Neural stem/precursor cells for the treatment of ischemic stroke

Marco Bacigaluppi [a,b], Stefano Pluchino [b], Gianvito Martino [b],
Ertugrul Kilic [a], Dirk M. Hermann [a,*]

[a] *Department of Neurology, University Hospital Zurich (USZ), Frauenklinikstr. 26, CH-8091 Zurich, Switzerland, CH-8091 Zurich, Switzerland*
[b] *Neuroimmunology Unit, DIBIT and Institute of Experimental Neurology, IRCCS San Raffaele*

Abstract

In ischemic stroke, the third most frequent cause of mortality in industrialized countries, therapeutic options have until now been limited to the first hours after disease onset. Cell transplantation has emerged in various neurological disorders, including experimental stroke, as a successful recovery-promoting approach also in the post-acute stroke phase. However, before envisaging any translation into humans of such promising cell-based approaches we still need to clarify: (i) the ideal cell source for transplantation, (ii) the most appropriate route of cell administration, and, last but not least, (iii) the best approach to achieve an appropriate and functional integration of transplanted cells into the host tissue. Here we discuss, with special emphasis on neural stem/precursor cells, potential mechanisms that may be involved in the action of cell-based therapies in stroke.
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1. Introduction

Ischemic stroke is the leading cause of long-term disability and the third most frequent cause of mortality in industrialized countries. With an annual incidence of approximately 250–400 in 100,000 inhabitants [1,2], about one million people in the EU suffer a stroke each year, many of them being struck by persistent long-term handicaps. Stroke is caused by the interruption of blood flow in a brain-supplying artery, in most cases by embolic vascular occlusion [3]. Despite considerable progress in recanalizing (*i.e.*, thrombolytic) [4] and secondary stroke prevention [5] therapies in recent years there are still no neuroprotective therapies available that allow to reduce brain damage and to improve neurological recovery once a stroke has occurred [6].

Following the discovery in the late sixties that neural stem/precursor cells (NPCs) are continuously generated in the adult rodent brain [7], it was recently shown that endogenous NPCs are activated in response to ischemia, both in rodents [8] and humans [9]. Stem/precursor cells residing within the germinal niches of the brain have the ability to migrate towards the stroke lesion, where they may induce recovery of the surrounding non-lesioned tissue [8–11]. While this observation has raised hopes that stem cells may be used for therapeutic purposes, it is still a matter of debate which mechanisms underlie the therapeutic efficacy of such cells.

2. NPCs for stroke

Different types of stem/precursor cells were used in experimental stroke in the past, among which neural, hematopoietic, bone marrow, and umbilical cord cells should be mentioned (see [12,13]; for NPCs see also Table 1). Several different delivery techniques were tested, including intraparenchymal transplantations, intrathecal injections or intravenous infusions (Table 1). As a consequence of the first encouraging results from experimental studies, pre-clinical phase I and II trials, using different types of stem cells, were tested in patients suffering from stroke (Table 2).

Although some of these trials could demonstrate neurological improvements and cell transplantations appeared to be

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* Corresponding author. Tel.: +41 44 255 5526; fax: +41 44 255 4507.
E-mail address: dirk.hermann@usz.ch (D.M. Hermann).

Table 1
Neural precursor cells in animal models of ischemic stroke

Animal	Stroke model	Cell type	Time of transplantation (after stroke)	Route of transplantation	Therapeutic action					Refs
					Cell replacement	Neuro-protection	Immuno-modulation	Plasticity	Functional outcome	
Rat	MCAO	Human NT2N teratocarcinoma-derived NPCs	1 month	Intraparenchymal	+		Not tested	Not tested	Not tested	Improved	[14]
Mouse	CCAO+ Hypoxia	Neonatal cerebellar C17.2-CD NPCs	7 days	Intraparenchymal	+		Not tested	+	Not tested	Not tested	[15]
Rat	MCAO	Immortalized murine neuroepithelial MHP36 cells	2–3 weeks	Intraparenchymal	+		Not tested	Not tested	Not tested	Improved	[16]
Rat	MCAO	Human fetal NPCs	7 days	Intraparenchymal	+		Not tested	Not tested	Not tested	Not tested	[17]
Rat	MCAO	Human fetal HB1.F3 NPCs	24 h	Intravenous	+	+	Not tested	Not tested	Improved	[18]
Gerbil	CCAO	Human fetal NPCs	96 h	Intraparenchymal	+		Not tested	Not tested	Not tested	Improved	[19]
Rat	MCAO	Rat NPCs	48 h	Intracisternal	Not tested	Not tested	Not tested	+	Not tested	[20]
Rat	MCAO	Human fetal NPCs	48 h	Intraarterial	Not tested	Not tested	Not tested	+	Improved	[21]
Rat	MCAO	Immortalized human CTX0E03 NPCs	3–4 weeks	Intraparenchymal	+		Not tested	Not tested	Not tested	Improved	[22]
Rat	MCAO	Murine ES cell-derived NPCs	7 days	Intraparenchymal	+		Not tested	Not tested	Not tested	Not tested	[23]
Mouse	MCAO	ES cell-derived NPCs	24 h	Intraparenchymal	+		Not tested	Not tested	Not tested	Not tested	[24]
Mouse	CCAO+ hypoxia	Neonatal cerebellar C17.2-CD NPCs	24 h, 72 h, 1, 2 and 5 weeks	Intraparenchymal	+	+	Not tested	Not tested	Not tested	[25]
Monkey	MCAO	Human NPCs	7 days	Intraparenchymal	+		Not tested	Not tested	Not tested	Not tested	[26]

Abbreviations: MCAO, middle cerebral artery occlusion; CCAO, common carotid artery occlusion. +, favourable influence found.

a safe procedure, the precise mechanisms underlying the restorative effects of stem cells were still poorly known at the time of these early studies. Furthermore, the lack of reliable surrogate marker to test the mechanism of action in patients with stroke and the non-existence of long-term observations regarding the influence of stem cells in the patients' brains, together with observations of tumor formation in mice with experimental stroke treated with local parenchymal implantations of embryonic stem (ES) cells [32], raised safety concerns, which slowed down further progress.

However, the scientific community has to intensify efforts in order to evaluate the efficacy of these strategies since in these days thousands of desperate patients are estimated to be on the waiting list to receive stem cell-based therapies in centers not rigorously following approved clinical trial protocols [33]. In the following, we shortly summarize (a) advantages and disadvantages of ES cells compared with adult NPCs, (b) concepts on the pathotropism of NPCs, as

well as (c) prevailing ideas about precursor cell actions in the stroke brain.

3. Stem cell sources: ES-derived *vs.* adult NPCs

The ideal cell for transplantation should meet first of all the criteria of safety for the receiver as well as offer the highest therapeutic potential. Unlike hematopoietic bone marrow reconstitution, where a single cell may be sufficient to replenish the whole body's stores, therapeutic preparations for stroke requires an adequate cell number, which raises the need to expand *in vitro* the putative precursor cell source.

Despite several groups reporting that ES cells have promising therapeutic potential [13,23,24], ethical and safety concerns (such as feeder-independent expansion and *in vivo* teratocarcinoma formation) still limit their translation to clinics. On the other hand, adult NPCs, which can be obtained

Table 2
Cell-based therapies tested in pre-clinical trials

Clinical trial	No. of patients	Disease	Cell type	Time of transplantation (after stroke)	Route of transplantation	Therapeutic action		Refs.
						Cell replacement	Functional outcome	
Phase I	12	Basal ganglia infarcts	Human NT2/D1 teratocarcinoma-derived NPCs	Mean: 27 months (range: 7–55)	Intraparenchymal	(+)	Some improvement	[27,28]
Phase II	18	Ischemic/hemorrhagic infarcts	Human NT2/D1 teratocarcinoma-derived NPCs	Mean: 3.5 years (range: 1–5)	Intraparenchymal	Not tested	Some improvement	[29]
Phase I/II	30	MCA infarcts	Autologous mesenchymal precursor cells	4–9 weeks	Intravenous	Not tested	Improved	[30]
Phase I	5	Basal ganglia infarcts	Fetal porcine cells	Mean 5 years	Intraparenchymal	Not tested	No improvement	[31]

Abbreviations: MCA, middle cerebral artery. (+), histological evidence in one single patient.

from different tissues and safely expanded *in vitro*, have shown promising therapeutical effects in several neurological disorders without causing serious side effects.

4. Pathotropism of NPCs

Whatever route of transplantation is chosen (local intra-parenchymal or systemic), NPCs, both embryonic and adult, have the capacity to migrate long distances along chemoattractive gradients induced in sites of brain injury [24,34].

It has been, in fact, shown that transplanted stem/precursor cells are able to follow, *via* the blood stream or cerebrospinal fluid circulation, gradients of pro-inflammatory cytokines and chemokines that are released at the site of brain lesions. While promoting interaction between transplanted NPCs and activated endothelial/ependymal cells around inflamed CNS tissues, this chemoattractive gradient leads to selective and specific homing of transplanted cells in inflamed CNS areas [35–37].

Although the specific homing of transplanted cells has been demonstrated in spinal cord injury, brain tumors, epilepsy as well as in stroke, the exact molecular mechanisms that sustain this phenomenon have been detailed in particular in experimental autoimmune enchepahlomyelits (EAE), the animal model of multiple sclerosis. Tethering, rolling and firm adhesion to inflamed endothelial cells and extravasation into inflamed CNS areas are sequentially mediated by the constitutive expression of functional cell adhesion molecules, integrins and chemokine receptors on the surface of NPCs [38,39].

5. Differentiation of stem cells: ES cell-derived precursors as sources for cell replacement

In experimental stroke, it has been shown that stem/precursor cells exhibit the capacity to differentiate into mature neurons and glial cells once migrated into lesioned brain areas [8,23]. Differentiated neurons may reveal histochemical characteristics of host cells, producing neurotransmitters such as serotonin, GABA, acetyl choline or substance P, and forming dendritic branches that grow out into the cell environment [23]. Electrophysiological studies of these neurons may show characteristics of resting potentials, membrane currents and action potentials very similar to mature neurons [23].

However, only a small percentage of transplanted cells undergo terminal differentiation in the host tissue [12] and the number of differentiated transplanted cells remains very small compared with the large number of cells that are lost following a stroke, even when optimistic stereological estimations are used. Thus, doubts have been raised whether the improvement of function may indeed be a consequence of a significant cell replacement phenomenon. Since not only neurons, but also the tissue matrix, is injured by a stroke, hopes that three-dimensional tissue architectures may easily be reconstructed by transplantation of loose cells have largely been abandoned in the meantime [12].

6. Supportive effects: direct and indirect neuroprotective actions of NPCs

Experimental evidence does not strongly support the possibility that recovery from brain damage *via* cell replacement is easily achievable in stroke *via* transplantation of both ES and adult NPCs [8,23]. However, these cells may promote the survival and remodeling of the injured brain *via* the so-called *bystander effect*, which defines the capacity of NPCs to exert direct neuroprotection through neutralization of free radicals, inflammatory cytokines, excitotoxins, lipases peroxidases and other toxic metabolites that are released following an ischemic event [40]. In addition to these effects, it has also been shown that adult NPCs may exert an *immunomodulatory* action, while in an undifferentiated state, causing a profound downregulation of inflammatory T cells and macrophages within inflamed brain areas [15,38,39,41–43].

7. Supportive effects: plasticity-promoting actions of NPCs

Once ischemic injury has developed, the affected tissue activates recovery processes aiming at the restoration of function. Factors released in a paracrine and/or endocrine way by injured as well as spared nervous tissue (neurons, glia, inflammatory cells) induce axonal sprouting of surviving neurons, facilitating the formation of new synaptic contacts that take over lost functions [44,45]. The timing and coordination of such events is crucial for the success of the regenerative process [44].

Synaptic plasticity can be enhanced by physical activity, as well as by experimental manipulations aiming at the antagonization of growth-repulsive influences [45]. NPCs might have important roles in augmenting recovery processes and scavenging inhibitory molecules that might limit the reorganization of the injured brain [46]. That cell-based therapies may indeed enhance the reorganization of white matter tracts surrounding an ischemic infarct has recently been shown by magnetic resonance imaging using fractional anisotropy (FA) and diffusion tensor imaging (DTI) sequences [21].

8. Genetically-modified NPCs as tool for drug delivery

Studies in experimental models of ischemic stroke have shown that a major limitation for the efficacy of neuroprotective therapies is the inability of drugs to reach their target tissue [6,47]. In view of their pathotropism and their long-term persistence in target tissues, NPCs represent a promising vehicle for targeted drug delivery. The innate capacity of stem cells to release protective molecules can further be increased by genetically transfecting cells to secrete additional neuroprotective peptides [48] or molecules that modify the transplanted stem cell fate [49]. In Parkinson's disease, NPC transplants secreting glial-derived neurotrophic factor (GDNF) and vascular-endothelial growth factor (VEGF) have shown beneficial results in experimental studies and are presently assessed in pre-clinical trials [50].

9. Recommendations for clinical trials

Recent evidence consistently challenges the sole and limited view that neural stem/precursor cells may protect the CNS from inflammatory damage leading to neurodegeneration exclusively throughout cell replacement. As a matter of fact, NPC transplantation may also promote CNS repair *via* intrinsic *neuroprotective* bystander capacities, mainly exerted by undifferentiated stem cells releasing, at the site of tissue damage, a milieu of *survival promoting* molecules whose *in situ* release is temporally and spatially orchestrated by environmental needs. Thus, the concept of 'therapeutic plasticity' is emerging since stem/precursor cells might adapt their fate and function(s) to specific environmental needs occurring as a result of different pathological conditions. The challenging ability of transplanted NPCs to protect the brain from several types of injuries using different and/or articulated *bystander* strategies is of pivotal importance for the future of stem cell-based therapeutic approaches in humans.

In preparation for such trials, molecular actions of NPCs should be evaluated more thoroughly in the stroke brain. Specifically, additional insight from animal experiments is urgently needed regarding the long-term effects of NPCs in the host organism, which should preferably be obtained also in primates, in order to test the safety of the cells used. In subsequent patient trials, control conditions should stringently be included in phase II studies, possibly in form of crossover designs, in which a verum and placebo are delivered at two different time points. This is necessary, as control conditions provide the only possibility to provide the proof-of-concept of the efficacy of NPCs. With concerted efforts on an EU basis, it should be possible soon to obtain reliable data about the efficacy of NPCs in ischemic stroke.

References

[1] Truelsen T, Piechowski-Jozwiak B, Bonita R, Mathers C, Bogousslavsky J, Boysen G. Stroke incidence and prevalence in Europe: a review of available data. Eur J Neurol 2006;13:581–98.

[2] Sacco S, Marini C, Sucapane P, Carolei A. Stroke in Italy: a disease to prevent. Clin Exp Hypertens 2006;28:199–203.

[3] Hossmann KA. Pathophysiology and therapy of experimental stroke. Cell Mol Neurobiol 2006;26(7–8):1057–83.

[4] Broderick JP, Hacke W. Treatment of acute ischemic stroke: Part I: recanalization strategies. Circulation 2002;106:1563–9.

[5] Sacco RL, Adams R, Albers G, Alberts MJ, Benavente O, Furie K, et al. Guidelines for prevention of stroke in patients with ischemic stroke or transient ischemic attack: a statement for healthcare professionals from the American Heart Association/American Stroke Association Council on Stroke. Circulation 2006;113:e409–49.

[6] Spudich A, Kilic E, Xing H, Kilic U, Rentsch KM, Wunderli-Allenspach H. Inhibition of multidrug resistance transporter-1 facilitates neuroprotective therapies after focal cerebral ischemia. Nat Neurosci 2006;9:487–8.

[7] Altman J, Das GD. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol 1965;124:319–35.

[8] 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.

[9] Jin K, Wang X, Xie L, Mao XO, Zhu W, Wang Y, et al. Evidence for stroke-induced neurogenesis in the human brain. Proc Natl Acad Sci U S A 2006;103:13198–202.

[10] Nakatomi H, Kuriu T, Okabe S, Yamamoto S, Hatano O, Kawahara N, et al. Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. Cell 2002;110:429–41.

[11] Zhang RL, Zhang ZG, Chopp M. Neurogenesis in the adult ischemic brain: generation, migration, survival, and restorative therapy. Neuroscientist 2005;11:408–16.

[12] Martino G, Pluchino S. The therapeutic potential of neural stem cells. Nat Rev Neurosci 2006;7:395–406.

[13] Lindvall O, Kokaia Z. Stem cells for the treatment of neurological disorders. Nature 2006;441:1094–6.

[14] 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;149:310–21.

[15] 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.

[16] 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;33:2270–8.

[17] Kelly S, Bliss TM, Shah AK, Sun GH, Ma M, Foo WC, et al. Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex. Proc Natl Acad Sci U S A 2004;101:11839–44.

[18] Chu K, Kim M, Park KI, Jeong SW, Park HK, Jung KH, et al. Human neural stem cells improve sensorimotor deficits in the adult rat brain with experimental focal ischemia. Brain Res 2004;1016:145–53.

[19] Ishibashi S, Sakaguchi M, Kuroiwa T, Yamasaki M, Kanemura Y, Shizuko I, et al. Human neural stem/progenitor cells, expanded in long-term neurosphere culture, promote functional recovery after focal ischemia in Mongolian gerbils. J Neurosci Res 2004;78:215–23.

[20] Jiang Q, Zhang ZG, Ding GL, Zhang L, Ewing JR, Wang L, et al. Investigation of neural progenitor cell induced angiogenesis after embolic stroke in rat using MRI. Neuroimage 2005;28:698–707.

[21] Jiang Q, Zhang ZG, Ding GL, Silver B, Zhang L, Meng H, et al. MRI detects white matter reorganization after neural progenitor cell treatment of stroke. NeuroImage 2006;32:1080–9.

[22] Pollock K, Stroemer P, Patel S, Stevanato L, Hope A, Miljan E, et al. A conditionally immortal clonal stem cell line from human cortical neuroepithelium for the treatment of ischemic stroke. Exp Neurol 2006;199:143–55.

[23] Bühnemann C, Scholz A, Bernreuther C, Malik CY, Braun H, Schachner M, et al. Neuronal differentiation of transplanted embryonic stem cell-derived precursors in stroke lesions of adult rats. Brain 2006;129(Pt 12):3238–48.

[24] Hayashi J, Takagi Y, Fukuda H, Imazato T, Nishimura M, Fujimoto M, et al. Primate embryonic stem cell-derived neuronal progenitors transplanted into ischemic brain. J Cereb Blood Flow Metab 2006;26:906–14.

[25] Park KI, Hack MA, Ourednik J, Yandava B, Flax JD, Stieg PE, et al. Acute injury directs the migration, proliferation, and differentiation of solid organ stem cells: evidence from the effect of hypoxia–ischemia in the CNS on clonal "reporter" neural stem cells. Exp Neurol 2006;199:156–78.

[26] Roitberg BZ, Mangubat E, Chen EY, Sugaya K, Thulborn KR, Kordower JH, et al. Survival and early differentiation of human neural stem cells transplanted in a nonhuman primate model of stroke. J Neurosurg 2006;105:96–102.

[27] Kondziolka D, Wechsler L, Goldstein S, Meltzer C, Thulborn KR, Gebel J, et al. Transplantation of cultured human neuronal cells for patients with stroke. Neurology 2000;55:565–9.

[28] Nelson PT, Kondziolka D, Wechsler L, Goldstein S, Gebel J, DeCesare S, et al. Clonal human (hNT) neuron grafts for stroke therapy:

neuropathology in a patient 27 months after implantation. Am J Pathol 2002;160:1201–6.

[29] Kondziolka D, Steinberg GK, Wechsler L, Meltzer CC, Elder E, Gebel L, et al. Neurotransplantation for patients with subcortical motor stroke: a phase 2 randomized trial. J Neurosurg 2005;103:38–45.

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

[31] 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.

[32] Erdo F, Buhrle C, Blunk J, Hoehn M, Xia Y, Fleischmann B, et al. Host-dependent tumorigenesis of embryonic stem cell transplantation in experimental stroke. J Cereb Blood Flow Metab 2003;23:780–5.

[33] Baker M. Stem cell therapy or snake oil? Nat Biotechnol 2005;23:1467–9.

[34] Hoehn M, Kustermann E, Blunk J, Wiedermann D, Trapp T, Wecker S, et al. 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 2002;99:16267–72.

[35] Aboody KS, Brown A, Rainov NG, Bower KA, Liu S, Yang W, et al. Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. Proc Natl Acad Sci U S A 2000;97:12846–51.

[36] Ben-Hur T, Einstein O, Mizrachi-Kol R, Ben-Menachem O, Reinhartz E, Karussis D, et al. Transplanted multipotential neural precursor cells migrate into the inflamed white matter in response to experimental autoimmune encephalomyelitis. Glia 2003;41:73–80.

[37] Imitola J, Raddassi K, Park KI, Mueller FJ, Nieto M, Teng YD, et al. Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. Proc Natl Acad Sci U S A 2004;101:18117–22.

[38] Pluchino S, Quattrini A, Brambilla E, Gritti A, Salani G, Dina G, et al. Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. Nature 2003;422:688–94.

[39] Pluchino S, Zanotti L, Rossi B, Brambilla E, Ottoboni L, Salani G, et al. Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. Nature 2005;436:266–71.

[40] Ourednik J, Ourednik V, Lynch WP, Schachner M, Snyder EY. Neural stem cells display an inherent mechanism for rescuing dysfunctional neurons. Nat Biotechnol 2002;20:1103–10.

[41] Chen SH, Chang FM, Tsai YC, Huang KF, Lin CL, Lin MT. Infusion of human umbilical cord blood cells protect against cerebral ischemia and damage during heatstroke in the rat. Exp Neurol 2006;199:67–76.

[42] Zhang J, Li Y, Chen J, Yang M, Katakowski M, Lu M, et al. Expression of insulin-like growth factor 1 and receptor in ischemic rats treated with human marrow stromal cells. Brain Res 2004;1030:19–27.

[43] Einstein O, Fainstein N, Vaknin I, Mizrachi-Kol R, Reihartz E, Grigoriadis N, et al. Neural precursors attenuate autoimmune encephalomyelitis by peripheral immunosuppression. Ann Neurol 2007;61:209–18.

[44] Wieloch T, Nikolich K. Mechanisms of neural plasticity following brain injury. Curr Opin Neurobiol 2006;16:258–64.

[45] Raineteau O, Schwab ME. Plasticity of motor systems after incomplete spinal cord injury. Nat Rev Neurosci 2001;2:263–73.

[46] Emsley JG, Mitchell BD, Magavi SS, Arlotta P, Macklis JD. The repair of complex neuronal circuitry by transplanted and endogenous precursors. NeuroRx 2004;1:452–71.

[47] Kilic E, Dietz GP, Hermann DM, Bähr M. Intravenous TAT-Bcl-Xl is protective after middle cerebral artery occlusion in mice. Ann Neurol 2002;52:617–22.

[48] Muller FJ, Snyder EY, Loring JF. Gene therapy: can neural stem cells deliver? Nat Rev Neurosci 2006;7:75–84.

[49] Park KI, Himes BT, Stieg PE, Tessler A, Fischer I, Snyder EY. Neural stem cells may be uniquely suited for combined gene therapy and cell replacement: evidence from engraftment of neurotrophin-3-expressing stem cells in hypoxic–ischemic brain injury. Exp Neurol 2006;199:179–90.

[50] Akerud P, Canals JM, Snyder EY, Arenas E. Neuroprotection through delivery of glial cell line-derived neurotrophic factor by neural stem cells in a mouse model of Parkinson's disease. J Neurosci 2001;21:8108–18.