing of donor cells was tracked through a high intensity signal (white), at serial MRI over a 28 days period. Transplanted cells that differentiated into glial, neuronal and endothelial cells were traced. In this work, the authors overcame the false positive signalling due to phagocytosed or dead engrafted cells positive for Gd-DTPA, by washing out the contrast agent within 24 hours [41].

Compared to Gd agents, the iron-containing agents have lower specificity, however, they maintain a lower detection threshold. In rats with MCA occlusion (MCAO), the transhemispheric migration of MHP36 cells (an immortalized cell line from the hippocampal proliferative zone) labelled with the bimodal contrast agent GRID was detected on MRI up to 4 weeks following transplantation. However, compared to MHP36 cells labelled with the red fluorescent dye PKH26, GRID-labelled transplants did not significantly improve behaviour and in terms of evolution of the anatomical damage, they actually increased the lesion's size, whereas PKH26-labelled cells significantly decreased lesion's size. It is required, though, to improve the half-life of the GRID labelling to rule out the possible gradual degradation inside the cells [42]. Additional support comes from positron-emission tomography (PET) studies showing a correlation between clinical improvement and increased uptake of [18F]-fluorodopa in the striatum. This gives credit to the idea that transplanted cells are the direct effectors for the beneficial response by replacing the loss of function of the degenerating dopaminergic cells of the host nigro-striatal pathway. Moreover, diffusion tensor imaging and fractional anisotropy (FA) provide qualitative and quantitative modifications in white matter by evidencing new axon connections [43, 44]. This technique has already been applied to demonstrate the restorative effect of neural

progenitor cells treatment on the stroke area in animal models [35].

One innovative and accessible technology for *in vivo* non-invasive analysis is the bioluminescence imaging (BLI) that is based on the detection of light emitted from luminescent enzymes, such as luciferases, to label genes and cells in living organisms. Combining this reporter system to the new generation of ultra-sensitive CCD cameras that detect the light transmitted through the animal's tissues, has opened up the possibility to explore the mammalian gene expression and other biological phenomena in living animals [45]. Lentiviral vectors encoding firefly luciferase in the mouse SVZ can stably mark endogenous neural stem/progenitor cells (NSCs), which can subsequently be monitored throughout their migration to the olfactory bulb [46]. Moreover, NPCs expressing firefly luciferase, were transplanted in ischemic MCA mice and were monitored by BLI. The donor cell fate was tracked during migration from the contralateral parenchyma to the site of infarct at 7 days, validating once again the potential of the bioluminescence technique [47]. Finally a novel system for *in vivo* analysis of astrocyte response in cerebral ischemia was recently developed using a gene reporter expressing luciferase under the control of the murine glial fibrillary acidic protein (GFAP) promoter (GFAP-luc mice) and BLI. Interestingly, this study revealed marked sex differences in astrocyte response to ischemic injury. In fact, the increase in GFAP signals showed cyclic, estrus-dependent variations in response to ischemic injury, suggesting that the early-phase brain inflammatory response to ischemia may be linked to sex-specific biomarkers of brain injury [48].

Mechanism of tissue repair

Endogenous neurogenesis

The existence of endogenous neurogenesis in adult vertebrate brain was described by Luskin and Alvarez-Buylla et al., who demonstrated for the first time the presence of NSCs in the adult rodent SVZ that migrate out to the olfactory bulb (rostral migratory stream, RMS) [49, 50]. Recently, similar cell populations were identified in the adult human SVZ, although the existence of the RMS still remains controversial in the human brain [51, 52]. There is evidence of neurogenesis in the mammalian SVZ and subgranular zone of the dentate gyrus [53, 54]. Recent studies have detected NSCs in other brain regions, such as striatum, spinal cord and neocortex [55, 56]. A stem cell population has been recently isolated from the adult carotid body. These cells are multipotent and self-renewing *in vitro* and generate new neuron-like carotid body glomus cells. Carotid body stem cells are glia-like sustentacular cells, which give rise to mature glomus cells with the appropriate chemosensory properties, such as dopamine and glial cell line-derived neurotrophic factor release. This neurogenic center could increase the possibility of autologous stem cell repair in adults [57]. Interestingly, the stroke-damaged adult rodent brain preserves some replacement capacity mediated by endogenous NSCs. For several months after stroke, NSCs can generate new striatal neurons that migrate to the site of damage [1]. There is also evidence of the beneficial impact of exercise on the functional plasticity after stroke, by providing neurotrophic support to the lesion's environment and promoting neural repair [58]. Exercise induced neurogenesis was confirmed in humans by measuring exercise specific changes in cerebral blood volume (CBV) in the adult human dentate gyrus [59]. Strikingly, stroke induced neurogenesis has recently been observed in the adult human brain, even among the elderly [60]. It is reported that in animals, stroke increases the number of newly generated cells in the SVZ [49, 50, 61, 62]. Stroke triggers early expansion of the progenitor pool increasing the fraction of proliferating SVZ cells and shortening the cell-cycle length [63]. In addition to NPCs, stroke induces adult ependymal cells to proliferate and acquire features of radial glial cells [64]. Different neurotrophic factors [65–69] are responsible for endogenous neurogenesis stimulation after stroke. Moreover, neuroblasts migrate to the tissue adjacent to the infarct [49, 50, 62] attracted by matrix metalloproteinases (MMPs), particularly MMP 2 [70, 71], produced by the compromised endothelial cells and by neuroblasts themselves in a loop of endothelial-neuronal interaction. The neuroblasts' protective role is reinforced by the fact that they express doublecortin (DCX), a marker of cell migration [72] that is shown to be neuroprotective [73]. In addition, chemotactic signals, particularly SDF/CXCR4 are known to contribute to cell migration.

Exogenous neurogenesis

Stroke affects multiple cell types including neurons, glia and endothelial cells. Reconstitution of the complex and widespread neuronal-glial-endothelial interrelationship may require access to a broader array of cell lineages. Ideally, cells need to maintain initially an immature state and differentiate into several specific cell types after engraftment. The host environment plays an important role in this issue by generating appropriate neurogenic signals. Lack of these may divert the cell fate predominantly toward the glial phenotype. Indeed, stem cell trans-

plantation could enhance clinically valuable improvements through several mechanisms, including growth factor production, endogenous damage-induced plasticity, and local re-innervation, partially due to neuronal replacement.

Cell types and transplantation

Up to now, the following populations have been tested for brain repair: embryonic stem cells (ESCs) from blastocysts, neuroepithelial or teratocarcinoma cell lines, NSCs from embryonic or adult brain, or stem cells from other tissues, e.g. bone marrow (Table 1).

Neural stem/progenitor cells

NSCs self-renew and produce the three major CNS cell types. The studies of Reynolds and Weiss demonstrated for the first time that cells could be isolated from the CNS of adult and embryonic mice and propagate in the presence of EGF to give rise to large spheres of cells, termed "neurospheres" [74]. Consequently, many efforts have been made to isolate and characterize mammalian NSCs [75–78]. Studies investigating the regenerative capacity of rodent or human, embryonic or fetal derived neural stem/progenitor cells reported appropriate differentiation of grafted NSCs into neurons and astroglia as well as functional recovery in stroke models after intracerebral, intracerebroventricular and intravascular administration [79, 80, 25, 81]. Intravascular delivery of NSCs compared to local injection showed limited efficiency. To improve systemic delivery Guzman et al. have selected a NSC population expressing CD49d, a surface integrin that is the receptor of vascular cell adhesion molecule-1 (VCAM-1), an endothelial adhesion molecule known to be upregulated early after stroke. This molecule is thought to be responsible for the adhesion of inflammatory CD49d(+) cells. They observed a significant increase in the number of NSCs within the ischemic hemisphere in animals receiving CD49d(+) NSCs, as compared with the ones treated with CD49d(-) NSCs, together with improved behavioural outcome [82]. A different source of cells, human olfactory ensheathing cells (OECs)/olfactory nerve fibroblasts (ONF) were intracerebrally implanted in ischemic rodents. The upregulation of SDF-1alpha and the enhancement of CXCR4 and PrP(C) interaction induced by hOEC/ONF mediated neuroplastic signals in response to hypoxia and ischemia [83]. Only one group investigated the effects of adult rat derived NSC transplantation after stroke. SVZ derived progenitors were transplanted intracisternally 48 hours after MCAO in rats. Transplanted cells survived and migrated toward the ischemic area as confirmed by histological and radiological analysis (MRI) [84]. Furthermore, MRI has demonstrated that NSCs, labelled with SPIO particles and transplanted either in intact or stroke-damaged rodent brain, follow distinct migration patterns, survive long-term and differentiate in an appropriate site-specific manner, driven by the lesion's microenvironment [85]. On the other hand, hypoxia-inducible factor-1 (HIF-1) plays important roles in the prevention of cerebral ischemia. Deferoxamine (DFX), an iron chelator, stabilizes the HIF-1alpha and upregulates target genes to compensate for ischemia. In a recent study, human DFX-treated NSCs and naive NSCs were transplanted in the striatum of adult rats following focal ischemia at 7 days. Infarct volumes were reduced in both NSCs-transplanted groups, compared with ischemia-only, but the range of reduction was more pronounced in DFX-treated NSCs group. The protective effects of NSCs were ablated when HIF-1alpha was silenced. HIF-1alpha protein levels were increased in both NSCs-transplanted groups, but more in the DFX-treated NSCs group. These findings provide evidence that HIF-1alpha stabilization in human NSCs can be achieved effectively by DFX, and that HIF-1alpha-stabilized NSCs protect against ischemia in a preventive mode [86]. While permanent CBF reduction results in stroke, transient ischemic stress seems to favour preconditioning, therefore it could ameliorate the extent of irreversible brain injury from subsequent ischemia by inducing the so called "ischemic tolerance", as suggested by the recent work of Maysami [87].

Embryonic stem cell (ESC) derived neural stem/progenitor cells

Unlike other sources of stem cells, human ESCs (hESCs) lines possess self-renewal capacity and the potential to differentiate into any cell types. As such, they constitute an ideal source of cells for the development of cell transplantation strategies in stroke. In the experiment of Buhnemann et al., murine ESC derived precursors were grafted into the brain of rats after endothelin-induced MCAO, underwent neuro-glial differentiation and demonstrated functional neuronal electrophysiological properties [88]. Monkey ESC derived progenitors were transplanted into the brains of stroke mice models and were able to re-establish connections with target areas [89] leading to improved motor function [90]. Further genetic modification of the stem cell population, for example by overexpressing anti-apoptotic genes, has been proposed to increase the therapeutic efficacy [91]. Hypoxic preconditioning (HP) preceding stem cell transplantation seems to increase the beneficial effects after ischemic stroke. HP-primed ES-NPCs

Table 1. Preclinical Studies of Stem-Cell Transplantation.

Study Reference	Type of stem cells	Host animal	Mode of delivery	Type of cerebral infarct	Mean time since onset of stroke	Results
[83]	h-olfactory ensheathing cells and olfactory nerve fibroblasts	Rat	Intracerebral	3 vessels ligation	24 hours	SDF1-CXCR4 induced neuroplastic signals from donor cells
[105]	BMSCs	Rat	Intravenous	MCAO	24 hours	SDF1-CXCR4 mediated cells migration toward olfactory-thalamus and hippocampus-cortex route
[39]	MSCs labelled with SPIO particles	Rat	Intraarterial Intravenous	MCAO	After MCAO and 30 minutes of reperfusion	Intraarterial delivered cells were visualized at MRI and induced high cerebral engraftment rate with impeded cerebral blood flow
[40]	HMSCs labeled with ferumoxides protamine sulfate complexes	Rat	Intracerebral	MCAO	1 week	Cells were visualized by MRI up to 10 weeks Cells showed great migration capacity and pathotropism
[109]	h-Neurogenin 1-expressing MSCs	Rat	Intracerebral	MCAO	3 days	Improved motor functions compared to the parental MSCs
[108]	Angiogenin-gene-modified-hMSCs	Rat	Intravenous	MCAO	6 hours	Neuvascularization and regional blood flow were greater compared to the use of unmodified hMSCs
[106]	h-umbilical cord derived MSCs	Rat	Intracerebral	MCAO	2 weeks	Reduced infarct volume; improvement of neurobehavioral function
[111]	h-adult BM derived somatic cells	Rat	Intracerebral	MCAO	1 week	Enhanced neuroplasticity; recovery of forelimb function
[86]	Human deferoxamine treated NSCs	Rat	Intracerebral	Focal ischemia	Stroke after 7 days from graft	Higher reduction of infarct volume compared to the use of naive NSCs
[92]	Hypoxia preconditioning ES-primed NPCs	Rat	Intracerebral	MCAO	48 hours	Cells had increased survival, differentiation and induced a phenotype improvement respect to control cells
[42]	MPH36 NSCs Labelled with GRID contrast agent	Rat	Intracerebral	MCAO	2 weeks	Transhemispheric migration; increased lesion size; it was hypnotised a degradation of contrast agent inside cells
[99]	Homogenous population of hNSCs (named SD56) from hESCs	Ischemic rat, naïve nude rat	Intracerebral	MCAO	1 week	hNSCs migrated toward the ischemic-injured adult brain parenchyma. Functional improvement after two months.
[82]	Mouse CD29d+/- NSCs	Mouse	Intracarotid injection	left-sided hypoxia-ischemia surgery	48 hours	More CD29+ cells into host brain than CD29- Treated mice with CD29+ cells showed better functional improvement than CD29-
[85]	NPCs labelled with SPIO particles	Rat	Intracerebral	Distal MCAO	Stroke after graft	Cells were activated in response to stroke and exhibited directional migration into the parenchyma, similar to the endogenous NPCs, without a niche environment (imaging by MRI)
[110]	MSCs and PMSCs	Rat	Intravenous	MCAO	6 hours	Reduced infarct volume; clinical improvement
[37]	ESCs and MSCs labelled with SPIO particles	Rat	Intracerebral/ Intravenous	Cortical photochemical lesion	1 week	Cells visible for more than 30 days; reduced lesion size compared to controls.
[41]	h-immortalized BMSCs labelled with Gd DTPA contrast agent	Rat	Intracerebral	MCAO and bilateral carotid arteries occlusion	1 week	Cells differentiated into glial, neural and endothelial phenotypes
[24]	Neurosphere derived cells form newborn mice	Mouse	Intracerebro-Ventricular	MCAO	4 hours 7 hours	Functional improvement Increase in mRNA of different trophic factors
[81]	h-fetal striatal and cortical NSCs	Rat	Intracerebral damaged striatal region	MCAO	1–2 weeks	Cell survival In the core region: immature cells positive for nestin, b-tubulin, DCX, calretinin. Outside: mature cells positive for HuD, calbindin, parvalbumin

Table 1 *(Continued)*

Study Reference	Type of stem cells	Host animal	Mode of delivery	Type of cerebral infarct	Mean time since onset of stroke	Results
[88]	Murine ESC derived precursors	Rat	Into the core lesion Into the periphery of lesion Contralateral to the lesion	Endothelin-induced MCAO	1 week	Survival at 12 weeks Glial and neuronal differentiation Electrophysiological properties of functional donor neurons
[89]	Neural progenitors derived from Monkey ESCs	Mouse	Intracerebral ischemic lateral striatum	MCAO	24 hours after the start of reperfusion	Survival and differentiation of cells into neuro-glial phenotypes at 28 days
[11]	Rat BMSCs	Rat	Intracarotid	MCAO	24 hours	At 28 days: Improved functional recovery Increased vessel sprouting Synaptophysin expression NG2+ cell density increase Enhance in proliferating cells
[10]	Rat BMSCs	Rat	Intracarotid	MCAO	24 hours	At 28 days: Improved functional recovery Increasing in proliferating cells Increased BMP 2/4, connexin 43, synaptophysin expression in the boundary zone
[90]	Cynomolgus monkey ESCs	Mouse	Intracerebral periventricular area	Hemiplegic mice mimicking stroke	1 week	Nestin+ cells after 2 days At 28 days mature neurons Gradual recovery of motor function
[91]	Mouse ESCs overexpressing human Bcl2	Rat	Into cortex area	MCAO	1 week	At 1–8 weeks: survival and differentiation into neurons, glia, endothelial cells Expression of neuropil and dendrites Enhanced functional recovery
[76]	Neurospheres from human fetal NSCs	Rat	Intracerebral ischemic region	MCAO	1 week	Cell survival at 4 weeks Migration distance: 1.2 mm Most neuronal phenotype: DCX and b-tubulin+ cells Some GFAP+ cells
[19]	Human CD34+	Mouse	Intravenous	MCAO	48 hours	Neovascularization Endogenous neurogenesis stimulation
[84]	Adult rat derived SVZ cells labelled by ferromagnetic particles	Rat	Intracisternal	MCAO	48 hours	Cell migration Improvement of function
[104]	GCSF mobilized rat PBPCs/ Human UCBs	Rat	Intravenous	MCAO	24 hours	Reduction of hyperactivity induced by stroke Extensive motor asymmetry prevention
[29]	Immortal neuroepithelial SC MHP36 line	Rat	Into hemisphere contralateral to the lesion	MCAO	2–3 weeks	Brain cell diffusion and into lesioned hemisphere Improvement of functional outcome
[101]	Adult rat BMSCs	Rat	Intracerebral ischemic boundary zone	MCAO	24 hours	Significant recovery of function Survival migration Differentiation into neuronal cells
[102]	BMSCs	Rat	Intravenous	MCAO	24 hours	Recovery of function Cell survival Few cells with neuronal phenotype
[103]	BMSCs	Rat	Intracarotid	MCAO	24 hours	Glial and neuronal differentiation Functional improvement
[97]	Human NT2N cells	Rat	Intracerebral	MCAO	1 month	At 1–6 months: functional improvement both with fresh and cryopreserved transplanted cells

MCAO: Middle Cerebral Artery Occlusion; SDF1-CXCR4: Stromal cell Derived Factor 1 and its receptor CXCR4; BMSCs: Bone Marrow Stromal Cells; SPIO: Super-Paramagnetic Iron Oxide; MRI: Magnetic Resonance Imaging; NSCs: Neural Stem Cells ; hESCs: human Embryonic Stem Cells; NPCs: Neural Progenitor Cells; PMSCs: Peripheral Marrow Stromal Cells; DCX: doublecortin; BMP 2/4: Bone Morphogenetic Protein 2/4; SVZ: subventricular zone;
GCSF: Granulocyte Colony Stimulating Factor; PBPCs: Peripheral Blood Progenitor Cells; UBCs: Umbilical Cord Blood Derived Stem Cells; NT2N: immortalized NT2 cell line derived from a human teratocarcinoma.

survived better 3 days after transplantation into the ischemic brain, exhibited extensive neuronal differentiation, accelerated and enhanced recovery of sensorimotor function when compared to non-HP-treated ES-NPCs [92]. Interestingly, recent studies suggest that fibroblasts can be reprogrammed to make ESCs by introduction of stem cell specific transcription factors [93–95]. These pluripotent stem cells (induced pluripotent stem cells) are genetically identical to the recipient and ethically approved. They could represent an innovative source of stem cells for different neurodegenerative diseases as well as for stroke.

Cell lines

Some laboratories have created stem cell lines from rodent CNS and human tissues as alternative source for transplantation. One example is the immortalized cell line NT2, which is derived from a human testicular germ cell tumour [96]. Several studies have demonstrated the efficacy of NT2 cells in focal cerebral ischemia rodent models [97, 98]. Another cell line, the MHP36 cells, have been demonstrated to reduce infarct volume and improve sensorimotor recovery after transplantation [29]. The creation of a self-renewing, non-tumorigenic human ES-derived neural stem cell line (SD56 line) was recently described. This line was shown to retain the capacity of symmetrical self division, to migrate toward the ischemic adult brain parenchyma and promote functional recovery in rodents [99]. A recent work has documented the protocols and methods for the production of immortalized cell lines of human fetal NSCs by using a retroviral vector encoding for v-myc oncogene. One of the human NSC lines (HB1.F3) showed extensive migration from the site of implantation to other anatomical sites and differentiation potential into neurons and glia after cerebral transplantation in a mouse model of stroke [100].

Other somatic cells

Numerous studies investigated the regenerative capacity of haematopoietic stem cells (HSCs: directly bone marrow derived cells, umbilical cord blood (UCB) and G-CSF mobilized peripheral blood cells positive for CD34 antigen) following ischemic brain damage either by mobilization of endogenous HSC pool or by HSCs transplantation [10, 11, 19, 101–104]. In a recent study, a systemic administration of BMSCs in rat ischemic model, cells migrated to the ischemic lesion driven by the locally produced SDF-1alpha [105]. Human umbilical cord derived MSCs (hUC-MSCs), after *in vitro* neuronal differentiation, were implanted into the damaged hemisphere of immunosuppressed ischemic stroke rats, improving neurobehavioral function and reducing infarct volume *vs.* controls. The improvement was attributed to their neuroprotective effects rather than the formation of a new network between host neurons and the donor cells [106]. Recently, it has been proposed that the neuroprotective effect of hUCB mononuclear cells (MNC) was due to anti-apoptotic mechanisms related to direct cell-cell contacts with injured neuronal cells [107]. hMSCs and angiogenin-gene modified hMSCs infused intravenously in rats after MCAO are beneficial in terms of neovascularization and regional cerebral blood flow [108]. Transplantation of neurogenin-1–expressing MSCs, suggests that in addition to the intrinsic paracrine functions of MSCs, motor dysfunctions can be remarkably improved by MSCs transdifferentiated into neuronal cells [109]. Intravenous delivery of MSCs from bone marrow and MSCs prepared from peripheral blood (PMSCs) in rats reduces infarction volume and produces clinical improvement [110]. Human adult bone marrow-derived somatic cells (hABM-SC) after ischemic stroke in adult rats determined recovery of forelimb function positively correlated with increased axonal outgrowth of the intact, uninjured corticorubral tract [111].

The role of growth factors in stroke

Haematopoietic growth factors, also known as colony stimulating factors (CSFs), modulate the lineage specific differentiation of bone marrow stem cells leading to generation of circulating red cells, white cells and platelets. Data from experimental studies support the evidence that CSFs could improve stroke outcome by reducing stroke damage and improving post-stroke brain repair [112]. G-CSF is responsible for bone-marrow-derived stem cell differentiation in circulating neutrophilic granulocytes. G-CSF pretreatment was shown to be neuroprotective in glutamate-induced excitotoxicity [113]. In animal models of focal cerebral ischemia, G-CSF administration after reperfusion was associated with neuroprotection mediated by different mechanisms such as activation of anti-apoptotic pathways [114], reduction of focal inflammatory response [115], neurogenesis and angiogenesis potentiation [116], enhancement of cell proliferation of the SGZ of the DG and promotion of stem cell mobilization and homing to brain [117]. The promising results in animal models led to the implementation of Phase I/II clinical trials [118–121]. GM-CSF promotes HSCs differentiation into circulating granulocytes, macrophage and dendritic cells. GM-CSF's pattern of expression in the brain is very similar to G-CSF and was shown to have a similar anti-

apoptotic, neuroprotective [122] and angiogenetic function [123]. The expression of GM-CSF and its receptors is induced by ischemia in neurons and provides neuroprotection in experimental stroke [122]. Erythropoietin (EPO) promotes the differentiation of bone-marrow stem cells into circulating mature red cells. EPO and its receptors were demonstrated to improve brain repair and functional recovery in an acute ischemic stroke animal model and a rat model of neonatal cerebral ischemia [124, 125]. EPO administration to patients with hyperacute stroke was reported to be effective in reducing early neurological impairment and lesion size without any significant effect on combined death and dependency [120, 126]. SCF and G-CSF favour neurogenesis and show a neuroprotective effect partly mediated by up-regulation of anti-inflammatory cytokines such as IL-10 [127]. In an animal model of brain ischemia SCF and G-CSF administration was associated with infarct volume reduction [128] and increased brain angiogenesis [129]. VEGF is an angiogenetic growth factor that can increase survival and proliferation of endothelial cells [130,131] and survival and proliferation of transplanted human NSCs. Similarly to other growth factors VEGF was associated to neuroprotection [132] and pro-angiogenesis [133], smaller infarct volume and better outcome [134, 135] in animal models of brain ischemia. IGF-1 has a well-established angiogenic, anti-inflammatory and anti-apoptotic properties [136]. IGF-1 treatment has neuroprotective effects associated with improved long-term clinical outcome in mice with ischemic stroke [137]. Stromal cell-derived factor (SDF)-1alpha is a chemokine involved in HSCs circulation and homing. Intracerebral injection of (SDF)-1alpha in rat models of cerebral ischemia results in stem cell mobilization and homing to the ischemic area by blocking apoptotic pathways and by up-regulating the anti-apoptotic protein Bcl-2 [138].

Hormones and stroke

Stroke occurs mostly in older individuals with multiple risk factors. Although the ischemic events occur with greater frequency in men, women have a higher lifetime risk of stroke because of the longer life expectancy. Men and women present different risk factor profiles for stroke, different response to treatments and outcomes. Moreover, women have unique risk factors for stroke that relate to events that modify endogenous hormone levels throughout their life cycle, such as pregnancy and menopause [139]. Animal models after ovariectomy, which induces changes in reproductive hormones similar to that observed in menopause/andropause, presented an increased blood-brain barrier (BBB) permeability leading to higher exposure to neuroinflammation. [140].

Otherwise, animal models of focal and global cerebral ischemia have shown that estrogens have a beneficial effect. On the other hand, exogenous estrogens, such as oral contraceptive pills and postmenopausal hormone therapy, lead to an increased stroke risk in clinical trials. This paradox is under investigation [139].

Up to now, the majority of preclinical studies are conducted on mixed sex cell culture systems and on young and one gender type models *in vivo*. These are experimental conditions that do not entirely reproduce the human stroke pathology. Moreover, it is well documented that differences in stroke risk exist between the sexes also independently of hormone exposure. In particular, a different cell death pathway is activated in response to ischemia that is independent of sex hormone levels. It has been described both *in vitro* and *in vivo*, that females are sensitive to caspase-mediated cell death, while men activate a different cell death pathway, involving apoptosis inducing factor (AIF) and poly (ADP-ribose) polymerase (PARP). In fact, the efficacy of neuroprotective agents such as caspase and PARP inhibitors in pre-clinical stroke models, could be different between the two genders [141].

In conclusion, both related and hormone independent factors can influence stroke occurrence and clinical outcome in men and women, that need to be taken into account in preclinical and clinical studies.

Clinical trials of stem cell transplantation for stroke patients

Currently, stem cell therapy in stroke patients is in its infancy. Five small human trials so far have evaluated the safety and the effect of different stem cells on stroke (Table 2) [25,142–145]. In 1998, the US Food and Drug Administration (FDA) approved a small phase I open-label trial in which neuroteratocarcinoma (NT2N) cells were transplanted in 12 stroke patients [143]. Patients had experienced a basal ganglia stroke 6 months to 4.5 years before implantation and received immunosuppression treatment for 8 weeks. Five years after the surgery no adverse events were reported related to the implant of 2 or 6 million cells. Autopsy on 1 patient, who died of myocardial infarction 27 months after the surgical procedure, revealed a population of immunoreactive grafted cells with no signs of inflammation or neoplasia, suggesting the prolonged survival of the grafts even in the

absence of a continuous immunosuppressant regimen [146]. PET, performed at 6 months, showed high uptake of F-18 fluorodeoxyglucose at the site of transplantation in six patients, suggesting either graft survival or inflammatory response [147]. Although this trial was not designed to evaluate efficacy, 6 patients presented improvement according to the European Stroke Scale (ESS) scores at 24 weeks and in some patients it correlated with increased PET metabolic activity. Subsequently, in 2005, Kondziolka et al. [25] presented a phase II randomized open-label trial to test the safety, the feasibility and the effectiveness of NT2N cell transplantation for stroke. 18 patients with stable deficit 1 to 6 years after ischemic or hemorrhagic basal ganglia stroke, were randomized to receive either 5 or 10 million implanted cells (7 patients per group) or to serve as non-surgical control group (4 patients). All patients attended a stroke rehabilitation program. One patient experienced a single seizure the day after the surgery and another one had a subdural hematoma evacuated 1 month after transplantation. No cell-related adverse events were observed. The primary outcome was evaluated based on the ESS motor score at 6 months. Secondary end points included the Fugl-Meyer Stroke Assessment, Action Reach Arm Test, the Stroke Impact Scale and the results of comprehensive cognitive testing. ESS motor score in transplanted patients did not differ from controls, instead, some secondary outcome measures were significantly improved in treated patients [25]. Furthermore, Savitz and colleagues [145] stereotactically injected fetal porcine cells into 5 patients who had experienced basal ganglia stroke between 1, 5 and 10 years before. The study was terminated by FDA because 2 patients developed adverse events. One patient presented aggravation of motor deficits 3 weeks after the intervention and one patient developed seizures 1 week after transplantation. At four years of clinical follow up, 2 patients showed functional improvement but none of the patients showed improvement according to the modified Rankin scale. In 2005 as well, Bang and colleagues [142] presented a randomized controlled phase I/II clinical trial of autologous MSCs transplantation in massive cortical infarct of MCA territory. Thirty patients, within 7 days of stroke, were randomly allocated to receive intravenous infusion of autologous MSCs (5 patients) or no intervention (25 patients). No adverse cell-related effects were reported. At one year follow up, the Barthel index and the modified Rankin score identified a non-significant trend toward improved scores in patients treated with MSCs. National Institutes of Health Stroke Scale (NIHSS) score changes were not substantial. Moreover, Rabinovich et al. injected human fetal cells into the subarachnoidal space of 10 patients between 4 and 24 months after their ischemic or hemorrhagic stroke of MCA territory. Some patients developed fever and meningism during 48 hours post-transplantation. The control group was studied retrospectively and the outcome measures were not clearly described [144]. These shortcomings preclude any conclusions on the efficacy of the treatment.

In conclusion, these small initial human studies cannot be comparable due to differences in target population, type of cells, timing of injection and mode of delivery, but they indicate that stem cell therapy may be technically feasible in stroke patients. The significance of these results is unclear, however, considering the small sample size and the lack of double-blinded controls. Side effects were observed in 3 out of 5 studies presented above [25, 144, 145], therefore safety is still a principal concern. Epilepsy, risk of bleeding or thrombosis at the site of injection and risk of malignant transformation are the major adverse effects observed. In Kondziolka et al. and Bang et al. studies there was no evidence of teratoma transformation but the follow-up was extended to only 1 year; long-term follow-up is required to rule out the tumorigenic potential of the cells [142,143]. Only recently, the use of NSCs has been taken into consideration for human trials. The first human NSCs clinical trial was approved for Batten disease, a paediatric lysosomal storage disease that leads to neuronal loss and death [148]. The rational is based on the properties of NSCs to serve as "Trojan Horses", as described interestingly by the authors, by providing other cells within the brain with the lost enzymatic activity. Up to now, six patients have been treated. One patient died apparently of the disease (www.stemcellsinc.com/). In the research area of stroke, ReNeuron Group, a UK-based stem cell therapy business, applied for an Investigational New Drug (IND) proposal to the FDA to commence initial clinical trials in the US with the ReN001 stem cell line for stroke therapy. The clonal hNSC line (ReN001) has been developed for clinical use in the treatment of stable disability after stroke. This cell line has been conditionally immortalized using the fusion transgene c-mycER to allow controlled expansion when cultured in the presence of 4-hydroxytamoxifen. *In vivo* studies demonstrated the survival of this cell line after implantation into the lesion of a rodent model of stroke damage and its efficacy in the reduction of chronic behavioural dysfunction [149]. At the moment, the FDA notified that the IND remained on clinical hold. (www.reneuron.com). Currently, two clinical studies are recruiting patients for autologous HSCs transplantation in stroke. The aim of the first study conducted in UK is to determine the safety and

Table 2. Clinical Studies of Stem-Cell Transplantation.

Study Reference	Type of study	Sample	Type of stem cells	Mode of delivery	Type of cerebral infarct	Mean time since onset of stroke	Results
[143]	Phase I trial	12	NT2N cells*	Stereotactic implantation	Ischemic involving basal ganglia (8 cases) or basal ganglia and cerebral cortex (4 cases)	27 months	No cell-related adverse effects 5 years after cell transplantation
[25]	Phase II randomized trial	18	NT2N cells*	Stereotactic implantation	Ischemic (9 cases) or hemorrhagic (9 cases, involving basal ganglia but no motor cortex	3.5 years	No evidence of a significant benefit in motor function but it indicate the safety and the feasibility of neuron transplantation
[145]	Phase I trial	5	Fetal Porcine cells	Stereotactic implantation	Ischemic involving the striatum	4.9 years	The study was terminated by the FDA§ after 2 patients developed adverse events
[142]	Phase I and II randomized trial	30 (5 cases)	MSC+	Intravenous injection	Ischemic in middle cerebral artery territory	46.6 days	No cell-related adverse effects 1 year after cell transplantation
[144]	Case series	10	Human Fetal cells	Subarachnoidal injection	Ischemic (7 cases) or hemorrhagic (3 cases) involving the middle cerebral artery territory	12.1 months	Some patients developed fever and meningism 48 hours after transplantation

*Immortalized NT2 cell line derived from a human teratocarcinoma; §FDA= Federal Drug Administration (U.S.); +mesenchymal stem cells.

tolerability of an autologous CD34+ subset of bone marrow stem cell infusion into the MCA in patients who have suffered acute total anterior circulation syndrome (TACS) (http://clinicaltrials.gov/ct2/show/NCT00535197?term=stroke+stem+cells&rank=2). The second study will be conducted in Brazil. The purpose of this study is to evaluate the safety and feasibility of intra-arterial injection of autologous bone marrow mononuclear cells in patients in the acute and sub-acute phase (> 3 and < 90 days after symptoms onset) of ischemic cerebral infarct in the MCA territory. (http://clinicaltrials.gov/ct2/show/NCT00473057?term=stroke+stem+cells&rank=3).

Conclusions

Cell replacement therapy in ischemic stroke from a clinical and experimental point of view presents considerable variability in the outcomes. However, there is an emerging evolution in the definition of experimental procedures. In particular, the properties of: a) stem cell type, b) the route of cell administration, and c) time interval following the ischemic insult, each influence functional improvement and long term outcome. Although animal stroke models differ from their human counterparts and cannot answer many of the questions, it is still necessary to continue with preclinical studies in order to define standardized protocols and guidelines for the subsequent planning of human trials. Despite the fact that many questions remain to be answered, early work suggests that stem cell transplantation may be adapted for use as a therapeutic option in ischemic stroke.

Acknowledgements. We wish to thank "Associazione Amici del Centro Dino Ferrari" for their support. The financial support of the following research grants to S.C., G.P.C. and N.B. is gratefully acknowledged: Italian Ministry PRIN 2007, "Molecular pathogenesis of motoneuron disorders as a tool for the identification of novel biomolecular and cellular therapeutic targets and Cariplo Foundation grant.

1 Lindvall, O. and Kokaia Z. (2006) Stem cells for the treatment of neurological disorders. Nature 441, 1094–1096.
2 Gates, M.A., Fricker-Gates, R.A. and Macklis, J.D. (2000) Reconstruction of cortical circuitry. Prog. Brain. Res. 127, 115–156.
3 Dunnett, S.B., Ryan, C.N., Levin, P.D., Reynolds, M. and Bunch S.T. (1987) Functional consequences of embryonic neocortex transplanted to rats with prefrontal cortex lesions. Behav. Neurosci. 101, 489–503.
4 Dirnagl, U., Iadecola, C. and Moskowitz, M.A. (1999) Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci. 22, 391–397.
5 Durukan, A. and Tatlisumak, T. (2007) Acute ischemic stroke: overview of major experimental rodent models, pathophysiology, and therapy of focal cerebral ischemia. Pharmacol. Biochem. Behav. 87, 179–197.
6 Li, Y., Chen, J. and Chopp, M. (2002) Cell proliferation and differentiation from ependymal, subependymal and choroid plexus cells in response to stroke in rats. J. Neurol. Sci. 193, 137–146.
7 Nakatomi, H., Kuriu, T., Okabe, S., Yamamoto, S., Hatano, O., Kawahara, N., Tamura, A., Kirino, T. and Nakafuku, M. (2002) Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. Cell 110, 429–441.

8 Hess, D.C. and Borlongan CV. (2008) Stem cells and neurological diseases. Cell Prolif. 41, Suppl 1, 94–114.
9 Chen, C.P., Lee, Y.J., Chiu, S.T., Shyu, W.C., Lee, M.Y., Huang, S.P. and Li, H. (2006) The application of stem cells in the treatment of ischemic diseases. Histol. Histopathol. 21, 1209–1216.
10 Zhang, C., Li, Y., Chen, J., Gao, Q., Zacharek, A., Kapke, A. and Chopp, M. (2006) Bone marrow stromal cells upregulate expression of bone morphogenetic proteins 2 and 4, gap junction protein connexin-43 and synaptophysin after stroke in rats. Neurosci. 141, 687–695.
11 Shen, L.H., Li, Y., Chen, J., Zhang, J., Vanguri, P., Borneman, J. and Chopp, M. (2006) Intracarotid transplantation of bone marrow stromal cells increases axon-myelin remodeling after stroke. Neurosci. 137, 393–399.
12 Plate, K.H. (1999) Mechanisms of angiogenesis in the brain. J. Neuropathol. Exp. Neurol. 58, 313–320.
13 Krupinski, J., Kaluza, J., Kumar, P., Kumar, S. and Wang, J.M. (1994) Role of angiogenesis in patients with cerebral ischemic stroke. Stroke 25, 1794–1798.
14 Wei, L., Erinjeri, J.P., Rovainen, C.M. and Woolsey, T.A. (2001) Collateral growth and angiogenesis around cortical stroke. Stroke 32, 2179–2184.
15 Rafii, S. and Lyden, D. (2003) Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. Nat. Med. 9, 702–712.
16 Thored, P., Wood, J., Arvidsson, A., Cammenga, J., Kokaia, Z., and Lindvall, O. (2007) Long-term neuroblast migration along blood vessels in an area with transient angiogenesis and increased vascularization after stroke. Stroke 38, 3032–3039.
17 Teng, H., Zhang, Z.G., Wang, L., Zhang, R.L., Zhang, L., Morris, D., Gregg, S.R., Wu, Z., Jiang, A., Lu, M., Zlokovic, B.V., and Chopp, M. (2008) Coupling of angiogenesis and neurogenesis in cultured endothelial cells and neural progenitor cells after stroke. J. Cereb. Blood Flow Metab. 28 [Epub ahead of print]
18 Zhang, R.L., LeTourneau, Y., Gregg, S.R., Wang, Y., Toh, Y., Robin, A.M., Zhang, Z.G:, and Chopp, M. (2007) Neuroblast division during migration toward the ischemic striatum: a study of dynamic migratory and proliferate characteristics of neuroblasts from the subventricular zone. J. Neurosci. 27, 3157–3162.
19 Taguchi, A., Soma, T., Tanaka, H., Kanda, T., Nishimura, H., Yoshikawa, H., Tsukamoto, Y., Iso, H., Fujimori, Y., Stern, D.M., Naritomi, H. and Matsuyama, T. (2004) Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. J. Clin. Invest. 114, 330–338.
20 Hess, D.C., Hill, W.D., Martin-Studdard, A., Carroll, J., Brailer, J. and Carothers, J. (2002) Bone marrow as a source of endothelial cells and NeuN-expressing cells after stroke. Stroke 33, 1362–1368.
21 Borlongan, C.V., Lind, J.G., Dillon-Carter, O., Yu, G., Hadman, M., Cheng, C., Carroll, J. and Hess, D.C. (2004) Bone marrow grafts restore cerebral blood flow and blood brain barrier in stroke rats. Brain Res. 1010, 108–116.
22 Caplan, A.I. and Dennis, J.E. (2006) Mesenchymal stem cells as trophic mediators. J. Cell. Biochem. 98, 1076–1084.
23 Akiyama, H., Kondoh, T., Kokunai, T., Nagashima, T., Saito, N. and Tamaki, N. (2000) Blood-brain barrier formation of grafted human umbilical vein endothelial cells in athymic mouse brain. Brain Res. 858, 172–176.
24 Capone, C., Frigerio, S., Fumagalli, S., Gelati, M., Principato, M.C., Storini, C., Montinaro, M., Kraftsik, R., De Curtis, M., Parati, E. and De Simoni, M.G. (2007) Neurosphere-derived cells exert a neuroprotective action by changing the ischemic microenvironment. PLOS ONE 4, 1–11.
25 Kondziolka, D., Steinberg, G.K., Wechsler, L., Meltzer, C.C., Elder, E., Gebel, J., De Cesare, S., Jovin, T., Zafonte, R., Lebowitz, J., Flickinger, J.C., Tong, D., Marks, M.P., Jamieson, C., Luu, D., Bell-Stephens, T. and Teraoka, J. (2005) Neurotransplantation for patients with subcortical motor stroke: a phase 2 randomized trial. J. Neurosurg. 103, 38–45.
26 Savitz, S.I., Rosenbaum, D.M., Dinsmore, J.H., Wechsler, L.R. and Caplan, L.R. (2002) Cell transplantation for stroke. Ann Neurol. 52, 266–275.
27 Jin, K., Sun, Y., Xie, L., Mao, X.O., Childs, J., Peel, A., Logvinova, A., Banwait, S. and Greenberg, D.A. (2005) Comparison of ischemia-directed migration of neural precursor cells after intrastriatal, intraventricular, or intravenous transplantation in the rat. Neurobiol. Dis. 18, 366–374.
28 Hadani, M., Freeman, T., Munsiff, A., Young, W. and Flamm, E. (1992) Fetal cortical cells survive in focal cerebral infarct after permanent occlusion of the middle cerebral artery in adult rats. J. Neurotrauma 9, 107–112.
29 Veizovic, T., Beech, J.S., Stroemer, R.P., Watson, W.P. and Hodges, H. (2001) Resolution of stroke deficits following contralateral grafts of conditionally immortal neuroepithelial stem cells. Stroke 32, 1012–1019.
30 Modo, M., Stroemer, R.P., Tang, E., Patel, S. and Hodges, H. (2002) Effects of implantation site of stem cell grafts on behavioral recovery from stroke damage. Stroke 33, 2270–2278.
31 Bliss, T., Guzman, R., Daadi, M. and Steinberg, G.K. (2007) Cell transplantation therapy for stroke. Stroke 38, Suppl 2, 817–826.
32 Hoehn, M., Küstermann, E., Blunk, J., Wiedermann, D., Trapp, T., Wecker, S., Föcking, M., Arnold, H., Hescheler, J., Fleischmann, B.K., Schwindt, W. and Bührle, C. (2002) 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. U.S.A. 99, 16267–16272.
33 Cohen, J., Kim, K., King, P., Seibert, M. and Schulten, K. (2005) Finding gas diffusion pathways in proteins: application to O2 and H2 transport in CpI [FeFe]-hydrogenase and the role of packing defects. Structure 13, 1321–1329.
34 Genove, G., DeMarco, U., Xu, H., Goins, W.F. and Ahrens, E.T. (2005) A new transgene reporter for in vivo magnetic resonance imaging. Nat. Med. 11, 450–454.
35 Jiang, Q., Zhang, Z.G., Ding, G.L., Silver, B., Zhang, L., Meng, H., Lu, M., Pourabdillah-Nejed, D.S., Wang, L., Savant-Bhonsale, S., Li, L., Bagher-Ebadian, H., Hu, J., Arbab, A.S., Vanguri, P., Ewing, J.R., Ledbetter, K.A. and Chopp, M. (2006) MRI detects white matter reorganization after neural progenitor cell treatment of stroke. Neuroimage 32, 1080–1089.
36 Neri, M., Maderna, C., Cavazzin, C., Deidda-Vigoriti, V., Politi, L.S., Scotti, G., Marzola, P., Sbarbati, A., Vescovi, A.L. and Gritti, A. (2008) Efficient in vitro labeling of human neural precursor cells with superparamagnetic iron oxide particles: relevance for in vivo cell tracking. Stem Cells 26, 505–516.
37 Sykova, E., Jendelova, P. (2007) In vivo tracking of stem cells in brain and spinal cord injury. Prog. Brain Res. 161, 367–383.
38 Hoehn, M., Wiedermann, D., Justicia, C., Ramos-Cabrer, P., Kruttwig, K., Farr, T., Himmelreich, U. (2007) Cell tracking using magnetic resonance imaging. J Physiol. 584, 25–30.
39 Walczak, P., Zhang, J., Gilad, A.A., Kedziorek, D.A., Ruiz-Cabello, J., Young, R.G., Pittinger, M.F., van Zijl, P.C., Huang, J., Bulte, J.W. (2008) Dual modality monitoring of targeted intraarterial delivery of mesenchymal stem cells after transient ischemia. Stroke 39, 1569–1574.
40 Kim, D., Chun, B.G., Kim, Y.K., Lee, Y.H., Park, C.S., Jeon, I, Cheong, C., Hwang, T.S., Chung, H., Gwag, B.J., Hong, K.S., Song, J. (2008) In vivo tracking of human mesenchymal stem cells in experimental stroke. Cell Transplant. 16, 1007–1012.
41 Shyu, W.C., Chen, C.P., Lin, S.Z., Lee, Y.J., Li, H. (2007) Efficient tracking of non-iron-labeled mesenchymal stem cells with serial MRI in chronic stroke rats. Stroke 38, 367–374.
42 Modo, M., Beech, J.S., Meade, T.J., Williams, S.C., Price, J. (2008) A chronic 1 year assessment of MRI contrast agent-labelled neural stem cell transplants in stroke. Neuroimage Jun 25 [Epub ahead of print]

43 Mori, S. and van Zijl, P.C. (2002) Fiber tracking: principles and strategies-a technical review. N.M.R. Biomed. 15, 468–480.
44 Watanabe, T., Honda, Y., Fujii, Y., Koyama, M., Matsuzawa, H. and Tanaka, R. (2001) Three-dimensional anisotropy contrast magnetic resonance axonography to predict the prognosis for motor function in patients suffering from stroke. J. Neurosurg. 94, 955–960.
45 Luker, G.D., Luker, K.E. (2008) Optical imaging: current applications and future directions. J. Nucl. Med. 49,1–4.
46 Reumers, V., Deroose, C.M., Krylyshkina, O., Nuyts, J., Geraerts, M., Mortelmans, L., Gijsbers, R., Van Den Haute, C., Debyser, Z., Baekelandt, V. Non-invasive and Quantitative Monitoring of Adult Neuronal Stem Cell Migration in Mouse Brain using Bioluminescence Imaging. Stem Cells Jul 3 [Epub ahead of print]
47 Kim, D.E., Schellingerhout, D., Ishii, K., Shah, K., Weissleder, R. (2004) Imaging of stem cell recruitment to ischemic infarcts in a murine model. Stroke 35, 952–957.
48 Cordeau, P. Jr., Lalancette-Hébert, M., Weng, Y.C., Kriz, J. (2008) Live imaging of neuroinflammation reveals sex and estrogen effects on astrocyte response to ischemic injury. Stroke 39, 935–942.
49 Luskin, M.B., Zigova, T., Soteres, B.J. and Stewart, R.R. (1997) Neuronal progenitor cells derived from the anterior subventricular zone of the neonatal rat forebrain continue to proliferate in vitro and express a neuronal phenotype. Mol. Cell. Neurosci. 8, 351–366.
50 Alvarez-Buylla, A., Herrera, D.G. and Wichterle, H. (2000) The subventricular zone: source of neuronal precursors for brain repair. Prog. Brain Res. 127, 1–11.
51 Sanai, N., Tramontin, A.D., Quiñones-Hinojosa, A., Barbaro, N.M., Gupta, N., Kunwar, S., Lawton, M.T., McDermott, M.W., Parsa, A.T., Manuel Garcìa Verdugo, J., Berger, M.S. and Alvarez Buylla, A. (2004) Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. Nature 427, 740–744.
52 Curtis, M.A., Kam, M., Nannmark, U., Anderson, M.F., Axell, M.Z., Wikkelso, C., Holtas, S., van Roon Mom, W.M., Bjork-Eriksson, T., Nordborg, C., Frisèn, J., Dragunow, M., Faull, R.L. and Eriksson, P.S. (2007) Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. Science 315, 1243–1249.
53 Kaplan, M.S. and Bell, D.H. (1984) Mitotic neuroblasts in the 9-day-old and 11-month-old rodent hippocampus. J. Neurosci. 4, 1429–1441.
54 Kuhn, H.G., Dickinson-Anson, H. and Gage, F.H. (1996) Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J. Neurosci. 16, 2027–2033.
55 Palmer, T.D., Markakis, E.A., Willhoite, A.R., Safar, F. and Gage, F.H. (1999) Fibroblast growth factor-2 activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS. J. Neurosci. 19, 8487–8497.
56 Yamamoto, S., Nagao, M., Sugimori, M., Kosako, H., Nakatomi, H., Yamamoto, N., Takebayashi, H., Nabeshima, Y, Kitamura, T., Weinmaster, G., Nakamura, K. and Nakafuku, M. (2001) Transcription factor expression and Notch-dependent regulation of neural progenitors in the adult rat spinal cord. J. Neurosci. 21, 9814–9823.
57 Pardal, R., Ortega-Sáenz, P., Durán, R., López-Barneo, J. (2007) Glia-like stem cells sustain physiologic neurogenesis in the adult mammalian carotid body. Cell 131, 364–377.
58 Vaynman, S. and Gomez-Pinilla, F. (2005) License to run: exercise impacts functional plasticity in the intact and injured central nervous system by using neurotrophins. Neurorehabil. Neural Repair 19, 283–295.
59 Pereira, A.C., Huddleston, D.E., Brickman, A.M., Sosunov, A.A., Hen, R., McKhann, G.M., Sloan, R., Gage, F.H., Brown, T.R., Small, S.A. (2007) An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. Proc. Natl. Acad. Sci. U. S. A. 104, 5638–5643.
60 Minger, S.L., Ekonomou, A., Carta, E.M., Chinoy, A., Perry, R.H. and Ballard, C.G. (2007) Endogenous neurogenesis in the human brain following cerebral infarction. Regen. Med. 2, 69–74.
61 Zhang, R.L., Zhang, Z.G., Chopp, M. (2008) Ischemic stroke and neurogenesis in the subventricular zone. Neuropharmacology. Jun 3 [Epub ahead of print]
62 Arvidsson, A., Collin, T., Kirik, D., Kokaia, Z. and Lindvall, O. (2002) Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat. Med. 8, 963–970.
63 Zhang, R.L., Zhang, Z.G., Roberts, C., Letourneau, Y., Lu, M., Zhang, L., Wang, Y. and Chopp, M. (2008) Lengthening the G(1) phase of neural progenitor cells is concurrent with an increase of symmetric neuron generating division after stroke. J. Cereb. Blood Flow Metab. 28, 602–611.
64 Zhang, R.L., Zhang, Z.G, Wang, Y., Letourneau, Y., Liu, X.S., Zhang, X., Gregg, S.R., Wang, L., and Chopp, M. (2007) Stroke induces ependymal cell transformation into radial glia in the subventricular zone of the adult rodent brain. J. Cereb. Blood Flow Metab. 27, 1201–1212.
65 Yoshimura, S., Takagi, Y., Harada, J., Teramoto, T., Thomas, S.S., Waeber, C., Bakowska, J.C., Breakefield, X.O. and Moskowitz, M.A. (2001) Fgf-2 regulation of neurogenesis in adult hippocampus after brain injury. Proc. Natl. Acad. Sci. U.S.A. 98, 5874–5879.
66 Nakatomi, H., Kuriu, T., Okabe, S., Yamamoto, S., Hatano, O., Kawahara, N., Tamura, A., Kirino, T. and Nakafuku, M. (2002) Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. Cell 110, 429–441.
67 Teramoto, T., Qiu, J., Plumier, J.C. and Moskowitz, M.A. (2003) EGF amplifies the replacement of parvalbumin-expressing striatal interneurons after ischemia. J. Clin. Invest. 111, 1125–1132.
68 Louissaint, A.Jr., Rao, S., Leventhal, C. and Goldman, S.A. (2002) Coordinated interaction of neurogenesis and angiogenesis in the adult songbird brain. Neuron 34, 945–960.
69 Palma, V., Lim, D.A., Dahmane, N., Sanchez, P., Brionne, T.C., Herzberg, C.D., Gitton, Y., Carleton, A., Alvarez-Buylla, A., Ruiz, I. and Altaba, A. (2005) Sonic hedgehog controls stem cell behavior in the post natal and adult brain. Development 132, 335–344.
70 Wang, L., Zhang, Z.G., Zhang, R.L., Gregg, S.R., Hozeska-Solgot, A., LeTourneau, Y., Wang, Y. and Chopp, M. (2006) Matrix metalloproteinase 2 (MMP2) and MMP9 secreted by erythropoietin-activated endothelial cells promote neural progenitor cell migration. J. Neurosci. 26, 5996–6003.
71 Lee, S.R., Kim, H.Y., Rogowska, J., Zhao, B.Q., Bhide, P., Parent, J.M. and Lo, E.M. (2006) Involvement of matrix metalloproteinase in neuroblast cell migration from the subventricular zone after stroke. J. Neurosci. 26, 3491–3495.
72 Feng, Y. and Walsh, C.A. (2001) Protein-protein interactions, cytoskeletal regulation and neuronal migration. Nat. Rev. Neurosci. 2, 408–416.
73 Koizumi, H., Higginbotham, H., Poon, T., Tanaka, T., Brinkman, B.C. and Gleeson, J.G. (2006) Doublecortin maintains bipolar shape and nuclear translocation during migration in the adult forebrain. Nat. Neurosci. 9, 779–786.
74 Reynolds, B.A. and Weiss, S. (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255, 1707–1710.
75 Corti, S., Nizzardo, M., Nardini, M., Donadoni, C., Locatelli, F., Papadimitriou, D., Salani, S., Del Bo, R., Ghezzi, S., Strazzer, S., Bresolin, N. and Comi, G.P. (2007) Isolation and characterization of murine neural stem/progenitor cells based on Prominin-1 expression. Exp. Neurol. 205, 547–562.
76 Corti, S., Locatelli, F., Papadimitriou, D., Donadoni, C., Salani, S., Del Bo, R., Strazzer, S., Bresolin, N. and Comi, G.P. (2006) Identification of a primitive brain-derived neural stem cell population based on aldehyde dehydrogenase activity. Stem Cells 24, 975–985.

77 Rietze, R.L. and Reynolds, B.A. (2006) Neural stem cell isolation and characterization. Methods Enzymol. 419, 3–23.
78 Barraud, P., Thompson, L., Kirik, D., Björklund, A. and Parmar, M. (2005) Isolation and characterization of neural precursor cells from the Sox1-GFP reporter mouse. Eur. J. Neurosci. 22, 1555–1569.
79 Kelly, S., Bliss, T.M., Shah, A.K., Sun, G.H., Ma, M., Foo, W.C., Masel, J., Yenari, M.A., Weissman, I.L., Uchida, N., Palmer, T. and Steinberg, G.K. (2004) Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex. Proc. Natl. Acad. Sci. U.S.A. 101, 11839–11844.
80 Mattsson, B., Sorensen, J.C., Zimmer, J. and Johansson, B.B. (1997) Neural grafting to experimental neocortical infarcts improves behavioral outcome and reduces thalamic atrophy in rats haused in enriched but not in standard environments. Stroke 28, 1225–1231.
81 Darsalia, V., Kallur, T. and Kokaia, Z. (2007) Survival, migration and neuronal differentiation of human fetal striatal and cortical neural stem cells grafted in stroke-damaged rat striatum. Eur. J. Neurosci. 26, 605–614.
82 Guzman, R., De Los Angeles, A., Cheshier, S., Choi, R., Hoang, S., Liauw, J., Schaar, B. and Steinberg, G. (2008) 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 39, 1300–1306.
83 Shyu, W.C., Liu, D.D., Lin, S.Z., Li, W.W., Su, C.Y., Chang, Y.C., Wang, H.J., Wang, H.W., Tsai, C.H., Li, H. (2008) Implantation of olfactory ensheathing cells promotes neuroplasticity in murine models of stroke. J. Clin. Invest. 118, 2482–2495.
84 Zhang, Z.G., Jiang, Q., Zhang, R., Zhang, L., Wang, L., Zhang, L., Arniego, P., Ho, K.L. and Chopp, M. (2003) Magnetic resonance imaging and neurosphere therapy of stroke in rat. Ann. Neurol. 53, 259–263.
85 Guzman, R., Bliss, T., De Los Angeles, A., Moseley, M., Palmer, T. and Steinberg, G. (2008) Neural progenitor cells ransplanted into the uninjured brain undergo targeted migration after stroke onset. J. Neurosci. Res. 86, 873–882.
86 Chu, K., Jung, K.H., Kim, S.J., Lee, S.T., Kim, J., Park, H.K., Song, E.C., Kim, S.U., Kim, M., Lee, S.K., Roh, J.K. (2008) Transplantation of human neural stem cells protect against ischemia in a preventive mode via hypoxia-inducible factor-1alpha stabilization in the host brain. Brain Res. 1207, 182–192.
87 Maysami, S., Lan, J.Q., Minami, M., Simon, R.P. (2008) Proliferating progenitor cells: a required cellular element for induction of ischemic tolerance in the brain. J. Cereb. Blood Flow Metab. 28, 1104–1113.
88 Bühnemann, C., Scholz, A., Bernreuther, C., Malik, C.Y., Braun, H., Schachner, M., Reymann, K.G. and Dihné, M. (2006) Neuronal differentiation of transplanted embryonic stem cell derived precursors in stroke lesions of adult rats. Brain 129, 3238–3248.
89 Hayashi, J., Takagi, Y., Fukuda, H., Imazato, T., Nishimura, M., Fujimoto, M., Takahashi, J., Hashimoto, N. and Nozaki, K. (2006) Primate embryonic stem cell-derived neuronal progenitors transplanted into ischemic brain. J. Cereb. Blood Flow Metab. 26, 906–914.
90 Ikeda, R., Kurokawa, M.S., Chiba, S., Yoshikawa, H., Ide, M., Tadokoro, M., Nito, S., Nakatsuji, N., Kondoh, Y., Nagata, K., Hashimoto, T. and Suzuki, N. (2005) 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. 20, 38–48.
91 Wei, L., Cui, L., Snider, B.J., Rivkin, M., Yu, S.S., Lee, C.S., Adams, L.D., Gottlieb, D.I., Johnson, E.M.Jr., Yu, S.P. and Choi, D.W. (2005) Transplantation of embryonic stem cells overexpressing Bcl-2 promotes functional recovery after transient cerebral ischemia. Neurobiol. Dis. 19, 183–193.
92 Theus, M.H., Wei, L., Cui, L., Francis, K., Hu, X., Keogh, C., Yu, S.P. (2008) 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. 210, 656–670.
93 Yamanaka, S. (2008) Induction of pluripotent stem cells from mouse fibroblasts by four transcription factors. Cell Prolif. 41, Suppl 1, 51–56.
94 Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., Yamanaka, S. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861–872.
95 Park, I.H., Zhao, R., West, J.A., Yabuuchi, A., Huo, H., Ince, T.A., Lerou, P.H., Lensch, M.W. and Daley, G.Q. (2008) Reprogramming of human somatic cells to pluripotency with defined factors. Nature 451, 141–146.
96 Andrews, P.W., Damjanov, I., Simon, D., Banting, G.S., Carlin, C., Dracopoli, N.C. and Fogh, J. (1984) Pluripotent embryonal carcinoma clones derived from the human teratocarcinoma cell line Tera-2. Differentiation in vivo and in vitro. Lab. Invest. 50, 147–162.
97 Borlongan, C.V., Tajima, Y., Trojanowski, J.Q., Lee, V.M. and Sanberg, P.R. (1998) Transplantation of cryopreserved human embryonal carcinoma-derived neurons (NT2N cells) promotes functional recovery in ischemic rats. Exp. Neurol. 149, 310–321.
98 Park, K.I. (2000) Transplantation of neural stem cells: cellular and gene therapy for hypoxic-ischemic brain injury. Yonsei Med. J. 41, 825–835.
99 Daadi, M.M., Maag, A.L. and Steinberg, G.K. (2008) Adherent self-renewable human embryonic stem cell-derived neural stem cell line: functional engraftment in experimental stroke model. PLOS ONE 3, e1644.
100 Kim, S.U., Nagai, A., Nakagawa, E., Choi, H.B., Bang, J.H., Lee, H.J., Lee, M.A., Lee, Y.B., Park, I.H. (2008) Production and characterization of immortal human neural stem cell line with multipotent differentiation property. Methods Mol. Biol. 438, 103–121.
101 Chen, J., Li, Y., Wang, L., Lu, M., Zhang, X. and Chopp, M. (2001) Therapeutic benefit of intracerebral transplantation of bone marrow stromal cells after cerebral ischemia in rats. J. Neurol. Sci. 189, 49–57.
102 Chen, J., Li, Y., Wang, L., Zhang, Z., Lu, D., Lu, M. and Chopp M. (2001) Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. Stroke 32, 1005–1011.
103 Li, Y., Chen, J., Wang, L., Lu, M. and Chopp, M. (2001) Treatment of stroke in rat with intracarotid administration of marrow stromal cells. Neurology 56, 1666–1672.
104 Willing, A.E., Vendrame, M., Mallery, J., Cassady, C.J., Davis, C.D., Sanchez-Ramos, J. and Sanberg, P.R. (2003) Mobilized peripheral blood cells administered intravenously produce functional recovery in stroke. Cell Transplant. 12, 449–454.
105 Wang ,Y., Deng, Y., Zhou, G.Q. (2008) SDF-1alpha/CXCR4-mediated migration of systemically transplanted bone marrow stromal cells towards ischemic brain lesion in a rat model. Brain Res. 1195, 104–112.
106 Koh, S.H., Kim, K.S., Choi, M.R., Jung, K.H., Park, K.S., Chai, Y.G., Roh, W., Hwang, S.J., Ko, H.J., Huh, Y.M., Kim, H.T., Kim, S.H. (2008) Implantation of human umbilical cord-derived mesenchymal stem cells as a neuroprotective therapy for ischemic stroke in rats. Brain Res. Jul 2 [Epub ahead of print].
107 Hau, S., Reich, D.M., Scholz, M., Naumann, W., Emmrich, F., Kamprad, M., Boltze, J. (2008) Evidence for neuroprotective properties of human umbilical cord blood cells after neuronal hypoxia in vitro. BMC Neurosci. 9, 30.
108 Onda, T., Honmou, O., Harada, K, Houkin, K., Hamada, H., Kocsis, J.D. (2008) Therapeutic benefits by human mesenchymal stem cells (hMSCs) and Ang-1 gene-modified hMSCs after cerebral ischemia. J. Cereb. Blood Flow Metab. 28, 329–340.

109 Kim, S.S., Yoo, S.W., Park, T.S., Ahn, S.C., Jeong, H.S., Kim, J.W., Chang, D.Y., Cho, K.G., Kim, S.U., Huh, Y., Lee, J.E., Lee, S.Y., Lee, Y.D., Suh-Kim, H. (2008) Neural Induction with Neurogenin1 Increases the Therapeutic Effects of Mesenchymal Stem Cells in the Ischemic Brain. Stem Cells Jul 10 [Epub ahead of print].

110 Ukai, R., Honmou, O., Harada, K., Houkin, K, Hamada, H., Kocsis, J.D. (2007) Mesenchymal stem cells derived from peripheral blood protects against ischemia. J. Neurotrauma 24, 508–520.

111 Andrews, E.M., Tsai, S.Y., Johnson, S.C., Farrer, J.R., Wagner, J.P., Kopen, G.C., Kartje, G.L. (2008) Human adult bone marrow-derived somatic cell therapy results in functional recovery and axonal plasticity following stroke in the rat. Exp Neurol. 211, 588–592.

112 Sprigg, N., Bath, P. (2005) Pharmacological enhancement of recovery from stroke. Stroke Review 8, 33–40.

113 Shabitz, W.R., Kollmar, R., Schwannger, M., Juettler, E., Bardutzky, J., Scholze, M.N., Sommer, C., Schwab, S. (2003) Neuroprotective Effect of Granulocyte Colony-Stimulating Factor After Focal Cerebral Ischemia. Stroke 34, 745–751.

114 Solaroglu, I., Tsubokawa, T., Cahill, J., Zhang, J.H. (2006) Anti-apoptotic effect of granulocyte-colony stimulating factor after focal cerebral ischemia in the rat. Neurosci. 143, 965–974.

115 Sehara, Y., Hayashi, T., Deguchi, K., Zhang, H., Tsuchiya, A., Yamashita, T., Lukic, V., Nagai, M., Kamiya, T., and Abe, K. (2007) Decreased Focal Inflammatory Response by G-CSF May Improve Stroke Outcome After Transient Middle Cerebral Artery Occlusion in Rats. J. Neurosci. Res. 85, 2167–2174.

116 Sehara, Y., Hayashi, T., Deguchi, K., Zhang, H., Tsuchiya, A., Yamashita, T., Lukic, V., Nagai, M., Kamiya, T., Abe, K. (2007) Potentiation of neurogenesis and angiogenesis by G-CSF after focal cerebral ischemia in rats. Brain Res. 1151, 142–149.

117 Shyu, W.C., Lin, S.Z., Yang, H.I., Tzeng, Y.S., Pang, C.Y., Yen, P.S. and Li, H. (2004) Functional recovery of stroke rats induced by granulocyte colony-stimulating factor-stimulated stem cells. Circulation 110, 1847–1854.

118 Sprigg, N, Bath, P.M., Zhao, L., Willmot, M.R., Gray, L.J., Walker, M.F., Dennis, M.S., Russell, N. (2006) Granulocyte-Colony–Stimulating Factor Mobilizes Bone Marrow Stem Cells in Patients With Subacute Ischemic Stroke. The Stem Cell Trial of Recovery EnhanceMent After Stroke (STEMS) Pilot Randomized, Controlled Trial (ISRCTN 16784092).

119 Shyu, W.C., Lin, S.Z., Lee, C.C., Liu, D.D., Li, H. (2006) Granulocyte colony-stimulating factor for acute ischemic stroke: a randomized controlled trial. C.M.A.J. 174, 927–933.

120 Bath, P.M.W., Sprigg, N. (2007) Colony stimulating factors (including erythropoietin, granulocyte colony stimulating factor and analogues) for stroke. Cochrane Database of Systematic Reviews 2007, Issue 2.

121 Schabitz, W.R., Schneider, A. (2006) Developing Granulocyte-Colony Stimulating Factor for the treatment of Stroke: Current Status of Clinical Trials. Stroke 37, 1654.

122 Schäbitz, W.R., Krüger, C., Pitzer, C., Weber, D., Laage, R., Gassler, N., Aronowski, J., Mier, W., Kirsch, F., Dittgen, T., Bach, A., Sommer, C., Schneider, A. (2008) A neuroprotective function for the hematopoietic protein granulocyte-macrophage colony stimulating factor (GM-CSF). J. Cereb. Blood Flow Metab. 28, 29–43.

123 Schneider, U.C., Schilling, L., Schroeck, H., Nebe, C.T., Vajkoczy, P., Woitzik, J. (2007) Granulocyte-Macrophage Colony-Stimulating Factor–Induced Vessel Growth Restores Cerebral Blood Supply After Bilateral Carotid Artery Occlusion. Stroke 38, 1320–1328.

124 Wang, L., Zhang, Z., Wang, Y., Zhang, R., Chopp, M. (2004) Treatment of Stroke With Erythropoietin Enhances Neurogenesis and Angiogenesis and ImprovesNeurological Function in Rats. Stroke 35, 1732–1737.

125 Weil, L., Han, B.H., Li, Y., Keogh, C.L., Holtzman, D.M. and Yu, S.P. (2006) Cell Death Mechanism and Protective Effect of Erythropoietin after Focal Ischemia in the Whisker-Barrel Cortex of Neonatal Rats. J. Pharm. Exp Ther. 317, 109- 116.

126 Ehrenreich, H., Hasselblatt, M., Deambowski, C., Cepek, I., Lewczuk, P., Stiefel, M., Rustenbeck, H.H., Breiter, N., Jacob, S., Knerlich, F., Bohn, M., Poser, W., Rüther, E., Kochen, M., Gefeller, O., Gleiter, C., Wessel, T.C., De Ryck, M., Itri, L., Prange, H., Cerami, A., Brines, M., Sirén, A.L. (2002) Erithropoietin therapy for acute stroke is both safe and beneficial. Mol. Med. 8, 495–505.

127 Morita, Y., Takizawa, S., Kamiguchi, H., Uesugi, T., Kawada, H., Takagi, S. (2007) Administration of hematopoietic cytokines increases the expression of anti-inflammatory cytokine (IL-10) mRNA in the subacute phase after stroke. Neurosci. Res. 58, 356–360.

128 Zhao, L.R., Singhal, S., Duan, W.M., Mehta, J., Kessler, J.A. (2007) Brain Repair by Hematopoietic Growth Factors in a Rat Model of Stroke. Stroke 38, 2584–2591.

129 Toth, Z.E., Leker, R.R., Shahar, T., Pastorino, S., Szalayova, I., Asemenew, B., Key, S., Parmelee, A., Mayer, B., Nemeth, K., Bratincsák, A., Mezey, E. (2008) The combination of granulocyte colony-stimulating factor and stem cell factor significantly increases the number of bone marrow-derived endothelial cells in brains of mice following cerebral ischemia. Blood. 111, 5544–5552.

130 Leung, D.W., Cachianes, G., Kuang, W.J., Goeddel, D.V., Ferrara, N. (1969) Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 246, 1306–1309.

131 Gerber, H.P., McMurtrey, A., Kowalski, J., Yan, M., Keyt, BA, Dixit, V., Ferrara, N. (1998) Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 39-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation. J. Biol. Chem. 273, 30336–30343.

132 Sun, Y., Jin, K., Xie, L., Childs, J., Mao, X.O., Logvinova, A., Greenberg, D.A. (2003) VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. J. Clin. Invest. 111, 1843–1851.

133 Zhang, Z.G., Zhang, L., Jiang, Q., Zhang, R., Davies, K., Powers, C., Bruggen, N., Chopp, M. VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. J Clin. Invest. 106, 829–838.

134 Wang, Y., Galvan, V., Gorostiza, O., Ataie, M., Jin, K., Greenberg, D.A. (2006) Vascular endothelial growth factor improves recovery of sensorimotor and cognitive deficits after focal cerebral ischemia in the rat. Brain Res. 1115, 186–193.

135 Wang, Y.Q., Guo, X., Qiu, M.H., Feng, X.Y., Sun, F.Y. (2007) VEGF overexpression enhances striatal neurogenesis in brain of adult rat after a transient middle cerebral artery occlusion. J. Neurosci. Res. 85, 73–82.

136 Conti, E., Carrozza, C., Capoluongo, E., Volpe, M., Crea, F., Zuppi, C., Andreotti, F. (2004) Insulin-like growth factor-1 as a vascular protective factor. Circulation 110: 2260–2265.

137 Zhu, W., Fan, Y., Frenzel, T., Gasmi, M., Bartus, R.T., Young, W.L., Yang, G.Y., Chen, Y. (2008) Insulin Growth Factor-1 Gene Transfer Enhances Neurovascular Remodeling and Improves Long-Term Stroke Outcome in Mice. Stroke 39: 1254–1261.

138 Shyu, W.C., Lin, S.Z., Yen, P.S., Su, C.Y., Chen, D.C., Wang, H.J., Li, H. (2008) Stromal Cell-Derived Factor-1 Promotes Neuroprotection, Angiogenesis, and Mobilization/Homing of Bone Marrow-Derived Cells in Stroke Rats. J. Pharmacol. Exp. Ther. 324, 834–849.

139 Bushnell, C.D. (2008) Stroke and the female brain. Nat. Clin. Pract. Neurol. 4, 22–33.

140 Wilson, A.C., Clemente, L., Liu, T., Bowen, R.L., Meethal, S.V., Atwood, C.S. (2008) Reproductive hormones regulate the selective permeability of the blood-brain barrier. Biochim. Biophys. Acta. 1782, 401–407.

141 Lang, J.T., McCullough, L.D. (2008) Pathways to ischemic neuronal cell death: are sex differences relevant? J. Transl. Med. 6,33.
142 Bang, O.Y., Lee, J.S., Lee, P.H. and Lee, G. (2005) Autologous mesenchymal stem cell transplantation in stroke patients. Ann. Neurol. 57, 874–882.
143 Kondziolka, D., Wechsler, L., Goldstein, S., Meltzer, C., Thulborn, K.R., Gebel, J., Jannetta, P., De Cesare, S., Elder, E.M., McGrogan, M., Reitman, M.A. and Bynum, L. (2000) Transplantation of cultured human neuronal cells for patients with stroke. Neurology 55, 565–569.
144 Rabinovich, S.S., Seledtsov, V.I., Banul, N.V., Poveshchenko, O.V., Senyukov, V.V., Astrakov, S.V., Samarin, D.M. and Taraban, V.Y. (2005) Cell therapy of brain stroke. Bull. Exp. Biol. Med. 139, 126–128.
145 Savitz, S.I., Dinsmore, J., Wu, J., Henderson, G.V., Stieg, P. and Caplan, L.R. (2005) Neurotransplantation of fetal porcine cells in patients with basal ganglia infarcts: a preliminary safety and feasibility study. Cerebrovasc. Dis. 20, 101–107.
146 Nelson, P.T., Kondziolka, D., Wechsler, L., Goldstein, S., Gebel, J., De Cesare, S., Elder, E.M., Zhang, P.J., Jacobs, A., McGrogan, M., Lee, V.M. and Trojanowski, J.Q. (2002) Clonal human (hNT) neuron grafts for stroke therapy: neuropathology in a patient 27 months after implantation Am. J. Pathol. 160, 1201–1206.
147 Meltzer, C.C., Kondziolka, D., Villemagne, V.L., Wechsler, L., Goldstein, S., Thulborn, K.R., Gebel, J., Elder, E.M., De Cesare, S. and Jacobs, A. (2001) Serial [18F] fluorodeoxyglucose positron emission tomography after human neuronal implantation for stroke. Neurosurg. 49, 586–591.
148 Taupin, P. (2006) HuCNS-SC (StemCells). Curr. Opin. Mol. Ther. 8, 156–163.
149 Stroemer, P., Hope, A., Patel, S., Pollock, K. and Sinden, J. (2008) Development of a human neural stem cell line for use in recovery from disability after stroke. Front. Biosci. 13, 2290–2292.